Synthesis and Cytotoxic Activity of Certain New Arylazothiazole Containing Compounds

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2-Amino-5-arylazo-4-(2-chlorophenyl)thiazoles **(2a-e)** were prepared by the coupling of aryldiazonium chlorides with 2-amino-4-(2-chlorophenyl) thiazole **(1)**. The thioureas **3a-e** were obtained by condensing the arylazothiazoles **2a-c** with the appropriate isothiocyanates. Reaction of **2d** with aromatic aldehydes afforded the chalcone analogues **4a-c**. The pyridone derivatives **5a,b** were synthesized by reacting the ketone **2d** with different aromatic aldehydes, ethyl cyanoacetate and ammonium acetate. On the other hand, **5b** was also prepared by cyclizing **4c** with ethyl cyanoacetate and ammonium acetate. Furthermore, 6-chloroimidazo[2,1-*b*]-thiazole **7** was obtained from the acid derivative **6b** by treatment with $POCl_3$. While, the imidazo[2,1-*b*]-thiazolones **9a-d** were produced by the cyclization of the chloroacetyl derivatives **8a-d** with DMAP/pyridine. Representative examples of the prepared compounds were tested for *in vitro* antitumor activity against two human tumor cell lines. Some compounds showed activity against brain tumor cell lines.

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A considerable number of thiazole derivatives have been found to exhibit a wide variety of biological activity [1-5]. In addition, some derivatives of 2-aminothiazoles bearing an arylazo moiety at position 5 have shown cytostatic and cytopathic activities [6]. Keeping this in view it was thought of interest to synthesize certain arylazothiazole containing compounds in a trial to obtain compounds of anticipated antitumor value (*cf.* Scheme 1).

2-Amino-4-(2-chlorophenyl)thiazole (**1**) was prepared [*cf.* 6] in 70% yield *via* reaction of 2-chloroacetophenone with thiourea in the presence of iodine. This compound was previously prepared in 49% yield by the reaction of 2-chlorophenyldiazomethylketone with thiourea in the presence of Cu powder or CuBr [7]. Coupling **1** with some diazotized arylamines furnished the required 2-amino-5-arylazo-4-(2-chlorophenyl)thiazoles (**2a-e)**. Preparation of compound **2a** has been mentioned [8] but the procedure lacked experimental details, physical and spectral data. Condensing 2-aminothiazoles **2a-c** with some arylisothiocyanates brought about the thioureas **3a-e**.

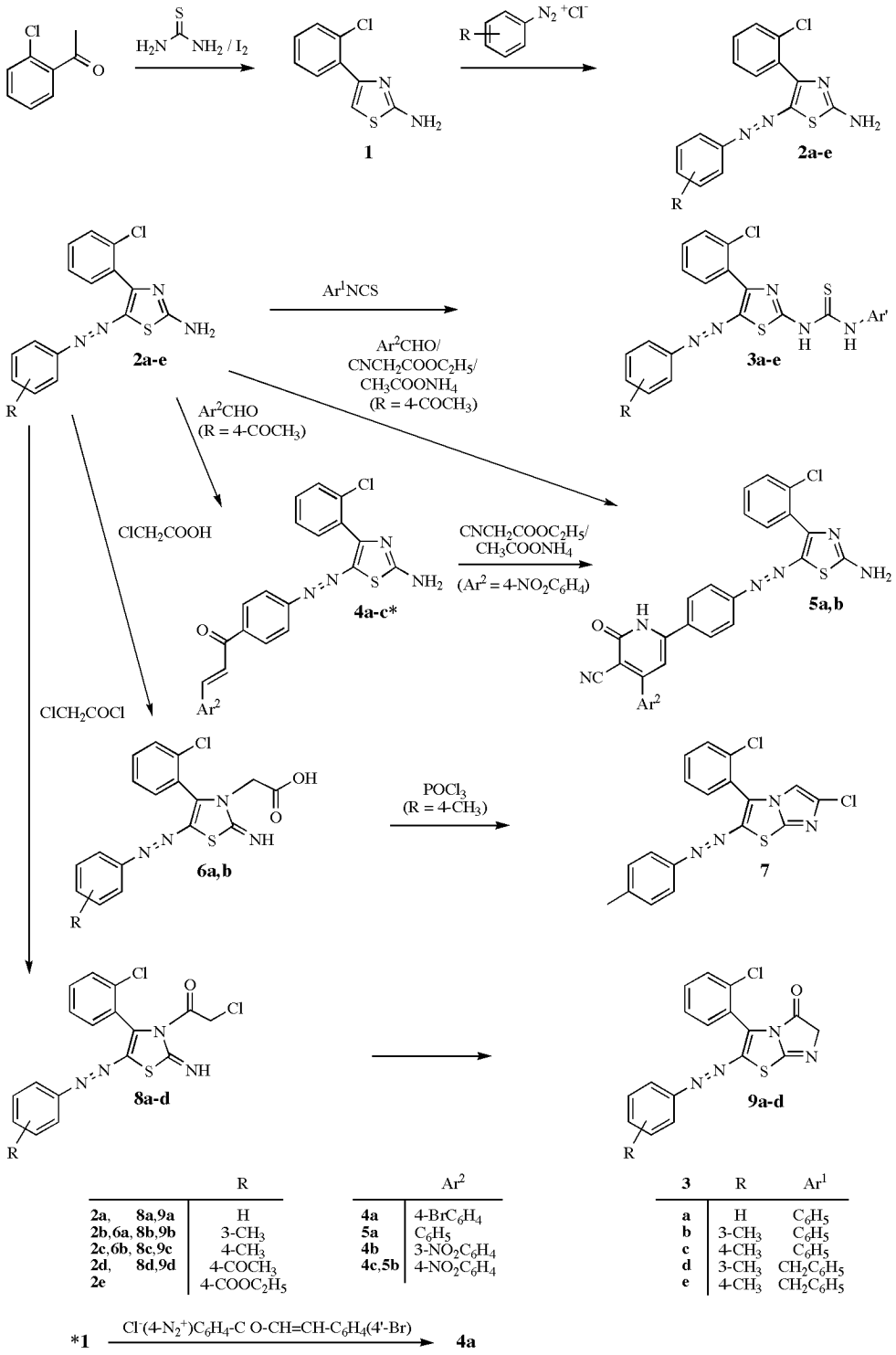
During this investigation attempts to obtain Schiff bases by heating 2-aminothiazole **2a** with the suitable aromatic aldehyde at reflux temperature in ethanol, 1-butanol or ethanol/acetic acid (3:1) for 25 hours were fruitless and the starting 2-aminothiazole **2a** was recovered. According to these experimental findings, the required chalcones **4a-c** were obtained *via* condensing the ketone **2d** with some aromatic aldehydes in ethanol/acetic acid (3:1). Moreover, a second method was undertaken for the preparation of **4a** by coupling of the diazonium salt of the prepared 1-(4-aminophenyl)-3-(4'-bromophenyl)-2-propen-1-one [9] with 2-aminothiazole **1**. Indeed, the overall yield in the two procedures was nearly equal.

In this work interacting **2d** with the suitable aromatic aldehyde, ethyl cyanoacetate and ammonium acetate in 1-butanol gave rise to the pyridones **5a,b**. Another approach for the preparation of the pyridone **5b** was *via* reacting the chalcone analogue **4c** with ethyl cyanoacetate and ammonium acetate in 1-butanol. Although, the latter is a two-step method yet the overall yield is higher than the former. It is interesting to note that the one-step synthesis produced compounds **5a,b** in 50-55% yield with the ketone **2d** which was detected by thin layer chromatography. Facile removal of the starting ketone **2d** was effected by silica gel column chromatography.

A search in the literature [10-13] showed that formation of imidazo[2,1-*b*]thiazoles could be obtained through reacting a 2-aminothiazole derivative with α-halocarbonyl compounds. It was also registered [11,13]that the reaction started by attack of the thiazole ring nitrogen on the α-halocarbon compound followed by cyclization. Accordingly, treating the arylazothiazoles **2b,c** with chloroacetic acid afforded 3-carboxymethyl-2-iminothiazolines **6a,b.** Reacting **6b** with $POCl_3$ [*cf.* 14] gave 6-chloroimidazo[2,1-*b*]thiazole **7** in a poor yield. The IR spectrum of **7** revealed the absence of the strong absorption due to NH/OH as well as the C=O function at 3450-2850 and 1690 cm^{-1} respectively. Also, the ^{1}H-NMR spectrum of **7** has confirmed its structure since it showed the absence of OH and NH signals.

In this study it was decided to interact arylazothiazoles **2a-d** with chloroacetyl chloride. This interaction was first conducted in dry benzene containing a few drops of triethylamine at room temperature but the 3-chloroacetyl derivatives **8a-d** were produced in good yields. When equimolar amounts of the two reactants were heated under reflux in dry benzene or dry pyridine for up to 10 hours, the open form intermediates **8a-d** (major products) alongside the imidazo[2,1-*b*]thiazoles **9a-d** (minor products) were obtained (TLC). Attempts to cyclize **8a,b** into the corresponding imidazo[2,1-*b*]thiazole by refluxing in 2 *M* HBr furnished **9a,b** as the hydrobromide salts which were

Scheme 1



	R
2a, **8a**,**9a**	H
2b,**6a**, **8b**,**9b**	3-CH_3
2c,**6b**, **8c**,**9c**	4-CH_3
2d, **8d**,**9d**	4-$COCH_3$
2e	4-$COOC_2H_5$

	Ar^2
4a	4-BrC_6H_4
5a	C_6H_5
4b	3-$NO_2C_6H_4$
4c,**5b**	4-$NO_2C_6H_4$

3	R	Ar^1
a	H	C_6H_5
b	3-CH_3	C_6H_5
c	4-CH_3	C_6H_5
d	3-CH_3	$CH_2C_6H_5$
e	4-CH_3	$CH_2C_6H_5$

*1 $\xrightarrow{Cl^-(4\text{-}N_2^+)C_6H_4\text{-}C\ O\text{-}CH=CH\text{-}C_6H_4(4'\text{-}Br)}$ **4a**

converted into the bases **9a,b** using NH_4OH [*cf.* 13]. By the application of this method ,the yield of both **9a** and **9b** was considerably low (15% and 17% yield respectively). However, the imidazo[2,1-*b*]thiazoles **9a-d** were produced in good yields *via* cyclizing the 3-chloroacetyl derivatives **8a-d** with dimethylaminopyridine (DMAP) in dry pyridine. The spectral data of **9a-d** ascertain their structure since IR spectra disclosed no absorption bands at 3450-3400 cm^{-1} for NH. Also, the absence of the NH group signal in the ^{1}H-NMR confirmed the imidazo[2,1-*b*] thiazole structure.

In Vitro Cytotoxic Activity.

Representative members of the synthesized compounds **2a-d**, **3b**, **4a**, **5b**, **8a** and **9a** were screened for potential anti-tumor activity against two human tumor cell lines namely brain tumor (U251) and breast tumor (MCF-7) cell lines.

In routine screening each compound is tested over a broad concentration range against every cell line. All lines are inoculated onto a series of standard 96-well microplates and incubated for 24 hours in humidified 5% carbon dioxide incubator. The inoculation densities employed depend upon the particular cell line and its growth characteristics. Tested compounds **2a-d**, **3b, 4a**, **5b**, **8a** and **9a** are routinely evaluated in range of 1-10 μg/mL solution in DMSO which is used for solubility of the compounds. Control cells were incubated with the same quantity of the DMSO solvent. After 24 hours of compound incubation, the cells are assayed by the sulphorhodamine B (SRB) assay according to the method of Skehan and co-workers [15].

IC50 which is the concentration (μg/mL) that inhibits 50% of the tumor growth is the parameter used for evaluation of each compound. The tested compounds will be either active or inactive.

Conclusion.

The tested thiazole derivatives showed no activity against both cell lines used even at high concentration (10μg/mL) except compounds **8a** and **9a** where they showed high activity against brain tumor cell line (U251) since IC50 was at 1.5 and 8 μg/mL respectively. Also, compound **2a** showed activity only at high concentration (IC50 was 10μg/mL) against the brain tumor cell line.

EXPERIMENTAL

Melting points were determined on a Griffin apparatus and are uncorrected . Microanalyses were performed by the Microanalytical Center, Cairo University, Egypt. IR spectra were recorded as KBr discs on Shimadzu IR 435 spectrophotometer and expressed in cm^{-1}. ^{1}H-NMR spectra were carried out on Varian Gemini 200 MHz and Jeol FXQ-90 MHz spectrophotometers with TMS as an internal standard (chemical shift values in ppm on δ scale) and EIMS spectra on Hewlett-Packard 5988 spectrometer. The progress of the reaction was monitored by TLC using aluminium sheets precoated with UV fluorescent silica gel (Merck 60 F254) and using benzene-methanol (8:2) as eluant.

2-Chloroacetophenone was obtained commercially from Aldrich fine chemicals. 1-(4-Aminophenyl)-3-(4'-bromophenyl)-2-propen-1-one [9] was prepared according to reported procedure.

2-Amino-4-(2-chlorophenyl)thiazole **(1)**.

A mixture of 2-chloroacetophenone (15.45 g; 0.1 mol), thiourea (15.20 g ; 0.2 mol) and iodine (25.40 g ; 0.1 mol) was heated 12 hours on a steam bath . The mixture was cooled and washed with ether. It was then diluted with water (200 mL), heated until most of the solid had gone into solution and filtered while hot. The filtrate was cooled and made alkaline with a strong solution of ammonia. The separated solid was collected by filtration, washed with water and recrystallized from acetone; yield (70 %), mp: 142-44 °C [7].

General Procedure for the Synthesis of 2-Amino-5-arylazo-4-(2-chlorophenyl)thiazoles (**2a-e**).

An ice-cold solution of sodium nitrite (1.38 g; 0.02 mol) in water (25 mL) was added slowly to a solution of the appropriate arylamine (0.02 mol) in HCl (6 mL) at 0 °C. To a well-cooled solution of **1** (4.21 g, 0.02 mol) and sodium acetate (10 g) in ethanol (50 mL) was gradually added the diazonium salt solution while stirring and cooling (-5 °C). The reaction mixture was stirred at –5 °C for 2 hours, and then diluted with cold water (50 mL). The separated solid was collected by filtration, washed with water and crystallized from ethanol/ether.

2-Amino-4-(2-chlorophenyl)-5-phenylazothiazole (**2a**).

This compound was prepared according to the general procedure above in a yield of 90%, mp 174-176 °C; IR: 3400-3300 (d of NH_2), 1620 (N=N), 1585, 1470, 1340, 1020 and 810 (st. of thiazole nucleus); ^{1}H-NMR (DMSO-d_6): δ 7.51-8.21(m, 9H, Ar-H), 8.80 (br, 2H, NH_2, D_2O exch.).

Anal. Calcd. for $C_{15}H_{11}ClN_4S$: C, 57.23; H, 3.49; N, 17.80. Found: C, 57.00; H, 3.70; N, 17.60.

2-Amino-4-(2-chlorophenyl)-5-(3-tolylazo)thiazole (**2b**).

This compound was prepared according to the general procedure above in a yield of 93%, mp 163-165 °C; IR: 3400-3300 (d of NH_2), 1610 (N=N), 1585, 1470, 1340, 1020 and 810 (st. of thiazole nucleus); ^{1}H-NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 7.37-8.16 (m, 8H, Ar-H), 8.62 (br, 2H, NH_2, D_2O exch.).

*Anal.*Calcd. for $C_{16}H_{13}ClN_4S$: C, 58.44; H, 3.95; N, 17.04. Found: C, 58.70; H, 3.90; N, 17.20.

2-Amino-4-(2-chlorophenyl)-5-(4-tolylazo)thiazole (**2c**).

This compound was prepared according to the general procedure above in a yield of 92%, mp 183-185 °C; IR: 3400-3300 (d of NH_2), 1630 (N=N), 1585, 1470, 1340, 1020 and 810 (st. of thiazole nucleus); ^{1}H-NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 7.29-8.19 (m, 8H, Ar-H), 8.62 (br, 2H, NH_2, D_2O exch.).

Anal. Calcd. for $C_{16}H_{13}ClN_4S$: C, 58.44; H, 3.95; N, 17.04. Found: C, 58.60; H, 4.20; N, 16.80.

5-(4-Acetylphenylazo)-2-amino-4-(2-chlorophenyl)thiazole (**2d**).

This compound was prepared according to the general procedure above in a yield of 95%, mp 244-245 °C; IR: 3400-3300 (d of NH_2), 1670 (C=O), 1630 (N=N), 1585, 1470, 1340, 1020 and 810 (st. of thiazole nucleus); ^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 7.51-8.17 (m, 8H, Ar-H), 8.66 (br, 2H, NH_2, D_2O exch.).

*Anal.*Calcd. for $C_{17}H_{13}ClN_4OS$: C,57.22; H, 3.64; N, 15.70. Found: C, 56.90; H, 3.90; N, 15.80.

2-Amino-5-(4-carbethoxyphenylazo)-4-(2-chlorophenyl)thiazole (**2e**).

This compound was prepared according to the general procedure above in a yield of 93%, mp 260-262 °C; IR: 3400-3300 (d of NH_2), 1710 (C=O), 1640 (N=N), 1585, 1470, 1340, 1020 and 810 (st. of thiazole nucleus); ^{1}H-NMR(DMSO-d_6): δ 1.33 (t, *J*=8

Hz, 3H, OCH_2 -C*H*$_3$), 4.31 (q, *J*=8 Hz, 2H, OC*H*$_2$$CH_3$), 7.50-8.25 (m, 8H, Ar-H), 8.74 (br, 2H, NH_2, D_2O exch.).

Anal. Calcd. for $C_{18}H_{15}ClN_4O_2S$: C, 55.88; H, 3.88 ; N, 14.48. Found: C, 56.00; H, 3.70; N, 14.30.

General Procedure for the Synthesis of 5-Arylazo-2-(3-arylthioureido)-4-(2-chlorophenyl)thiazoles (**3a-e**).

A solution of the appropriate isothiocyanate (0.002 mol) and the selective arylazothiazole **2a-c** (0.002 mol) in benzene (20 mL) was refluxed for 13 hours on a steam bath. The solvent was distilled off and the residue was treated with petroleum ether 40-60 °C and then with ether. The crystalline product so obtained was recrstallized from DMF/ethanol.

4-(2-Chlorophenyl)-5-phenylazo-2-(3-phenylthioureido)thiazole (**3a**).

This compound was prepared according to the general procedure above in a yield of 62%, mp 114-116 °C; IR: 3450,3250 (NH); 1640 (N=N), 1370 (C=S); ^{1}H-NMR (DMSO-d_6): δ 7.12-8.21 (m, 14H, Ar-H), 8.38 (s, 1H, NH of NH-C_6H_5, D_2O exch.); EIMS for **3a**: m/z 449 (1%, $M^{+\cdot}$).

Anal. Calcd. for $C_{22}H_{16}ClN_5S_2$: C, 58.73; H, 3.55; N, 15.57. Found: C,59.00; H, 3.70; N, 15.50.

4-(2-Chlorophenyl)-2-(3-phenylthioureido)-5-(3-tolylazo)-thiazole (**3b**).

This compound was prepared according to the general procedure above in a yield of 65%, mp 142-144 °C; IR: 3450, 3300 (NH); 1640 (N=N), 1340 (C=S); ^{1}H-NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 7.37-8.22 (m, 13H, Ar-H), 8.38 (s, 1H, NH of NH-C_6H_5, D_2O exch.), 11.04 (S, 1H, NH of –NHCS, D_2O exch.).

Anal. Calcd. $C_{23}H_{18}ClN_5S_2$: C, 59.54; H, 3.88; N, 15.10. Found: C, 59.80; H, 4.00; N, 14.80.

4-(2-Chlorophenyl)-2-(3-phenylthioureido)-5-(4-tolylazo)-thiazole (**3c**).

This compound was prepared according to the general procedure above in a yield of 63%, mp 200-201 °C; IR: 3450, 3300 (NH); 1640 (N=N), 1340 (C=S); ^{1}H-NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 7.13-8.17 (m, 13H, Ar-H), 8.38 (s, 1H, NH of NH-C_6H_5, D_2O exch.).

Anal. Calcd. for $C_{23}H_{18}ClN_5S_2$: C, 59.54; H, 3.88; N, 15.10. Found: C, 59.70; H, 3.60; N, 15.20.

2-(3-Benzylthioureido)-4-(2-chlorophenyl)-5-(3-tolylazo)-thiazole (**3d**).

This compound was prepared according to the general procedure above in a yield of 58%, mp 185-186 °C; IR: 3450, 3300 (NH); 1640 (N=N), 1340 (C=S); ^{1}H-NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 7.21-8.23 (m, 13H, Ar-H), 8.38 (s, 1H, NH of NH-C_6H_5, D_2O exch.).

Anal. Calcd. for $C_{24}H_{20}ClN_5S_2$: C, 60.31; H, 4.18; N, 14.65. Found: C, 60.10; H, 3.90; N, 14.90.

2-(3-Benzylthioureido)-4-(2-chlorophenyl)-5-(4-tolylazo)-thiazole (**3e**).

This compound was prepared according to the general procedure above in a yield of 55%, mp 206-207 °C; IR: 3450, 3300 (NH); 1640 (N=N), 1340 (C=S); ^{1}H-NMR (DMSO-d_6): δ 2.39 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 7.32-8.04 (m, 13H, Ar-H), 8.38 (s, 1H, NH of NH-C_6H_5, D_2O exch.), 11.80 (S, 1H, NH of –NHCS, D_2O exch.).

Anal. Calcd. $C_{24}H_{20}ClN_5S_2$: C, 60.31; H, 4.18; N, 14.65. Found: C, 60.10; H, 4.40; N, 14.50.

General Procedures for the Synthesis of 2-Amino-4-(2-chlorophenyl)-5-[4-(substituted cinnamoyl)phenylazo]thiazoles **(4a-c)**.

Method A: for Compounds **4a-c**.

To a solution of **2d** (1.07 g, 0.003 mol) in EtOH/AcOH (3:1) (30mL), the appropriate aromatic aldehyde (0.003 mol)was added with stirring . The reaction mixture was stirred and refluxed for 8 hours, then cooled and poured onto cold water. The formed precipitate was collected by filtration and crystallized from dioxane.

Method B: for Compound **4a**.

An ice-cold solution of sodium nitrite (0.14 g, 0.002 mol) in water (2.5 mL) was added slowly to a solution of 1-(4-aminophenyl)-3-(4'-bromophenyl)-2-propen-1-one (0.60 g, 0.002 mol) in HCl (1 mL) at 0 °C. To a well cooled solution of compound **1** (0.42 g, 0.002 mol) and sodium acetate (1 g) in ethanol (25 mL) was gradually added the diazonium salt solution while stirring and cooling (-5 °C). The reaction mixture was stirred at -5 °C for 4 hours and then diluted with cold water (30 mL). The separated solid was collected by filtration, washed with water and crystallized from dioxane.

2-Amino-5-[4-(4-bromocinnamoyl)phenylazo]-4-(2-chlorophenyl)thiazole (**4a**).

This compound was prepared according to the general procedures above in method A and method B in a yield of 80% and 78% respectively, mp 262-264 °C; IR: 3400-3300(d of NH_2) 1650 (C=O); ^{1}H-NMR (DMSO-d_6): δ 6.72 (d, *J*=18 Hz, 1H, CH=), 7.35-7.84 (m, 12H, Ar-H), 8.00 (d, *J*=18 Hz, 1H, CH=), 8.40 (br, 2H, NH_2, D_2O exch.).

Anal. Calcd. $C_{24}H_{16}BrClN_4OS$: C, 55.01; H, 3.05; N, 10.69. Found: C, 55.30; H, 3.20; N, 10.40.

2-Amino-4-(2-chlorophenyl)-5-[4-(3-nitrocinnamoyl)phenylazo]thiazole (**4b**).

This compound was prepared according to the general procedure above in method A in a yield of 82%, mp 250-252 °C; IR: 3400-3250(d of NH_2), 1660 (C=O), 1520 & 1340 (asym. & sym. St. of NO_2); ^{1}H-NMR (DMSO-d_6): δ 7.75 (d, *J*=18 Hz, 1H, CH=), 7.24-8.25 (m, 12H, Ar-H), 8.34 (d, *J*=18 Hz, 1H, CH=), 8.90 (br, 2H, NH_2 , D_2O exch.).

Anal. Calcd. for $C_{24}H_{16}ClN_5O_3S$: C, 58.83; H, 3.26; N, 14.30. Found: C, 59.10; H, 3.50; N, 14.60.

2-Amino-4-(2-chlorophenyl)-5-[4-(4-nitrocinnamoyl)phenylazo]thiazole (**4c**).

This compound was prepared according to the general procedure above in method A in a yield of 85%, mp 256-258 °C; IR: 3400-3300(d of NH_2), 1660 (C=O), 1530 & 1350 (asym. & sym. St. of NO_2); ^{1}H-NMR (DMSO-d_6): δ 7.75 (d, *J*=18 Hz, 1H, CH=), 7.54-8.26 (m, 12H, Ar-H), 8.36 (d, *J*=18 Hz, 1H, CH=), 8.81 (br, 2H, NH_2 , D_2O exch.).

Anal. Calcd. for $C_{24}H_{16}ClN_5O_3S$: C, 58.83; H, 3.26; N, 14.30. Found: C, 59.10; H, 3.50; N, 14.10.

General Procedures for the Synthesis of 2-Amino-5-[4-(4-aryl-5-cyano-6-oxo-1,6-dihydropyrid-2-yl)phenylazo]-4-(2-chlorophenyl)thiazoles **(5a,b)**.

Method A: for Compounds **5a,b**.

A solution of the arylazothiazole **2d** (1.07 g, 0.003 mol), the appropriate aromatic aldehyde (0.003, mol), ethyl cyanoacetate (0.34 g, 0.003 mol) and ammonium acetate (1.85 g, 0.024 mol) in 1-butanol (15 mL) was heated at reflux for 22 hours and then cooled in an ice-bath. The separated solid was collected by filtration and eluted through a column, packed with silica gel, with a mixture of benzene–ethyl acetate (9:1) in 40-60 mL fractions. The arylazothiazole **2d** was eluted at first (in fractions 3-8) followed by the pyridone derivatives **5a,b** (in fractions 12-18). A total volume of about 200 mL was used for the elution of each product. Eluted 1 and 2 on removal of the solvent systems under high vacuum and at 30 °C gave **2d** and **5a,b** respectively.

Method B: for Compound **5b**.

A solution of the chalcone analogue **4c** (1.47 g, 0.003 mol), ethyl cyanoacetate (0.34 g, 0.003 mol) and ammonium acetate (1.85 g, 0.024 mol) in 1-butanol (10 mL) was heated at reflux for 18 hours and then cooled. The separated solid was collected by filtration, washed with ethanol and crystallized from acetic acid.

Compounds **5a,b** are insoluble in the available solvents of ^{1}H-NMR.

2-Amino-4-(2-chlorophenyl)-5-[4-(5-cyano-6-oxo-4-phenyl-1,6-dihydropyrid-2-yl)phenylazo]thiazole (**5a**).

This compound was prepared according to the general procedure above in method A in a yield of 50%, mp 268-270 °C; IR: 3450-3300 (d of NH_2), 3100 (NH), 2200 (CN), 1640 (C=O); EIMS: m/z 510 (3.2%, $M+2^{+\cdot}$) & 508 (9.72%, $M^{+\cdot}$).

Anal. Calcd. for $C_{27}H_{17}ClN_6OS$: C, 63.71; H, 3.34; N, 16.51. Found: C, 63.80; H, 3.50; N, 16.30.

2-Amino-4-(2-chlorophenyl)-5-[4-(5-cyano-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrid-2-yl)phenylazo]thiazole (**5b**).

This compound was prepared according to the general procedures above in method A and method B in a yield of 55% and 72% respectively, mp 320-321 °C; IR: 3450-3300 (d of NH_2), 3100 (NH), 2200 (CN), 1640 (C=O), 1520 & 1340 (asym. & sym. St. of NO_2); EIMS: m/z 555 (15.10%, $M+2^{+\cdot}$) & 553 (45.45%, $M^{+\cdot}$).

Anal. Calcd. for $C_{27}H_{16}ClN_7O_3S$: C, 58.53; H, 2.89; N, 17.70. Found: C, 58.80; H, 2.80; N, 17.60.

General Procedure for the Synthesis of 5-Arylazo-3-carboxymethyl-4-(2-chlorophenyl)-2-iminothiazolines (**6a,b**).

A mixture of the arylazothiazole **2b** or **2c** (0.003 mol), sodium hydroxide (0.12 g , 0.003 mol), chloroacetic acid (0.28 g , 0.003 mol) and absolute ethanol (25 mL) was refluxed for 8 hours. The reaction mixture was poured onto water (30 mL) and the formed precipitate was collected by filtration, washed with water and crystallized from dioxane.

3-Carboxymethyl-4-(2-chlorophenyl)-2-imino–5-(3-tolylazo)-thiazoline (**6a**).

This compound was prepared according to the general procedure above in a yield of 85%, mp 190-192 °C; IR: 3450-2850 (NH/OH), 1690 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 4.81(s, 2H, CH_2), 7.14-7.94 (m, 8H, Ar-H), 9.98 (br, 1H, OH, D_2O exch.), 11.67(s, 1H, NH, D_2O exch.).

Anal. Calcd. for $C_{18}H_{15}ClN_4O_2S$: C, 55.88; H, 3.88; N, 14.48. Found: C, 56.10; H, 4.10; N, 14.20.

3-Carboxymethyl-4-(2-chlorophenyl)-2-imino–5-(4-tolylazo)thiazoline (**6b**).

This compound was prepared according to the general procedure above in a yield of 82%, mp 214-216 °C; IR: 3450-2850 (NH/OH), 1690 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 4.80 (s, 2H, CH_2), 7.20 –8.21(m,8H,Ar-H), 10.08 (br, 1H, OH, D_2O exch.).

Anal. Calcd. for $C_{18}H_{15}ClN_4O_2S$: C, 55.88; H, 3.88; N, 14.48. Found: 55.60; H, 4.10; N, 14.20.

6-Chloro-3-(2-chlorophenyl)–2-(4-tolylazo)imidazo[2,1-*b*]-thiazole(**7**).

A mixture of **6b** (1.16 g, 0.003 mol) and $POCl_3$ (6 mL) was heated under reflux for 4 hours during this time solution occurred. The excess $POCl_3$ was removed by evaporation under vacuum, leaving a dark syrupy residue. This syrup was poured with stirring into an ice-water mixture and the obtained solution was basified with aqueous NaOH. The separated solid was collected by filtration, dried and crystallized from petroleum ether 60-80 °C. Yield of 35%**,** mp 174-175 °C; IR: 1640 (N=N), 1630 (C=N); ^{1}H-NMR ($CDCl_3$): δ 2.39 (s, 3H , CH_3), 7.21-8.16 (m, 9H, Ar-H and imidazo proton).

Anal. Calcd. for $C_{18}H_{12}Cl_2N_4S$: C, 55.81; H, 3.10; N, 14.47. Found: C, 55.80; H, 3.10; N, 14.20.

General Procedure for the Synthesis of 5-Arylazo-3-chloromethylcarbonyl-4-(2-chlorophenyl)-2-iminothiazolines (**8a-d**).

To a solution of the appropriate arylazothiazole **2a-d** (0.005 mol) in dry benzene (10 mL) containing triethylamine (3 drops), chloroacetyl chloride (0.57 g, 0.005 mol) in dry benzene (5 mL) was added. The reaction mixture was stirred at room temperature for 2 hours and then filtered. The filtrate was evaporated under vacuum and the separated solid was crystallized from dioxane.

3-Chloromethylcarbonyl-4-(2-chlorophenyl)-2-imino-5-phenylazo-thiazoline (**8a**).

This compound was prepared according to the general procedure above in a yield of 85%, mp 210-211 °C; IR: 3400 (NH), 1700 (C=O); ^{1}H-NMR (DMSO-d_6): δ 4.50 (s, 2H, CH_2), 7.33-8.26 (m, 9H, Ar-H), 13.03 (s, 1H, NH, D_2O exch.).

Anal. Calcd. for $C_{17}H_{12}Cl_2N_4OS$: C, 52.17; H, 3.06; N, 14.32. Found: C, 52.10; H, 3.20; N, 14.20.

3-Chloromethylcarbonyl-4-(2-chlorophenyl)-2-imino-5-(3-tolylazo)thiazoline (**8b**).

This compound was prepared according to the general procedure above in a yield of 89%, mp 186-188 °C; IR: 3400 (NH), 1720 (C=O); ^{1}H-NMR (DMSO-d_6): δ 2.39 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 7.33-8.26 (m, 8H, Ar-H), 13.03 (s, 1H, NH, D_2O exch.).

Anal. Calcd. for $C_{18}H_{14}Cl_2N_4OS$: C, 53.33; H, 3.45; N, 13.82. Found: C, 53.10; H, 3.40; N, 13.70.

3-Chloromethylcarbonyl-4-(2-chlorophenyl)-2-imino-5-(4-tolylazo)thiazoline (**8c**).

This compound was prepared according to the general procedure above in a yield of 89%, mp 197-199 °C; IR: 3400 (NH),

1700 (C=O); ^{1}H-NMR (DMSO-d_6): δ 2.40 (s, 3 H, CH_3), 4.48 (s, 2H, CH_2), 7.37-8.25 (m, 8H, Ar-H), 12.8 (br, 1H, NH, D_2O exch.).

Anal. Calcd. for $C_{18}H_{14}Cl_2N_4OS$: C, 53.33; H, 3.45; N, 13.82. Found: C, 53.20; H, 3.70; N, 14.00.

5-(4-Acetylphenylazo)-3-chloromethylcarbonyl-4-(2-chlorophenyl)-2-iminothiazoline (**8d**).

This compound was prepared according to the general procedure above in a yield of 88%, mp 280-281 °C; IR: 3450 (NH), 1700 (C=O); 1665 (C=O); ^{1}H-NMR (DMSO-d_6): δ 2.51 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 7.54-8.24 (m, 8H, Ar-H), 13.09 (s ,1H , NH ,D_2O exch.).

Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O_2S$: C, 52.65; H, 3.23; N, 12.93. Found: C, 52.90; H, 3.20; N, 12.80.

General Procedure for the Synthesis of 2-Arylazo-3-(2-chlorophenyl)-6*H*-imidazo[2,1-*b*]thiazol-5-ones (**9a-d**).

A solution of the appropriate **8a-d** (0.002 mol), pyridine (0.16 g, 0.002 mol) and DMAP (0.24g, 0.002 mol) in THF (10 mL) was stirred at room temperature for 24 hours. The formed precipitate of pyridinium salt was filtered off and the filtrate was evaporated under vacuum. The remaining residue was crystallized from acetone.

3-(2-Chlorophenyl)-2-phenylazo-6*H*-imidazo[2,1-*b*]thiazol-5-one (**9a**).

This compound was prepared according to the general procedure above in a yield of 78%, mp 110-111 °C; IR: 1700 (C=O); ^{1}H-NMR ($CDCl_3$): δ 4.82 (s, 2H, CH_2), 7.36-8.18 (m, 9H, Ar-H).

Anal. Calcd. for $C_{17}H_{11}ClN_4OS$: C, 57.54; H, 3.10; N, 15.79. Found: C, 57.20; H, 3.30; N, 15.70.

3-(2-Chlorophenyl)-2-(3-tolylazo)-6*H*-imidazo[2,1-*b*]thiazol-5-one (**9b**).

This compound was prepared according to the general procedure above in a yield of 80%, mp 144-145 °C; IR: 1700 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 4.76 (s, 2H, CH_2), 7.37-8.26 (m, 8H, Ar-H).

Anal. Calcd. for $C_{18}H_{13}ClN_4OS$: C, 58.61; H, 3.52; N, 15.19. Found: C, 58.60; H, 3.50; N, 15.00.

3-(2-Chlorophenyl)-2-(4-tolylazo)-6H-imidazo[2,1-*b*]thiazol-5-one (**9c**).

This compound was prepared according to the general procedure above in a yield of 82%, mp 106-107 °C; IR: 1700 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 4.75 (s, 2H, CH_2), 7.23-8.20 (m, 8H, Ar-H); EIMS: m/z 368 (1.15%, $M^{+\cdot}$).

Anal. Calcd. for $C_{18}H_{13}ClN_4OS$: C, 58.61; H, 3.52; N, 15.19. Found: C, 58.90; H, 3.80; N, 15.30.

2-(4-Acetylphenylazo)-3-(2-chlorophenyl)-6*H*-imidazo[2,1-*b*]-thiazol-5-one (**9d**).

This compound was prepared according to the general procedure above in a yield of 82%, mp 216-218 °C; IR: 1700 (C=O), 1660(C=O); ^{1}H-NMR ($CDCl_3$): δ 2.61(s, 3H, CH_3), 4.83 (s, 2H, CH_2), 7.41-8.33 (m, 8H, Ar-H).

Anal. Calcd. for $C_{19}H_{13}ClN_4O_2S$: C, 57.50; H, 3.27; N, 14.12. Found: C, 57.20; H, 3.40; N, 13.90.

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