NEW APPROACHES FOR THE SYNTHESIS AND ANTITUMOR EVALUATION OF PYRIDINE, THIENO[3,4-*c*]PYRIDINE, PYRAZOLO[3,4-*b*]PYRIDINE AND PYRIDO[3,4-*d*]PYRIDAZINE DERIVATIVES

Karam A. El-Sharkawy[a] Rehab A. Ibrahim[b]

Keywords: Arylidenes, pyridines, thieno[*c*]pyridines, benzo[*c*]pyridines, antitumor activity

This work has been carried out to investigate the utility of arylidene acetoacetanilides **3a, b** in heterocyclic synthesis. Thus, the synthesis of pyridine derivatives **6a, b**, **8a-d**, **12a, b** were done by the reaction of arylidine derivatives **3a, b** with activated methylene groups **4a, b** also synthesis of compounds **15**, **21a**, **b**, **22a**, **b** were carried out via the reaction of compound **12a** with benzaldehyde **(2a)** and reaction of arylidine derivatives **3a, b** with ethylcyanoacetate **(4b)** in addition to reaction of compound **21a** with either benzaldehyde **(2a)** or salicylaldehyde **(2c)** respectively. Isoquinoline derivative **14** was afforded via the reaction of compound **12a** with malononitrile **(4a)**. Thieno[3,4-*c*]pyridine derivatives **10**, **19** were afforded via the reaction of 2-pyridone derivatives either **8a** or **12a** with elemental sulphur respectively**,** pyrazolo[3,4-*b*]pyridine drivatives **18a, b** were synthesized through the reaction of compound **12a** with either hydrazine hydrate or phenylhydrazine **16a**, **b.** Finally pyrido[3,4-*d*]pyridazine derivatives **25a, b** were produced via the reaction between compound **21a** and aryldiazonium chlorides **23a**, **b**. The biological potentialities of the prepared compounds has been examined and evaluated as antitumor agents. All compounds showed significant growth inhibitory effect.

* Corresponding Authors
Fax: Tel:20201004712543
E-Mail: karamsyn@yahoo.com
[a] Department of Organic Chemistry, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), El-Wahat Road, 6 October City, A. R. Egypt.
[b] Higher Institute of Engineering and Technology, El-Tagammoe El-Khames, New Cairo, Egypt.

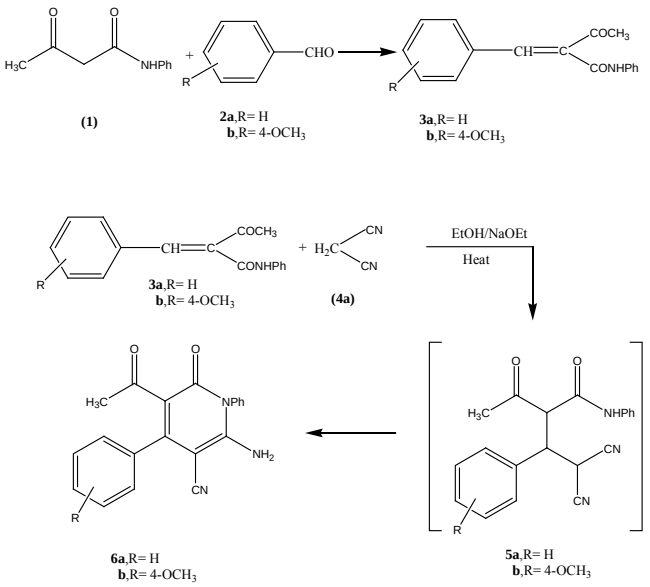
Introduction

During the last two decades, a large number of substituted pyridines have been reported to have several biological activities.[1-8] The antifungal and antibacterial properties of these compounds have opened up the possibility of their potential to try to add a novel class of synthetic antitumor active compounds against three different human tumor cell lines MCF-7, NCI-H460 and SF-268. Moreover, fused pyridines comprise a very interesting class of compounds because of their significant and versatile biological and pharmacological activities such as anticonvulsant, anti-malarial, antiproliferative, antiviral and antimicrobial.[9-15]

Results and discussion

Condensation of acetoacetanilide **(1)** with either benzaldehyde **(2a)** or *p*-anisaldehyde **(2b)** was reported to give the arylidene derivatives **3a** and **3b**, respectively[16] (cf. Scheme 1).

In this work, we reported the uses of compounds **3a** and **3b** in heterocyclic synthesis to give pyridine derivatives with expected broad spectrum biological activity, among which, antimalarial,[17] antitumor[18] and anti-inflammatory agents.[19] Thus, the reaction of **3a** and **3b** with equimolar amounts of malononitrile **(4a)** in ethanolic sodium-ethoxide solutions gave the corresponding 5-Acetyl-2-amino-6-oxo-1,4-diphenyl-1,6-dihydro-pyridine-3-carbonitrile **(6a)** and 5-acetyl-2-amino-4-(4-methoxy-phenyl)-6-oxo-1-phenyl-1,6-dihydro-pyridine-3-carbonitrile **(6b)**, respectively (cf. Scheme 1). Structural assignment of the latter products was based on analytical and spectral data. Thus, the ^{1}HNMR (DMSO-d_6) spectrum of compound **6a**, as an example, showed, a singlet at δ 2.39 (3H) ppm corresponding to CH_3 group, a singlet at δ 4.20 (2H) corresponding to NH_2 group (D_2O-exchangeable) and a multiplet at δ 6.95-7.42 (10H) ppm corresponding to aromatic protons. Formation of **6a** and **6b** took place via intermediacy of the acyclic Michael adducts **5a, b**. However, carrying the reaction of **3a, b** with either malononitrile **(4a)** or ethyl cyanoacetate **(4b)** in the presence of anhydrous ammonium acetate and heating in an oil bath at 140 ^{o}C gave the pyridine derivatives **8a-d**, respectively (cf. Scheme 2). Assignment of the structure of latter products was based on analytical and spectral data. The reaction of **8a** with elemental sulfur in ethanol in the presence of a catalytic amount of triethylamine gave the corresponding thieno[3,4-*c*]pyridine derivative **10** (cf. Scheme 2). Formation of **10** is explained in terms of the acidic nature of the CH_3 group neighbouring to the CN group present in **8a** which is capable of the first formation of the intermediate –SH derivative **9**. Such activity of CH_3 group neighboring to the CN group was reported before in literature.[20] The reaction of compounds **3a** and **3b** with malononitrile **(4a)** in ethanolic/Et_3N gave the corresponding 5-benzylidene-2-hydroxy-4-methyl-6-oxo-5,6-dihydro-pyridine-3-carbonitrile **(12a)** and 2-hydroxy-5-(4-methoxy-benzylidene)-4-methyl-6-oxo-5,6-dihydro-pyridine-3-carbonitrile **(12b)** respectively (cf. Scheme 3). The reaction took place via the intermediate formation of **11a, b** followed by elimination of aniline. Analytical and spectral data of the reaction products were in analogy with the proposed structure. Thus, the ^{1}HNMR (DMSO-d_6) spectrum of the compound **12a**, as an example, showed a singlet at δ 2.25 (3H) ppm corresponding to CH_3 group, a singlet at δ 4.15



Scheme 1. Synthesis of compounds **3a, b-6a, b**

(1H) ppm corresponding to OH group (D_2O- exchangeable), a singlet at δ 6.88 (1H) ppm corresponding to CH=C group and a multiplet at δ 7.13-7.44 (5H) ppm corresponding to the aromatic protons. Further conformation for the structure of **12a** was obtained through studying it's reactivity towards a variety of chemical reagents. Thus, the reaction of compound **12a** with malononitrile **(4a)** gave the corresponding isoquinoline derivative **14** (cf. Scheme 3).

Formation of **14** assumed to took place via the intermediate formation of **13**. Structure of compound **14** was established based on analytical and spectral data.

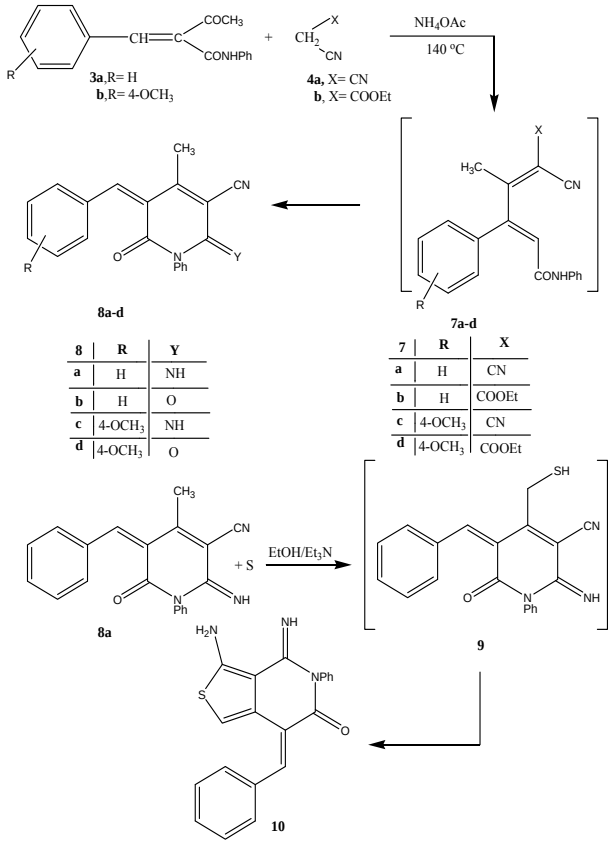
The methyl group in compound **12a** condensed with benzaldehyde **(2a)** in the presence of a catalytic amount of piperidine and heating in an oil bath at 140 ^oC to afford the corresponding benzal derivative **15** (cf. Scheme 3). The reactivity of compound **12a** towards hydrazines was studied in the aim of formation of pyrazolopyridine derivatives with potential biological activity[10,12]. Thus, the reaction of **12a** with either hydrazine hydrate **(16a)** or phenylhydrazine **(16b)** give the corresponding pyrazolo[3,4-*b*]pyridine derivatives **18a** and **18b**, respectively (cf. Scheme 4). Analytical and spectral data of **18a** and **18b** were in agreement with the proposed structure. Compound **12a** reacted with elemental sulfur in ethanol to give the corresponding thieno[3,4-*c*]pyridine derivative **19** as the Gewald's product[21-24] (cf. Scheme 4). The analytical and spectral data were in agreement with the proposed structure.

The arylidene derivatives **3a** and **3b** reacted with equimolar amount of ethyl cyanoacetate **(4b)** in the presence of triethylamine to give the pyridine derivatives **21a** and **21b**, respectively (cf. Scheme 4). The reaction took place via the intermediate formation of **20a, b** followed by intramolecular heterocyclization. Structure of **21a** and **21b** were based on analytical and spectral data. Thus, the ^{1}HNMR (DMSO-d_6) spectrum of **21a,** as an example, showed a singlet at δ 2.29 (3H) ppm corresponding to CH_3 group, a singlet at δ 3.40 (2H) ppm corresponding to NH_2 group (D_2O-exchangeable), a singlet at δ 6.76 (1H) ppm corresponding to CH=C group and a multiplet at δ 7.13-7.58 (10H) ppm corresponding to the aromatic protons. The reaction of **21a** with either benzaldehyde **(2a)** or salicylaldehyde **(2c)** gave the arylidene derivatives **22a** and **22b**, respectively (cf. Scheme 5).

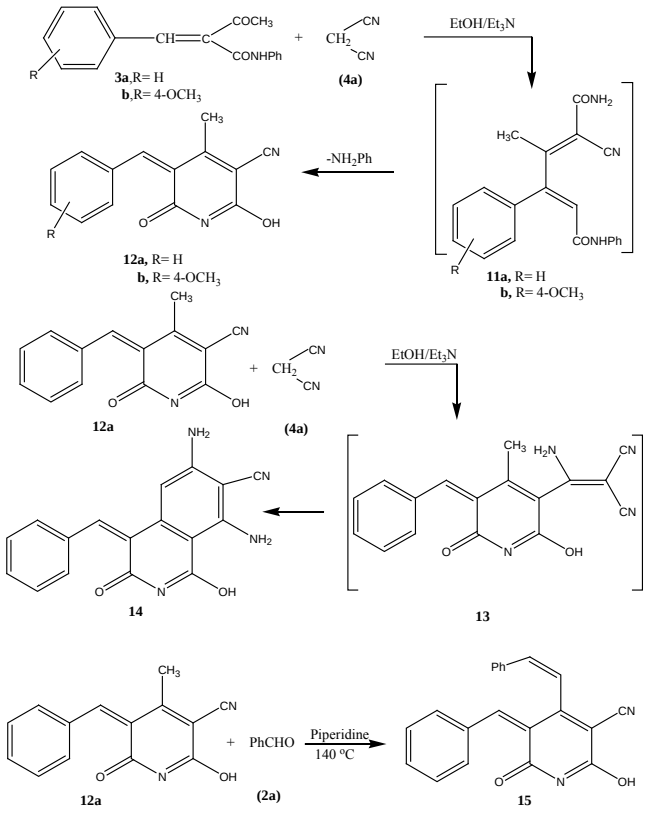
Coupling of **21a** with equimolar amounts of arenediazonium chlorides **23a, b** gave the corresponding pyrido[3,4-*d*]pyridazine derivatives **25a, b** (cf. Scheme 5). The synthesis of compounds **25a, b** was based on the first formation of hydrazone derivatives **24a, b** followed by cyclization. Structure of **25a** and **25b** were based on analytical and spectral data. Thus, the ^{1}HNMR (DMSO-d_6) spectrum of **25a** as an example, showed a singlet at δ 6.99 (1H) ppm corresponding to CH=C group, a singlet at δ 7.09 (1H) ppm corresponding to pyridazine H-3 and a multiplet at δ 7.18-7.58 (15H) ppm corresponding to the aromatic protons.

Effect on the growth of human tumor cell lines.

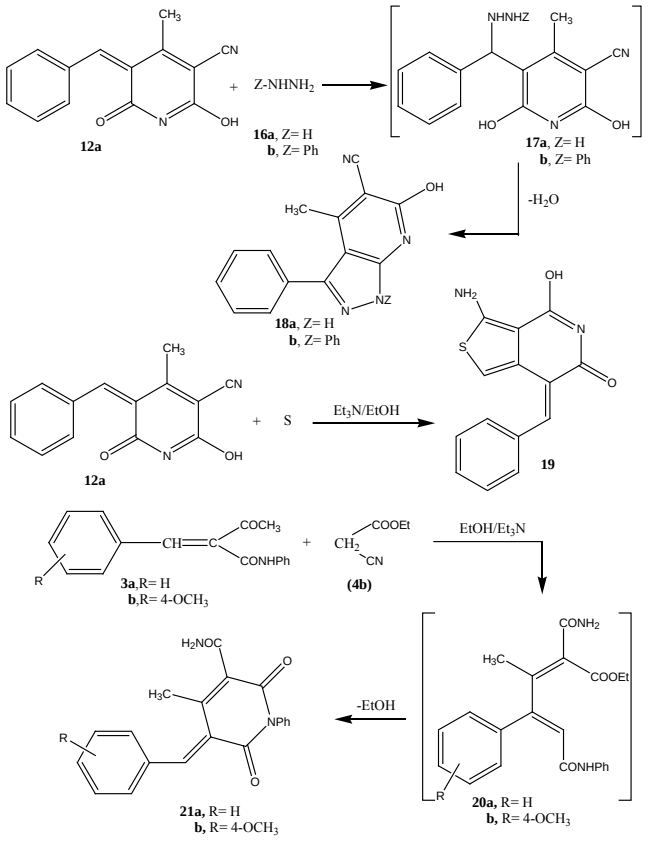
The effect of compounds **3a-25b** on the *in vitro* growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' which uses the protein-binding dye sulforhodamine B to assess cell growth.[25]



Scheme 2. Synthesis of compounds **8a-d** and **10**.

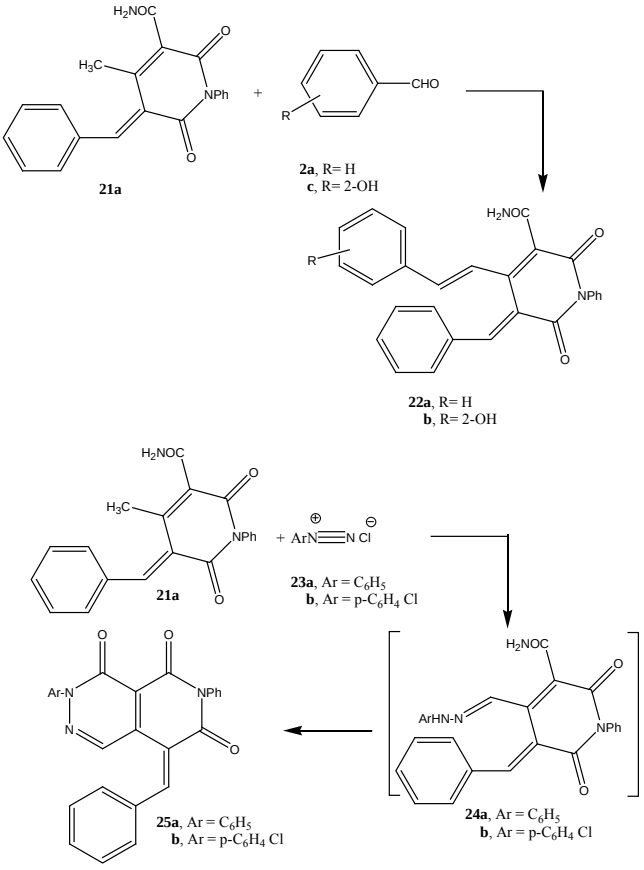


Scheme 3. Synthesis of compounds **12a, b-15.**



Scheme 4. Synthesis of compounds **18a, b-21a, b**

Briefly, cells growing exponentially in 96-well plates were then exposed for 48 h. The results are summarized in Table 1. GI_{50} concentrations of the synthesized compounds were calculated as described before compared to positive control Doxorubicin against three human tumor cell lines.[26]



Scheme 5. Synthesis of compounds **22a, b-25a, b**

All the compounds were able to inhibit the growth of the human tumor cell lines in a dose-dependent manner from the data represented in table 1, benzylidine derivative **3b**, pyridine derivatives **8a, 12a, 15, 21b** and **22a,** pyrazolo[3,4-*b*]pyridine derivative **18b** and thieno[3,4-*c*]pyridine derivative **19** were showed the highest inhibitory effect towards the three cell lines relative to the other tested compounds . On the other hand, pyridine derivatives **8b, 12b** and **22b,** thieno[3,4-*c*]pyridine derivative **10,** isoquinoline derivative **14,** pyrido[3,4-*d*]pyridazine derivatives **25a** and **25b** showed moderated growth inhibitory effect. Comparing the activities of **3a** and **3b** it is observed that compound **3b** with its substituted 4-methoxybanzal moiety showed stronger inhibitory effect than compound **3a** with its benzal moiety towards the three cell lines. Comparing the inhibitory effect of compounds **8a**, **8b**, **8c** and **8d**, it is obvious that 5-benzylidene-2-imino-4-methyl-6-oxo-1-phenyl-1,2,5,6-tetrahydro-pyridine-3-carbo-nitrile **(8a)** showed highest inhibitory effect among the four compounds it may be due to NH group, moreover, substituting the C=NH group by C=O group decreases the inhibitory effect as in the case of **8b**. Compounds **6a, 6b, 8c, 8d, 18a** and **21a** showed the lowest inhibitory effect towards the three cell line where their GI_{50} is higher than 30 μmol L^{-1}.

Table 1. Effect of compounds **3a, b-25a, b** on the growth of three human tumor cell lines

Compd. No.	GI_{50}, µmol L^{-1}		
	MCF-7	**NCI-H460**	**SF-268**
3a	3.0±0.6	17.3±1.4	22.3±1.5
3b	0.8±0.04	1.3±0.3	0.6±0.04
6a	30.6±16.9	28.9±10.8	10.8±8.6
6b	40.6±12.2	32.6±8.6	60.4±14.8
8a	0.4±0.2	0.1±0.02	0.3±0.06
8b	11.8±0.6	14.5±0.8	16.7±1.6
8c	72.7±17.5	40.2±12.8	50.0±9.01
8d	50.1±0.7	23.2±4.8	18.4±1.8
10	22.0±0.2	30.6±1.4	38.4±0.6
12a	0.02±0.001	0.06±0.02	0.05±0.02
12b	20.0±0.6	22.0±0.4	31.5±8.0
14	11.9±0.6	14.1±0.6	20.3±0.5
15	0.09±0.019	0.06±0.02	0.02±0.008
18a	70.9±0.9	43.6±1.8	56.8±0.8
18b	0.4±0.2	0.1±0.08	0.9±0.08
19	0.3±0.04	0.5±0.08	0.1±0.02
21a	38.0±1.8	44.0±0.8	20.5±1.1
21b	0.01±0.5	0.5±0.6	0.3±0.4
22a	2.6±10.0	4.6±8.6	2.4±0.8
22b	10.8±0.6	12.5±0.8	16.7±1.6
25a	14.3±0.7	16.1±4.9	22.5±1.2
25b	15.6±14.9	26.9±10.8	10.8±6.6
Doxorubicine	0.04±0.008	0.09±0.008	0.09±0.007

From the results listed in Table 1 its very clear that compounds (5-benzylidene-2-hydroxy-4-methyl-6-oxo-5,6-dihydro-pyridine-3-carbonitrile **(12a)**, 5-benzylidene 2-hydroxy-6-oxo-4-styryl-5,6-dihydro-pyridine-3-carbonitrile **(15)** and 5-(4-methoxy-benzylidene)-4-methyl-2,6-dioxo-1-phenyl-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid amide **(21b)** showed the maximum inhibitory effect among the three cell lines.

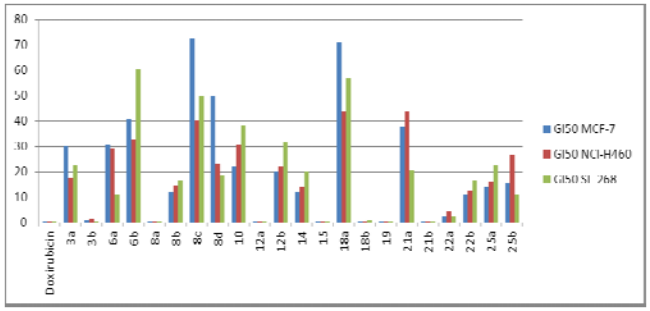


Figure 1. GI_{50} concentrations of the synthesized compounds compared to positive control Doxorubicin against MCF-7, NCI-H460 and SF-268.

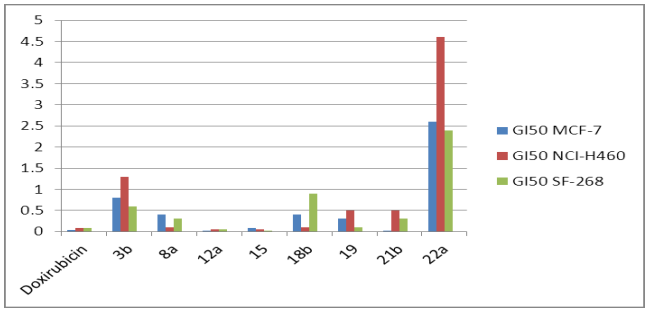


Figure 2 GI_{50} concentrations of the highly active synthesized compounds compared to positive control Doxorubicin against MCF-7, NCI-H460 and SF-268.

The growth inhibitory of the newly synthesized products against the cancer cell lines are presented above through Figures 1 and 2.

Experimental

All melting points were determined in open capillaries and are uncorrected. IR spectra were measured using (KBr) discs on a Pye Unicam Sp-1000 Spectro- photometer. ^{1}HNMR spectra were measured on a Varian EM-390-200 MHz instrument in (DMSO-d_6) as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. Mass spectra were measured on a Shimadzu GCMS QP- 1000 EX mass spectrometer at 70 eV. Synthetic pathways are presented in Schemes 1-5.

General procedure for synthesis of 2-benzylidene-3-oxo-N-phenylbutyramide (3a) and 2-(4-methoxy-benzylidene)-3-oxo-N-phenyl-butyramide (3b)

To a mixture of acetoacetanilide **(1)** (5.31 g, 0.03 mol) and either benzaldehyde **(2a)** (3.18 g, 0.03 mol) or *p*-anisaldehyde (**2b)** (4.08 g, 0.03 mol), a catalytic amount of piperdine (0.5 ml) was added. The reaction mixture was fused at 140 °C for 15 minute. Then left to cool, whereby, the solid product formed after boiling in ethanol was collected by filtration, dried and crystallized from ethanol.

Compound **3a**: Dark yellow crystals, 6.44 g. (81%), mp.108 °C [16].

Compound **3b**: Dark orange crystals, 7.52 g. (85%), mp.72-74 °C [16].

General procedure for synthesis of 5-acetyl-2-amino-6-oxo-1,4-diphenyl-1,6-dihydropyridine-3-carbonitrile (6a) and 5-acetyl-2-amino-4-(4-methoxyphenyl)-6-oxo-1-phenyl-1,6-dihydropyridine-3-carbonitrile (6b)

To a suspension of either **3a** (0.53 g, 0.002 mol) or **3b** (0.59 g, 0.002 mol) in sodium ethoxide solution [prepared by dissolving sodium metal (0.046 g, 0.002 mol) in absolute ethanol (50 ml)], malononitrile **(4a)** (0.132 g, 0.002 mol) was added. The reaction mixture in each case was heated for 4 h, then poured on an ice/water mixture containing few drops of hydrochloric acid. The formed solid product, in each case, was collected by filtration, recrystallized from ethanol.

Compound **6a**: 0.33 g (50%), m.p. 82-84 °C , IR (KBr, cm^{-1}): 3450, 3298 (NH_2), 3059 (CH aromatic), 2920 (CH_3), 2218, (CN), 1714, 1673 (2C=O), 1656, (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.39 (s, 3H, CH_3), 4.20 (s, 2H, NH_2, D_2O-exchangeable), 6.95-7.42 (m, 10H, $2C_6H_5$). MS: *m/z* (%) 329 (M^+, 15.2), Anal.Calcd. for $C_{20}H_{15}N_3O_2$ (329.35): C, 72.93; H, 4.59; N, 12.76. found: C, 72.68; H, 4.82; N, 12.66.

Compound **6b**: 0.596 g (83%), m.p. 85-86 °C, IR (KBr, cm^{-1}): 3363, 3250 (NH_2), 3035 (CH aromatic), 2921, (CH_3), 2210, (CN), 1698, 1685 (2C=O), 1633(C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.23, 3.22 (2s, 6H, $2CH_3$), 3.80 (s, 2H, NH_2, D_2O-exchangeable), 6.85-7.46 (m, 9H, C_6H_5,

C_6H_4). MS: *m/z* (%) 359 (M^+, 11.4), Anal.Calcd. for $C_{21}H_{17}N_3O_3$ (359.376): C, 70.17; H, 4.76; N, 11.69. found: C, 70.46; H, 4.98; N, 11.38.

General procedure for synthesis of 5-benzylidene-2-imino-4-methyl-6-oxo-1-phenyl-1,2,5,6-tetrahydropyridine-3-carbonitrile (8a), 5-benzylidene-4-methyl-2,6-dioxo-1-phenyl-1,2,5,6-tetrahydropyridine-3-carbonitrile (8b), 2-imino-5-(4-methoxy-benzylidene)-4-methyl-6-oxo-1-phenyl-1,2,5,6-tetrahydropyridine-3-carbonitrile (8c) and 5-(4-Methoxy-benzylidene)-4-methyl-2,6-dioxo-1-phenyl-1,2,5,6-tetrahydro-pyridine -3-carbonitrile (8d)

To a mixture of either **3a** (1.06 g, 0.004 mol) or **3b** (1.18 g, 0.004 mol), either malononitrile **(4a)** (0.264 g, 0.004 mol) or ethyl cyanoacetate **(4b)** (0.452 g, 0.004 mol) and anhydrous ammonium acetate (0.5 g) were added. The reaction mixture in each case was fused at 140 °C for 20 minute. Then left to cool, the solid product so formed, in each case, dried, recrystallized from ethanol.

Compound **8a**: 1.07 g (86%), m.p. 118-120 °C , IR (KBr, cm^{-1}): 3490-3320 (NH), 3059 (CH aromatic), 2924, (CH_3), 2198 (CN), 1690 (C=O), 1660 (exocyclic C=N), 1599 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.32 (s, 3H, CH_3), 6.48 (s, 1H, CH=C), 7.24-7.46 (m,10H, $2C_6H_5$), 9.39 (s, 1H, NH, D_2O-exchangeable). MS: *m/z* (%) 313 (M^+, 10.8), Anal.Calcd. for $C_{20}H_{15}N_3O$ (313.35): C, 76.65; H, 4.82; N, 13.41. found: C, 76.43; H, 4.91; N, 13.14.

Compound **8b**: 0.654 g (52%), m.p. 140-141 °C , IR (KBr, cm^{-1}): 3060 (CH aromatic), 2928 (CH_3), 2194 (CN), 1685, 1680 (2C=O), 1673 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.21 (s, 3H, CH_3), 6.85 (s, 1H, CH =C), 7.23-7.46 (m, 10H, $2C_6H_5$). MS: *m/z* (%) 314 (M^+, 27.1), Anal.Calcd. for $C_{20}H_{14}N_2O_2$ (314.332): C, 76.41; H, 4.48; N, 8.91 found: C, 76.30; H, 4.34; N, 8.73.

Compound **8c**: 1.03 g (75%), m.p. 72-74 °C , IR (KBr, cm^{-1}): 3480–3366 (NH), 3035 (CH aromatic), 2933 (CH_3), 2199 (CN), 1687 (C=O), 1660 (C=N), 1633 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.18, 3.11 (2s, 6H, $2CH_3$), 6.72 (s, 1H, CH=C), 6.86-7.37 (m, 9H, C_6H_5 , C_6H_4), 9.90 (s, 1H, NH, D_2O-exchangeable). MS: *m/z* (%) 343 (M^+, 23.5), Anal.Calcd. for $C_{21}H_{17}N_3O_2$ (343.376): C, 73.45; H, 4.99; N, 12.24 found: C, 73.33; H, 4.66; N, 11.99.

Compound **8d**: 0.84 g (61%), m.p. 80-81 °C, IR (KBr, cm^{-1}): 3033 (CH aromatic), 2931(CH_3), 2202 (CN), 1688, 1685 (2C=O), 1640 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.44, 3.31 (2s, 6H, $2CH_3$), 6.71 (s, 1H, CH=C), 6.86-7.36 (m, 9H, C_6H_5, C_6H_4). MS: *m/z* (%) 344 (M^+, 9.8), Anal.Calcd. for $C_{21}H_{16}N_2O_3$ (344.358): C, 73.24; H, 4.68; N, 8.13 found: C, 73.40; H, 4.87; N, 7.83.

3-Amino-7-benzylidene-4-imino-5-phenyl-4,5-dihydro-7*H*-thieno[3,4-*c*]pyridin-6-one (10)

To a solution of compound **8a** (0.313 g, 0.001 mol) in ethanol (50 ml) containing a catalytic amount of triethylamine (0.5 ml), elemental sulfur (0.032 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 3 h. It was allowed to cool then poured on an ice/water mixture containing few drops of hydrochloric acid. The reaction mixture was left overnight to settle and the formed solid product so formed was collected by filtration, recrystallized from ethanol.

0.29 g (84%), m.p. 125-126 °C, IR (KBr, cm^{-1}): 3540–3312 (NH_2, NH), 3030 (CH aromatic) , 1690 (C=O), 1673 (exocylcic C = N), 1635 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 4.1 (s, 2H, NH_2 , D_2O-exchangeable), 6.98, 7.02 (s, br, 2H, thiophene H-5, CH=C), 7.21–7.58 (m, 10H, $2C_6H_5$), 9.80 (s, br, 1H, NH, D_2O-exchangeable). MS: *m/z* (%) 345 (M^+, 23.4), Anal.Calcd. for $C_{20}H_{15}N_3OS$ (345.42): C, 69.53; H, 4.37; N, 12.16; S, 9.28 found: C, 69.43; H, 4.09; N, 11.89; S, 9.02.

General procedure for synthesis 5-benzylidene-2-hydroxy-4-methyl-6-oxo-5,6-dihydro-pyridine-3-carbonitrile (12a) and 2-hydroxy-5-(4-methoxy-benzylidene)-4-methyl-6-oxo-5,6-dihydro-pyridine-3-carbonitrile (12b)

To a solution of either **3a** (0.53 g, 0.002 mol) or **3b** (0.59 g, 0.002 mol) in ethanol (50 ml) containing a catalytic amount of triethylamine (0.5 ml), malononitrile **(4a)** (0.132 g, 0.002 mol) was added. The reaction mixture in each case was heated under reflux for 4 h, then poured on an ice/water mixture containing few drops of hydrochloric acid. The solid product, so formed in each case, was collected by filtration, dried and recrystallized from ethanol.

Compound **12a**: 0.391g (82%), m.p. 98-100 °C, IR (KBr, cm^{-1}): 3460-3381 (OH), 3059 (CH aromatic), 2927 (CH_3), 2204 (CN), 1688 (C=O), 1660 (C=N), 1635 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.25 (s, 3H, CH_3), 4.15 (s, 1H, OH D_2O-exchange- able), 6.88 (s, 1H, CH=C), 7.13-7.44 (m, 5H, C_6H_5). MS: *m/z* (%) 238 (M^+, 33.6), Anal. Calcd. for $C_{14}H_{10}N_2O_2$ (238.24): C, 70.57; H, 4.23; N, 11.76; found: C, 70.60; H, 4.14; N, 11.52.

Compound **12b**: 0.241g (90%), m.p. 102-104 °C , IR (KBr, cm^{-1}): 3450–3361 (OH), 3061 (CH aromatic), 2933 (CH_3), 2206 (CN), 1685 (C=O), 1667 (C=N), 1634 (C=C). 1H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.3, 2.95 (2s, 6H, $2CH_3$), 4.08 (s, 1H, OH, D_2O-exchangeable), 6.62 (s, 1H, CH=C), 7.12–7.45 (m, 4H, C_6H_4). MS: *m/z* (%) 268 (M^+, 37.9), Anal. Calcd. for $C_{15}H_{12}N_2O_3$ (268.266): C, 67.15; H, 4.50; N, 10.44; found: C, 67.10; H, 4.81; N, 10.23.

6,8-Diamino-4-benzylidene-1-hydroxy-3-oxo-3,4-dihydro-isoquinoline-7-carbonitrile (14)

To a solution of compound **12a** (0.238 g, 0.001 mol) in ethanol (50 ml) containing a catalytic amount of triethylamine (0.5 ml), malononitrile **(4a)** (0.066 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 6 h, then poured on an ice/water mixture containing few drops of hydrochloric acid. The solid product, so formed, was collected by filtration, dried, recrystallized from ethanol.

Yield: 0.18 g (59%), m.p. 136-137 °C, IR (KBr, cm^{-1}): 3566-3323(OH), 3450-3222 ($2NH_2$), 3060 (CH aromatic), 2240 (CN),1687 (C=O), 1658 (C=N), 1648 (C=C).^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 3.47, 3.76 (2s, 4H, $2NH_2$ D_2O-exchangeable), 4.15 (s,1H, OH, D_2O- exchangeable),

6.48, 6.64 (2s, 2H, CH=C, isoquinoline H5), 7.23–7.62 (m, 5H, C_6H_5). MS: *m/z* (%) 304 (M^+, 18.3), Anal. Calcd . for $C_{17}H_{12}N_4O_2$ (304.306): C, 67.09; H, 3.97; N, 18.41; found: C, 66.89; H, 4.22; N,18.21.

5-(Benzylidine)2-Hydroxy--6-oxo-4-styryl-5,6-dihydro-pyridine -3-carbonitrile (15)

To a mixture of compound **12a** (0.476 g, 0.002 mol) and benzaldehyde **(2a) (**0.212 g, 0.002 mol), a catalytic amount of piperidine (0.5 ml) was added. The reaction mixture was fused at 140 °C for 35 minute, then left to cool. The solid product formed after boiling in ethanol was poured on an ice/water mixture containing few drops of hydrochloric acid, collected by filtration, recrystallized from ethanol.

0.379 g (58%), m.p. 65-66 °C, IR (KBr, cm^{-1}): 3540-3200 (OH), 3060 (CH aromatic), 2928 (CH=CH), 2199 (CN), 1685 (C=O), 1663(C=N), 1636 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.85(s, 1H, OH, D_2O-exchangeable), 7.18-7.21 (m, 3H, CH=CH, CH=C), 7.26–7.43 (m, 10H, $2C_6H_5$). MS: *m/z* (%) 326 (M^+, 41.2), Anal. Calcd. for $C_{21}H_{14}N_2O_2$ (326.342): C, 77.28; H, 4.32; N, 8.58; found: C, 77.14; H, 4.17; N, 8.32.

General procedure for synthesis of 6-hydroxy-4-methyl-3-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (18a) and 6-hydroxy-4-methyl-1,3-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (18b)

To a solution of compound **12a** (0.238 g, 0.001 mol) in ethanol (50 ml), either hydrazine hydrate **(16a)** (0.05 ml, 0.001 mol) or phenylhydrazine **(16b)** (0.108 ml, 0.001 mol) was added. The reaction mixture in each case was heated under reflux for 6 h, left to cool at room temperature, then poured on an ice/water mixture containing few drops of hydrochloric acid. The formed solid product, in each case, was collected by filtration, dried, recrystallized from ethanol.

Compound **18a**: 0.18 g (72%), m.p. 130-131 °C, IR (KBr, cm^{-1}): 3545-3320 (OH), 3480-3240 (NH), 3045 (CH aromatic), 2930 (CH_3), 2225 (CN), 1630 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.45 (s, 3H, CH_3), 4.53 (s, 1H, OH, D_2O-exchangeable), 7.05–7.39 (m, 5H, C_6H_5,) 9.43 (s, 1H, NH, D_2O-exchangeable). MS: *m/z* (%) 250 (M^+, 19.1), Anal. Calcd. for $C_{14}H_{10}N_4O$ (250.26): C, 67.18; H, 4.02; N, 22.39; found: C, 66.89; H, 4.18; N, 22.07.

Compound **18b**: 0.222 g (68%), m.p. 110-112 °C, IR (KBr, cm^{-1}): 3540-3250 (OH), 3060 (CH aromatic), 2942 (CH_3), 2232 (CN), 1644 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.39 (s, 3H, CH_3), 4.32 (s, 1H, OH, D_2O-exchangeable), 7.16–7.48 (m, 10H, $2C_6H_5$). MS: *m/z* (%) 326 (M^+, 28.8), Anal. Calcd. for $C_{20}H_{14}N_4O$ (326.352): C, 73.6; H, 4.32; N, 17.17; found: C, 73.51; H, 4.12; N, 17.01.

3-Amino-7-benzylidene-4-hydroxy-7*H*-thieno[3,4-*c*]pyridin-6-one (19)

To a solution of compound **12a** (0.476 g, 0.002 mol) in ethanol (50 ml) containing a catalytic amount of triethylamine (0.5 ml), elemental sulfur (0.064 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 3h. It was allowed to cool, then poured on an ice/water mixture containing few drops of hydrochloric acid. The reaction mixture was left overnight to settle and the formed solid product was collected by filtration, dried, recrystallized from ethanol.

Yield: 0.378 g (70%), m.p. 147-149 °C, IR (KBr, cm^{-1}): 3550-3150 (OH), 3383-3314 (NH_2), 3058 (CH aromatic), 1688 (C=O), 1670 (C=N), 1634 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.89 (s, 2H, NH_2, D_2O-exchangeable), 3.32(s, 1H, OH, D_2O-exchangeable), 7.17(s, 2H, CH=C, thiophene H-5), 7.20-7.49 (m, 5H, C_6H_5). MS: *m/z* (%) 270 (M^+, 30.7), Anal. Calcd. for $C_{14}H_{10}N_2O_2S$ (270.31): C, 62.20; H, 3.72; N, 10.36; S, 11.86 found: C, 62.34; H, 3.51; N, 10.13; S, 11.71.

General procedure for synthesis of 5-benzylidene-4-methyl-2,6-dioxo-1-phenyl-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid amide (21a) and 5-(4-methoxy-benzylidene)-4-methyl -2,6-di-oxo-1-phenyl-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid amide (21b):

To a solution of either **3a** (0.265 g, 0.001 mol) or **3b** (0.295 g, 0.001 mol) in ethanol (50 ml) containing a catalytic amount of triethylamine (0.5 ml), ethyl cyanoacetate **(4b)** (0.113 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 3h, then poured on an ice/water mixture containing few drops of hydrochloric acid. The formed solid product, in each case was collected by filtration, dried, recrystallized from ethanol.

Compound **21a**: Yield: 0.269 g (81%), m.p. 93-95 °C, IR (KBr, cm^{-1}): 3583–3132 (NH_2), 3060 (CH aromatic), 2924 (CH_3), 1690, 1687, 1680 (3C=O), 1654 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.29 (s, 3H, CH_3), 3.40 (s, 2H, NH_2 D_2O-exchangeable), 6.76 (s, 1H, CH=C), 7.13–7.58 (m, 10H, $2C_6H_5$). MS: *m/z* (%) 332 (M^+, 17.9), Anal. Calcd. for $C_{20}H_{16}N_2O_3$ (332.342): C, 72.27; H, 4.85; N, 8.43; found: C, 71.96; H, 4.64; N, 8.51.

Compound **21b**: Yield: 0.221g (61%), m.p. 53-54 °C, IR (KBr, cm^{-1}): 3434–3200 (NH_2), 3034 (CH aromatic), 2932 (CH_3), 1688, 1685, 1680 (3C=O), 1645(C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.12, 3.28 (2s, 6H, $2CH_3$), 4.21 (s, 2H, NH_2 D_2O-exchangeable), 6.85 (s, 1H, CH=C), 7.17-7.36 (m, 9H, C_6H_5, C_6H_4). MS: *m/z* (%) 362 (M^+, 13.4), Anal. Calcd. for $C_{21}H_{18}N_2O_4$ (362.374): C, 69.59; H, 5.00; N, 7.73; found: C, 69.24; H, 4.78; N, 7.87.

5-Benzylidene-2,6-dioxo-1-phenyl-4-styryl-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid amide (22a):

To a mixture of compound **21a** (0.332 g, 0.001 mol) and benzaldehyde (**2a)** (0.106 g, 0.001 mol), a catalytic amount of piperdine (0.5 ml) was added. The reaction mixture was fused at 140°C for 30 minute, then left to cool. The solid product formed after boiling in ethanol was collected by filtration, dried, recrystallized from ethanol.

Compound **22a**: Yield: 0.32g (76%), m.p. 88-89 °C, IR (KBr, cm^{-1}): 3450–3167 (NH_2) , 3058 (CH aromatic) ,1695, 1689, 1675(3C=O), 1638 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 3.32 (s, 2H, NH_2, D_2O-exchangeable),

6.95-7.22(m, 3H, CH=CH, CH=C), 7.28-7.43 (m, 15H, $3C_6H_5$). MS: *m/z* (%) 420 (M^+, 38.7), Anal. Calcd. for $C_{27}H_{20}N_2O_3$ (420.45): C, 77.12; H, 4.79; N, 6.66; found: C, 77.33; H, 4.53; N, 6.63.

5-Benzylidene-4-[2-(2-hydroxy-phenyl)-vinyl]-2,6-dioxo-1-phenyl-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid amide (22b):

A mixture of compound **21a** (0.332 g, 0.001 mol) and salicylaldehyde **(2c)** (0.122 g, 0.001 mol) in dry dimethylformamide (30 ml) containing a catalytic amount of piperdine (0.5 ml), was heated under reflux for 5 h, then poured on an ice/water mixture containing few drops of hydrochloric acid. The solid product, so formed, was collected by filtration, dried, recrystallized from ethanol.

Compound **22b**: Yield: 0.271 g (62%), m.p. 69-70 °C, IR (KBr, cm^{-1}): 3480–3259 (OH), 3367-3151(NH_2), 3057 (CH aromatic), 1714, 1685, 1682 (3C=O), 1635(C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 3.88 (s, 2H, NH_2, D_2O-exchangeable), 6.79-7.11(m, 3H, CH=CH, CH=C), 7.16-7.54 (m, 14H, $2C_6H_5$, C_6H_4), 10.09(s, 1H, OH, D_2O-exchangeable). MS: *m/z* (%) 436 (M^+, 22.2), Anal. Calcd. for $C_{27}H_{20}N_2O_4$ (436.45): C, 74.29; H, 4.61; N, 6.41; found: C, 74.03; H, 4.32; N, 6.31.

General procedure for synthesis of 8-benzylidene-3,6-diphenyl-3*H*, 8*H*-pyrido[3,4-*d*]pyridazine-4,5,7-trione (25a) and 8-benzylidene-3-(4-chloro-phenyl)-6-phenyl-3*H*,8*H*-pyrido[3,4-*d*] pyridazine-4,5,7-trione (25b):

To a cold solution (0-5 °C) of compound **21a** (0.332 g, 0.001mol) in ethanol (50 ml) containing sodium hydroxide (0.04 g, 0.001 mol), arenediazonium chlorides **23a, b** (0.001 mol) [prepared by adding an aqueous sodium nitrite solution (0.069 g, 0.001 mol) to a cold solution of the appropriate primary aromatic amine (0.001 mol) in the appropriate amount of conc. HCl at (0-5 °C) with continuous stirring] was added with continuous stirring, the reaction mixture was stirred at room temperature for an additional 4 h and the solid product, so formed, was collected by filtration, dried, recrystallized from ethanol.

Compound **25a**: Yield: 0.31 g (74%), m.p. 105-107 °C, IR (KBr, cm^{-1}): 3055 (CH aromatic), 1695, 1687, 1682 (3C=O), 1677 (C=N), 1640 (C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.99(s, 1H, CH=C), 7.09(s, 1H, pyridazine H-3), 7.18-7.58 (m, 15H, $3C_6H_5$). MS: *m/z* (%) 419 (M^+, 40.1), Anal. Calcd. for $C_{26}H_{17}N_3O_3$ (419.426): C, 74.44; H, 4.08; N, 10.02; found: C, 74.35; H, 3.82; N, 9.92.

Compound **25b**: Yield: 0.349 g (77%), m.p. 116-118 °C, IR (KBr, cm^{-1}): 3059 (CH aromatic), 1692, 1685, 1680 (3C=O), 1670 (C=N), 1643(C=C). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.87 (s, 1H, CH=C), 7.13 (s, 1H, pyridazine H-3), 7.15-7.60 (m, 14H, $2C_6H_5$, C_6H_4). MS: *m/z* (%) 453 (M^+, 35.5), Anal. Calcd. for $C_{26}H_{16}ClN_3O_3$ (453.868): C, 68.79; H, 3.55; N, 9.26; found: C, 68.66; H, 3.44; N, 9.02.

Antitumor Activity

Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethylsulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA). Samples: Stock solutions of new compounds through **3a-25b** were prepared in DMSO and kept at -20 °C. Appropriate dilutions of the compounds were freshly prepared just prior the assays. Final concentrations of DMSO did not interfere with the cell growth.

Cell cultures, three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 μg mL^{-1}, streptomycin 100 μg mL^{-1}), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 X 10^5 cells/mL for MCF-7 and SF-268 and 0.75 X 10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

Conclusion

A convenient method was described for the synthesis of heterocyclic arylideneaceto- acetanilides. They have been shown to be useful building blocks for the synthesis of pyridine, thieno[3,4-*c*]pyridine, pyrazolo-[3,4-*b*]pyridine and pyrido[3,4-*d*]-pyridazine derivatives, for which we might expect a wide spectrum of bioresponses. Antitumor evaluation of the newly synthesized products showed in table1.

Acknowledgment

The authors would like to thank the research group at the National Research center "Hormones Department" and the National Cancer Institute at Cairo University for recording the antitumor evaluations for the newly synthesized products.

References

[1]Gaston, M. A., Osaria, D. L. S., Freitas, C. A. C., Miranda, P. A. and Barreiro, J. E., *Pharm. Acta. Helv.,* **1996,** *71,* 213.

[2]El-Gamal, M. I., Jung, M. H., Lee, W. S., Sim, T. Y. K. H. and Oh, C. H., *Eur. J. Med. Chem.,* **2011**, *46,* 3218.

[3]Foks, H., Ksepko, D. P., Kędzia, A., Zwolska, Z., Janowiec, M. and Kopeć, E. A., *Il Farmaco.*, **2005**, *60*, 513.

[4]Ali, T. E., *Eur. J. Med. Chem.,* **2009,** *44,* 4385.

[5]Lacerda, R. B., De Lima, C. K. F., Da Silva, L. L., Romeiro, N. C., Miranda, A. L. P., Barreiro, E. J. and Fraga, C. A. M., *Biol. Med. Chem*., **2009,** *17,* 74.

[6]Dias, L. R. S., Alvim, M. J. F., Freitas, A. C. C., Barreiro, E. J. and Miranda, A. L. P., *Pharm. Acta Helv.,* **1994,** *69*, 163.

[7]Queiroz, M. R. P., Calhelha, R. C., Silva, L. A., Pinto, E., Lima, R. T. and Vasconcelos, M. H., *Eur. J. Med. Chem*., **2010,** *45*, 5628.

[8]Bahekar, R. H., Jain, M. R., Jadav, P. A., Prajapati, V. M., Patel, D. N., Gupta, A. A., Sharma, A., Tom, R., Bandyopadhya, D., Modi, H. and Patel, P. R., *Bioorg. Med. Chem.,* **2007,** *15*, 6782.

[9]De Sarro, A., De Sarro, G., Chimirri, A., Grasso, S., Monforte, A. M. and Zappala, M., *Gen. Pharm: The Vascular System*., **1994,** *25,* 1027.

[10]Carla, M. S. M., Carlos, M. R. S. A., Carlos, R. R. and Eliezer, J. B., *J. Mol. Struct. (Theochem.),***2002,** *579,* 31.

[11]Poreba, K., Opolski, A., Wietrzyk, J. and Kowalska, M., *Arch. Pharm . (Weinheim),* **2001,** *334,* 219.

[12]Poreba, K., Opolski, A. and Wietrzyk, J., *J. Acta. Pol. Pharm.,* **2002,** *59,* 215.

[13]Copo, J., Sanchez, A., Nogueras, M. and De Clercq, E., *Tetrahedron,* **1997,** *53*, 8225.

[14]Zhuravel', I. O., Kovalenko, S. M., Ivachtchenko, A. V., Balakin, K. V. and Kazmirchuk, V. V., *Biol. Med. Chem. Lett.,* **2005,** *15,* 5483.

[15]Elkholy, M. Y., *Heterocyclic Comm*., **2011,** *11,* 89.

[16]Khalil, Z. H. and Yanni, A. S., *J. Indian Chem. Soc.,* **1981,** *58,* 168.

[17]Lima, P. C., Avery, M. A., Tekwani, B. L., Alves, H. D. M., Barreiro, E. J. and Fraga, C. A. M., *Il Farmaco.,* **2002,** *57*, 825.

[18]Stuart, T. H., Lisa, P., Juiwanna, K., Kathryn, W., Thomas, H. C. and Jerome, P. H., *Bioorg. Med. Chem.,* **2006,** *Part 5*, *14,* 2462.

[19]Murata, T., Shimada, M., Sakakibara, S., Yoshino, T., Masuda, T., Shintani, T., Sato, H., Koriyama,Y., Fukushima, K., Nunami, N., Yamauchi, M., Fuchikami, K., Komura H., Watanabe, A., Ziegelbauer, K., Bacon, K. B. and Lowinger, T. B., *Bioorg. Med. Chem.,* **2004,** *14*, 4019.

[20]Elnagdy, M. H., Mohareb, R. M., Abd El-Aal, F. A. and Mohamed, H. A., *Phosphorus, Sulfur Silicon Related Elements*, **1993,** *82*,195.

[21]Gewald, K., Schinke, E. and Böttcher, H., *Chem. Ber*., **1966,** *99,* 94.

[22]Gewald, K., Schnbert, A. and Martin, G., *J. Prakt. Chem*., **1975,** *317,* 561.

[23]Sabnis, R. W., *Sulfur Rep.,* **1994,** *16,* 1.

[24]Sabnis, R. W., Rangnekar, D. W. and Sonawane, N. D., *J. Heterocyclic Chem.,* **1999,** *36,* 333.

[25]Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenny, S. and Boyd, M. R., *J. Natl. Cancer Inst.,* **1990,** *82*, 1107.

[26]Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paul, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J. and Boyd, J. M., *J. Natl. Cancer Inst.,* **1991,** *83,* 757.

Received: 02.03.2013.
Accepted: 31.03.2013.