Full Paper

Synthesis and Biological Evaluation of Some Polymethoxylated Fused Pyridine Ring Systems as Antitumor Agents

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A series of 3,5-*bis*(arylidene)-4-piperidones like chalcone analogues carrying variety of methoxylated aryl groups, pyrazolo[4,3-*c*]pyridines, pyrido[4,3-*d*]pyrimidines, and pyrido[3,2-*c*]pyridines, carrying an arylidene moiety, and some pyrano[3,2-*c*]pyridines, like flavone and coumarin isosteres, were synthesized and screened for their *in-vitro* antitumor activity at the National Cancer Institute (NCI, USA). The tested compounds **7**, **9**, **10**, **12, 13, 15, 17**, and **19** exhibited a broad spectrum of antitumor activity. Compounds belonging to the pyrazolo[4,3-*c*]pyridine series proved to be more active than those of the pyrido[3,2-*c*]pyridine and pyrano[3,2-*c*]pyridine analogues, in which the monomethoxylated derivatives showed better antitumor activity when compared with their corresponding dimethoxylated congeners. Compound **7** is considered to be the most active member identified in this study with a broad spectrum of activity against 22 different tumor cell lines belonging to the nine subpanels employed, and a particular effectiveness against the breast cancer T-47D cell line (GI 54.7%). The pyrano[3,2-*c*]pyridine heterocyclic system **19** proved to be the most active antitumor agent among the six-membered fused pyridines, with variable activity against 18 different tumor cell lines, and special activity against the non-small cell lung cancer Hop-92 and ovarian cancer OVCAR-4 cell lines (GI values 63.9 and 48.5%, respectively).

Keywords: Antitumor activity / Chalcones / Fused pyridines / Synthesis

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Introduction

Cancer poses a serious human health problem despite much progress in understanding its biology and pharmacology. Consequently, the design of new lead structures employed as antitumor agents is one of the most urgent research areas in contemporary medicinal chemistry. Among the wide range of pharmacologically active heterocycles, pyridines and some pyridine fused ring systems have attracted great attention as potential chemotherapeutic agents [1 – 7]. In particular, the cytotoxic activity of (*E*)-3,5-*bis*(benzylidene)-4-piperidones and their specificity toward leukemia cell lines with IC_{50} values less than 10 μM have been reported [8 – 11]. This type of compounds is a combination of cyclic α,β-unsaturated ketone (chalcone) and β-amino ketone in one structure and is known as "curcuminoids" being structurally related to curcumin (1,7-*bis*-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa* [12]. Several studies revealed that curcumin and its congeners manifest antiproliferative activity against various cancers, however, the actual mechanism of the antitumor potential of curcuminoids are still far from being under-

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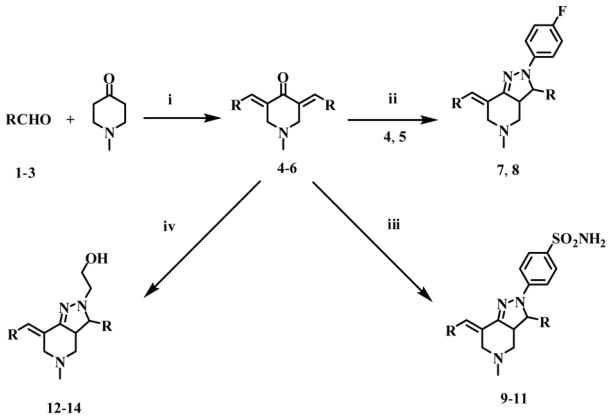
stood [13]. With this in mind, it was reported that such compounds of Michael acceptor-type could inhibit cancer cell proliferation *in vitro* and *in vivo* via promotion of apoptosis in cancer cells by suppressing cyclin D and endothelial growth factor receptor (EGFR) function, while simultaneously decreasing levels of phosphokinase B (AKT), c-myc, and phospho-AKT (pAKT) [14]. In one of the more significant findings on the anticancer activity of compounds inspired by curcumin, Adams *et al*. [15] announced the superior activity of 2,6-*bis*(2-fluorobenzylidene)piperidone (EF24) in anti-angiogenesis, cell cycle arrest, and apoptosis of cancer cells. These authors observed that the *bis*-benzylidenepiperidone, pyrone, and cyclohexanone derivatives, containing the α,β-unsaturated ketone unit, exhibit much greater anticancer and anti-angiogenesis activities than curcumin, with its 1,3-diketone unit [15].

During our ongoing studies aimed at the discovery of new heterocycles endowed with antitumour activity, we have reported on the synthesis and antitumor activities of a series of (*E*)-3,5-*bis*(arylidene)-4-piperidones carrying a variety of aryl and heteroaryl groups [16]. Some 2-aminoquinazolines, their aza analogues, and the other fused pyridine analogues such as 5-deazaaminopetrin [17] and quinolones [18] interfere with the folic acid synthesis. These findings rationalized the design and synthesis of some pyrazolo[4,3-*c*]pyridine, pyrido[4,3-*d*]pyrimidine, pyrano[3,2-*c*]pyridine, and pyrido[3,2-*c*]pyridine targets carrying an arylidene moiety to be evaluated as antitumor agents which may exert their activity through folic acid synthesis inhibition. The results revealed that compounds exhibited a broad spectrum of antitumor activity. In addition, some members proved to be of moderate selectivity toward leukemia cell lines. Among the investigated heterocycles, the pyrano[3,2-*c*]pyridine heterocyclic system proved to be the most active antitumor agents [16]. On the other hand, we have identified some related members belonging to the same fused heterocyclic ring systems as potential antioxidants with remarkable oxygen free-radical scavenger activity [19]. Careful literature survey revealed that incorporation of alkoxy substituents (methoxy and / or aryloxy moieties) results in significant enhancement of several biological activities due to the magnification of the compounds' lipophilicity [20 – 23].

Motivated by the above-mentioned findings, the present study deals with the synthesis of a series of (*E*)-3,5-*bis*(arylidene)-4-piperidones and some derived pyrazolo[4,3-*c*]pyridine, pyrido[4,3-*d*]pyrimidine, pyrano[3,2-*c*]pyridine, and pyrido[3,2-*c*]pyridine ring systems carrying methoxylated aryl groups. The variation in the nature and size of substituents at such structures was thought to be of interest representing variable electronic, lipophilic, and steric environment that would influence the anticipated biological activity. The *in-vitro* antitumor activity of the newly synthesized compounds was evaluated according to the current one-dose protocol of the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay.

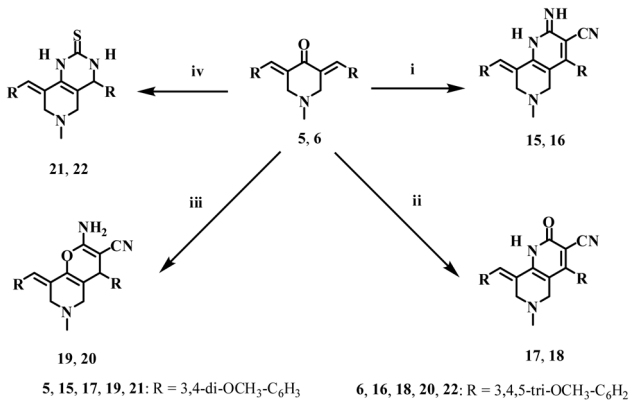


Scheme 1. Synthesis of compounds **4**–**14**.

Results and discussion

Chemistry

The designed target compounds depicted in Schemes 1 and 2 were obtained by reacting the starting material 1-methyl-4-piperidone with variety of methoxylated aromatic aldehydes **1**–**3** under aldol condensation conditions to produce the diarylidene ketone analogues **4**–**6** according to a previously described procedure [16]. These chalcones were employed as key intermediates for further synthesis of the other target molecules. They were subjected to cycloaddition condensation reactions using 4-fluoro- or 4-sulfamylphenylhydrazine hydrochlorides to give the corresponding 2-(4-fluorophenyl or 4-aminosulfonylphenyl)pyrazolo[4,3-*c*]pyridines **7**–**11**. Analogously, condensation of the same diarylidene derivatives **4**–**6** with 2-hydrazinoethanol in the presence of sodium metal afforded the corresponding 2-(2-hydroxyethyl)pyrazolo[4,3-*c*]pyridines **12-14** (Scheme 1). On the other hand, the chalcones **5** and **6** were further utilized for another cyclocondensation reactions using either malononitrile or ethyl cyanoacetate in the presence of ammonium acetate in refluxing ethanol to afford the 2-iminopyrido[3,2-*c*]pyridine and pyrido[3,2-*c*]pyridin-2-one derivatives **15**, **16** and **17**, **18**, respectively. It is worth mention-



Reagents and reaction conditions: i) Malononitrile, ammonium acetate, EtOH, reflux, 4h; **ii)** ethyl cyanoacetate, ammonium acetate, EtOH, reflux, 8 h; **iii)** malononitrile, *n*-butanol, reflux, 5-6 h; **iv)** thiourea, NaOH, EtOH, reflux, 8-10 h.

Scheme 2. Synthesis of compounds **15**–**22**.

ing that reaction of **5** and **6** with malononitrile in refluxing *n*-butanol produced the pyrano[3,2-*c*]pyridine analogues **19** and **20**, respectively. Finally, the targeted pyrido[4,3-*d*]pyrimidine derivatives **21** and **22** could be successfully obtained via the reaction of **5** and **6** with thiourea in the presence of sodium hydroxide in refluxing ethanol (Scheme 2).

Preliminary *in-vitro* anticancer screening

Out of the newly synthesized compounds, eight derivatives **7**, **9**, **10**, **12**, **13**, **15**, **17**, and **19** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. The effective one-dose assay has been added to the NCI 60 cell screen in order to increase the compound throughput and reduce data turnaround time to suppliers while maintaining efficient identification of active compounds [24–26]. In this protocol, all compounds submitted to the NCI 60 Cell screen, are tested initially at a single high dose (10 μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would progress to the five-dose screen. The threshold inhibition criteria for progression to the five-dose screen were designed to efficiently capture compounds with antiproliferative activity and are based on careful analysis of historical Development Therapeutic Program (DTP) screening data. The data are reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI%) caused by the test compounds (Table 1).

The obtained data revealed that some of the tested subpanel tumor cell lines exhibited variable sensitivity profiles against most of the tested compounds. Among these, the non-small cell lung cancer Hop-92 cell line exhibited a wide range of sensitivity towards all of the tested analogues, particularly compound **19** (GI 63.9%). Moreover, compounds **7**, **12**, **15**, and **17** showed moderate growth inhibitory activity against the same cell line with GI values of 40.8, 49.5, 46.3, and 46.0%, respectively. Furthermore, the growth of the breast cancer T-47D cell line was variably affected by the presence of all the tested compounds except the analog **10**. Particular effectiveness against this cell line was shown by compound **7** with a GI value of 54.7%, whereas the analogues **15** and **17** exhibited moderate activity against the same cell line (GI 34.7 and 38.6%, respectively). Among the ovarian cancer subpanel, the OVCAR-4 cell line showed moderate sensitivity towards compound **19** (GI 48.5%), whereas the MCF7 cell line was mildly affected by the presence of seven out of the tested compounds with a GI range of 15.0 to 29.2%. Regarding the activity against the leukemia subpanel, the growth of the RPMI-8226 cell line was found to be affected by the presence of the eight tested compounds with a reliable sensitivity to compounds **7** and **9** (GI 43.1 and 52.0%, respectively). Meanwhile, the leukemia MOLT-4 cell line showed moderate to weak sensitivity towards five of the tested compounds especially the compounds **9** and **10** (GI 39.2 and 37.7%, respectively). Additionally, compounds **12** and **19** showed noticeable activity against the leukemia SR cell line with GI values of 43.9 and 37.5%, respectively. Referring to the renal cancer subpanel, the UO-31 cell line showed moderate to mild sensitivity to all the tested compounds, especially compound **7** (GI 43.6%). On the other hand, the growth of the melanoma SK-MEL-2 and CNS cancer (SF-268, SNB-75 and U251) cell lines were mildly affected by the presence of most of the tested compounds with a GI range of 11.0 to 26.3%. It is worth-mentioning that only compounds **7** and **9** were able to inhibit the growth of the prostate cancer PC-3 cell line with GI values of 25.0 and 23.3%, respectively.

Structurally, the biologically active compounds belong to two series: five-membered fused pyridines namely pyrazolo[4,3-*c*]pyridines **7**, **9**, **10**, **12**, and **13** and six-membered fused pyridines namely pyrido[3,2-*c*]pyridines (**15**, **17**) and a pyrano[3,2-*c*]pyridine (**19**). In general, compounds belonging to the first series proved to be biologically more active than those of the second one. Among the derivatives of the first series, the monomethoxylated compounds **7**, **9,** and **12** exerted better antitumor activity when compared with their corresponding dimethoxylated analogues **10** and **13**, besides, the 4-fluorophenyl moiety seemed to be the most favorable substituent. In this view, compound **7** (R = OCH_3) is considered to be the

Table 1. *In-vitro* percentage growth inhibition (GI%) caused by the test compounds against some selected tumor cell lines at the single-dose assay.[a)]

Cpd No.	NSC No.	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
7	**746578**	Non-Small Cell Lung Cancer	HOP-92 (40.8)
		Colon Cancer	HCC-2998 (22.9), HCT-116 (31.1)
		Breast Cancer	HS-578T (23.1), MCF7 (29.2), T-47D (54.7), MDA-MB-231/ATCC (31.4)
			OVCAR-5 (17.3)
		Ovarian Cancer	CCRF-CEM (26.6), K-562 (15.0), RPMI-8226 (43.1), SR (13.7)
		Leukemia	786-0 (27.4), ACHN (26.4), CAKI-1 (26.8), UO-31 (43.6)
			LOX-IMVI (19.2), MALME-3M (29.4), SK-MEL-2 (16.2)
		Renal Cancer	PC-3 (25.0)
			SE-268 (24.8), U251(19.8)
		Melanoma	
		Prostate Cancer	
		CNS Cancer	
9	**746577**	Non-Small Cell Lung Cancer	HOP-92 (18.3)
		Breast Cancer	MCF7 (15.0), T-47D (24.3)
		Leukemia	CCRF-CEM (18.0), HL-60 (TB) (49.4), K-562 (17.8), MOLT-4 (39.2),
			RPMI-8226 (52.0)
			CAKI-1 (46.4), UO-31 (29.6), ACHN (12.6)
		Renal Cancer	SK-MEL-2 (20.0)
			PC-3 (23.3)
		Melanoma	SE-268 (19.8), SNB-75 (23.4), U251(24.3)
		Prostate Cancer	
		CNS Cancer	
10	**746572**	Non-Small Cell Lung Cancer	HOP-92 (15.7)
		Breast Cancer	MCF7 (16.9), T-47D (15.3)
		Leukemia	K-562 (34.3), MOLT-4 (37.7), RPMI-8226 (21.0)
			UO-31 (37.9)
		Renal Cancer	LOX IMVI (19.7)
		Melanoma	U251 (21.5)
		CNS Cancer	
12	**746579**	Non-Small Cell Lung Cancer	HOP-92 (49.5)
		Breast Cancer	MCF7 (17.4), T-47D (16.2)
		Leukemia	MOLT-4 (20.2), RPMI-8226 (30.9), SR (43.9)
			CAKI-1 (34.7), UO-31 (33.5)
		Renal Cancer	MALME-3M (16.0), SK-MEL-2 (20.7)
		Melanoma	SNB-75 (14.2), U251 (13.7)
		CNS Cancer	
13	**746573**	Non-Small Cell Lung Cancer	HOP-92 (25.1)
		Breast Cancer	T-47D (22.6)
		Ovarian Cancer	OVCAR-3 (11.6), OVCAR-5 (12.9)
		Leukemia	MOLT-4 (25.2), RPMI-8226 (20.1)
		Renal Cancer	786-0 (14.6), ACHN (13.3), UO-31 (25.8)
			MALME-3M (16.5), SK-MEL-2 (16.2)
		Melanoma	SNB-75 (12.2)
		CNS Cancer	
15	**746575**	Non-Small Cell Lung Cancer	HOP-92 (46.3)
		Breast Cancer	HS-578T (16.9), MCF7 (17.2), T-47D (34.7)
			RPMI-8226 (29.3), SR (19.9)
		Leukemia	CAKI-1 (28.2), UO-31 (28.8)
		Renal Cancer	MALME-3M (26.5), SK-MEL-2 (20.7)
		Melanoma	SF-268 (26.0), SNB-75 (26.3), U251 (12.8)
		CNS Cancer	
17	**746574**	Non-Small Cell Lung Cancer	HOP-92 (46.0)
		Breast Cancer	HS-578T (25.1), MCF7 (15.1), T-47D (38.6)
			CCRF-CEM (18.2), HL-60 (TB) (21.0), K-562 (20.0), MOLT-4 (18.2),
		Leukemia	RPMI-8226 (11.9)
			UO-31 (18.0)
			LOX IMVI (12.1), SK-MEL-2 (17.2)
		Renal Cancer	SF-268 (15.4), SNB-75 (11.0), U251 (16.6)
		Melanoma	
		CNS Cancer	

Table 1. Continued.

Cpd No.	NSC No.	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
19	**746576**	Non-Small Cell Lung Cancer	HOP-92 (63.9)
		Breast Cancer	HS-578T (39.3), MCF7 (16.5), T-47D (27.5)
			OVCAR-3 (21.3), OVCAR-4 (48.6),
		Ovarian Cancer	OVCAR-5 (22.6)
			RPMI-8226 (11.6), SR (37.5)
		Leukemia	786-0 (21.0), ACHN (19.2), CAKI-1 (16.0), UO-31 (37.0)
		Renal Cancer	LOX IMVI (15.5), SK-MEL-2 (24.3)
			SF-268 (28.4), SNB-75 (26.0), U251 (16.5)
		Melanoma	
		CNS Cancer	

[a] The data obtained from NCI's *in-vitro* disease-oriented human tumor cell screen at 10 μM conc.

Table 2. Physicochemical and analytical data of compounds **7**–**22**.

Compound	R	M.p. (°C) (Cryst. solv.)[a]	Yield (%)	Molecular Formula (Mol. Weight)[b]
7	4-OCH_3-C_6H_4	248-9 (E)	60	$C_{28}H_{28}FN_3O_2$ (457.54)
8	3,4-di-OCH_3-C_6H_3	183-5 (E)	54	$C_{30}H_{32}FN_3O_4$ (517.59)
9	4-OCH_3-C_6H_4	202-3 (E / B)	86	$C_{28}H_{30}N_4O_4S$ (518.63)
10	3,4-di-OCH_3-C_6H_3	264-4 (E / B)	84	$C_{30}H_{34}N_4O_6S$ (578.68)
11	3,4,5-tri-OCH_3-C_6H_2	148-9 (E)	73	$C_{32}H_{38}N_4O_8S$ (638.73)
12	4-OCH_3-C_6H_4	125-6 (E / W)	48	$C_{24}H_{29}N_3O_3$ (407.51)
13	3,4-di-OCH_3-C_6H_3	86-8 (E / W)	83	$C_{26}H_{33}N_3O_5$ (467.56)
14	3,4,5-tri-OCH_3-C_6H_2	133-35 (M / W)	14	$C_{28}H_{37}N_3O_7$ (527.61)
15	3,4-di-OCH_3-C_6H_3	246-8 (DMF / W)	20	$C_{27}H_{28}N_4O_4$ (472.54)
16	3,4,5-tri-OCH_3-C_6H_2	174-6 (DMF / W)	11	$C_{29}H_{32}N_4O_6$ (532.59)
17	3,4-di-OCH_3-C_6H_3	273-5 (E)	21	$C_{27}H_{27}N_3O_5$ (473.52)
18	3,4,5-tri-OCH_3-C_6H_2	157-9 (E)	13	$C_{29}H_{31}N_3O_7$ (533.57)
19	3,4-di-OCH_3-C_6H_3	168-9 (E)	31	$C_{27}H_{29}N_3O_5$ (475.54)
20	3,4,5-tri-OCH_3-C_6H_2	160-2 (E / PE)	50	$C_{29}H_{33}N_3O_7$ (535.59)
21	3,4-di-OCH_3-C_6H_3	180-2 (E)	27	$C_{25}H_{29}N_3O_4S$ (467.58)
22	3,4,5-tri-OCH_3-C_6H_2	161-3 (E / W)	14	$C_{27}H_{33}N_3O_6S$ (527.63)

[a] Crystallization solvent(s): B: benzene, E: ethanol, DMF: *N*,*N*-dimethylformamide, M: methanol, PE: petroleum ether (60:80), W: water.

[b] The found values were within ± 0.4% of the theoretical values.

most active member identified in this study with a broad spectrum of activity against about 22 different tumor cell lines belonging to the nine subpanels employed, with particular effectiveness against the breast cancer T-47D cell line (GI 54.7%). Replacement of the fluorine atom with a sulfonamide functional group resulted in two less active compounds **9** and **10**, of which the monomethoxylated analog (**9**, R = OCH_3) exhibited a better potential and spectrum of activity especially against the leukemia subpanel tumor cell lines. On the other hand, substitution of the pyrazole moiety with a hydroxyethyl fragment (as in **12** and **13**) resulted in an obvious reduction in activity. Among these, the monomethoxylated analog **12** (R = OCH_3) showed noticeable activity against the non-small cell lung cancer Hop-92 and leukemia SR cell lines (GI values 49.5 and 43.9%, respectively). Shifting to the second series (Scheme 2), the most impressive growth inhibitory potential was displayed by the pyranopyridine analog **19** which was active against 18 different tumor cell lines with special remarkable activity against the non-small cell lung cancer Hop-92 and ovarian cancer OVCAR-4 cell lines (GI values 63.9 and 48.5%, respectively).

These heterocycles could be considered as useful templates for future development and further derivatization or modification to obtain more potent and selective antitumor agents. The physicochemical and analytical data of compounds **7**–**22** are presented in Table 2.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK) and were uncorrected. The ^{1}H-NMR spectra were recorded on a Varian EM 360 spectrometer using tetramethylsilane as the internal standard (Varian Inc., Palo Alto, CA, USA; Chemical shifts in δ, ppm). Splitting patterns were designated as follows: s: singlet; brs: broad singlet; d: doublet; m: multiplet. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia, and the found values were within ± 0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck, Germany) and the spots were detected by exposure to UV-lamp at λ = 254 nm.

*General procedure for preparation of (E)-2-(4-fluorophenyl)-3-aryl-5-methyl-7-arylidene-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-c]pyridines **7, 8** and (E)-2-(4-aminosulfonylphenyl)-3-aryl-5-methyl-7-arylidene-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-c]pyridines **9–11***

A mixture of the appropriate diarylidene derivative **4**–**6** (0.01 mol), and the corresponding 4-substituted phenylhydrazine hydrochloride (0.015 mol) in EtOH (30 mL) was heated under reflux for 8 – 10 h. The solvent was then removed under reduced pressure and the remaining residue was triturated with water, filtered, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 2.

^{1}H-NMR ($CDCl_3$, ppm) δ:

7: 2.23 (s, 3H, N-C*H*$_3$), 2.97 – 3.06 (m, 2H, N-C*H*$_2$), 3.18 – 3.21 (m, 2H, C*H*$_2$-N), 3.87 (s, 3H, OC*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 4.67 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.78 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.79 (s, 1H, C*H*=C), 6.89–7.27 (m, 12H, Ar*H*).

8: 2.20 (s, 3H, NC*H*$_3$), 3.02 – 3.11 (m, 2H, N-C*H*$_2$), 3.20 – 3.27 (m, 2H, C*H*$_2$-N), 3.90 (s, 6H, 2 OC*H*$_3$), 3.96 (s, 6H, 2 OC*H*$_3$), 4.71 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.77 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.69 (s, 1H, C*H*=C), 7.04 – 7.31 (m, 10H, Ar*H*).

9: 2.15 (s, 3H, NC*H*$_3$), 2.91–2.96 (m, 2H, N-C*H*$_2$), 3.04 – 3.12 (m, 2H, C*H*$_2$-N), 3.79 (s, 3H, OC*H*$_3$), 3.86 (s, 3H, OC*H*$_3$), 4.63 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.68 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.67 (s, 1H, C*H*=C), 6.81 – 7.19 (m, 12H, Ar*H*), 10.64 (brs, 2H, N*H*$_2$).

10: 2.16 (s, 3H, NC*H*$_3$), 2.93 – 2.97 (m, 2H, N-C*H*$_2$), 3.03–3.08 (m, 2H, C*H*$_2$-N), 3.81 (s, 6H, 2 OC*H*$_3$), 3.89 (s, 6H, 2 OC*H*$_3$), 4.65 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.74 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.73 (s, 1H, C*H*=C), 6.89 – 7.26 (m, 10H, Ar*H*), 10.5 (brs, 2H, N*H*$_2$).

11: 2.13 (s, 3H, NC*H*$_3$), 2.96 – 2.99 (m, 2H, N-C*H*$_2$), 3.04–3.10 (m, 2H, C*H*$_2$-N), 3.87 (s, 9H, 3 OC*H*$_3$), 3.94 (s, 9H, 3 OC*H*$_3$), 4.71 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.78 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.70 (s, 1H, C*H*=C), 6.81 – 7.25 (m, 8H, Ar*H*), 10.57 (brs, 2H, N*H*$_2$).

*(E)-2-(2-Hydroxyethyl)-3-aryl-5-methyl-7-arylidene-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-c] pyridines **12–14***

A solution of the appropriate diarylidene derivative **4**–**6** (0.01 mol) in ethanol (20 mL) was heated under reflux with 2-hydrazinoethanol (1.52 g, 0.02 mol) and Na metal (0.5 g, 0.022 mol) for 8 – 10 h. The volume of the reaction mixture was concentrated to half and the separated solid product was filtered, washed with cold ethanol, and recrystallized. Physicochemical and analytical data are recorded in Table 2.

^{1}H-NMR ($CDCl_3$, ppm) δ:

12: 2.17 (s, 3H, NC*H*$_3$), 2.95 (m, 4H, C*H*$_2$C*H*$_2$), 3.0 – 3.09 (m, 2H, N-C*H*$_2$), 3.16 – 3.25 (m, 2H, C*H*$_2$-N), 3.89 (s, 3H, OC*H*$_3$), 3.93 (s, 3H, OC*H*$_3$), 4.74 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.88 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.04 (brs, 1H, O*H*), 6.81 (s, 1H, C*H*=C), 6.96 – 7.18 (m, 8H, Ar*H*).

13: 2.19 (s, 3H, NC*H*$_3$), 2.99 (m, 4H, C*H*$_2$C*H*$_2$), 3.06 – 3.11 (m, 2H, N-C*H*$_2$), 3.17 – 3.26 (m, 2H, C*H*$_2$-N), 3.92 (s, 6H, 2 OC*H*$_3$), 3.97 (s, 6H, 2 OC*H*$_3$), 4.77 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.90 (d, *J* = 8 Hz, 1H, CH-C*H*), 6.09 (brs, 1H, O*H*), 6.78 (s, 1H, C*H*=C), 7.08 – 7.23 (m, 6H, Ar*H*).

14: 2.20 (s, 3H, NC*H*$_3$), 2.97 (m, 4H, C*H*$_2$C*H*$_2$), 3.03 – 3.11 (m, 2H, N-C*H*$_2$), 3.19 – 3.24 (m, 2H, C*H*$_2$-N), 3.88 (s, 9H, 3 OC*H*$_3$), 3.95 (s, 9H, 3 OC*H*$_3$), 4.75 (d, *J* = 8 Hz, 1H, C*H*-CH), 4.91 (d, *J* = 8 Hz, 1H, CH-C*H*), 5.96 (brs, 1H, O*H*), 6.79 (s, 1H, C*H*=C), 7.05 – 7.21 (m, 4H, Ar*H*).

*General procedure for preparation of (E)-3-cyano-4-aryl-2-imino-6-methyl-8-arylidene-5,6,7,8-tetrahydro-1H-pyrido[3,2-c]pyridines **15, 16** and (E)-3-cyano-4-aryl-6-methyl-8-arylidene-5,6,7,8-tetrahydro-1H-pyrido[3,2-c]pyridin-2-ones **17, 18***

A mixture of the diarylidene compound **5** or **6** (0.01 mol), ammonium acetate (4.7 g, 0.06 mol) and malononitrile (for **15**, **16**) or ethyl cyanoacetate (for **17**, **18**) (0.01 mol) was refluxed in ethanol (50 mL) for 4 – 8 h. Solvent was removed under reduced pressure and the obtained residue was triturated with water, filtered, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 2.

^{1}H-NMR (DMSO-d_6, ppm) δ:

15: 2.19 (s, 3H, NC*H*$_3$), 2.49–2.55 (m, 4H, C*H*$_2$NC*H*$_2$), 3.86 (s, 6H, 2 OC*H*$_3$), 3.90 (s, 6H, 2 OC*H*$_3$), 6.87 (s, 1H, C*H*=C), 7.07 – 8.09 (m, 8H, Ar*H* + 2 N*H*).

16: 2.18 (s, 3H, NC*H*$_3$), 2.47 – 2.52 (m, 4H, C*H*$_2$NC*H*$_2$), 3.85 (s, 9H, 3 OC*H*$_3$), 3.89 (s, 9H, 3 OC*H*$_3$), 6.87 (s, 1H, C*H*=C), 7.21 – 8.13 (m, 6H, Ar*H* + 2 N*H*).

17: 2.22 (s, 3H, NC*H*$_3$), 2.58 – 2.64 (m, 4H, C*H*$_2$NC*H*$_2$), 3.79 (s, 6H, 2 OC*H*$_3$), 3.84 (s, 6H, 2 OC*H*$_3$), 6.98 (s, 1H, C*H*=C), 7.23 – 8.12 (m, 7H, Ar*H* + N*H*).

18: 2.23 (s, 3H, NC*H*$_3$), 2.51 – 2.57 (m, 4H, C*H*$_2$NC*H*$_2$), 3.81 (s, 9H, 3 OC*H*$_3$), 3.88 (s, 9H, 3 OC*H*$_3$), 6.94 (s, 1H, C*H*=C), 7.19 – 8.09 (m, 5H, Ar*H* + N*H*).

*(E)-2-Amino-3-cyano-4-aryl-6-methyl-8-arylidene-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridines **19, 20***

To a solution of the diarylidene compound **5** or **6** (0.01 mol) in *n*-butanol (50 mL), was added malononitrile (0.7 g, 0.01 mol), and the reaction mixture was heated under reflux for 5 – 6 h. After cooling, the precipitated product was filtered off and recrystallized. Physicochemical and analytical data are recorded in Table 2.

^{1}H-NMR (DMSO-d_6, ppm) δ:

19: 2.16 (s, 3H, NCH_3), 2.68 – 2.81 (m, 4H, CH_2NCH_2), 3.79 (s, 6H, 2 OCH_3), 3.84 (s, 6H, 2 OCH_3), 4.19 (s, 1H, pyran-C$_4$-*H*), 6.83 (brs, 2H, NH_2), 6.90 (s, 1H, C*H*=C), 7.12 – 7.27 (m, 6H, Ar*H*).

20: 2.14 (s, 3H, NCH_3), 2.65 – 2.79 (m, 4H, CH_2NCH_2), 3.80 (s, 9H, 3 OCH_3), 3.87 (s, 9H, 3 OCH_3), 4.21 (s, 1H, pyran-*H*), 6.75 (brs, 2H, NH_2), 6.92 (s, 1H, C*H*=C), 7.15 – 7.31 (m, 4H, Ar*H*).

(E)-4-Aryl-6-methyl-8-arylidene-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-d]pyrimidine-2-thiones ***21, 22***

A mixture of the diarylidene derivative **5** or **6** (0.01 mol), thiourea (0.8 g, 0.01 mol), and NaOH (0.4 g, 0.01 mol) in ethanol (50 mL), was heated under reflux for 8 – 10 h. After cooling to room temperature, water (20 mL) was added and the mixture was neutralized to pH 6 using 10% HCl solution. The separated solid was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 2.

^{1}H-NMR (DMSO-d_6, ppm) δ:

21: 2.27 (s, 3H, NCH_3), 3.07 – 3.15 (dd, *J* = 8.0 Hz, 2H, NCH_2), 3.59–3.71 (m, 2H, CH_2N), 3.86 (s, 6H, 2 OCH_3), 3.90 (s, 6H, 2 OCH_3), 5.19 (s, 1H, C*H*-NH), 6.68 (s, 1H, C*H*=C), 7.13 – 7.42 (m, 6H, Ar*H*), 8.13 (brs, 1H, N*H*), 8.44 (brs, 1H, N*H*).

22: 2.25 (s, 3H, NCH_3), 3.11 – 3.24 (dd, *J* = 8.0 Hz, 2H, NCH_2), 3.55 – 3.69 (m, 2H, CH_2N), 3.84 (s, 9H, 3 OCH_3), 3.91 (s, 9H, 3 OCH_3), 5.12 (s, 1H, C*H*-NH), 6.64 (s, 1H, C*H*=C), 7.08 – 7.36 (m, 4H, Ar*H*), 8.08 (brs, 1H, N*H*), 8.29 (brs, 1H, N*H*).

Preliminary *in-vitro* anticancer screening

Out of the newly synthesized compounds, eight derivatives, namely **7**, **9**, **10**, **12**, **13**, **15**, **17**, and **19** were selected by the National Cancer Institute (NCI) for the *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* anticancer activity. A primary *in-vitro* one-dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda, MD, USA [24 – 26]. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48 h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth.

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