Synthesis and Antitumor Activity of 9-Anilino, Phenylhydrazino, and Sulphonamido Analogs of 2- or 4-Methoxy-6-nitroacridines

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Summary

Synthesis of several new 9-anilino, phenylhydrazino, and sulphonamido analogs of 2- or 4-methoxy-6-nitroacridine derivatives is described. The prepared compounds were tested for their *in vitro* antitumor activity against 60 human tumor cell lines by the National Cancer Institute (NCI) and showed a potential anticancer activity. Compounds 9-(phenylhydrazino)-2-methoxy-6-nitroacridine (**8a**) and 9-(4-chlorophenylhydrazino)-4-methoxy-6-nitroacridine (**9b**) exhibited a broad spectrum antitumor activity with full panel (MG-MID) median growth inhibition (GI_{50}), of 16.1 and 10.9 μM and total growth inhibition (TGI) of 66.7 and 37.9 μM, respectively. Meanwhile, compounds **15a** and **15b** showed moderate selectivity toward leukemia cell lines. As a trial to explore the mode of action of their antitumor activity, the 6-nitroacridine analogs were evaluated for their inhibitory effect on major cell cycle control proteins cdc2 kinase and cdc25 phosphatase as possible molecular targets that may account for antimitotic potency. None of the tested compounds proved to exert their activity *via* this antimitotic mode of action.

Introduction

The effectiveness of DNA intercalators in cancer treatment has been known for some time and well documented [1,2]. Many of the clinically used acridine derivatives have been shown to exhibit their anticancer activity by intercalating with the DNA duplex such as m-Amsacrine (m-AMSA, **A**) and nitracrine (**C**) [3–5]. m-Amsacrine (**A**) has a short plasma half-life due to its oxidation into the inactive diiminoquinone structure (m-AQDI, **B**) [6,7]. It was found that more potent derivatives with prolonged plasma half-life could be obtained if the formation of such diiminoquinone is inhibited[7,8] (Figure 1).

The nitroacridine analog, nitracrine (**C**) has been used clinically for the treatment of mammary, lung, ovarian, and colon cancers [9] the hypoxia selective-cytotoxicity of nitracrine justifies the therapeutic potential of the nitroacridines. Hypoxic cells are refractory to ionizing radiation and to many chemotherapeutic agents, in addition to their pronounced existence in solid tumors rather than in normal tissues. These findings provide an environmental difference which may be exploited in the design of novel antitumor nitroacridines [10,11].

Nitracrine (**C**) screening results proved its *in vitro* rather than *in vivo* potency, this could be attributed to the formation of its inactive hydroxylamine analog **D** [12,13]. The introduction of 4-methoxy group as in **E** (Figure 1), was found to provide an improved metabolic stability and a decreased

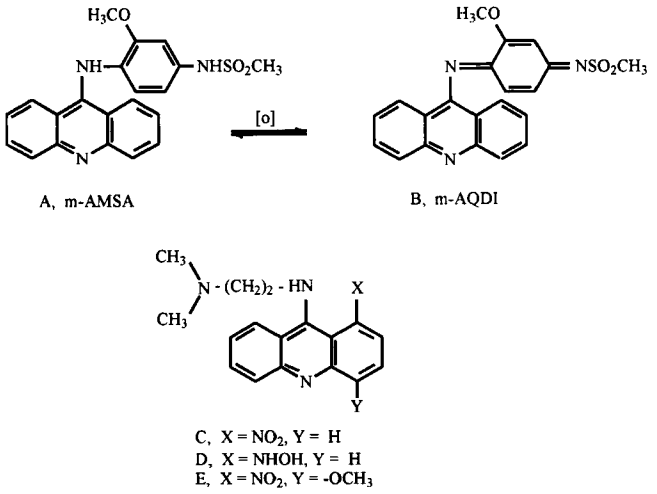

Figure 1.

oxygen sensitivity to nitracrine, preventing the formation of such hydroxylamine derivative. Furthermore, methoxy group is found in the structure of most of the natural antimitotic agents such as colchicine, podophyllotoxin, steganacin, and combrestatin-A4; all contain a trimethoxybenzene moiety [14].

In the present study, certain series of 6-nitroacridines with expected intercalation and hypoxia selective-cytotoxicity was synthesized. The newly prepared nitroacridines were substituted at position 2- or 4-with a methoxy group borrowed from the known naturally occurring antitumor agents [14]. Meanwhile, 2- or 4-methoxyanilino, 4-substituted phenylhydrazino and substituted sulphonamido moities were placed at position 9. Unlike m-Amsacrine (**A**), the 2- or 4-methoxyanilino and sulphonamido moieties were individually introduced to the 9-position of the new acridines, in two different structures, to allow the study of their influence on activity in the absence of each other. Also, the methylsulfonylaminophenyl ($phNHSO_2CH_3$) of **A** is reversed in the new compounds to be $phSO_2NH$-R in order to prevent the formation of the inactive diiminoquinone structure. Recent data [15,16] suggested that inhibition of major cell cycle regulator proteins such as cdc2 Kinase and cdc25 phosphatase might be a possible mechanism by which some anticancer agents exhibit their antimitotic activity. As a trial to explore the compounds possible antimitotic mode of action, the affinity of the new nitroacridine derivatives toward cdc2 kinase and cdc25 phosphatase enzymes was investigated.

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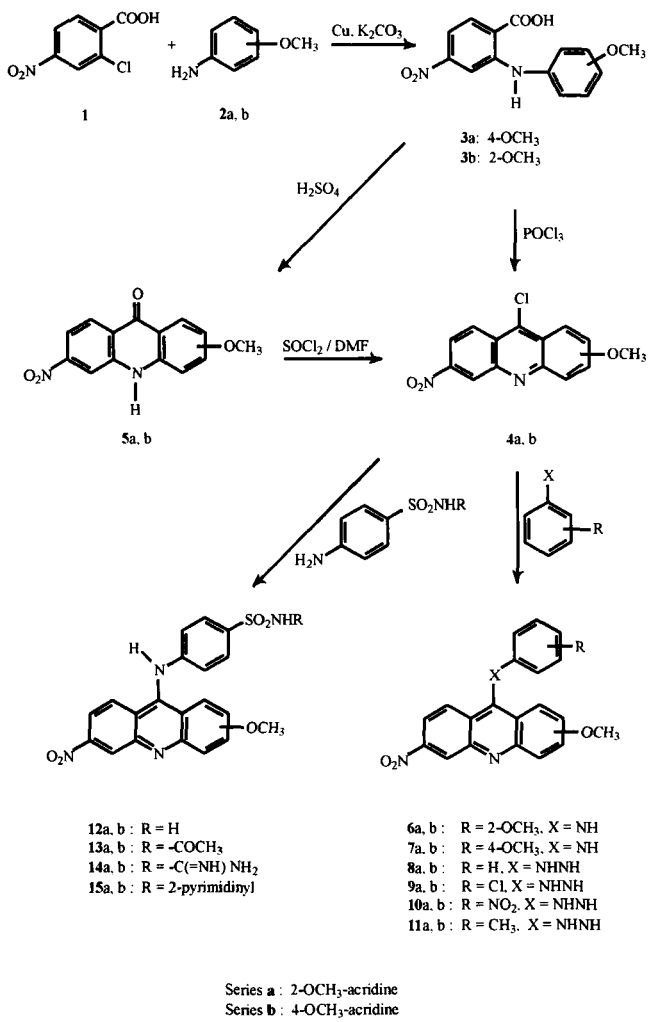
Scheme 1:



Chemistry

The synthesis of the new 6-nitroacridine derivatives is depicted in Scheme 1. The anthranilic acid analogues **3a** and **3b** were prepared by direct nucleophilic displacment of 2-chloro-4-nitrobenzoic acid (**1**) with *o.* or *p.* anisidine (**2a,b**) using Ullmann [17] reaction condition. Treatment of **3a** and **3b** with phosphorous oxychloride afforded the 6-nitro-9-chloroacridine derivatives **4a** and **4b**, respectively in nearly quantitative yields. Compounds **4a,b** could be obtained using an alternate route; **3a,b** was cyclized into 9-(10H)-acridinones (**5a,b**) using conc. H_2SO_4 followed by their chlorination into **4a,b** using $SOCl_2$/DMF and used in the next reaction without further purification. The 9-chloro moiety was then displaced with *o-* or *p*-anisidine; phenylhydrazine, and substituted sulphonamide derivatives to give the 9-substituted acridine analogs **6–15**. The physical and analytical data of the newly prepared compounds are listed in Table 1.

Biology: Results and Discussion

Antitumor Testing

Compounds **8a**, **8b**, **9a**, **9b**, **10a**, **13a**, **13b**, **14a**, **14b**, **15a**, and **15b** were subjected to the NCI *in vitro* disease-oriented human cells screening panel assay [18–21]. About 60 cell lines of nine tumor subpanels were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log concentration-% growth inhibition curves. Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value corresponds to the compound's concentration causing 50% decrease in net cell growth, the TGI value is the compound's concentration resulting in total growth inhibition and the LC_{50} value is the compound's concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI or LC_{50} values of all cell lines in the subpanel or the full panel, respectively [18], the NCI antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor compounds and tumor or subpanel-selective agents [19]

In the present study, the 2- or 4-methoxy-6-nitroacridine analogs **8a–15b** showed a distinctive potential pattern of selectivity as well as broad spectrum antitumor activity (Tables 2–6). With regard to sensitivity against individual cell lines, compound **8a** is particularly effective against non-small cell lung NCI-H226 with GI_{50}, TGI values of 0.2, 6.7 μM, respectively. Compound **9a** is effective against non-small cell lung NCI-H460 with GI_{50}, TGI values of 0.9, 20.5 μM, respectively; and ovarian OVCAR-3 with GI_{50}, TGI values of <0.01 and 0.9 μM, respectively. Compounds **8a** and **9a** were sensitive against melanoma UACC-257 with GI_{50} values of 1.2 and 0.6 μM; and against melanoma UACC-62 with GI_{50} values of 0.8 and 1.8 μM, respectively. Compounds **8a**, **9a**, and **10a** were also effective against renal A-498 with GI_{50} values of 0.07, 0.02, and 0.04 μM and against renal CAKI-1 with GI_{50} values of 0.2, 0.07, and 2.1 μM, respectively (Table 2). Comparing these data with m-AMSA (**A**) data available from NCI against the aforementioned individual cell lines proved that compounds **8a** and **9a** with inhibitory concentrations GI_{50} 1.2, 0.6 μM; respectively are more potent than **A** (GI_{50} 3.1 μM) toward UACC-257 melanoma cell line. Compound **9a** (GI_{50} 0.01 μM, TGI 0.9 μM) is also more active than **A** (GI_{50} 1.1 μM, TGI 4.9 μM) against OVCAR3 ovarian cancer. Compound **8a**, **9a**, and **10a** showed inhibitory concentration GI_{50} 0.07, 0.02, 0.04 μM and cytostatic concentration TGI 0.4, 0.1, 0.2 μm; respectively toward A498 renal cancer while **A** showed only GI_{50} and TGI 0.33 and 3.6 μM, respectively.

With regard to broad spectrum antitumor activity, most of the tested compounds showed (GI_{50}, TGI, and $LC_{50} \leq$ 100 μM) against leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer subpanel cell lines (Tables 3–5). Compounds **9a**, **9b**, **13b**, and **14a** showed GI_{50}, TGI, and LC_{50} values < 100 μM. These four compounds exhibited effective growth inhibition GI_{50} values (MG-MID) of 9.5, 10.9, 39.6, and 23.1 μM, cytostatic activity TGI values (MG-MID) of 47.8, 37.9, 92.2, and

Table 1: Physical and analytical data of compounds **3a–15b**.

Cpd.	Crystallization solvent	Mp (°C)	Yield (%)	Molecular formula	IR (KBr, cm^{-1})	^1H NMR (DMSO-d_6)
3a	EtOH-H_2O	238–40	50	$C_{14}H_{12}N_2O_5$	3400–2400, 1680	4.0 (s, 3H, OCH_3), 6.84 (s, 1H, NH). 7.5–8.85 (m, 7H, aromatic), 11.05 (s, 1H, COOH)
3b	EtOH-H_2O	187–89	45	$C_{14}H_{12}N_2O_5$	3400–2400, 1660	3.92 (s, 3H, OCH_3), 7.35–8.80 (m, 8H, aromatic + NH), 11.05 (s, 1H, COOH)
5a	EtOH	> 340	80	$C_{14}H_{10}N_2O_4$	3060, 2960, 1960	3.98 (s, 3H, OCH_3), 7.4–8.85 (m, 7H, aromatic)
5b	ETOH	> 340	83	$C_{14}H_{10}N_2O_4$	3040,2960, 1980	3.97 (s, 3H, OCH_3), 7.38–8.83 (m, 7H, aromatic)
6a	Acetone-H_2O	168–70	39	$C_{21}H_{17}N_3O_4$	3300, 3020, 2960	3.95 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.4–8.75 (m, 11H, aromatic + NH)
6b	Acetone-H_2O	235–36	35	$C_{21}H_{17}N_3O_4$	3240, 3040, 2970	3.95 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.41–8.82 (m, 11 H, aromatic + NH)
7a	Acetone-H_2O	238–40	36	$C_{21}H_{17}N_3O_4$	3260, 3040, 2980	3.98 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 7.43–8.85 (m, 11 H, aromatic + NH)
7b	Acetone-H_2O	213–15	35	$C_{21}H_{17}N_3O_4$	3250, 3030, 2980	3.89 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 7.42–8.85 (m, 10 H, aromatic), 9.23 (brs, 1H, NH)
8a	CH_3OH-H_2O	125–27	40	$C_{20}H_{16}N_4O_3$	3300, 3020, 2970	3.94 (s, 3H, OCH_3), 6.3 (brs, 2H, NH), 7.35–8.89 (m, 11H, aromatic)
8b	CH_3OH-H_2O	148–50	40	$C_{20}H_{16}N_4O_3$	3250, 3030, 2970	3.95 (s, 3H, OCH_3), 4.2 (brs, 1H, NH), 7.35–8.88 (m, 12H, aromatic + NH)
9a	CH_3OH-H_2O	128–29	35	$C_{20}H_{15}ClN_4O_3$		4.06 (s, 3H, OCH_3), 5.4 (brs, 1H, NH), 7.34–8.99 (m, 11H, aromatic + NH)
9b	CH_3OH-H_2O	119–21	36	$C_{20}H_{15}ClN_4O_3$	3220, 3040, 2950	3.95 (s, 3H, OCH_3), 5.9 (brs, 1H, NH) 7.15 –8.76 (m, 11H, aromatic + NH)
10a	DMF-H_2O	136–7	40	$C_{20}H_{15}N_5O_5$		3.98 (s, 3H, OCH_3), 4.3 (brs, 1H, NH), 7.3 3 –8.90 (m, 10H, aromatic), 9.2 (brs, 1H, NH)
10b	DMF-H_2O	146–8	35	$C_{20}H_{15}N_5O_5$	3240, 3040, 2970	4.05 (s, 3H, OCH_3),5.2 (brs, 2H, NH), 7.3 5–8.90 (m, 10H, aromatic)

Table 1: *Continued.*

Cpd.	Crystallization solvent	Mp (°C)	Yield (%)	Molecular formula	IR (KBr, cm^{-1})	^1H NMR (DMSO-d_6)
11a	CH_3OH-H_2O	120–1	35	$C_{21}H_{18}N_4O_3$		2.1 (s, 3H, CH_3), 4.01 (s, 3H, OCH_3), 7.27–8.91 (m, 12H, aromatic + NH)
11b	CH_3OH-H_2O	138–40	35	$C_{21}H_{18}N_4O_3$	3260, 3040, 2960	2.1 (s, 3H, CH_3),4.01 (s, 3H, OCH_3),7.37–8.99 (m, 12H, aromatic + NH)
12a	DMF-H_2O	250–1	42	$C_{20}H_{16}N_4O_5S$	3280, 3250, 3040,2980	4.05 (s, 3H, OCH_3),5.2 (brs, 1H, NH), 7.35–8.90 (m, 12H, aromatic + NH)
12b	DMF-H_2O	239–41	30	$C_{20}H_{16}N_4O_5S$		4.0 (s, 3H, OCH_3), 7.37–8.91 (m, 13H, aromatic + NH)
13a	DMF-H_2O	202–5	40	$C_{22}H_{18}N_4O_6S$	3230, 3030, 2970	1.9 (s, 3H, CH_3), 4.03 (s, 3H, OCH_3), 7.11–8.92 (m, 12H, aromatic + NH)
13b	Acetone-H_2O	136–38	45	$C_{22}H_{18}N_4O_6S$	3240, 3030, 2960	2.0 (s, 3H, CH_3),4.00 (s, 3H, OCH_3),7.13–8.91 (m, 12H, aromatic + NH)
14a	Acetone-H_2O	133–35	30	$C_{21}H_{18}N_4O_5S$	3250, 3010, 2970	4.04 (s, 3H, OCH_3),5.6 (brm, 2H, NH), 7.33–8.92 (m, 13H, aromatic + NH)
14b	Acetone	188–90	38	$C_{21}H_{18}N_4O_5S$	3230, 3030, 2980	4.06 (s, 3H, OCH_3), 4.9 (bn-n, 2H, NM, 7.29–8.91 (m, 13H, aromatic + NH)
15a	Acetone-H_2O	223–25	34	$C_{24}H_{18}N_6O_5S$	3260, 3040, 2980	4.03 (s, 3H, OCH_3), 5.2 (brs, 1H, NH), 7.30–8.95 (m, 14H, aromatic + NH)
15b	DMF-H_2O	284–86	30	$C_{24}H_{18}N_6O_5S$	3250, 3020, 2960	4.03 (s, 3H, OCH_3), 5.8 (brs, 1H, NH), 7.32–8.96 (m, 14H, aromatic + NH)

Table 2: Growth inhibitory concentration (GI_{50}, TGI) of some selected in vitro tumor cell lines (μM).[a]

Cpd	Non-small cell lung cancer				Melanoma				Ovarian		Renal			
	CI-H226		NCI-H460		UACC-257		UACC-62		OVCAR-3		A-498		CAKI-1	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
8a	0.2	6.7	11.8	42.3	1.2	–[b]	0.8	19.5	26.6	–	0.07	0.4	0.2	NT[c]
8b	24.9	–	32.3	–	29.9	–	6.4	–	50.2	–	43.4	–	12.5	–
9a	3.9	–	0.9	20.5	0.6	NT	1.8	28.7	<0.01	0.9	0.02	0.1	0.07	1.2
9b	13.9	36.8	4.9	17.2	19.2	55.2	8.4	26.3	12.5	36.3	13.0	33.2	2.3	15.2
10a	7.9	–	NT	NT	2.3	–	5.7	–	32.8	–	0.04	0.2	2.1	62.2
13a	–	–	40.7	–	–	–	73.4	–	38.1	–	–	–	–	–
13b	42.2	–	32.3	–	47.6	–	63.5	–	49.2	–	95.8	–	NT	NT
14a	20.4	–	9.8	25.0	51.2	–	14.6	46.9	16.5	72.6	–	–	23.3	–
14b	38.8	–	4.3	–	17.4	56.1	6.0	32.8	20.3	–	19.1	44.9	5.6	32.1
15a	78.0	–	61.5	–	–	–	–	–	–	–	–	–	–	–
15b	38.7	–	27.7	–	–	–	–	–	–	–	–	–	48.0	–

[a]Data obtained from NCI's *in vitro* disease-oriented human tumor cell screen (see reference [18,19] for detail);
[b]–, GI_{50} or TGI values > 100 μM.; [c]NT, not tested.

Table 3: Median growth inhibitory concentration (GI_{50}, μM) of *in vitro* subpanel tumor cell lines.

Cpd[c]	Subpanel Tumor Cell Lines[a]									MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
8a	17.1	21.9	17.6	37.3	24.7	38.0	23.0	37.5	26.5	16.1
8b	24.5	36.9	27.8	41.0	36.6	39.7	27.8	44.2	30.1	27.1
9a	19.2	14.2	16.7	44.8	22.5	20.1	11.5	21.2	18.3	9.5
9b	6.3	11.2	14.0	14.6	15.8	15.6	10.1	9.4	18.8	10.9
10a	18.7	24.2	36.5	35.1	28.8	34.3	24.3	27.0	34.3	17.0
13a	25.3	83.0	55.8	89.0	85.4	84.5	82.8	68.1	65.3	65.0
13b	22.9	77.5	36.8	64.6	58.5	65.1	50.9	60.9	63.8	39.6
14a	9.3	22.2	23.5	34.7	24.7	50.8	35.8	20.4	25.7	23.1
14b	6.0	17.6	17.5	11.6	15.4	25.9	19.8	52.7	25.0	12.5
15a	19.4	87.8	64.0	90.0	83.1	82.4	76.7	78.4	90.7	62.4
15b	11.3	66.9	44.1	77.3	65.5	79.2	49.3	30.1	50.5	39.6
m-AMSA	0.1	1.0	0.6	0.2	1.5	1.6	0.4	0.1	2.1	3.0

[a]I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer. [b] GI_{50} (μM) full panel mean-graph midpoint. [c]Compound **10b** was not tested.

Table 4: Total growth inhibitory concentration (TGI, μM) of *in vitro* subpanel tumor cell lines.

Cpd[c]	Subpanel Tumor Cell Lines[a]									MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
8a	78.0	57.1	56.7	95.0	75.1	–[d]	83.5	–	88.5	66.7
8b	81.8	–	92.3	–	–	–	91.8	–	95.9	92.2
9a	59.4	74.6	67.9	92.2	67.7	57.5	40.9	68.3	88.1	47.8
9b	42.9	23.3	60.1	32.3	57.7	47.9	34.9	39.8	67.1	37.9
10a	88.5	–	–	89.0	–	–	83.7	–	–	84.7
13b	77.9	–	77.9	97.6	–	–	–	–	–	92.2
14a	31.6	82.7	78.3	–	69.5	93.2	–	–	97.9	73.7
14b	77.8	79.9	97.9	–	57.6	88.6	62.5	–	73.0	72.6
15a	67.5	–	–	–	–	–	–	–	–	92.2
15b	80.6	–	–	–	–	–	–	–	–	96.4
m-AMSA	85.0	4.0	4.6	7.4	9.5	5.4	3.7	9.0	11.5	6.8

[a]For subpanel tumor cell lines see footnote (a) of table 3. [b]TGI (μM) full panel mean-graph midpoint. [c]Compound **10b** was not tested.
[d]-Compound showed subpanel TGI values > 100 μM.

73.7 μM, and cytotoxic activity LC_{50} values (MG-MID) of 87.0, 77.2, 98.5, and 96.5 μM, respectively. Compounds **8a**, **8b**, **10a**, **14b**, **15a**, and **15b** showed GI_{50} and TGI values < 100 μM. These compounds exhibited growth inhibition GI_{50} values (MG-MID) of 16.1, 27.1, 17.0, 12.5, 62.4, and 39.6 μM, cytostatic activity TGI values (MG-MID) of 66.7, 92.2, 84.7, 72.6, 92.2, and 96.4 μM, respectively. Compound **13a** is the least effective member of this series with GI_{50} (MG-MID) 65.0 μM (Tables 3–5).

The ratio obtained by dividing the compound's full panel MG-MID (μM) by its individual subpanel MG-MID concentration (μM) is considered as a measure for the compound selectivity[19]. Ratios between 3 and 6 refer to moderate selectivity, while ratios greater than 6 indicate selectivity towards the corresponding cell line-subpanel. All of the tested compounds proved to be nonselective with broad spectrum antitumor activity against the nine tumor subpanels used, except compounds **13a**, **14a**, **14b**, **15a**, and **15b**. Compounds **15a** and **15b** showed moderate selectivity at the GI_{50} level towards leukemia cell lines with ratios of 3.2 and 3.5, respectively; while compounds **13a**, **14a**, and **14b** showed only mild selectivity towards the same leukemia cell lines with ratios of 2.6, 2.5, and 2.1, respectively. Compound **14a**, besides its selectivity at the GI_{50} level, it showed the same effectiveness at the TGI level with ratio of 2.3. The *in vitro* antitumor evaluation of the new 9-anilino, phenylhydrazino, and sulphonamido-2 or 4-methoxy-6-nitroacridines (**6a–15b**) revealed the potentiality of this class of compounds as antitumor agents as shown by their activity against a panel of human tumor cell lines at the GI_{50} level.

Table 5: Median lethal concentration (LC_{50}, μM) of *in vitro* subpanel tumor cell lines.

Cpd[c]	Subpanel Tumor Cell Lines[a]									MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
9a	94.9	–[d]	87.1	–	94.6	–	82.1	–	–	87.0
9b	74.8	61.2	87.0	72.3	96.1	87.5	77.9	85.4	89.0	77.2
13b	–	–	87.0	–	–	–	–	–	–	98.5
14a	82.2	94.8	97.3	–	92.8	–	–	–	–	96.5
m-AMSA	–	71.5	50.2	–	82.4	12.5	9.8	–	–	48.0

[a]For subpanel tumor cell lines see footnote (a) of table 3. [b]LC_{50} (μM) full panel mean-graph midpoint. [c]Compounds **8a, 8b, 10a, 13a, 14b, 15a**, and **15b** showed LC_{50} values > 100 μM. [d]– subpanel LC_{50} value > 100 μM.

The activity of the tested compounds could be correlated to structure variations and modifications. In the 9-phenylhydrazino analogs, compounds with a 2-methoxy substituent (series a) seems to be more active than those with a 4-methoxy substituent (series b) as shown in case of **8a**, **9a** which are more active than **8b**, **9b**, GI_{50} (MG-MID) 16.1, 9.5, 27.1, 10.9 μM, respectively. Also, the introduction of a Cl atom to the 4-position of the phenylhydrazine **8a,b** produced **9a,b** with increased activity, while the introduction of a 4-NO_2 group produced **10a**, with no change in activity (Table 3).

In the 9-sulphonamido analogs, compounds with a 4-methoxy substituent (series b) are more active than their 2-methoxy substituted analogs (series a) as shown by **13b** > **13a**; **14b** > **14a**; **15b** > **15a**; GI_{50} (MG-MID) 39.6, 65.0; 12.5, 23.1; 39.6, 62.4 μM, respectively. Also, the use of basic sulphonamides e.g. sulfaguanidine, rather than acidic sulphonamide e.g. sulfacetamide will increase the acitivity as shown by **14a** > **13a**, **14b** > **13b**, GI_{50} (MG-MID) 23.1, 65.0; 12.5; 39.6 μM, respectively.

Cyclization of the guanidino group of **14a** and **14b** into pyrimidine nucleus as in **15a** and **15b**; decreased the spectrum of activity but increased the selective effectiveness toward leukemia cell lines (Table 3, 6). Compounds **14a** GI_{50}, TGI, and LC_{50} (MG-MID) 23.1, 73.7, and 96.5 μM, respectively, and **14b** GI_{50}, TGI and LC_{50} (MG-MID) 12.5, 72.6, and > 100 μM, respectively are the most active members of the 9-sulphonamido-2 or 4-methoxy-6-nitroacridines. Also, it is important to note that these sulphonamido derivatives are incapable to form the inactive diiminoquinone structure as in case of m-AmsA (**A**).

Comparing the obtained results with the NCI data of m-AMSA (**A**) showed that m-AMSA is more potent than the investigated compounds **8a–15b** at the full panel GI_{50}, TGI, and LC_{50} (MG-MID) with values of 3.0, 6.8, and 48.0 μM; respectively. On the subpanel level, compounds **9a**, **9b**, **14a**, and **15a** with TGI (MG-MID) values of 59.4, 42.9, 31.6, and 67.5 μM; respectively, proved to be more active than **A** with TGI (MG-MID) value of 85.0 μM against leukemia subpanel (Table 4). Compounds **9a**, **9b**, and **14a** with LC_{50} (MG-MID) values of 94.9, 74.8, and 82.2 μM; respectively proved to be more potent than **A** with LC_{50} (MG-MID) value of > 100 μM against leukemia subpanel (Table 5). Compound **9b** with LC_{50} (MG-MID) values of 61.2, 72.3, 85.4, and 89.0 μM; respectively against non-small cell lung, CNS, prostate, and breast cancer subpanels, proved to be more active than **A** which showed LC_{50} (MG-MID) value of > 100 μM toward the 5 mentioned subpanels (Table 5).

In conclusion, compounds such as **8a**, **9a**, and **10a** exhibited remarkable activity against some individual cell lines such as UACC-257 melanoma, OVCAR3 ovarian, and A498 renal cancers, and more potent than the parent m-AMSA (**A**) in this respect. Compound **8a** was chosen by the NCI for further *in vivo* evaluation using hollow fiber assay and xenograft models, its result will be reported later.

Inhibition of cdc2 Kinase and cdc25 Phosphatase

Although DNA intercalation and/or inhibition of topoisomerase II seems to be the most two reliable mechanisms by which acridines exhibit their antitumor effect [3–5], recent data [15,16] showed that major cell cycle regulator proteins such as cyclin-dependent kinases (cdk's) play an important role in cell division cycle regulation and human tumor development. The inhibition of such regulators e.g. cdc2 kinase and cdc25 phosphatase may provide a novel approach for the discovery of new antitumor agents. A compound is considered an inhibitor to these enzymes if its IC_{50} < 10 μM. Thus, all of the prepared compounds (**3a–15b**) were tested for their ability to inhibit cdc2 and cdc25. None of these compound was found to exhibit such an inhibitory effect which implies that some other mechanism(s) of action may account for their antitumor activities.

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Table 6: Selectivity ratios of the tested compounds towrds leukemia subpanel (full panel MG-MID / leukemia MG-MID).

Cpd	8a	8b	9a	9b	10a	13a	13b	14a	14b	15a	15b
Full panel MG-MID (μM)	16.1	27.1	9.5	10.9	17.0	65.0	39.6	23.1	12.5	62.4	39.6
Leukemia MG-MID (μM)	17.1	24.5	19.2	6.3	18.7	25.3	22.9	9.3	6.0	19.4	11.3
Selectivity ratio[a]	0.9	1.1	0.5	1.7	0.9	2.6	1.7	2.5	2.1	3.2	3.5

[a] Obtained by dividing the compound's full panel MG-MID (μM) by its leukemia subpanel MG-MID (μM).

Experimental Part

Synthesis

Melting points were determined in open capillaries on Electrothermal melting-point apparatus and are uncorrected. IR spectra were made on Perkin-Elmer IR 297 spectrophotometer. ^{1}H NMR spectra were recorded on Varian 80 MHZ spectrometers, chemical shifts are given in δ (ppm) values downfield from Me_4Si as an internal standard. Elemental analyses were carried out by the Central Laboratory of the College of Pharmacy, King Saud University, on Perkin-Elmer 2400 elemental analyzer, results are within ± 0.4% of the theoretical values. Thin layer chromatography (TLC) was performed on Merck 5 × 10 cm plates, precoated with silica gel GF254, with short wavelength UV light for visualization.

N-(4'-Methoxyanilino)-4-nitroanthranilic acid **(3a)** *and N-(2'-methoxyanilino)-4-nitroanthranilic acid* **(3b)**

A mixture of 2-chloro-4-nitrobenzoic acid (20.5 g, 0.012 mol), appropriate aniline derivative (1.52 g, 0.012 mol), anhydrous K_2CO_3 (3.42 g, 0.025 mol), Cu powder (200 mg), KI (100 mg), and DMF (50 ml) were refluxed for 16 h. The solvent was evaporated *in vacuo*, the obtained residue was extracted with water and filtered. The filtrate was decolorized with charcoal, filtered while hot and acidified with dilute HCl. The obtained red precipitate was filtered, dried, and recrystallized (Table 1).

2-Methoxy-6-nitro-9(10H)acridinone **(5a)** *and 4-methoxy-6-nitro-9(10H)-acridinone* **(5b)**

A mixture of **3b** or **3a** (3 g, 0.01 mol) and 20 ml conc. H_2SO_4 was heated at 100 °C for 4 h, the mixture was cooled, and poured onto ice-water. The formed yellow precipitate was collected, washed with water, dried, and recrystallized from the proper solvent to provide **5a** and **5b**, respectively (Table 1).

9-Chloro-2-methoxy-6-nitroacridine **(4a)** *and 9-chloro-4-methoxy-6-nitroacridine* **(4b)**

Method A: A suspension of **5a** or **5b** (1 g) in thionyl chloride (5 ml) containing 2 drops of DMF, was heated gently under reflux until a homogeneous solution was obtained then heating was continued for further 2 h. The obtained solution was evaporated to dryness, the residue was azeotroped twice with dry benzene (5 ml) and the remained residue was used without further purification in the next step.

Method B: A mixture of **3a** or **3b** (1 g) and $POCl_3$ (10 ml) was refluxed for 3 h in an oil bath. Excess $POCl_3$ was then removed under reduced pressure, the mixture was cooled to room temperature and the content were slowly added to a large excess of ice-ammonium hydroxide mixture. The resulting reaction product was extracted with $CHCl_3$, the chloroformic layer was promptly separated, dried with Na_2SO_4 and concentrated to give **4a** and **4b**. The obtained products were used in the next step without further purification.

9-Substituted-2 or 4-methoxy-6-nitroacridines **(6a-15b)**

A mixture of **4a** or **4b** (1.0 g, 0.0035 mol) and an equimolar amount of the appropriate aniline, phenylhydrazine or sulphonamide in dry DMF (10 ml) containing 3 drops TEA was stirred at room temperature for 2 h, then refluxed for 16 h. The mixture was then allowed to cool and water was added dropwise while stirring and cooling. The obtained precipitate was filtered, dried and recrystallized (Table 1).

Biological testing

Antitumor screening

Compounds **6a–15b** were subjected to the NCI *in vitro* screening panel assays as described elsewhere [20,21].

Inhibition of cdc2 kinase and cdc25 phosphatase

The cdc2 activity is assayed, in the presence of potential inhibitors, using histone H1 and ^{32}P-labelled ATP, as described elsewhere [22], highly purified recombinant glutathione-S-transferase / cdc25 fusion protein, assayed colorimetrically for p-nitrophenylphosphate phosphatase activity in microtitration plates [23].

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