Synthesis and Antitumor Activity of Some New Substituted Quinolin-4-one and 1,7-Naphthyridin-4-one Analogs

H.I. El-Subbagh*, A.H. Abadi, I.E. Al-Khawad, and K.A. Al-Rashood

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

***Key Words**: Synthesis; quinolin-4-one; 1,7-naphthyridin-4-one; antitumor testing; cdc kinase; phosphatase*

Summary

The synthesis of some new analogs of quinolin-4-one and 1,7-naphthyridin-4-one is described. The prepared compounds were tested for their *in vitro* antitumor and cdc2 kinase or cdc25 phosphatase inhibitory activity. Compound ethyl 7-oxo-2,3-dihydro-7*H*-pyrido [1,2,3-*de*][2,3-*b*]pyrido-1,4-thiazine-6-carboxylate (**6b**) showed antitumor activity against CNS SNB-75, breast T-47D, and lung NCI-H522 cancer cell lines with GI_{50} values of 8.3, 17.6, and 22.7 μM, respectively. Meanwhile, the compounds ethyl 4-oxo-8-phenylthio-1*H*,4*H*-quinoline-3-carboxylate (**11a**) and 4-oxo-8-phenylthio-1*H*,4*H*-1,7-naphthyridine-3-carboxylic acid (**12b**) have proved to be cdc25 phosphatase inhibitors at IC_{50} values of 11 and 5 μM, respectively.

Introduction

Quinolinones and their isosteric counterpart, naphthyridinones are known for their antimicrobial and antitumor activity.[1–9] Nalidixic acid **(A)**, the first prototypic "quinolinone" and its modified analoge ofloxacin **(B)** were believed to exert their activities through DNA intercalation and alteration of the normal functions of bacterial gyrase and its corresponding enzyme in human, namely topoisomerase II [7,10,11]. Quinolinones and naphthyridinones were also found to inhibit tubulin polymerization resulting in the disruption or the suppression of both microtubule structure and normal functions of the eukaryotic cells with consequent arrest of mitosis [12]. Several natural flavones *e.g.* 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxy-flavone **(C)** isolated from *Polanisia dodecandra* [13], its synthetic amino analog NSC 664171 **(D)**, and its naphthyridinone isostere **(E)** (Figure 1), showed remarkable *in vitro* cytotoxicity against panels of cell lines, with GI_{50} values in the low micromolar or nanomolar concentration range in most of the human tumor cell lines tested by the National Cancer Institute (NCI). Also they possess inhibitory activity against tubulin polymerization comparable to that of the potent antimitotic natural product colchicine [9].

The cell division cycle comprises two major phases, the S phase, during which DNA is replicated, and the M phase, during which the replicated DNA is equally distributed among the two daughter cells. These two phases are separated by the "gap" periods G_1 and G_2 and are controlled by cyclin-dependent kinases (cdk's) such as cdc2 kinase and its activator cdc25 phosphatase [14–16]. Increasing evidence supports the importance of cdk's in human tumor development, as manifested by their overexpression which is always associated with various types of tumors [17–19]. Thus, the inhibitions of cdc2 kinase and cdc25 phosphatase may provide a novel approach for the discovery of new and selective antitumor agents.

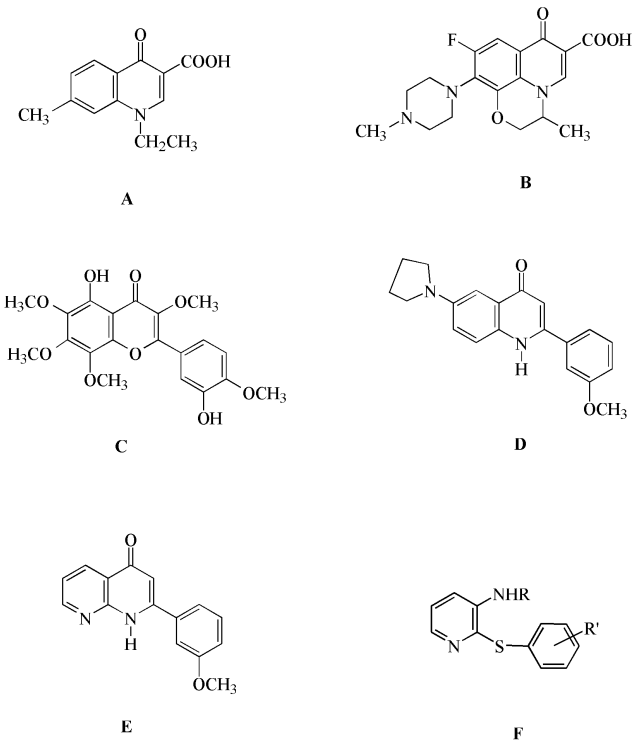


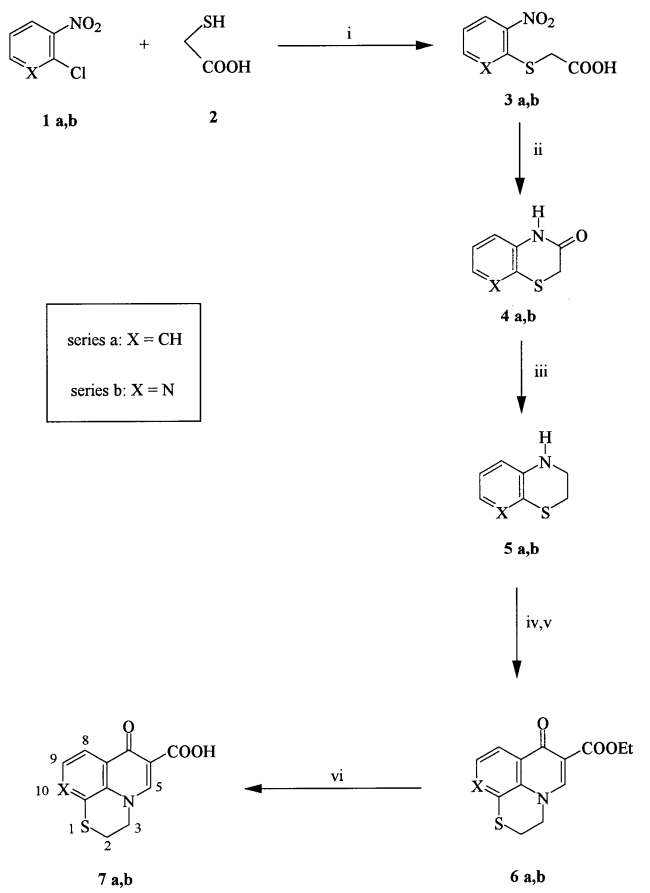
Figure 1

In a previous paper [20], we reported the antitumor potency of some diaryl sulfide analogs; the activity of this type of compound, e.g. **F** (Figure 1), was attributed to the presence of the thioether function. In the present study, a new series of quinolinone and its isosteric congeners, naphthyridinone, were synthesized. The thioether function was introduced to those heteroaromatic nuclei either in the form of a fused tricyclic ring structure as in the pyrido[1,2,3-*de*]-1,4-benzothiazine and its 10-aza isostere (**6a,b** and **7a,b**) or as a phenylthio substituent at position 8 of both heterocycles (quinolinone and naphthyridinone) as in **11a,b** and **12a,b**. Such combination has been designed in order to explore its effect on antitumor activity as well as antimitotic potency as cdc2 kinase and cdc25 phosphatase inhibitors.

Chemistry

The synthesis of the target compounds ethyl 7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzothiazine-6-carboxylate (**6a**), ethyl 7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][2,3-*b*]pyrido-1,4-thiazine-6-carboxylate (**6b**) and their corre-

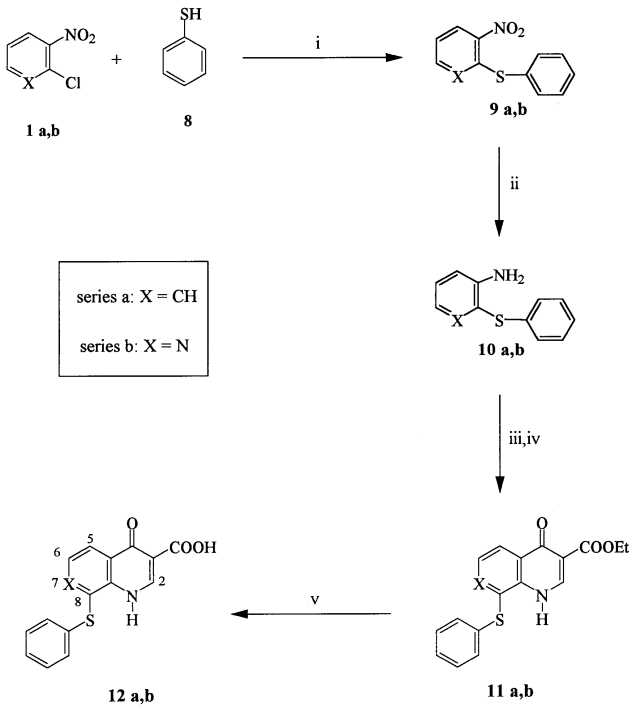
  0365-6233/99/0101/0019 $17.50 +.50/0



Scheme 1. i – NaOH, H_2O; ii – H_2 (g), 5% Pd/C, EtOH; iii – $LiAlH_4$, THF; iv – EMMF, 120 °C, neat 2h; v – PPA, 160 °C, 1 h; vi – 20% aq. NaOH.

sponding acids **7a,b** are depicted in Scheme 1. Reaction of 2-chloronitrobenzene (**1a**) or 2-chloro-3-nitropyridine (**1b**) with thioglycolic acid (**2**) in NaOH solution afforded the corresponding thioethers **3a,b** in good yield (84, 80%, respectively). The $-NO_2$ function of **3a,b** was then subjected to a reductive cyclization process using catalytic hydrogenation (H_2, 5% Pd/C) to give the 1,4-thiazinone analogs **4a,b**. Metallic reduction of **4a,b** using $LiAlH_4$ yielded the 1,4-thiazine derivatives **5a,b** in reasonable yields (86, 80%, respectively). The secondary amine moiety of **5a,b** was further utilized by allowing its reaction with diethyl(ethoxymethylene) malonate (EMME) and the products were subsequently cyclized using polyphosphoric acid (PPA) to afford the tricyclic ester compounds **6a,b**. The ester function of **6a,b** was then hydrolyzed using 20% NaOH to give their corresponding acids **7a,b** in poor yields (20, 17%, respectively).

The synthesis of the target compounds ethyl 4-oxo-8-phenylthio-1*H*,4*H*-quinoline-3-carboxylate (**11a**), ethyl 4-oxo-8-phenylthio-1*H*,4*H*-1,7-naphthyridine-3-carboxylate (**11b**) and their corresponding acids **12a,b** is outlined in Scheme 2. Compound **1a** or **1b** was reacted with thiophenol (**8**) to give the nitrothioether analogs **9a,b**. Catalytic hydrogenation of **9a,b** afforded the corresponding aminothioether derivatives **10a,b**. The amino function of **10a,b** was allowed to react with EMME and the products were cyclized to afford the quinolinone **11a** and the naphthyridinone **11b.** Hydrolysis of the ester group of **11a,b** gave the free acids **12a,b** in poor



Scheme 2. i – NaOH, H_2O; ii – H_2(g), 5% Pd/C, EtOH; iii – EMME, 120 °C, neat, 2 h; iv – PPA, 160 °C, 1 h; v – 20% aq. NaOH.

yields (15, 30% respectively). The physical and analytical data of the newly prepared compounds are listed in Table 1.

Table 1: Physicochemical properties of compounds **3a–12b**.

Compd. No.	X	Solvent	Mp (°C)	Yield %	Molecular formula[a]
3a	CH	EtOH/H_2O	150–152	84	$C_8H_7NO_4S$
3b	N	EtOH	98–100	80	$C_7H_6N_2O_4S$
4a	CH	EtOH/H_2O	172–174	45	C_8H_7NOS
4b	N	EtOH	206–208	60	$C_7H_6N_2OS$
5a	CH	EtOH	198–200	86	$C_8H_9NS{\cdot}HCl$
5b	CH	EtOH	Oil	80	$C_7H_8N_2S$
6a	CH	EtOH/$CHCl_3$	148–150	40	$C_{14}H_{13}NO_3S$
6b	N	EtOH/CH_2Cl_2	85–87	60	$C_{13}H_{12}N_2O_3S$
7a	CH	DMF	195–198	20	$C_{12}H_9NO_3S$
7b	N	DMF/H_2O	136–137	17	$C_{11}H_8N_2O_3S$
9a	CH	Toluene/Pet.ether	73–74	79	$C_{12}H_9NO_2S$
9b	N	Toluene/Pet.ether	90–92	72	$C_{11}H_8N_2O_2S$
10a	CH	EtOH	213–214	50	$C_{12}H_{11}NS{\cdot}HCl$
10b	N	EtOH/H_2O	147–148	45	$C_{11}H_{10}N_2S$
11a	CH	EtOH	120–122	70	$C_{18}H_{15}NO_3S$
11b	N	EtOH/H_2O	105–106	35	$C_{17}H_{14}N_2O_3S$
12a	CH	DMF	202–204	15	$C_{16}H_{11}NO_3S$
12b	N	DMF/H_2O	194–196	30	$C_{15}H_{10}N_2O_3S$

[a] All compounds were analyzed for their C, H, N contents, results were within ±0.4% of the theoretical values.

Biology: Results and Discussion

Antitumor Testing

The prepared compounds were subjected to the NCI *in vitro* disease-oriented human cells screening panel assay [21–24]. About 60 cell lines of nine tumor subpanels were incubated with five concentrations (0.01–100μM) 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).

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

Cell lines	**6a**	**6b**	**11b**	**12b**
Leukemia				
CCRF-CEM	–[b]	48.6	22.3	46.9
HL-60 (TB)	–	–	27.5(83.2[c])	78.8
MOLT-4	–	–	17.8	–
Non-Small Cell Lung Cancer				
A549/ATCC	–	69.9	87.6	–
HOP-92	–	66.4	46.9	–
NCI-H23	–	32.1	32.3	–
NCI-H522	91.5	22.7(74.4[c])	19.7(74.0[c])	76.7
Colon Cancer				
KM12	–	40.9	44.6	39.9
CNS Cancer				
SNB-75	–	8.3	71.1	–
U251	–	43.8	44.4	–
Melanoma				
LOX IMVI	–	57.4	31.0	44.2
UACC-62	–	51.7	36.7	–
Ovarian Cancer				
IGROV1	–	40.5	21.7	–
Renal Cancer				
UO-31	77.7	57.6	13.2	–
Breast Cancer				
BT-549	42.8	62.2	30.7	33.0
T-47D	–	17.6	18.3	–

[a] Data obtained from NCI's *in vitro* disease-oriented human tumor cell screen. [b] –, GI_{50} value > 100 μM. [c] TGI values in μM.

In the present study, only compounds **6a**, **6b**, **11b**, and **12b** showed GI_{50} values at concentrations < 100 μM (Table 2). Those four compounds showed a distinctive potential pattern of sensitivity against individual cell lines. Compound **6b** proved to be active against non-small cell lung NCI-H522 with GI_{50} and TGI values of 22.7 and 74.4 μM, respectively; CNS SNB-75 with GI_{50} value of 8.3 μM and breast cancer T-47D with GI_{50} value of 17.6 μM. Compound **11b** proved to be active against leukemia cell lines CCRF-CEM (GI_{50} 22.3 μM), HL-60 (TB) (GI_{50} 27.5μM, TGI 83.2 μM), MOLT-4 (GI_{50} 17.8 μM). Compound **11b** also proved active against non-small cell lung NCI-H522 with GI_{50} and TGI values of 19.7, 74.0 μM, respectively, and against ovarian IGROV1, Renal UO-31, breast T-47D cancers with GI_{50} values of 21.7, 13.2, and 18.3, respectively. Compounds **6a** and **12b** showed weak to moderate activity against many of the used tumor cell lines.

Structure activity correlations of the obtained results revealed that the isosteric replacement of CH in the quinoline ring by N in the naphthyridine analogs increased the antitumor activity as evidenced by the four folds increase in potency of **6b** (GI_{50} 22.7 μM) against non-small cell lung cancer NCI-H 522 when compared by **6a** (GI_{50} 91.5 μM). This is also noticed in the case of CNS SNB-75 cancer (**6b**, GI_{50} 8.3 μM *vs* **6a**, GI_{50} > 100μM) and breast T-47D (**6b**, GI_{50} 17.6 μM *vs* **6a**, GI_{50} > 100 μM).

Comparing the activity of the cyclized thioether analog **6b** to the 8-phenylthio derivative **11b** showed that the inclusion of the S atom in a ring structure shifted the threshold of

Table 3: Inhibitory concentration (IC50, μM) of the new compounds on cell cycle control enzymes.

Compound	cdc2 kinase	cdc25 phosphatase
3a	–[a]	300
3b	–	300
4a	400	–[a]
4b	280	–
5a	–	–
5b	–	–
6a	500	–
6b	550	30
7a	350	–
7b	–	–
9a	220	55
9b	350	310
10a	350	310
10b	400	300
11a	130	11
11b	300	120
12a	nt[b]	nt[b]
12b	–	5

[a] – , IC_{50} value of > 1000 μM. [b] nt, compound was not tested.

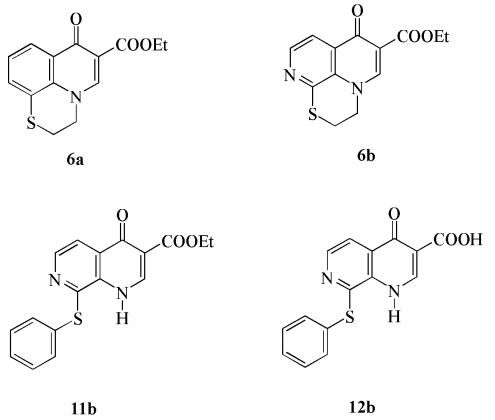


Figure 2

potency from the inactive side towards activity particularly against the leukemia cell lines CCRF-CEM, HL-60 (TB), and MOLT-4 (**6b**, GI_{50} 48.6, >100 and >100 μM *vs* **11b,** GI_{50} 22.3, 27.5, and 17.8 μM, respectively) (Table 2). This is also found in case of Renal UO-31 (**6b**, GI_{50} 57.6 μM while **11b**, GI_{50} 13.2 μM). Hydrolysis of the ester function of **11b** produced the free acid **12b** with an appreciable decrease in the antitumor activity.

Inhibition of cdc2 Kinase and cdc25 Phosphatase

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 its activator cdc25 phosphatase, may provide a novel approach for the discovery of new antitumor agents. A compound is considered an inhibitor to these two enzymes if its IC_{50} < 50 μM [25]. Table 3 shows a list of the IC_{50}'s of the prepared compounds. All of the tested compounds showed no inhibitory effect toward cdc2 kinase. On the other hand compounds **6b**, **11a**, and **12b** proved to be cdc25 phosphatase inhibitors at a concentrations of 30, 11, and 5 μM, respectively. These results indicate that the antitumor activity of **6b** and **12b** might be through an antimitotic mode of action while **11b** may exert its potency through some other mechanism(s). A close examination of the obtained data suggests that the 3-COOH group in **12b** (IC_{50}, 5 μM) is essential for cdc25 phosphatase inhibition if compared with its ester precursor **11b** (IC_{50}, 120 μM).

In conclusion, the obtained data suggests that the 1,7-naphthyridinone series is more potent antitumors than the quinolinone series (**6b** > **6a**, Figure 2 and Table 2). The introduction of S atom at position 8 as a thioether function yielded a more potent analogue rather than its inclusion in the form of a fused 1,4-thiazine ring (**11b** > **6b**, Figure 2 and Table 2). The 3-COOEt function seemed to be more contributing to the antitumor activity than the 3-COOH (**11b** > **12b**, Figure 2); on the contrary, the 3-COOH seemed to be in the favor of the cdc25 phosphatase inhibition activity rather than the 3-COOEt group. Future research efforts will focus on the derivatization of compounds **11b** and **12b** as antitumor and cdc25 phosphatase inhibitor, respectively.

Acknowledgement

The authors are deeply grateful to the authority of the National Cancer Institute, USA, for the antitumor screening and the Centre National de la Recherche Scientifique, Station Biologique, Roscoff, France, for carrying out the inhibitory effect of the prepared compounds on cdc2 kinase and cdc25 phosphatase.

Experimental Part

Synthesis

Melting points were determined in open capillaries on an Electrothermal melting-point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Varian XL-400 MHz spectrometer, chemical shifts are given in δ (ppm) values down field from Me_4Si as an internal standard. Elemental analyses (C,H,N) were carried out by the Central Laboratory of the College of Pharmacy, King Saud University, on Perkin-Elmer 2400 elemental analyzer, results were within ± 0.4% of the theoretical values. Thin layer chromatography (TLC) was performed on Merck 5 × 10 cm plates, precoated with silica gel GF_{254}. Chromatographic separations were carried out on Chromatotron model No. 7924 T, Harrison Research, Palo Alto, CA.

*S-(2-Nitrophenyl)-thioglycolic Acid (**3a**) and S-(3-Nitropyridin-2-yl)-thioglycolic Acid (**3b**)*

A solution of NaOH (1.76 g, 0.44 mol) in water (15 ml) was cooled to 10 °C and stirred. Thioglycolic acid (**2**, 2.0 g, 0.022 mol) was then added followed by a solution of either 2-chloro-nitrobenzene (**1a**) or 2-chloro-3-nitropyridine (**1b**) (0.022 mol) in ethanol (100 ml) while temperature was maintained below 20 °C. The mixture was then refluxed for 1 h then cooled and poured into ice water. The precipitated solid obtained upon acidification with dilute HCl was collected by filtration, dried and recrystallized (Table 1). ^{1}H-NMR (DMSO-d_6), **3a**: δ 4.75 (S, 2H, CH_2), 8.50–9.00 (m, 4H, ArH), 11.80 (s, 1H, COOH). **3b**: δ 4.0 (S, 2H, CH_2), 7.40–8.80 (m, 3H, pyridine-H), 12.10 (s, 1H, COOH).

*3-Oxo-3,4-dihydro-2H-1,4-benzothiazine (**4a**) and 3-Oxo-3,4-dihydro-2H-pyrido[2,3-b]-1,4-thiazine (**4b**)*

A slurry of **3a** or **3b** (0.001 mol) and 350 mg of 5% Pd/C in ethanol (200 ml) was subjected to hydrogenation using a Parr hydrogenator at 50 psi for 5 h. Catalyst was then removed by filtration and filtrate was evaporated *in vacuo* to give crude **4a** or **4b**. An analytically pure sample was obtained by recrystallization (Table 1). ^{1}H-NMR (DMSDO-d_6) , **4a**: δ 3.60 (S, 2H, CH_2), 7.40–7.60 (m, 4H, ArH), 10.60 (brs, 1H, NH). **4b**: δ 3.60 (S, 2H, CH_2), 7.20–7.40 (m, 2H, pyridine-H), 7.92–8.10 (m, 1H, pyridine-H), 10.60 (brs, 1H, NH).

*3,4-Dihydro-2H-1,4-benzothiazine (**5a**) and 3,4-Dihydro-2H-pyrido[2,3-b]-1,4-thiazine (**5b**)*

A solution of **4a** or **4b** (0.05 mol) in dry THF (100 ml) was added dropwise to a suspension of $LiAlH_4$ (2.4 g, 0.06 mol) in dry THF (50 ml). The reaction mixture was stirred at room temperature for 2 h, then dilute HCl was added to destroy the excess of $LiAlH_4$. The mixture was filtered and the solution was alkalinized with 10% NaOH and extracted with $CHCl_3$. The chloroform extract was washed with water, dried, and evaporated. The obtained residue was chromatographed to provide **5a** and **5b** (Table 1). ^{1}H NMR (DMSO-d_6), **5a**: δ 3.38–3.42 (m, 2H, CH_2NH), 3.65–3.80 (t, 2H, CH_2S), 6.70–8.60 (m, 5H, ArH, and NH). **5b**: δ 3.35–3.46 (m, 2H, CH_2NH), 3.75–3.82 (t, 2H, CH_2S), 6.80–8.80 (m, 3H, pyridine-H), 9.20 (brs, 1H, NH).

*Ethyl 7-Oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzothiazine-6-carboxylate (**6a**) and Ethyl 7-Oxo-2,3-dihydro-7H-pyrido [1,2,3-de][2,3-b]pyrido-1,4-thiazine-6-carboxylate (**6b**)*

A mixture of **5a** or **5b** (0.025 mol) and 10.0 g of diethyl(ethoxymethylene) malonate (EMME) was heated at 120°C for 2 h. Polyphosphoric acid (35 g) was added, and the mixture was then gradually heated to 160°C and kept at that temperature for 1 h. After cooling, the reaction mixture was poured into

ice-water and the separated solid was collected by filtration, washed with 10% $NaHCO_3$, water and then recrystallized to afford **6a** and **6b** (Table 1). ^{1}H NMR (DMSO-d_6), **6a**: δ 1.48–1.50 (t, *J* = 7.2Hz, 3H, *CH*$_3$-CH_2), 3.40–3.52 (m, 2H, CH_2S), 4.25–4.32 (q, *J* = 7.2 Hz,2H,CH_3*CH*$_2$), 4.95–5.10 (m, 2H, CH_2N), 7.80–8.10 (m, 3H, ArH), 9.20 (s, 1H, N-CH=C). **6b**: δ 1.50–1.53(t, *J* = 7.3 Hz, 3H, *CH*$_3$$CH_2$), 3.65–3.70 (m, 2H, CH_2S) 4.25–4.30 (q, *J* = 7.3 Hz, 2H, CH_3*CH*$_2$), 5.10–5.20 (m, 2H, CH_2N), 7.10–8.00 (m, 2H, pyridine-H), 9.10 (s, 1H, N-CH=C).

*7-Oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzothiazin-6-carboxylic Acid. (**7a**) and 7-Oxo-2,3-dihydro-7H-pyrido[1,2,3-de][2,3-b]pyrido-1,4-thiazine-6-carboxylic Acid (**7b**)*

A suspension of **6a** or **6b** (1.0 g) in 20% NaOH solution (50 ml) was stirred for 3 h. The cooled mixture was acidified with HCl till pH 5–6 and the precipitate formed was filtered and washed with water then recrystallized to afford **7a** and **7b** (Table 1). ^{1}H NMR (DMSO-d_6), **7a**: 3.30–3.5 (m, 2H, CH_2S), 4.8–5.0 (m, 2H, CH_2N), 7.90–8.20 (m, 3H, ArH), 9.10 (s, 1H, N-CH=C), 11.10 (s, 1H, COOH). **7b**: 3.69–3.75 (m, 2H, CH_2S), 5.15–5.30 (m, 2H, CH_2N), 7.20–7.9 (m, 2H, pyridine-H), 9.30 (s, 1H, N-CH=C), 11.30 (s, 1H, COOH).

*2-Phenylthio-nitrobenzene (**9a**) and 2-Phenylthio-3-nitropyridine (**9b**)*

A solution of NaOH (8.0 g, 0.2 mol) in water (100 ml) was cooled to 10–15 °C and stirred. Thiophenol (**8**, 11.0 g, 0.1 mol) was then added followed by a solution of either 2-chloronitrobenzene (**1a**) or 2-chloro-3-nitropyridine (**1b**) (0.1 mol) in ethanol (100 ml) and continued as mentioned under **3a,b** to afford **9a** and **9b** (Table 1). ^{1}H NMR (DMSO-d_6), **9a**: δ 7.15–7.48 (m, 5H, ArH), 7.62–8.35 (m, 3H, ArH), 8.56–8.92 (m, 1H, ArH). **9b**: δ 7.20–7.52 (m, 5H, ArH), 7.64–8.50 (m, 1H, ArH), 8.73–9.30 (m, 2H, ArH).

*2-Phenylthio-aniline (**10a**) and 2-Phenylthio-3-aminopyridine (**10b**)*

A slurry of **9a** or **9b** (0.001 mol) and 350 mg of 5% Pd/C in ethanol (200 ml) was subjected to catalytic hydrogenation using a Parr hydrogenator at 50 psi for 3 h, and continued as mentioned under **4a,b** to give **10a** and **10b** (Table 1). ^{1}H NMR (DMSO-d_6), **10a**: δ 7.10–7.50 (m, 7H, ArH), 7.55–7.83 (m, 4H, ArH, and NH_2). **10b**: δ 7.21–7.54 (m, 5H, ArH), 7.56–7.91 (m, 3H, pyridine-H), 8.10 (brs, 2H, NH_2).

*Ethyl 4-oxo-8-phenylthio-1H, 4H-quinoline-3-carboxylate (**11a**) and Ethyl 4-oxo-8-phenylthio-1H,4H-1,7-naphthyridine-3-carboxylate (**11b**)*

A mixture of **10a** or **10b** (0.025 mol) and 10 g of diethyl(ethoxymethylene) malonate (EMME) was heated at 120 °C for 2 h. Polyphosphoric acid (35 g) was then added, and the mixture was gradually heated to 160°C and kept at that temperature for 1 h. The reaction mixture was then worked up as mentioned under **6 a,b** to afford **11a** and **11b** (Table 1). ^{1}H NMR (DMSO-d_6), **11a**: δ 1.40–1.42 (t, *J* = 7.2Hz, 3H, *CH*$_3$$CH_2$), 4.25 –4.30(q, *J* = 7.2 Hz, 2H, CH_3*CH*$_2$), 7.20–7.90 (m, 8H, ArH), 8.20 (d, *J* = 2Hz, 2H, NH-*CH*=C), 11.4 (brm, 1H, NH). **11b**: δ 1.49–1.53 (t, *J* = 7.3 Hz, 3H, *CH*$_3$$CH_2$), 4.32–4.37 (q, *J* = 7.3 Hz, 2H, CH_3*CH*$_2$), 7.25–8.4 (m, 8H, ArH, and pyridine-H), 11.0 (brm, 1H, NH).

*4-Oxo-8-phenylthio-1H,4H-quinoline-3-carboxylic acid (**12a**) and 4-Oxo-8-phenylthio-1H,4H-1,7-naphthyridine-3-carboxylic acid (**12b**)*

A suspension of **11a** or **11b** (1.0 g) in 20% NaOH solution (50 ml) was stirred at r.t. for 3 h, and the procedure continued as mentioned under **7 a,b** to give **12a** and **12b** (Table 1).^{1}H- NMR (DMSO-d_6), **12a:** δ 7.3–7.95 (m, 8H, ArH), 8.40 (d, *J* = 2Hz, 1H,NH-*CH*=C), 11.0 (brm, 1H, NH), 11.90 (s, 1H, COOH). **12b**: δ 6.70 (brm, 1H, NH), 7.4–8.9 (m, 8H, ArH), 12.2 (s, 1H, COOH).

Biological Testing

Antitumor Screening

Compound **3a**–**12b** were subjected to the NCI *in vitro* screening panel assays. Sixty different cell lines of nine tumor subpanels were incubated with five concentrations (0.01–100 μM) of each tested compound for 48 h. Dose response parameters were calculated as given previously [21–24].

Inhibition of cdc2 Kinase and cdc25 Phosphatase

The cdc2 kinase activity is assayed, in the presence of potential inhibitors, using histone H1 and ^{32}P-labelled ATP, as described elsewhere [25], highly purified recombinant glutathione-S-transferase/cdc25 fusion protein, assayed colorimetrically for *p*-nitrophenylphosphate activity in microtitration plates [26,27].

References

[1] D.S. Reeves, *Eur. J. Clin. Microbiol*. **1984**, *3*, 327.

[2] V. Cecchetti, A. Fravolini, R. Fringuelli, G. Mascellani, P. Pagella, M. Palmicoli, G. Serge, P. Terni, *J. Med. Chem*. **1987,** *30*, 465,

[3] L. Li, H. Wang, S. Kuo, T. IUu, D. Lednicer, C. Lin, E. Hame, K. Lee, *J. Med. Chem.* **1994**, *37*, 1126.

[4] M. Liu, T. Lin, P. Penketh, A. Sartorelli, *J. Med. Chem.* **1995,** *38*, 4234.

[5] A. Bridges, H. Zhou, D. Cody, G. Rewcastle, A. McMichael, H. Showalter, D. Fry, A. Kraker, W. Denny, *J. Med. Chem.*, **1996,** *39*, 267.

[6] I. Antonini, P. Polucci, T. Jenkins, L. Kelland, E. Menta, N. Pescalli, B. Stefanska, J. Mazerski, S. Martelli, *J. Med. Chem*. **1997,** *40*, 3749.

[7] K. Ohemeng, B. Podlogar, V. Nguyen, J. Bernstein, H. Krause, J. Hilliard, J. Barrett, *J. Med. Chem*. **1997**, *40*, 3292.

[8] B. Siim, G. Atwell, R. Anderson, P. Wardmann, S. Pullen, W. Wilson, W. Denny, *J. Med. Chem.* **1997,** *40*, 1381.

[9] K. Chen, S. Kuo, M. Hsieh, A. Mauger, C. Lin, E. Hamel, K. Lee, *J. Med. Chem*. **1997,** *40***,** 2266.

[10] T.M. Seay, S. J. Peretsman, P.S. Dixon, *J. Urol.* **1966,** *155*, 757.

[11] L. Shen, L. Mitscher, P. Sharma, T. O'Donnell, D. Chu, C. Cooper, T. Rosen, A. Parnet, *Biochem.* **1989,** *32***,** 3886.

[12] C. Lin, S. Singh, P. Chu, R. Dempcy, J. Schmidt, G. Pettit, E. Hamel, *Mol. Pharmacol.* **1988**, *34*, 200.

[13] G. Shi, K. Chen, L. Li, J. Chang, C. Autry, M. Kozuka, T. Konoshima, J. Estes, C. Lin, E. Hamel, A. McPhail, K. Lee, *J. Nat. Prod.*, **1995,** *58*, 475.

[14] P. Fantes, R. Brooks, The Cell Cycle–A Practical Approach, IRL Press, Oxford, U.K. **1994**.

[15] D. Glover, A. Hall, N. Hastie, *J. Cell. Sci*. **1994,** *18*, 672.

[16] V.W. Hu, The Cell Regulators, Targets and Clinical Application, Plenum Press, New York **1994**.

[17] T. Motokura, A. Arnold, *Biochem. Biophys. Acta*, **1993,** *115*, 63.

[18] C. Cordon, *Amer. J. Pathol*. **1995,** *147*, 545.

[19] J.E. Krap, S. Broder, *Nature Medicine* **1995,** *1***,** 309.

[20] H.I. El-Subbagh, M.A. El-Sherbeny, M.N. Nasr, F.E. Goda, F.A. Badria, *Boll. Chim. Farmaceutico* **1995,** *134*, 80.

[21] M.R. Grever, S.A. Schepartz, B.A. Chabner, *Seminars Oncol*. **1992,** *19***,** 622.

[22] M.R. Boyd, K.D. Paull, *Drug Rev. Res.* **1995,** *34***,** 91.

[23] M.R. Boyd, K.D. Paull, L.R. Rubinstein, Data Display and Analysis Strategies for the NCI Disease-Oriented *in vitro* Antitumor Drug Screen, Klaur Academic Publisher, Amsterdam **1992**.

[24] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronsie, A. Viagro-Wolff, M. Gray-Goodrich, H. Campell, M. Boyd, *J. Natl. Cancer Inst.* **1991,** *83*, 757.

[25] M. Koufaki, V. Polychroniou, T. Cologeropoulo, A. Tsotinis, M. Drees, H. Fiebig, A. Makriyannis, *J. Med. Chem.* **1996,** *39*, 2609.

[26] V. Rialet, L. Meijer, *Anticancer Res.* **1991,** *11*, 1581.

[27] B. Baratte, L. Meijer, K. Galaktionov, D. Beach, *Anticancer Res.*, **1992,** *12*, 873.

Received: September 29, 1998 [FP334]