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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A facile synthesis of some 3-cyano-1,4,6-trisubstituted-2(1*H*)-pyridinones and their biological evaluation as anticancer agents

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Abstract The synthesis of some new 3-cyano-1,4,6-trisubstituted-2(1*H*)-pyridinones supported with various pharmacophores and functionalities at positin-1 is described. The in vitro anticancer activity of 24 of the newly synthesized compounds was evaluated according to the protocol of the NCI in vitro disease-oriented human cells screening panel assay. The results revealed that five compounds **4a**–**c**, **7b**, and **12b** were able to display moderate antitumor potential against some of the tested subpanel tumor cell lines at the GI_{50} and TGI levels, however, with marginal or no cytotoxic (LC_{50}) activity. The obtained data suggested that better antitumor activity was linked to derivatives with either 4-bromophenyl or 3,4-dimethoxyphenyl moieties, together with a 1-methyl-1*H*-pyrrol-2-yl counter part at positions 6 and 4, respectively. Consequently, the 3-cyano-4-(1-methyl-1*H*-pyrrol-2-yl)-6-(4-bromophenyl or 3,4-dimethoxyphenyl)-2(1*H*)-pyridinones **4a** and **4b**, could be considered as the most active members identified in this investigation as evidenced from their relative higher growth inhibitory (GI_{50} (MG-MID) 77.6 and 67.6 μM, respectively) and cytostatic (TGI (MG-MID) 85.1 and 95.5 μM, respectively) activities, when compared with the substituted thiocarbamoyl analog **7b** and the bicyclic [1,2,4]triazolo[3,4-a]pyridine derivative **12b**.

Keywords Synthesis · Chalcones · Pyridinones · Pyrrole · Thiophene · Anticancer activity

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The worldwide-ongoing efforts of research on treatment of malignancy are focused on the discovery of novel potent and effective antineoplastic agents, particularly those interacting with novel biological targets. Nevertheless, in spite of the large number of available chemotherapeutic agents, the medical need is still largely unmet due to many factors among which the lack of selectivity of conventional drugs leading to toxicity, the metastatic spreading, and the intrinsic or acquired resistance to chemotherapy developed after few therapeutic cycles (multi-drug resistance; MDR) (Braña and Ramos, 2001; Cozzi, 2003). At the same time, random screening remains one of the essential means to discover new structure leads with antineoplastic activity. The National Cancer Institute (NCI), Bethesda, USA, is still playing an articular role in this field, with special emphasis on novel chemical structures that have not had extensive clinical evaluation (Cocco *et al.*, 2000). In this respect, among the most important pharmacologically active heterocycles, pyridine-containing compounds have attracted much attention as versatile chemotherapeutic agents owing to their reported potential antimicrobial (Abdel-Aziz *et al.*, 2005; Srivastava *et al.*, 2007; Aridoss *et al.*, 2007), antitubercular (Mamolo *et al.*, 1999; Ranft *et al.*, 1999), antiamoebic (Abid *et al.*, 2005), antiparasitic (Goebel *et al.*, 2008), and antiviral activities (Dragovich *et al.*, 2002; Gudmundsson *et al.*, 2005; Allen *et al.*, 2006). As far as the antineoplastic potential is concerned, pyridine derivatives were reported to possess cytotoxic

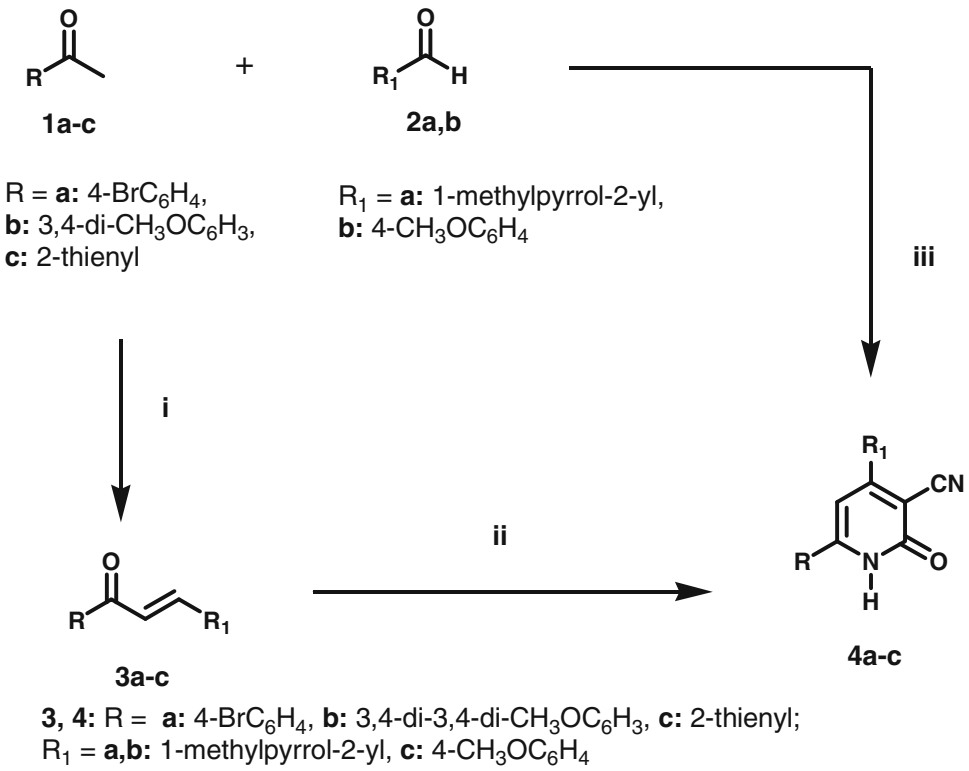
(Perez-Rebolledo *et al.*, 2005; Kamal *et al.*, 2007), antiproliferative (Poreba *et al.*, 2001; Kim *et al.*, 2010), anticancer (Segapelo *et al.*, 2009; Abadi *et al.*, 2009), CDK kinase (Huang *et al.*, 2007; Jacquemard *et al.*, 2008) and topoisomerase I, II inhibitory activities (Thapa *et al.*, 2010). Penclomedine (PEN1, 3,5-dichloro-4,6-dimethoxy-2-(trichloromethyl)pyridine), is an antitumor agent which was selected for clinical development by the NCI based on its selective antitumor activity against some carcinomas (Tiwari *et al.*, 2002). Furthermore, particular interest has been focussed on the anticancer activity of cyanopyridine derivatives, for instance, some 2-cyanopyridylurea, 3-cyano-2,6-dihydropyridine, and dicyanopyridine derivatives have been claimed for their antitumor properties in treating hyperproliferative and angiogenesis disorders (Cocco *et al.*, 2005), whereas other substituted cyanopyridines were reported to possess remarkable antitumor as well as anti-HCV activities (El-Hawash *et al.*, 2006).

During our ongoing studies aimed at the discovery of new structure leads endowed with diverse chemotherapeutic activities (Fahmy *et al.*, 2002, 2003; Rostom *et al.*, 2003a, b; Al-Saadi *et al.*, 2005a; Rostom, 2006; Faid-Allah *et al.*, 2007; Al-Saadi *et al.*, 2008a, b; Rostom *et al.*, 2009a, b), much concern has been given to the antimicrobial and antitumor potentials of some pyridines (Al-Saadi *et al.*, 2005b; Faid Allah *et al.*, 2008; Rostom *et al.*, 2009c), among which those comprising the 3-cyano-4,6-disubstituted-2(1*H*)-pyridinone scaffold (Al-Saadi *et al.*, 2005b). Some of these compounds were selected by the NCI to be evaluated for their antitumor potentials, where they exhibited promising broad-spectrum antitumor activity against several subpanel tumor cell lines. The results obtained (reported herein for the first time) prompted the design and synthesis of new analogs with further structure modification of the above-mentioned scaffold by introducing various pharmacophores and functionalities at positin-1 that are believed to be responsible for the biological significance of some relevant anticancer agents such as the formyl, acetyl, nitroso, benzenesulfonyl, thioureido, and alkyl groups. The substitution profile of the main pyridine ring was designed so as to comprise some biologically active counterparts such as the pyrrolyl, thienyl, and methoxylated phenyl rings, together with other pharmacophoric groups that would confer different electronic, lipophilic, and steric environment, which would influence the targeted anticancer activity. Moreover, it was considered worthwhile to utilize the *N*-acetyl derivatives as precursors for the synthesis of the fused-ring system triazolo[3,4-*a*]pyridine as an interesting structural variation, hoping to improve the anticipated chemotherapeutic activity. The in vitro antitumor activity of the newly synthesized compounds has been evaluated according to the protocol of the NCI (Grever *et al.*, 1992; Boyd and Paull, 1995; Monks *et al.*, 1991).

Chemistry

The synthetic routes adopted for the preparation of the intermediate and target compounds are described in Schemes 1 and 2. In the first part, two synthetic strategies were adopted to synthesize the target cyanopyridinones **4a–c** according to a reported procedure (Al-Saadi *et al.*, 2005b). The first method involved the formation of the intermediate 1,3-disubstituted-2-propen-1-ones **3a–c** (chalcones) via Claisen–Schmidt condensation of the appropriate aryl or hetaryl aldehydes **2a**,**b** with the appropriate ketones **1a–c** using ethanolic potassium hydroxide. These chalcones, in their turn, were allowed to react with ethyl cyanoacetate and ammonium acetate to yield the target 3-cyano-4,6-disubstituted-2(1*H*)-pyridinones **4a–c.** On the other hand, the same compounds **4a–c** could be directly prepared via one-pot multicomponent reaction (MCR) of the appropriate ketones **1a–c**, the aldehydes **2a**,**b**, an excess of ammonium acetate and ethyl cyanoacetate in boiling ethanol. Such type of reactions has received considerable interest since it is easier to perform, gives higher yields and less time consuming. Therefore, a comparison of the data obtained from the above-mentioned synthetic methods revealed that the one-pot reaction was better in terms of yield percentage.

In their turn, the pyridinones **4a-c** obtained from Scheme 1 were utilized as key intermediates for the synthesis of the target compounds in the second part (Scheme 2). In this respect, heating **4a–c** with formic acid resulted in the formation of the *N*-formyl pyridinones **5a–c**. Whereas, reacting the same starts with sodium nitrite in the presence of cold acetic acid was used to synthesize the *N*-nitroso derivatives **6a–c**. Condensation of **4a–c** with the appropriate aryl isothiocyanate in alkaline medium afforded the corresponding *N*-arylthiocarbamoyl pyridinones **7a-f**. Furthermore, when compounds **4a–c** were alkylated with either ethyl iodide or benzyl chloride in the presence of sodium hydroxide, the targeted *N*-alkyl pyridinones **8a–f** were formed, but in low yields. However, better yields were obtained when the reaction was carried out in pyridine as a basic solvent. At this stage, it was thought of interest to study the effect of applying different conditions that affect the alkylation of such type of pyridinones. In this respect, compounds **4a–c** were treated with the same alkyl halides in the presence of ethanolic silver nitrate according to a reported procedure (Hopkins *et al.*, 1966) in an attempt to obtain the *O*-alkyl derivatives **9**, nevertheless, such method failed to produce the targeted compounds. Furthermore, adopting another reported procedures that utilize sodium ethoxide as a basic catalyst instead of silver nitrate (Kornblum *et al.*, 1963a, b) were also insufficient to afford the *O*-alkylated compounds **9**. On the other hand,



Scheme 1 Reagents and reaction conditions: *i* potassium hydroxide, ethanol, r.t., 6-8 h, *ii* ethyl cyanoacetate, ammonium acetate, ethanol, reflux, 6–8 h, *iii* (one-put) ethyl cyanoacetate, ammonium acetate, ethanol, reflux, 3–6 h

reacting compounds **4a–c** with benzenesulfonyl chloride in the presence of pyridine resulted in the introduction of a phenylsulfonyl moiety at position-1 to yield compounds **10a–c**. Warming the starting compounds **4a–c** with acetic anhydride in the presence of anhydrous sodium acetate afforded the *N*-acetyl derivatives **11a–c**. In their turn, when compounds **10a–c** were reacted with hydrazine hydrate, the targeted 1,2,4-triazolo[3,4-a]pyridines **12a–c** were successfully obtained.

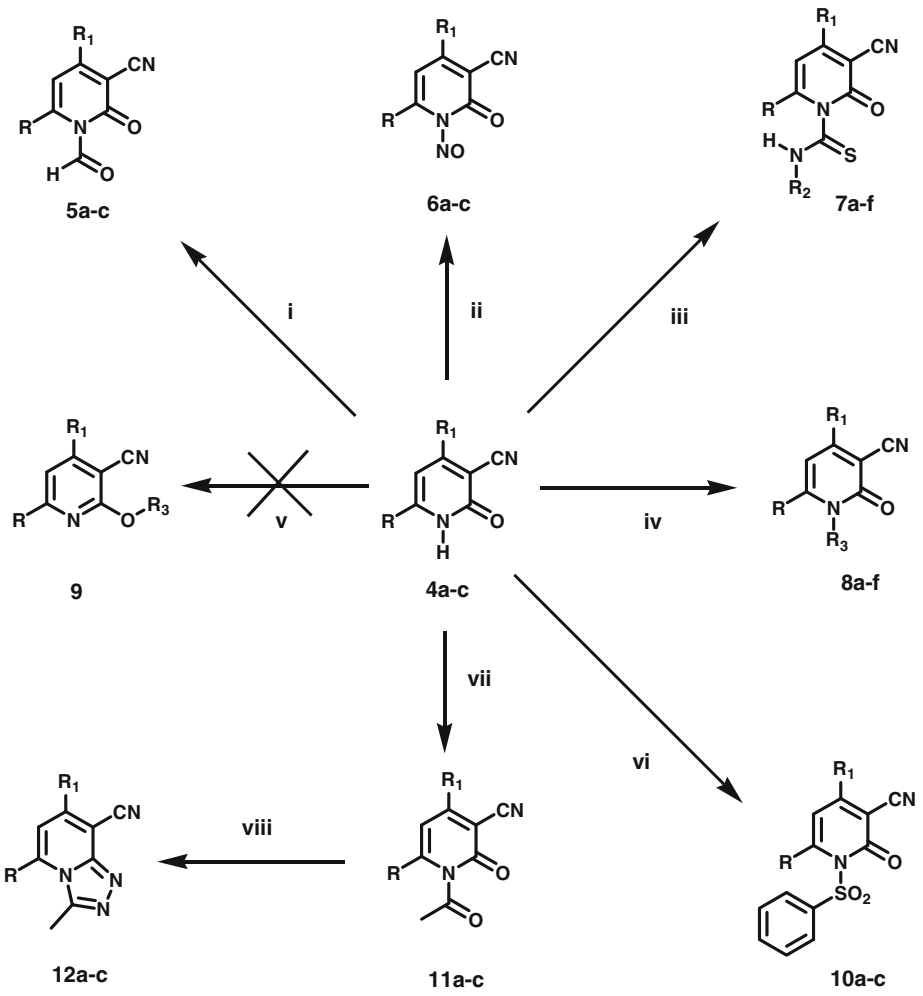
In vitro antitumor screening

Primary in vitro one-dose assay

Out of the newly synthesized compounds, 24 derivatives were selected for the evaluation of their antitumor activity as per National Cancer Institute (NCI) in vitro protocol (Grever *et al.*, 1992; Boyd and Paull, 1995; Monks *et al.*, 1991).

Effective one-dose assay has been added to the NCI 60 Cell screen in order to increase compound throughput and reduce data turnaround time to suppliers while maintaining efficient identification of active compounds. All compounds submitted to the NCI 60 Cell screen, are now 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 5-dose screen. The threshold inhibition criteria for progression to the 5-dose screen was designed to efficiently capture compounds with anti-proliferative activity and is 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 some sensitivity profiles against most of the tested compounds. Among these, the non-small cell lung cancer HOP-92 cell line exhibited a variable degree of sensitivity toward 17 out of the tested compounds, with particular sensitivity toward compounds **8b** and **12b** (GI values 44.7 and 48.0%, respectively). Meanwhile, moderate activity toward the same cell line was displayed by compounds **6c**, **7b**, and **12a** (GI values 36.2, 39.6, and 30.0%, respectively). Concerning the Leukemia subpanel, most of the tested compounds revealed considerable growth inhibitory activities against most of the tested cell lines, especially the CCRF-CEM cell line. Particular high activities against this cell line were shown by compounds **8a**, **10a**, **10c**, and **12a** with GI values of 62.5, 55.9, 56.2, and 66.0%, respectively. Whereas, compounds **5b**, **6c**, **7b**, **8b**, **8c**, and **12b** proved to exert moderate growth inhibition on the same cell line with values GI



Scheme 2 Reagents and reaction conditions: *i* formic acid, reflux, 3 h, *ii* sodium nitrite, acetic acid, 0°C, 2 h, *iii* R2NCS, pyridine, reflux, 6–8 h, *iv* R3X, pyridine, reflux, 3–4 h, *v* R3X, ethanol, silver nitrate or sodium ethoxide, reflux, 3–10 h, *vi* benzenesulfonyl chloride, pyridine, reflux, 4–6 h, *vii* acetic anhydride, anhyd. sodium acetate, reflux, 4 h, *viii* hydrazine hydrate 98%, ethanol, reflux, 6–8 h

5, 6, 10, 11, 12: R = **a:** 4-BrC_6H_4, **b:** 3,4-di-$CH_3OC_6H_3$, **c:** 2-thienyl;
R_1 = **a,b:** 1-methylpyrrol-2-yl, **c:** 4-$CH_3OC_6H_4$

7: R = **a:** 4-BrC_6H_4, **b:** 3,4-di-$CH_3OC_6H_3$, **c:** 2-thienyl;
R_1 = **a,b:** 1-methylpyrrol-2-yl, **c:** 4-$CH_3OC_6H_4$;
R_2 = **a, c, e:** C_6H_5, **b, d, f:** 4-FC_6H_4

8: R = **a:** 4-BrC_6H_4, **b:** 3,4-di-$CH_3OC_6H_3$, **c:** 2-thienyl;
R_1 = **a,b:** 1-methylpyrrol-2-yl, **c:** 4-$CH_3OC_6H_4$;
R_3 = **a, c, e:** C_2H_5, **b, d, f:** $CH_2C_6H_5$

of 47.1, 42.0, 48.3, 39.5, 43.7, and 39.2%, respectively. Furthermore, remarkable sensitivity profile was shown by the Leukemia K-562 cell line toward compounds **5a**, **6c**, **7b**, **8b**, **8c**, **8f**, and **12b** with GI values of 54.8, 65.8, 60.0, 45.4, 63.5, 50.4, and 41.5%, respectively. In relation to the Leukemia HL-60(TB), the growth of this cell line was potentially inhibited by the presence of compound **7b** (GI value 82.2%), whereas, compounds **5b**, **10a**, and **10c** showed appreciable growth inhibition on the same cell line with values of 57.1, 59.7, and 55.0%, respectively. Additionally, Leukemia RPMI-8226 cell line showed mild sensitivity toward compounds **8a**, **8b**, **12a**, and **12b** (GI% range 32.1–43.2), meanwhile, compounds **7b** and **8d** proved to exert a recognizable inhibitory activity against the same cell line (GI values 59.2 and 64.1%). It is worth-mentioning that, both of the prostate cancer cell lines were resistant to most of the tested compounds, except for compounds **8a**, **8b**, **12a**, and **12b**, which showed weak activity against the PC-3 cell line, with GI values of 28.0, 32.5, 24.0, and 23.8%, respectively.

Further interpretation of the results revealed that, the 4-fluorophenyl thiocarbamoyl 2(1*H*)-pyridinone analog **7b** proved to be the most active member in this primary bioassay, with a broad spectrum of growth inhibitory activity

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

Cpd no.	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
5a	Non-small cell lung cancer	HOP-92 (24.2)
	Ovarian cancer	OVCAR-5 (26.9)
	Leukemia	K-562 (54.8)
	Renal Cancer	UO-31 (29.7)
5b	Leukemia	CCRF-CEM (47.1), HL-60 (TB) (57.1), K-562 (21.4), MOLT-4 (28.5)
6a	Non-small cell lung cancer	HOP-92 (28.2), BT-549 (32.8)
	Leukemia	SR (37.5)
	Renal Cancer	A498 (25.7)
6b	Non-small cell lung cancer	HOP-92 (26.8)
	Leukemia	K-562 (26.6)
6c	Non-small cell lung cancer	HOP-92 (36.2)
	Leukemia	CCRF-CEM (42.0), K-562 (65.8)
7b	Non-small cell lung cancer	A549/ATCC (47.8), EKVX (29.7), HOP-62 (34.5), HOP-92 (39.6), NCI-H460 (31.6),
	Colon cancer	COLO 205 (45.6), HCC–2998 (27.6)
	Breast cancer	MDA-MB-435 (35.1)
	Leukemia	CCRF-CEM (48.3), HL-60 (TB) (82.3), K-562 (60.0), RPMI-8226 (59.2)
	Renal cancer	786-0 (28.8), A498 (27.6), CAKI-1 (30.3),
	Melanoma	LOX IMVI (23.0), M14 (29.1), SK-MEL-5 (33.5)
	CNS cancer	SF-295 (40.2), SNB-75 (42.7), U251 (26.3)
7d	Leukemia	K-562 (26.9), SR (27.8)
	Renal cancer	CAKI-1 (31.1)
8a	Non-small cell lung cancer	HOP-92 (28.3)
	Breast cancer	MDA-MB-468 (30.0)
	Leukemia	CCRF-CEM (62.5), RPMI-8226 (35.0)
	Prostate cancer	PC-3 (28.0)
8b	Non-small cell lung cancer	HOP-92 (44.7)
	Leukemia	CCRF-CEM (39.5), K-562 (45.4), PRMI-8226 (38.2)
	Prostate cancer	PC-3 (32.5)
8c	Non-small cell lung cancer	HOP-92 (29.0)
	Leukemia	CCRF-CEM (43.7), K-562 (63.5)
	Renal cancer	A498 (44.0)
8d	Leukemia	RPMI-8226 (64.1)
8f	Non-small cell lung cancer	HOP-92 (26.8), NCI-H23 (21.0), NCI/ADR-
	Leukemia	K-562 (50.4)
	Renal cancer	UO-31 (23.5)
10a	Non-small cell lung cancer	HOP-92 (26.0), NCI-H23 (22.2)
	Leukemia	CCRF-CEM (55.9), HL-60 (TB) (59.7), K-562 (22.5), MOLT-4 (26.6)
10b	Non-small cell lung cancer	HOP-92 (25.8),
	Breast cancer	BT-549 (21.0)
	Leukemia	SR (31.4)
10c	Non-small cell lung cancer	HOP-92 (21.3), NCI-H23 (20.0)
	Leukemia	CCRF-CEM (56.2), HL-60 (TB) (55.0), K-562 (27.7), MOLT-4 (28.1), RPMI-8226 (22.0)
11a	Non-small cell lung cancer	HOP-92 (24.6)
11b	Leukemia	K-562 (25.3)
11c	Non-small cell lung cancer	HOP-92 (29.6)
	Leukemia	SR (30.5)

Table 1 continued

Cpd no.	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
12a	Non-small cell lung cancer	HOP-92 (30.0)
	Leukemia	CCRF-CEM (66.0), RPMI-8226 (43.2)
	Prostate Cancer	PC-3 (24.0)
12b	Non-small cell lung cancer	HOP-92 (48.0)
	Breast cancer	T-47D (34.3), MCF7 (29.0)
	Leukemia	CCRF-CEM (39.2), K-562 (41.5), RPMI-8226 (32.1)
	Prostate cancer	PC-3 (23.8)
	CNS Cancer	SNB-75 (25.8)
12c	Non-small cell lung cancer	HOP-92 (23.5)

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

against seven of the nine tested subpanel tumor cell lines, and particular effectiveness against the Leukemia subpanel tumor cell lines CCRF-CEM, HL-60 (TB), K-562, and RPMI-8226 (GI% 48.3, 82.3, 60.0, and 59.2, respectively). In addition, the bicyclic [1,2,4]triazolo[3,4-*a*]pyridine derivative **12b** was also able to exhibit a noticeable spectrum of antitumor activity against five different subpanels, with special moderate growth inhibitory activity on the Leukemia tumor cell lines CCRF-CEM, K-562, and RPMI-8226 (GI% values 39.2, 41.5, and 32.1, respectively). Consequently, compounds **7b** and **12b** passed this primary anticancer assay and were carried over to the 5-dose screen against a panel of about 60 different tumor cell lines.

In vitro full panel (five-dose) 60-cell line assay for compounds 4a–c, 7b, and 12b

Five compounds namely, **4a–c** and **7b** and **12b**, were selected to be evaluated for their antitumor activities according to the in vitro full panel (five-dose) 60-cell line assay. (Grever *et al.*, 1992; Boyd and Paull, 1995; Monks *et al.*, 1991).

About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines, 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 (growth inhibitory activity) corresponds to the concentration of the compounds causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition and the LC_{50} value (cytotoxic activity) is the concentration of the compounds causing 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. The NCI antitumor drug discovery was designed to distinguish between broad-spectrum antitumor compounds and tumor or subpanel-selective agents.

In this part of the present study, the active five compounds including the previously prepared **4a** and **4b** (Al-Saadi *et al.*, 2005b), together with the new analogs; **4c**, **7b**, and **12b**, exhibited variable degree of antitumor activities against some of the tested subpanel tumor cell lines (GI_{50}, TGI, and LC_{50} values <100 μM). These compounds showed particular sensitivity toward some individual cell lines, as well as a broad spectrum (MG-MID) of antitumor activity (Table 2).

With regard to the sensitivity against some individual cell lines, compound **4a** revealed mild to moderate growth inhibitory activity against 14 tumor cell lines belonging to 7 different subpanels, with special influence on the non-small cell lung Hop-92 and CNS SNB-75 cell lines (GI_{50} 27.5 and 25.7 μM, respectively). Furthermore, compound **4b** was also able to inhibit the growth of 14 different cell lines, with a remarkable activity against the leukemia CCRF-CEM and SR cell lines (GI_{50} 5.01 and 0.48 μM, respectively) and a distinctive sensitivity to the non-small cell lung HOP-92 cell line at both the GI_{50} (0.05 μM) and the TGI (9.77 μM) levels. Moreover, it revealed moderate antitumor activity against the leukemia RPMI-8226, non-small cell lung EKVX, CNS SNB-75 and breast T-47D cell lines with GI_{50} values of 22.9, 30.9, 23.9, and 34.7 μM, respectively. Meanwhile, compound **4c** exhibited weak antitumor profile against 6 cell lines, nevertheless, with considerable equipotent activity against leukemia CCRF-CEM and RPMI-8226 cell lines (GI_{50} 23.4 and 22.4 μM, respectively). Shifting to the analog **7b**, it showed observable antitumor spectrum against 10 different cell lines, with a distinctive growth inhibitory and cytostatic potentials against the non-small cell lung HOP-92 cell line (GI_{50} 0.01 and TGI 0.03 μM, respectively), and a reliable

Table 2 Growth inhibitory concentration (GI_{50}, μM) of some selected in vitro tumor cell lines of compounds **4a–c**, **7b**, and **12b**

Cell lines	**4a**	**4b**	**4c**	**7b**	**12b**
Leukemia					
CCRF-CEM	47.3	5.01	23.4	–[a]	–
HL-60(TB)	–	–	45.7	14.5	–
RPMI-8226	57.5	22.9	22.4	–	19.0 (45.7)[b]
SR	–	0.48	60.2	–	–
Non-small cell lung cancer					
EKVX	34.7	30.9	–	37.1	37.1
HOP-62	–	–	–	–	–
HOP-92	27.5	0.05 (9.77)	–	0.01 (0.03)	0.01 (0.03)
EKVX	34.7	30.9	–	37.1	37.1
Colon cancer					
HCC-2998	–	89.1	–	–	–
CNS cancer					
SF-268	–	–	–	20.4	–
SF-539	89.1	67.6	–	–	–
SNB-75	25.7	23.9	39.8	38.0	44.7
Melanoma					
UACC-62	48.9	66.1	–	72.4	95.5
Ovarian cancer					
IGROV1	77.6	91.2	–	22.9	–
OVCAR-3	85.1	–	–	–	–
OVCAR-4	–	–	69.2	–	–
Renal cancer					
A498	54.9	56.2	–	17.8	25.7
UO-31	69.2	85.1	–	13.8 (27.5) (54.9)[c]	–
Breast cancer					
MDA-MB-231/ATCC	83.2	–	–	–	–
BT-549	85.1	–	–	–	–
T-47D	–	34.7	–	–	–
GI_{50} (MG-MID)[d]	77.6	67.6	91.2	83.2	89.1
TGI (MG-MID)[e]	85.1	95.5	–	95.5	–
LC_{50} (MG-MID)[f]	–	–	–	–	–

Data obtained from NCI's in vitro disease-oriented human tumor cell screen

[a] Values >100 μM

[b] Total growth inhibitory concentration value (TGI, μM)

[c] Lethal concentration 50 value (LC_{50}, μM)

[d] GI_{50} (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines toward the test agent

[e] TGI (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines toward the test agent

[f] LC_{50} (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines toward the test agent

activity against the leukemia HL-60(TB) and renal A498 cell lines (GI_{50} 14.5 and 17.8 μM, respectively). Meanwhile, the same compound displayed significant growth inhibitory, cytostatic, and cytotoxic activities on the renal UO-31 cell line with GI_{50}, TGI, and LC_{50} values of 13.8, 27.5, and 54.9 μM, respectively. Finally, the bicyclic derivative **12b** was able to affect the growth of 7 cell lines, with particular activity against the non-small cell lung HOP-92 and leukemia RPMI-8226 cell lines at both the GI_{50} (0.01 and 19.0 μM) and the TGI (0.03 and 45.7 μM) levels.

Concerning the broad-spectrum antitumor activity, the results revealed that the five active compounds; **4a**, **4b**, **4c**, **7b**, and **12b**, showed effective growth inhibition GI_{50} (MG-MID) values of 77.6, 67.6, 91.2, 83.2, and 89.1 μM, respectively. Moreover, three compounds namely; **4a**, **4b**,

and **7b** were able to display a cytostatic activity with TGI (MG-MID) values of 85.1, 95.5, and 95.5 μM, respectively (Table 2). However, all the tested compounds were deprived of any cytotoxic efficacy (LC_{50} (MG-MID) values >100 μM).

Further interpretation of the obtained results indicated that, better antitumor activity was confined to those derivatives comprising either the 4-bromophenyl or 3,4-dimethoxyphenyl moieties, together with a 1-methyl-1*H*-pyrrol-2-yl counterpart at positions 6 and 4, respectively, at the main 3-cyano-2(1*H*)-pyridinone scaffold. In this view, the 3-cyano-4-(1-methyl-1*H*-pyrrol-2-yl)-6-(4-bromophenyl or 3,4-dimethoxyphenyl)-2(1*H*)-pyridinones **4a** and **4b**, could be considered as the most active members identified in this investigation as evidenced from their relative higher growth inhibitory (GI_{50} (MG-MID) 77.6 and 67.6 μM, respectively) and cytostatic (TGI (MG-MID) 85.1 and 95.5 μM, respectively) potentials, when compared with the substituted thiocarbamoyl analog **7b** and the bicyclic [1,2,4]triazolo[3,4-a]pyridine derivative **12b**. In addition, the active compounds revealed special effectiveness against the leukemia subpanel, particularly the CCRF-CEM and RPMI-8226 cell lines, beside their pronounced activity toward the non-small cell lung cancer HOP-92 cell line (Table 2).

All these favorable features make such type of pyridine derivatives the appropriate candidates for further derivatization in order to explore the scope and limitations of their potential, hoping to find more selective and active anticancer and/or antileukemic agent(s).

Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H-NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet; q: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer 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) and the spots were detected by exposure to UV-lamp at λ 254. The synthesis, physicochemical, and analytical data of compounds **3a**,**b** and **4a**,**b** are reported in reference Al-Saadi *et al.* (2005b).

3-(4-Methoxyphenyl)-1-(2-thienyl)-2-propen-1-one **3c**

A solution of the 4-methoxybenzaldehyde **2c** (1.36 g, 10 mmol) in ethanol (20 ml) was added to a stirred solution of 2-acetylthiophene **1c** (1.26 g, 10 mmol) in ethanolic potassium hydroxide (20%) (20 ml), and stirring was maintained at room temperature for 6 h. The reaction mixture was then poured onto water (200 ml) and set aside for an overnight. The precipitated solid product was collected by filtration, washed with water, dried, and recrystallized from ethanol. Yield: 92%; m.p. 80–82°C; IR (cm^{-1}): 1665 (C=O). ^{1}H-NMR (δ-ppm): 3.67 (s, 3H, OCH_3), 6.58–7.41 (m, 6H, 2 olefinic H, and 4 Ar–H), 7.58–7.72 (m, 3H, 3, and thiophen-H). Analysis for $C_{14}H_{12}O_2S$ (244.31): C: 68.83; H: 4.95. Found: C: 68.96; H: 4.79.

3-Cyano-4-(4-methoxyphenyl)-6-(2-thienyl)-2(1H)-pyridinone **4c**

Method A

A mixture of the chalcone **3c** (2.44 g, 10 mmol), ethyl cyanoacetate (1.1 g, 10 mmol), and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (30 ml) was heated under reflux for 8 h. After being cooled to room temperature, the solid product formed was filtered, washed with water, dried, and recrystallized from the DMF containing few drops of water (Yield: 36%)

Method B

A one-pot mixture of 2-acetylthiophene **1c** (1.26 g, 10 mmol), 4-methoxybenzaldehyde **2c** (1.36 g, 10 mmol), ethyl cyanoacetate (1.1 g, 10 mmol), and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (50 ml) was refluxed for 6 h. The reaction mixture was allowed to cool, and the formed precipitate was filtered, washed with water, dried, and recrystallized (Yield: 51%).

M.p.: 244–246°C; IR (cm^{-1}): 3400–3250 (NH), 2210 (CN), 1675 (C=O). ^{1}H-NMR (δ-ppm): 3.82 (s, 3H, OCH_3), 6.24 (s, 1H, pyridine C_5-H), 6.81–7.64 (m, 7H, 3 thiophen-H, and 4 Ar–H), 8.21 (s, 1H, NH). Analysis for $C_{17}H_{12}N_2O_2S$ (308.35): C: 66.22; H: 3.92; N: 9.08. Found: C: 65.96; H: 4.13; N: 9.27.

3-Cyano-1-formyl-4,6-disubstituted-2(1H)-pyridinones **5a–c**

A solution of the appropriate pyridinone **4a–c** (3 mmol) in formic acid (5 ml) was heated under reflux for 3 h. The reaction mixture was poured on crushed ice and the

separated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 2220–2210 (CN), 1678–1670 (C=O pyridone), 1663–1657 (C=O aldehyde). ^{1}H-NMR (δ-ppm) for **5b** (R = 3,4-diOCH_3-C_6H_3, R_1 = 1-methylpyrrol-2-yl): 3.46 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.74–7.66 (m, 7H, 3 Ar–H, 3 pyrrole-H, and pyridine C_5-H), 8.37 (s, 1H, aldehyde-H).

3-Cyano-1-nitroso-4,6-disubstituted-2(1*H*)-pyridinones **6a–c**

To an ice-cooled stirred solution of the appropriate start **4a–c** (3 mmol) in acetic acid (10 ml), was added dropwise a solution of sodium nitrite (0.28 g, 4 mmol) in water (5 ml) over a period of 2 h. Stirring was maintained for further 2 h, then the reaction mixture was left aside at room

Table 3 Physicochemical and analytical data of compounds **5–12**

Cpd. no.	R	R_1	R_2 or R_3	M.p. °C (Solvent)[a]	Yield %	Mol. formula (mol. weight)	Analysis		
								% Calcd.	Found
5a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	–	111–113 (E)	60	$C_{18}H_{12}BrN_3O_2$ (382.21)	C	56.56	56.19
							H	3.16	2.83
							N	10.99	11.24
5b	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	–	109–110 (E)	78	$C_{20}H_{17}N_3O_4$ (363.37)	C	66.11	65.87
							H	4.72	4.97
							N	11.56	11.31
5c	2-thienyl	4-OCH_3-C_6H_4	–	221–227 (D/W)	75	$C_{18}H_{12}N_2O_3S$ (336.36)	C	64.27	64.39
							H	3.6	3.41
							N	8.33	8.12
6a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	–	108–110 (E)	87	$C_{17}H_{11}BrN_4O_2$ (383.21)	C	53.28	53.13
							H	2.89	3.07
							N	14.62	14.46
6b	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	–	112–114 (E)	39	$C_{19}H_{16}N_4O_4$ (364.35)	C	62.63	62.37
							H	4.43	4.58
							N	15.38	15.04
6c	2-thienyl	4-OCH_3-C_6H_4	–	231–232 (D/W)	79	$C_{17}H_{11}N_3O_3S$ (337.35)	C	60.52	60.16
							H	3.29	3.44
							N	12.46	12.72
7a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	C_6H_5	155–157 (E)	74	$C_{24}H_{17}BrN_4O$ S (489.39)	C	58.9	59.22
							H	3.5	3.41
							N	11.45	11.63
7b	4-BrC_6H_4	1-methyl-pyrrol-2-yl	4-F-C_6H_4	103–105 (E)	70	$C_{24}H_{16}BrFN_4OS$ (507.38)	C	56.81	57.26
							H	3.18	3.4
							N	11.04	10.88
7c	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	C_6H_5	102–104 (E/W)	60	$C_{26}H_{22}N_4O_3S$ (470.54)	C	66.37	66.41
							H	4.71	4.54
							N	11.91	12.06
7d	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	4-F-C_6H_4	106–108 (E/W)	55	$C_{26}H_{21}FN_4O_3S$ (488.53)	C	63.92	64.05
							H	4.33	4.17
							N	11.47	11.59
7e	2-thienyl	4-OCH_3-C_6H_4	C_6H_5	218–220 (DMF/W)	72	$C_{24}H_{17}N_3O_2S_2$ (443.54)	C	64.99	65.28
							H	3.86	3.61
							N	9.47	9.76
7f	2-thienyl	4-OCH_3-C_6H_4	4-F-C_6H_4	226–227 (DMF/W)	65	$C_{24}H_{16}FN_3O_2S_2$ (461.53)	C	62.46	62.25
							H	3.49	3.67
							N	9.1	9.23

Table 3 continued

Cpd. no.	R	R_1	R_2 or R_3	M.p. °C (Solvent)[a]	Yield %	Mol. formula (mol. weight)	Analysis		
								% Calcd.	Found
8a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	C_2H_5	101–103 (B/PE)	79	$C_{19}H_{16}BrN_3O$ (382.25)	C	59.7	59.54
							H	4.22	4.46
							N	10.99	11.07
8b	4-BrC_6H_4	1-methyl-pyrrol-2-yl	CH_2–C_6H_5	108-110 (E/B)	59	$C_{24}H_{18}BrN_3O$ (444.32)	C	64.88	64.51
							H	4.08	3.96
							N	9.46	9.73
8c	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	C_2H_5	107–109 (E)	67	$C_{21}H_{21}N_3O_3$ (363.41)	C	69.41	69.3
							H	5.82	6.02
							N	11.56	11.37
8d	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	CH_2–C_6H_5	99–101 (B/PE)	45	$C_{26}H_{23}N_3O_3$ (425.48)	C	73.39	73.56
							H	5.45	5.13
							N	9.88	10.09
8e	2-thienyl	4-OCH_3-C_6H_4	C_2H_5	233–235 (A/W)	79	$C_{19}H_{16}N_2O_2S$ (336.41)	C	67.84	67.98
							H	4.79	4.43
							N	8.33	8.26
8f	2-thienyl	4-OCH_3-C_6H_4	CH_2–C_6H_5	229–231 (A/W)	68	$C_{24}H_{18}N_2O_2S$ (398.48)	C	72.34	72.06
							H	4.55	4.71
							N	7.03	6.89
10a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	–	101–103 (E)	67	$C_{23}H_{16}BrN_3O_3S$ (494.36)	C	55.88	56.04
							H	3.26	3.13
							N	8.5	8.27
10b	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	–	97–99 (E/B)	53	$C_{25}H_{21}N_3O_5S$ (475.52)	C	63.15	62.86
							H	4.45	4.63
							N	8.84	8.55
10c	2-thienyl	4-OCH_3-C_6H_4	–	166–168 (E)	66	$C_{23}H_{16}N_2O_4S_2$ (448.51)	C	61.59	61.4
							H	3.6	3.72
							N	6.25	6.01
11a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	–	145–147 (E)	72	$C_{19}H_{14}BrN_3O_2$ (396.24)	C	57.59	57.41
							H	3.56	3.63
							N	10.6	10.27
11b	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	–	128–130 (E)	68	$C_{21}H_{19}N_3O_4$ (377.39)	C	66.83	67.01
							H	5.07	4.92
							N	11.13	11.27
11c	2-thienyl	4-OCH_3-C_6H_4	–	256–258 (DMF/W)	61	$C_{19}H_{14}N_2O_3S$ (350.39)	C	65.13	64.88
							H	4.03	3.81
							N	7.99	8.14
12a	4-BrC_6H_4	1-methyl-pyrrol-2-yl	–	123–125 (E)	34	$C_{19}H_{14}BrN_5$ (392.25)	C	58.18	58.29
							H	3.6	3.43
							N	17.85	17.61
12b	3,4-di-CH_3O-C_6H_3	1-methyl-pyrrol-2-yl	–	117–119 (E)	20	$C_{21}H_{19}N_5O_2$ (373.41)	C	67.55	67.31
							H	5.13	5.34
							N	18.76	18.32
12c	2-thienyl	4-OCH_3-C_6H_4	–	224–226 (D/W)	27	$C_{19}H_{14}N_4OS$ (346.41)	C	65.88	65.97
							H	4.07	3.82
							N	16.17	16.24

[a] Crystallization solvents: *E* ethanol, *B* benzene, *W* water. *DMF* *N*,*N*-dimethylformamide, *D* 1,4-dioxan, *PE* petroleum ether (60/80)

temperature for overnight. The formed solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 2223–2217 (CN), 1675–1667 (C=O pyridone). ^{1}H-NMR (δ-ppm) for **6c** (R = 2-thienyl, R_1 = 4-OCH_3-C_6H_4): 3.71 (s, 3H, OCH_3), 6.71–7.54 (m, 8H, 4 Ar–H, 3 thiophen-H, and pyridine C_5-H).

3-Cyano-1-(substituted thiocarbamoyl)-4,6-disubstituted-2(1H)-pyridinones **7a–f**

A mixture of the appropriate starting compound **4a–c** (3 mmol) and the appropriate isothiocyanate (4 mmol) in pyridine (10 ml) was heated under reflux for 6–8 h. After being cooled to room temperature, the reaction mixture was poured on ice cold water and the separated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 2230–2215 (CN), 1678–1665 (C=O pyridone), 1232–1216 (C=S). ^{1}H-NMR (δ-ppm) for **7a** (R = 4-Br-C_6H_4, R_1 = 1-methylpyrrol-2-yl, R_2 = C_6H_5): 3.49 (s, 3H, CH_3), 5.87–6.24 (m, 4H, 3 pyrrole-H and pyridine C_5-H), 6.68–7.27 (m, 9H, 9 Ar–H), 8.51 (s, 1H, NH). For **7c** (R = 3,4-diOCH_3-C_6H_3, R_1 = 1-methylpyrrol-2-yl, R_2 = C_6H_5): 3.45 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.41 (s, 1H, pyridine C_5-H), 6.73–7.84 (m, 11H, 8 Ar–H, 3 pyrrole-H), 8.47 (s, 1H, NH). For **7f** (R = 2-thienyl, R_1 = 4-OCH_3-C_6H_4, R_2 = 4-F-C_6H_4): 3.83 (s, 3H, OCH_3), 6.47 (s, 1H, pyridine C_5-H), 6.85–7.61 (m, 11H, 8 Ar–H, 3 thiophen-H), 8.55 (s, 1H, NH).

3-Cyano-1-(benzyl or ethyl)-4,6-disubstituted-2(1H)-pyridinones **8a–f**

To a solution of the appropriate pyridone **4a–c** (3 mmol) in pyridine (10 ml), was added the appropriate alkyl halide (3 mmol) and the mixture was heated under reflux for 3–4 h. The reaction mixture was allowed to attain room temperature, poured on ice cold water and the separated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 2222–2216 (CN), 1675–1668 (C=O pyridone). ^{1}H-NMR (δ-ppm) for **8a** (R = 4-Br-C_6H_4, R_1 = 1-methyl-pyrrol-2-yl, R_3 = CH_2CH_3): 1.49 (t, J = 4 Hz, 2H, CH_3), 2.94 (q, J = 4 Hz, 2H, CH_2), 3.44 (s, 3H, CH_3), 6.59 (s, 1H, pyridine C_5-H), 6.88–7.94 (m, 7H, 4 Ar–H, and 3 pyrrole-H). For **8d** (R = 3,4-diOCH_3-C_6H_3, R_1 = 1-methylpyrrol-2-yl, R_3 = CH_2-C_6H_5): 3.39 (s, 3H, CH_3), 3.71 (s, 6H, 2 OCH_3), 4.46 (s, 2H, CH_2), 6.51 (s, 1H, pyridine C_5-H), 6.92–7.88 (m, 11H, 8 Ar–H, and 3 pyrrole-H). For **8e** (R = 2-thienyl, R_1 = 4-OCH_3-C_6H_4, R_3 = CH_2CH_3): 1.45 (t, J = 4 Hz, 2H, CH_3), 2.87 (q, J = 4 Hz, 2H, CH_2), 3.76 (s, 3H, OCH_3), 6.65–7.72 (m, 8H, 4 Ar–H, 3 thiophen-H, and pyridine C_5-H).

3-Cyano-1-phenylsulfonyl-4,6-disubstituted-2(1H)-pyridinones **10a–c**

A mixture of the appropriate start **4a–c** (3 mmol) and benzenesulfonyl chloride (3 mmol) in pyridine (10 ml) was heated under reflux for 4–6 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 2230–2221 (CN), 1670–1665 (C=O pyridone), 1388–1370 and 1197–1175 (SO_2). ^{1}H-NMR (δ-ppm) for **10b** (R = 3,4-diOCH_3-C_6H_3, R_1 = 1-methylpyrrol-2-yl): 3.42 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.69–7.95 (m, 12H, 8 Ar–H, 3 pyrrole-H, and pyridine C_5-H). For **10c**: (R = 2-thienyl, R_1 = 4-OCH_3-C_6H_4): 3.75 (s, 3H, OCH_3), 6.64 (s, 1H, pyridine C_5-H), 6.82-7.88 (m, 12H, 9 Ar–H, and 3 thiophen-H).

1-Acetyl-3-cyano-4,6-disubstituted-2(1H)-pyridinones **11a–c**

To a solution of the appropriate **4a–c** (3 mmol) in acetic anhydride (10 ml), was added anhydrous sodium acetate (0.37 g, 4.5 mmol). The reaction mixture was heated under reflux for 4 h, allowed to cool, then poured on crushed ice with vigorous stirring. The formed solid product was filtered, thoroughly washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 2226-2215 (CN), 1725–1718 (C=O acetyl), 1678–1672 (C=O pyridone). ^{1}H-NMR (δ-ppm) for **11b** (R = 3,4-diOCH_3-C_6H_3, R_1 = 1-methylpyrrol-2-yl): 2.48 (s, 3H, CH_3CO), 3.51 (s, 3H, N-CH_3), 3.77 (s, 6H, 2 OCH_3), 6.74–7.28 (m, 7H, 3 Ar–H, 3 pyrrole-H and pyridine C_5-H). For **11c**: (R = 2-thienyl, R_1 = 4-OCH_3-C_6H_4): 2.51 (s, 3H, CH_3CO), 3.46 (s, 3H, N-CH_3), 3.85 (s, 3H, OCH_3), 6.69–7.68 (m, 8H, 4 Ar–H, 3 thiophen-H, and pyridine C_5-H).

8-Cyano-5,7-disubstituted-[1,2,4]-triazolo[3,4-a]pyridines **12a–c**

A mixture of the appropriate 1-acetylpyridinone **11a–c** (3 mmol) and hydrazine hydrate (0.3 g, 5 mmol) in ethanol (15 ml) was heated under reflux for 6–8 h. The reaction mixture was allowed to attain room temperature, poured on crushed ice and the precipitated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data were recorded in Table 3. IR (cm^{-1}): 2220–2215 (CN). ^{1}H-NMR (δ-ppm) for **12a** (R = 4-Br-C_6H_4, R_1 = 1-methyl-pyrrol-2-yl): 2.61

(s, 3H, triazole CH_3), 3.46 (s, 3H, N-CH_3), 6.69–7.44 (m, 8H, 4 Ar–H, 3 pyrrole-H, and pyridine C_5-H). For **12b** (R = 3,4-diOCH_3-C_6H_3, R_1 = 1-methylpyrrol-2-yl): 2.55 (s, 3H, triazole CH_3), 3.49 (s, 3H, N-CH_3), 3.74 (s, 6H, 2 OCH_3), 6.81–7.52 (m, 7H, 3 Ar–H, 3 pyrrole-H, and pyridine C_5-H).

In vitro antitumor screening

Preliminary in vitro one-dose antitumor screening

Out of the newly synthesized compounds, 21 derivatives were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro anticancer activity. 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. 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. Two compounds that passed this primary anticancer assay were consequently carried over to the 5-dose screen against a panel of about 60 different tumor cell lines.

Full in vitro five-dose antitumor assay

Compounds were subjected to the NCI in vitro disease-oriented human cells screening panel assay to screen their antitumor activities (Grever *et al.*, 1992; Boyd and Paull, 1995; Monks *et al.*, 1991) using about 60 cell lines consisting of nine tumor subpanels such as leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines (NCI protocol). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg ml^{-1} gentamicin. Additional four, 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 ml of these different drug dilutions were added to the appropriate microtiter wells already containing 100 ml of medium, resulting in the required final drug concentrations. Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line.

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