

Synthesis and Biological Evaluation of Certain α,β -Unsaturated Ketones and Their Corresponding Fused Pyridines as Antiviral and Cytotoxic Agents[†]

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A new series of 3,5-bis(arylidene)-4-piperidones, as chalcone analogues carrying variety of aryl and heteroaryl groups, pyrazolo[4,3-*c*]pyridines, pyrido[4,3-*d*]pyrimidines, and pyrido[3,2-*c*]pyridines, carrying an arylidene moiety, and a series of pyrano[3,2-*c*]pyridines, as flavone and coumarin isosteres, were synthesized and screened for their *in vitro* antiviral and antitumor activities at the National Cancer Institute (NCI). Compounds **9** and **18** proved to be active against herpes simplex virus-1 (HSV-1), while compound **13** showed moderate activity against human immunodeficiency virus-1 (HIV-1). Compounds **14**, **26**, **28**, **33**, and **35** exhibited a broad spectrum antitumor activity. In addition, compounds **26**, **33**, and **35** proved to be of moderate selectivity toward leukemia cell lines. The pyrano[3,2-*c*]pyridines heterocyclic system proved to be the most active antitumors among the investigated heterocycles.

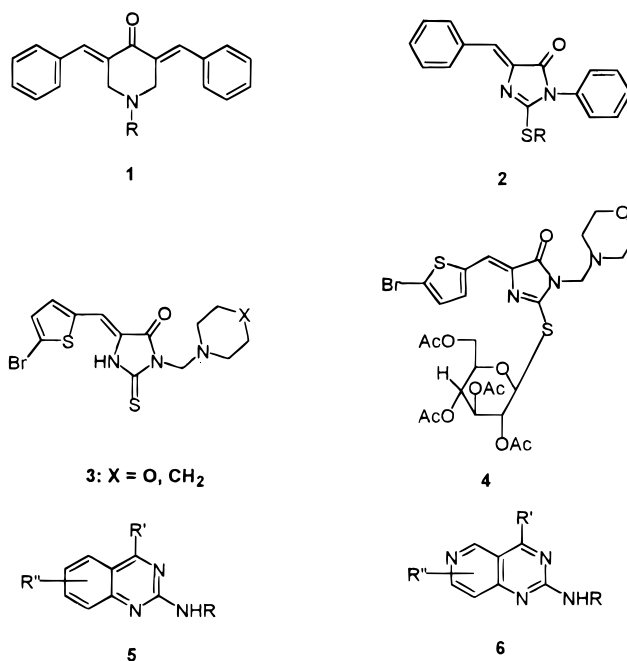
Introduction

The cytotoxic activity of (*E*)-3,5-bis(benzylidene)-4-piperidones **1** (Chart 1) and their specificity toward leukemia cell lines with IC₅₀ values less than 10 μ M have been reported.¹ This type of compound is a combination of cyclic α,β -unsaturated ketone (chalcone) and β -amino ketone in one structure. Those compounds proved to possess marked affinities for thiols but not for amino or hydroxy functions found in nucleic acids; hence mutagenic and carcinogenic side effects should be absent.^{2,3}

The practice of incorporating chalcones into heterocyclic nitrogenous ring has been noticed recently⁴ represented by the potential antiviral compounds *S*-glucosylated hydantoin **2** (Chart 1). This combination rationale has been used in our laboratory^{5,6} to synthesize some 5-substituted-2-thiohydantoin analogues as a novel class of antitumor agents. This latter study showed that compounds such as **3** and its *S*-glucosylated analogue **4** (Chart 1) are potential broad-spectrum antitumor agents⁶ based on the anticancer screening data obtained from the National Cancer Institute (NCI) antitumor drug discovery screen.

The present study is a continuation of our previous efforts^{5,6} aiming to locate novel synthetic lead compound(s) for future development as antiviral and antitumor agents. A new series of (*E*)-3,5-bis(arylidene)-4-piperidones (**8–14**) were designed carrying a variety of aryl and heteroaryl groups to explore the scope and limitations of their antiviral and antitumor activities. To determine the influence of the pharmacophoric function (α,β -unsaturated ketone) on activity, a selective

Chart 1



reduction of the unsaturated centers of **8–14** produced either the saturated ketone analogues **15–17** or the unsaturated alcohols **18–20**. This type of tailored synthesis is aiming to discover the vital part of the pharmacophore, whether the ketonic moiety or the olefinic function.

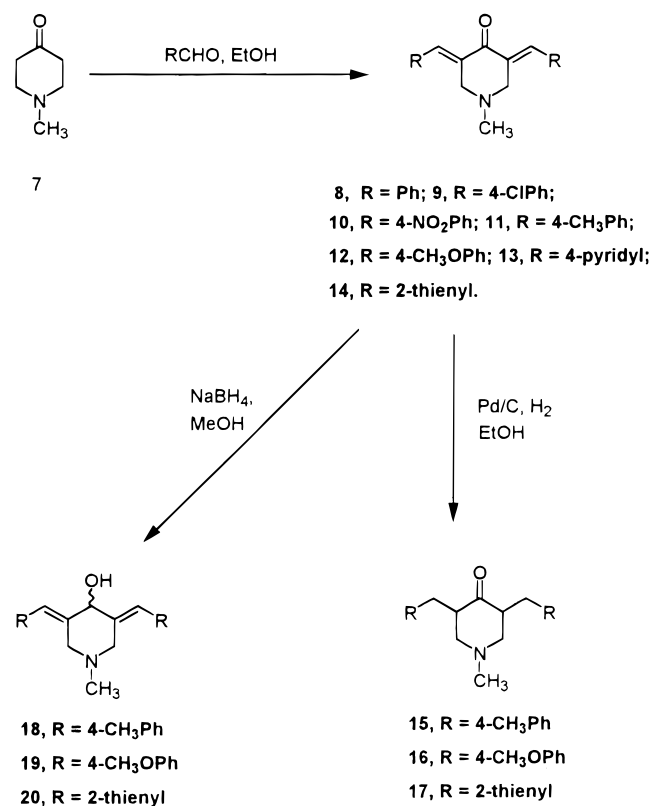
The finding that some 2-aminoquinazolines **5**, their aza analogues **6**⁷ (Chart 1), and the other fused pyridine analogues such as 5-deazaaminopetrin⁸ and quinolones⁹ interfere with folic acid synthesis rationalized the design and synthesis of the pyrazolo[4,3-*c*]pyridine (**21–26**), pyrido[4,3-*d*]pyrimidine (**27–29**), and pyrido[3,2-*c*]pyridine (**30–32**) targets carrying an arylidene moiety to be evaluated as antitumor agents which may exert their

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Scheme 1

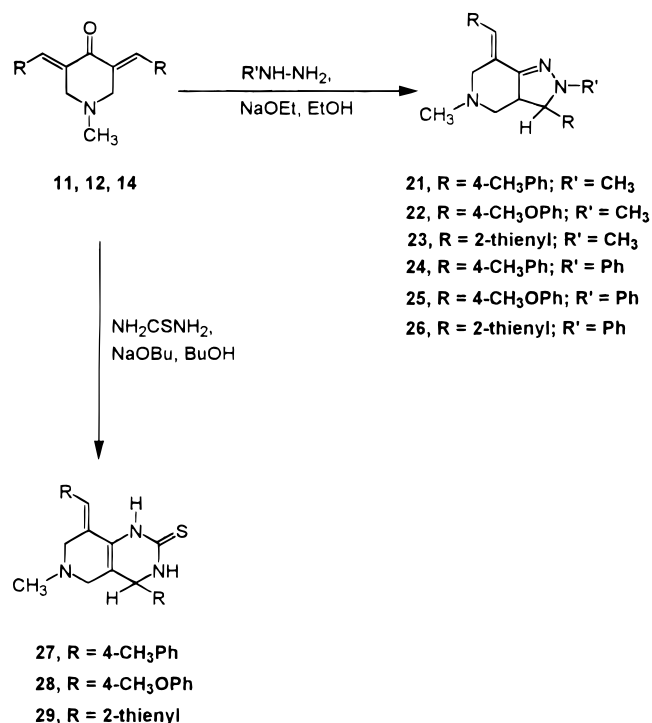
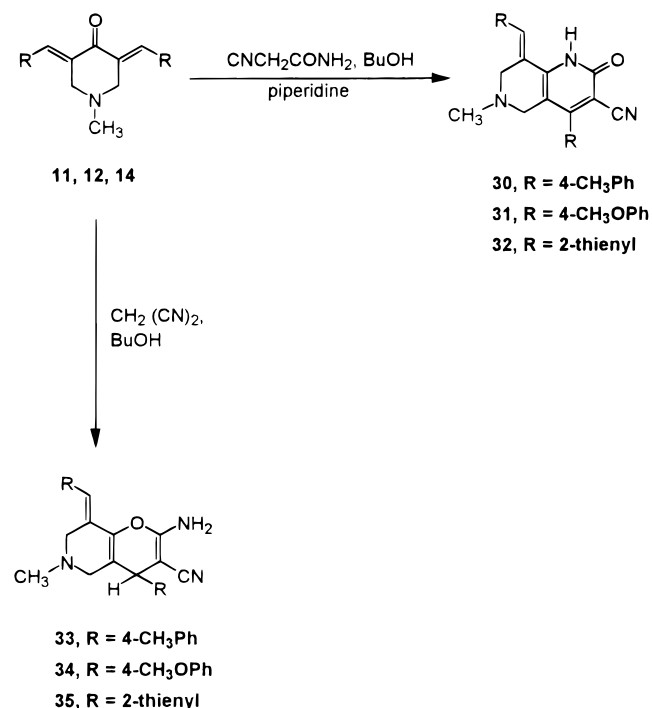
activity through folic acid synthesis inhibition. Several reports stated the ability of flavonoids to reduce the rate of colon,^{10–20} breast,^{21,22} and ovarian²³ cancers. Flavonoids²⁴ and coumarins^{25,26} are also proved to be HIV-integrase inhibitors. These literature facts suggested the synthesis of their isosteric analogues, the pyrano[3,2-*c*]pyridines **33–35**.

The main objective of the present investigation, aiming toward the synthesis of the target compounds **8–35**, is to reach a new active antiviral and antitumor agent(s) with potential activity toward cancerous cells and less toxicity toward normal cells.

Chemistry

The designed target compounds depicted in Schemes 1–3 were obtained by reacting the starting material 1-methyl-4-piperidone (**7**) with variety of aromatic aldehydes under aldol condensation conditions to produce the α,β -unsaturated ketone analogues **8–14**. The chalcone derivatives **8**, **9**, and **13** ($R = Ph$, 4-*Cl*Ph, and 4-pyridyl, respectively) were previously reported.^{27,28} The chalcone analogues **11**, **12**, and **14** ($R = 4-CH_3Ph$, 4-*CH*₃OPh, and 2-thienyl, respectively), representing aryl and heteroaryl examples, were selected to be used for further synthesis of the other target molecules.

Compounds **11**, **12**, and **14** were subjected to catalytic hydrogenation using a Parr hydrogenator (Pd/C , $H_2(g)$, 50 psi) to reduce the olefinic function yielding the 3,5-bis(arylmethyl) derivatives **15–17**. The ketonic groups in the chalcones **11**, **12**, and **14** were exclusively reduced into their corresponding racemic alcohols **18–20**, quantitatively using $NaBH_4$ in methanol (Scheme 1). The α,β -unsaturated derivatives **11**, **12**, and **14** were subjected to cycloaddition condensation reactions using methyl-

Scheme 2**Scheme 3**

or phenylhydrazine to give their corresponding pyrazolo[4,3-*c*]pyridine analogues **21–26** (Scheme 2). The interaction of **11**, **12**, and **14** with thiourea in refluxing NaOBu in butanol gave the pyrido[4,3-*c*]pyrimidine derivatives **27–29** (Scheme 2). The chalcone function of **11**, **12**, and **14** was further utilized for another cyclocondensation reaction using cyanoacetamide in refluxing butanol containing catalytic amount of piperidine to afford the pyrido[3,2-*c*]pyridine derivatives **30–32** (Scheme 3). Reacting **11**, **12**, and **14** with malononitrile in refluxing butanol produced the pyrano[3,2-*c*]pyridine analogues **33–35** (Scheme 3).

Table 1. In Vitro Anti-HSV-1 Testing Results

compd	% reduction ^a	MAC ^b		IC ₅₀ ^c	
		$\mu\text{g/mL}$	μM	$\mu\text{g/mL}$	μM
9	93	0.02	0.07	0.2	0.69
14	33	0.1	3.3	0.2	0.66
18	82	0.02	0.06	0.2	0.63
19	58	0.02	0.06	0.04	0.12
27	45	0.1	0.27	0.07	0.19
29	27	0.1	0.29	0.15	0.58
aphidicolin ^d	100	0.005	0.02	0.2	0.59

^a Percent (%) reduction in the number of viral plaques. ^b Minimum antiviral concentration (minimum molar concentration proved to be lethal to the viral population). ^c Cytotoxicity (compound concentration caused 50% loss of the monolayer present around the viral plaques). ^d Positive control.

Results and Discussion

In Vitro Anti-Herpes Simplex-1 Virus (HSV-1).

The antiviral testing was performed using Vero cells, HSV-1, and aphidicolin (0.005 $\mu\text{g/mL}$) as a positive control.²⁹ Compound **9** showed the highest activity among the tested compounds and was able to reduce the number of viral plaques by 93%. Replacing the 4-chloro atom in **9** either by hydrogen atom as in **8** or by electron-donating functions (CH_3 , CH_3O) as in **11** and **12** abolished the antiviral activity. Meanwhile, replacing the entire aromatic residue (benzylidene) by a hetero-aromatic moiety such as thienylidene (**14**) decreased the antiviral potency to 33% reduction of the viral plaques. These results imply that the aryl part attached to the α,β -unsaturated ketone moiety is an essential pharmacophoric site. Saturation of the olefinic function of the α,β -unsaturated ketones **8**–**14** produced the arylmethyl analogues **15**–**17**, which proved to be devoid of any antiviral activity. On the other hand, reduction of the ketonic function produced the racemic α,β -unsaturated alcohols **18**–**20** with reduced magnitude of activity and reversed concept of substitution. Compound **18** with an electron-donating substituent ($\text{R} = \text{CH}_3\text{Ph}$) showed 82% reduction in the HSV-1 plaques, and replacing the 4-methylphenyl function by a 4-methoxyphenyl as in **19** lowered the antiviral activity to 58% reduction of viral plaques. Cyclization of the chalcone analogues to produce the pyrido[4,3-*d*]pyrimidine-2-thione preserved the antiviral potency with a dramatic decrease in activity as shown in **27** ($\text{R} = 4\text{-CH}_3\text{Ph}$) and **29** ($\text{R} = 2\text{-thienyl}$). Compounds **9**, **14**, and **18** showed cytotoxicity (IC_{50}) to Vero cells equal to that of aphidicolin (0.2 $\mu\text{g/mL}$), while compounds **19**, **27**, and **29** proved to be cytotoxic (Table 1).

In Vitro Anti-Human Immunodeficiency-1 Virus (HIV-1). The procedure used to evaluate the anti-HIV-1 potency is designed to detect agents acting at any stage of the virus reproductive cycle.³⁰ Compound **13** proved to possess moderate activity against HIV-1 cytopathic effect. Compounds **23**–**25**, **30**, and **32** did not show any activity or toxicity at the highest concentration used (200.0 μM), probably because of a solubility problem in the used culture media. Compounds **14**, **20**, **26**–**29**, **31**, and **33**–**35**, however, were highly cytotoxic to the uninfected T4 cells at the concentrations which showed marginal antiviral activity in the infected cultures (Table 2).

Antitumor Screening. Compounds **14**, **20**, **23**–**31**, and **33**–**35** were subjected to the NCI's in vitro disease-

Table 2. In Vitro Anti-HIV-1 Testing Results

compd	IC ₅₀ ^a (μM)	EC ₅₀ ^b (μM)	TI ₅₀ ^c (IC ₅₀ /EC ₅₀)
13	>200.0	64.7	>3.09
14	15.2	<i>d</i>	<i>d</i>
20	126.0	<i>d</i>	<i>d</i>
23	>200.0	<i>d</i>	<i>d</i>
24	>200.0	<i>d</i>	<i>d</i>
25	>200.0	<i>d</i>	<i>d</i>
26	9.1	<i>d</i>	<i>d</i>
27	27.6	<i>d</i>	<i>d</i>
28	6.8	<i>d</i>	<i>d</i>
29	34.0	<i>d</i>	<i>d</i>
30	>200.0	<i>d</i>	<i>d</i>
31	104.0	<i>d</i>	<i>d</i>
32	>200.0	<i>d</i>	<i>d</i>
33	11.1	<i>d</i>	<i>d</i>
34	30.4	<i>d</i>	<i>d</i>
35	9.0	<i>d</i>	<i>d</i>
AZT ^e	35.6	0.0007	50.59

^a 50% Inhibitory concentration (molar concentration of compounds which cause 50% inhibition of cell growth). ^b 50% Effective concentration (molar concentration of compounds which cause 50% protection against HIV cytopathic effects). ^c Therapeutic index (the ratio of 50% inhibitory concentration to 50% effective concentration for each of the active compounds). ^d Inactive compounds. ^e Positive control.

oriented human cells screening panel assay.^{31,32} Three response parameters, median growth inhibition (GI_{50}), total growth inhibition (TGI), and median lethal concentration (LC_{50}), were calculated for each cell line. The NCI antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor and tumor or subpanel-selective compounds.³³

In the present study, the tested analogues showed a distinctive potential pattern of selectivity as well as broad-spectrum antitumor activity. With regard to sensitivity against individual cell lines, compounds **26**, **28**, **29**, **34**, and **35** showed effectiveness toward HL-60 (TB) leukemia cell line at a concentration range of 0.8–2.2 μM . Non-small-cell lung HOP-92 cell line proved to be sensitive toward compounds **23**, **27**, **33**, and **35** at a concentration range of 0.08–2.0 μM . Compound **35** proved to be effective against renal ACHN, breast MCF7, and T-47D cell lines at concentrations of 0.3, 0.2, and 0.9 μM , respectively.

With regard to broad-spectrum antitumor activity, compounds **14**, **26**, **28**, and **33**–**35** showed GI_{50} , TGI, and LC_{50} (MG-MID) < 100 μM against leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer subpanel cell lines. Compounds **20**, **23**, **27**, **29**, and **31** showed (MG-MID) values < 100 μM at only the GI_{50} and TGI levels. Compounds **24** and **25** are the least effective members of this series with GI_{50} (MG-MID) values, while compound **30** proved to be inactive (Tables 3, 4).

The ratio obtained by dividing the compounds full panel MG-MID concentration (μM) by its individual subpanel MG-MID concentration (μM) is considered as a measure for the compound selectivity.³³ Ratios between 3 and 6 refer to moderate selectivity, while ratios greater than 6 indicate selectivity toward the corresponding cell line subpanel. Compounds **26** and **33**–**35** showed moderate selectivity at the GI_{50} level toward leukemia cell lines (Table 3). Compounds **33** and **35**, beside their selectivity at the GI_{50} level, showed the same effectiveness at the TGI level. The in vitro antitumor evaluation of the newly synthesized com-

Table 3. Median Growth Inhibitory Concentration (GI₅₀, μ M) of in Vitro Subpanel Tumor Cell Lines

compd ^a	subpanel tumor cell lines ^b									MG-MID ^c
	I	II	III	IV	V	VI	VII	VIII	IX	
14	10.6 (2.2) ^d	36.5	23.7	36.6	34.6	23.5	24.2	23.9	28.7	23.5
20	32.5 (2.4)	83.9	85.8	<i>e</i>	92.5	81.4	96.2	<i>e</i>	84.3	77.1
23	31.8 (2.1)	86.4	79.2	80.7	85.0	74.4	89.7	59.5	86.0	68.0
24	37.4 (2.2)	97.4	<i>e</i>	77.0	77.9	92.8	83.7	69.5	<i>e</i>	81.0
25	<i>e</i> (0.7)	82.1	<i>e</i>	63.1	77.7	80.7	54.7	<i>e</i>	91.8	72.9
26	2.2 (3.9)	20.8	3.8	35.7	42.5	24.2	65.3	19.3	23.6	8.5
27	19.2 (1.8)	59.6	37.1	53.7	58.8	51.2	60.8	38.3	39.4	34.5
28	2.0 (2.5)	6.5	4.0	30.0	11.7	5.0	5.9	9.1	11.1	4.9
29	22.4 (2.4)	67.2	70.5	76.1	60.5	65.5	75.0	<i>e</i>	45.1	53.5
31	35.0 (1.5)	66.6	60.5	44.9	79.9	51.8	61.2	70.9	66.9	52.5
33	5.1 (3.3)	(3.3)	16.6	37.7	26.6	15.5	18.1	29.4	30.7	16.9
34	9.1 (3.7)	36.7	41.2	60.5	70.7	38.1	40.4	79.4	48.6	33.3
35	3.2 (3.6)	16.3	17.3	42.2	36.3	25.0	32.5	13.5	27.9	11.6

^a Compound **30** showed GI₅₀ values > 100 μ M. ^b 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. ^c GI₅₀ full panel mean-graph midpoint (μ M). ^d GI₅₀ selectivity ratios obtained by dividing the compound's full panel MG-MID (μ M) by its leukemia subpanel MG-MID (μ M) are shown in parentheses. ^e Compound showed GI₅₀ values > 100 μ M.

Table 4. Total Growth Inhibitory Concentration (TGI, μ M) of in Vitro Subpanel Tumor Cell Lines

compd ^a	subpanel tumor cell lines ^b									MG-MID ^c
	I	II	III	IV	V	VI	VII	VIII	IX	
14	51.6	90.1	72.2	82.7	84.1	81.4	70.4	<i>d</i>	75.4	74.0 (97.7) ^e
20	78.1	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	96.8	97.7
23	87.8	<i>d</i>	<i>d</i>	<i>d</i>	97.7	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	97.7
26	52.2	<i>d</i>	87.1	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	72.9 (95.8)
27	62.3	89.9	83.6	94.1	<i>d</i>	94.0	96.9	<i>d</i>	88.8	86.5
28	36.3	93.6	76.3	81.9	83.1	76.4	97.2	30.1	68.1	56.3 (91.8)
29	69.8	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	98.1	95.8
31	80.7	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	87.8	<i>d</i>	<i>d</i>	95.8
33	17.9	54.1	43.9	83.6	59.5	55.7	54.5	66.2	70.1	51.1 (97.3)
34	49.9	95.5	97.4	<i>d</i>	93.9	<i>d</i>	96.9	<i>d</i>	93.8	85.6 (97.3)
35	27.6	75.5	87.3	<i>d</i>	87.0	73.2	81.6	90.9	57.7	57.8 (91.1)

^a Compounds **24**, **25**, and **30** showed TGI values > 100 μ M. ^b For subpanel tumor cell lines, see footnote b of Table 3. ^c TGI full panel mean-graph midpoint (μ M). ^d Compound showed TGI values > 100 μ M. ^e Median lethal concentrations (LC₅₀, μ M) are shown in parentheses.

pounds revealed the potential of this class of compounds as antitumor agents.

The α,β -unsaturated ketone found in compounds **8–14** proved to be essential for antitumor activity. Compound **14** proved to be 3-fold more active than its racemic α,β -unsaturated alcohol counterpart **20**. Cyclocondensation of **14** with methyl- or phenylhydrazines afforded the pyrazolo[4,3-*c*]pyridine analogues **21–26** at which the olefinic residue, remaining from the chalcone function, is kept at position 7 in addition to the introduced heterocyclic ring. The 2-methyl derivative **23** exhibited one-third the potency of **14**, while the 2-phenyl analogue **26** showed a remarkable increase in activity by almost 3-fold, compared with **14**, in addition to a particular selectivity toward leukemia cell lines.

Cyclocondensation of the other two chalcones **11** (R = CH₃Ph) and **12** (R = CH₃OPh) with phenylhydrazine produced the 2-phenyl analogues **24** and **25** with almost a 10-fold decrease in activity compared with their 2-thienylidene derivative **26**.

Cyclocondensation of the mentioned chalcones with thiourea yielded the pyrido[4,3-*c*]pyrimidine analogues **27–29**, which showed various antitumor potencies depending on the type of substituent. Compound **28** (R = CH₃OPh) is the most active member among the pyrimidine analogues. Replacing the methoxy function of **28** by a methyl group gave compound **27** with reduced activity. Changing the arylidene moiety of **27** and **28** by the heteroaromatic function 2-thienyl at position 4 and 2-thienylidene at position 8 produced compound **29**

with 10-fold reduced activity. On the other hand, cyclizing the chalcone analogues with cyanoacetamide yielded the pyrido[3,2-*c*]pyridine analogues **30** and **31**. Compound **30** ($R = \text{CH}_3\text{Ph}$) proved to be inactive toward the used tumor cell lines, and changing the methyl function by a methoxy group as in **31** moved the potency toward the active side with GI_{50} and TGI values. Reacting the chalcone analogues **11**, **12**, and **14** with malononitrile produced the pyrano[3,2-*c*]pyridines **33**–**35**. Compound **35** ($R = 2\text{-thienyl}$) proved to be the most active member among this group followed by compounds **33** ($R = 4\text{-CH}_3\text{Ph}$) and **34** ($R = 4\text{-CH}_3\text{OPh}$). Compounds **33**–**35** proved to possess potential selectivity toward leukemia cell lines.

Structure–Activity Correlation. The attempt to connect the variations in the backbone of the synthesized heterocycles and the obtained antiviral and antitumor data facilitated the deduction of the following general considerations:

(1) The obtained screening results showed that the antiviral activity is embedded in only three out of the seven designed main structure formulas found in compounds **8**–**35**: namely (*E*)-3,5-bis(arylidene)-4-piperidone, their (\pm)-(*E*)-4-hydroxypiperidine analogues, and pyrido[4,3-*d*]pyrimidine-2-thione.

(2) The pyrano[3,2-*c*]pyridine heterocyclic system proved to be the most active antitumor agent among the investigated heterocycles represented by compounds **33**–**35** followed by the pyrido[4,3-*d*]pyrimidines **27**–**29** and pyrazolo[4,3-*c*]pyridines **23**–**26**, then the α,β -unsaturated ketones represented by compound **14**.

(3) The pattern of substitution on those heterocyclic ring systems suggested that for the pyrazolo[4,3-*c*]pyridines, the introduction of a phenyl group at position 2 (**24**–**26**) favored the antitumor activity rather than a methyl function (**23**), while the introduction of a 2-thienylidene moiety at position 7 produced more active compounds $> 4\text{-(CH}_3\text{O)benzylidene} > 4\text{-(CH}_3\text{)benzylidene}$: **26** $>$ **25** $>$ **24**, respectively. For the pyrido[4,3-*d*]pyrimidine, the order of substitution at position 8 is $4\text{-(CH}_3\text{O)benzylidene} > 4\text{-(CH}_3\text{)benzylidene} > 2\text{-thienylidene}$: **28** $>$ **27** $>$ **29**, respectively, while for the pyrano[3,2-*c*]pyridine the order of substitution is $2\text{-thienylidene} > 4\text{-(CH}_3\text{)benzylidene} > 4\text{-(CH}_3\text{O)benzylidene}$: **35** $>$ **33** $>$ **34**, respectively.

Conclusion

The α,β -unsaturated ketone **9** and the racemic alcohol **18** showed antiviral activity against HSV-1 and HIV-1, respectively. The heterocyclic systems pyrano[3,2-*c*]pyridine, pyrido[4,3-*d*]pyrimidine, and pyrazole[4,3-*c*]pyridine exhibited remarkable cytotoxic activity with selectivity toward leukemia cell lines possibly through interfering with folic acid synthesis. These heterocycles could be considered as useful templates for future development and further derivatization or modification to obtain more potent and selective antitumor agents. It is worth mentioning that compounds **14**, **26**, **28**, **33**, and **35** have been selected by the NCI biological evaluation committee for performing hollow fiber assay in vivo testing.

Experimental Section

Melting points were determined on a Mettler FP80 melting point apparatus and are uncorrected. Microanalyses were performed on a Perkin-Elmer 240 elemental analyzer at the

Central Research Laboratory, College of Pharmacy, King Saud University. All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within $\pm 0.4\%$ of the theoretical values. ^1H and ^{13}C NMR spectra were recorded on a Varian XL 400 MHz FT spectrometer; chemical shifts are expressed in δ ppm with reference to TMS. Mass spectral data were obtained on a Shimadzu GC/MS QP 5000 apparatus. Thin-layer chromatography was performed on precoated (0.25-mm) silica gel plates; compounds were detected with a 254-nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. The synthesis of compounds **8**, **9**, and **13** were previously reported in refs 27 and 28. The synthesized compounds were tested in vitro for their antiviral activity using HSV-1 at College of Pharmacy, University of Mansoura, Mansoura, Egypt. In vitro anti-human HIV-1 and antitumor activity were conducted at the NCI, Bethesda, MD.

General Procedure for Preparation of (*E*)-1-Methyl-3,5-bis(arylidene)-4-piperidones 10–12 and 14. A mixture of 1-methyl-4-piperidone (**7**; 0.01 mol) and the appropriate aldehyde (0.02 mol) in alcoholic NaOH (50 mL, 10%) was stirred at room temperature for 10 min. The separated solid was filtered and recrystallized from the suitable solvents.

(*E*)-1-Methyl-3,5-bis(4-nitrobenzylidene)-4-piperidone (10). The crude product was recrystallized from BuOH to give **10** (65%): mp 204–5 °C; ^1H NMR (CDCl_3) δ 2.25 (s, 3H, NCH_3), 2.85–3.56 (m, 4H, CH_2NCH_2), 6.55–8.89 (m, 10H, $\text{CH}=\text{C}$ & ArH); MS m/e (379, 20%). Anal. ($\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_5$) C, H, N.

(*E*)-1-Methyl-3,5-bis(4-methylbenzylidene)-4-piperidone (11). The obtained crude product was recrystallized from EtOH/ H_2O to give **11** (95%): mp 195–6 °C; MS m/e (317, 100%). Anal. ($\text{C}_{22}\text{H}_{23}\text{NO}$) C, H, N.

(*E*)-1-Methyl-3,5-bis(4-methoxybenzylidene)-4-piperidone (12). The crude product was recrystallized from EtOH to yield **12** (70%): mp 193–4 °C; MS m/e (349, 100%). Anal. ($\text{C}_{22}\text{H}_{23}\text{NO}_3$) C, H, N.

(*E*)-1-Methyl-3,5-bis(2-thienylidene)-4-piperidone (14). The obtained crude product was recrystallized from EtOH/ H_2O to produce **14** (84%): mp 114–5 °C; MS m/e (301, 100%). Anal. ($\text{C}_{16}\text{H}_{15}\text{NOS}_2$) C, H, N.

General Procedure for Preparation of 1-Methyl-3,5-bis(arylmethyl)-4-piperidones 15–17. (*E*)-1-Methyl-3,5-bis(arylidene)-4-piperidone (**11**, **12**, or **14**; 0.01 mol) was dissolved in ethanol (50 mL) and subjected to catalytic hydrogenation (H_2 , 10% Pd/C, 50 psi) for 10 h. The reaction mixture was filtered through Celite and filtrate was evaporated to dryness in vacuo. The obtained residue was chromatographed on silica gel (20% EtOAc, CHCl_3) to give an analytically pure samples of compounds **15**–**17**, which were then recrystallized from the appropriate solvents.

1-Methyl-3,5-bis(4-methylphenylmethyl)-4-piperidone (15). The obtained product was recrystallized from EtOH/ H_2O to give **15** (32%): mp 167–8 °C; ^1H NMR (CDCl_3) δ 2.18 (s, 3H, NCH_3), 2.34 (s, 6H, ArCH_3), 2.85–3.56 (m, 10H, $(\text{CH}_2\text{CHCH}_2)_2\text{N}$), 7.20–7.62 (m, 8H, ArH); MS m/e (321, 10%). Anal. ($\text{C}_{22}\text{H}_{27}\text{NO}$) C, H, N.

1-Methyl-3,5-bis(4-methoxyphenylmethyl)-4-piperidone (16). The obtained product was recrystallized from MeOH to yield **16** (20%): mp 200–2 °C; MS m/e (353, 15%). Anal. ($\text{C}_{22}\text{H}_{27}\text{NO}_3$) C, H, N.

1-Methyl-3,5-bis(2-thienylmethyl)-4-piperidone (17). The obtained product was recrystallized from EtOH to yield **17** (15%): mp 156–7 °C; MS m/e (305, 12%). Anal. ($\text{C}_{16}\text{H}_{19}\text{NOS}_2$) C, H, N.

General Procedure for Preparation of (\pm)-(*E*)-1-Methyl-3,5-bis(arylidene)-4-hydroxypiperidines 18–20. (*E*)-1-Methyl-3,5-bis(arylidene)-4-piperidone (**11**, **12**, or **14**; 0.01 mol) was dissolved in methanol (50 mL) and stirred. NaBH_4 (1.0 g) was added portionwise over a period of 0.5 h and stirred at room temperature for another 2 h. Solvent was removed under reduced pressure, 20 mL of water was then added, and the undissolved solid was filtered and recrystallized from the appropriate solvent.

(±)-(E)-1-Methyl-3,5-bis(4-methylbenzylidene)-4-hydroxypiperidine (**18**). The prepared compound was recrystallized from EtOH to yield **18** (96%): mp 129–30 °C; ¹H NMR (CDCl₃) δ 2.34 (s, 3H, NCH₃), 2.37 (s, 6H, ArCH₃), 3.31–3.76 (dd, 4H, *J* = 15 Hz, CH₂NCH₂), 3.40 (s, 1H, OH), 4.66 (s, 1H, CHOH), 6.59 (s, 2H, CH=C), 7.16 (s, 8H, ArH); ¹³C NMR δ 21.7, 44.9, 56.0, 78.9, 125.9, 129.3, 129.5, 134.4, 137.1, 137.8; MS *m/e* (319, 100%). Anal. (C₂₂H₂₅NO) C, H, N.

(±)-(E)-1-Methyl-3,5-bis(4-methoxybenzylidene)-4-hydroxypiperidine (**19**). The synthesized compound was recrystallized from EtOH to give **19** in (90%): mp 138–9 °C; MS *m/e* (351, 65%). Anal. (C₂₂H₂₅NO₃) C, H, N.

(±)-(E)-1-Methyl-3,5-bis(2-thienylidene)-4-hydroxypiperidine (**20**). The obtained compound was recrystallized from EtOH to produce **20** (87%): mp 141–2 °C; MS *m/e* (303, 67%). Anal. (C₁₆H₁₇NOS₂) C, H, N.

General Procedure for Preparation of (E)-2-(Methyl or phenyl)-3-aryl-5-methyl-7-arylidene-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridines 21–26. A mixture of **11**, **12**, or **14** (0.01 mol), the appropriate hydrazine derivative (0.04 mol) and Na metal (0.5 g) in ethanol (20 mL) was heated under reflux for 10 h. Solvent was then removed under reduced pressure and the remained residue was chromatographed on silica gel (CHCl₃) to furnish compounds **21–26**.

(E)-2-Methyl-3-(4-methylphenyl)-5-methyl-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridine (**21**). The prepared compound was recrystallized from EtOAc to produce **21** (28%): mp 118–20 °C; ¹H NMR (CDCl₃) δ 2.35 (s, 3H, NCH₃), 2.37 (s, 3H, ArCH₃), 2.42 (s, 3H, ArCH₃), 2.79 (s, 3H, NCH₃), 3.05–3.12 (m, 2H, N–CH₂), 3.25–3.34 (m, 2H, CH₂–N), 4.00 (d, 1H, *J* = 11 Hz, CH–CH), 4.12 (d, 1H, *J* = 14 Hz, CH–CH), 7.03–7.13 (m, 8H, ArH), 7.29 (s, 1H, CH=C); MS *m/e* (345, 100%). Anal. (C₂₃H₂₇N₃) C, H, N.

(E)-2-Methyl-3-(4-methoxyphenyl)-5-methyl-7-(4-methoxybenzylidene)-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridine (**22**). The produced compound was recrystallized from EtOAc to give **22** (35%): mp 182–3 °C; MS *m/e* (377, 85%). Anal. (C₂₃H₂₇N₃O₂) C, H, N.

(E)-2-Methyl-3-(2-thienyl)-5-methyl-7-(2-thienylidene)-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridine (**23**). The obtained product was recrystallized from EtOH to yield **23** (20%): mp 154–5 °C; MS *m/e* (329, 100%). Anal. (C₁₇H₁₉N₃S₂) C, H, N.

(E)-2-Phenyl-3-(4-methylphenyl)-5-methyl-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridine (**24**). The synthesized compound was recrystallized from EtOH to give **24** (53%): mp 175–6 °C; ¹H NMR (DMSO-*d*₆) δ 2.36 (s, 3H, NCH₃), 2.39 (s, 6H, Ar–CH₃), 2.99–3.03 (dd, 2H, *J* = 7.0, 6.5 Hz, NCH₂), 3.66–3.75 (m, 2H, CH₂N), 5.21 (d, 1H, *J* = 5 Hz, CH–CH), 5.24 (d, 1H, *J* = 10 Hz, CH–CH), 6.54–7.39 (m, 14H, CH=C & ArH); ¹³C NMR 21.5, 21.7, 42.8, 64.4, 113.9, 119.6, 121.3, 126.2, 126.9, 129.4, 129.9, 130.3, 133.0, 134.4, 137.6, 138.5, 139.9, 144.8, 149.0; MS *m/e* (407, 10%). Anal. (C₂₈H₂₉N₃) C, H, N.

(E)-2-Phenyl-3-(4-methoxyphenyl)-5-methyl-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridine (**25**). The produced compound was recrystallized from CHCl₃/EtOH to yield **25** (51%): mp 192–3 °C; MS *m/e* (439, 48%). Anal. (C₂₈H₂₉N₃O₂) C, H, N.

(E)-2-Phenyl-3-(2-thienyl)-5-methyl-7-(2-thienylidene)-3,3a,4,5,6,7-hexahydro-2H-pyrazolo[4,3-*c*]pyridine (**26**). The obtained product was recrystallized from CHCl₃/Hexane to give **26** (36%): mp 169–70 °C; MS *m/e* (391, 100%). Anal. (C₂₂H₂₁N₃S₂) C, H, N.

General Procedure for Preparation of (E)-4-Aryl-6-methyl-8-arylidene-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-*d*]pyrimidine-2-thiones 27–29. A mixture of the diarylidene compound **11**, **12**, or **14** (0.01 mol), thiourea (0.8 g, 0.01 mol), and Na metal (0.5 g) in butanol (50 mL) was heated under reflux for 10 h. Solvent was evaporated in vacuo, water (20 mL) was then added, and the mixture was neutralized to pH 6. The separated solid was filtered, washed, dried and recrystallized from the appropriate solvent.

(E)-4-Methylphenyl-6-methyl-8-(4-methylbenzylidene)-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-*d*]pyrimidine-2-thione (**27**). The obtained residue was recrystallized from EtOH/H₂O to produce **27** (91%): mp 151–2 °C; ¹H NMR (DMSO-*d*₆) δ 2.37 (s, 3H, NCH₃), 2.40 (s, 6H, ArCH₃), 3.00–3.05 (dd, 2H, *J* = 7.0 Hz, NCH₂), 3.68–3.78 (m, 2H, CH₂N), 5.28 (s, 1H, CHNH), 6.60–7.42 (m, 9H, CH=C & ArH), 8.04 (brs, 1H, NH), 8.39 (s, 1H, NH); MS *m/e* (375, 43%). Anal. (C₂₃H₂₅N₃S) C, H, N.

(E)-4-Methoxyphenyl-6-methyl-8-(4-methoxybenzylidene)-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-*d*]pyrimidine-2-thione (**28**). The produced compound was recrystallized from EtOH/ether to give **28** (80%): mp 197–8 °C; MS *m/e* (407, 42%). Anal. (C₂₃H₂₅N₃O₂S) C, H, N.

(E)-4-(2-Thienyl)-6-methyl-8-(2-thienylidene)-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-*d*]pyrimidine-2-thione (**29**). The prepared compound was recrystallized from EtOH to produce **29** (83%): mp 217.8 °C; MS *m/e* (359, 100%). Anal. (C₁₇H₁₇N₃S₂) C, H, N.

General Procedure for Preparation of (E)-3-Cyano-4-aryl-6-methyl-8-arylidene-5,6,7,8-tetrahydro-1H-pyrido[3,2-*c*]pyridin-2-ones 30–32. A mixture of the diarylidene compound **11**, **12**, or **14** (0.01 mol) and cyanoacetamide (0.8 g, 0.01 mol) was refluxed in butanol (50 mL) in the presence of a catalytic amount of piperidine for 10 h. Solvent was removed under reduced pressure and the obtained residue was washed with water, dried and recrystallized from appropriate solvents.

(E)-3-Cyano-4-(4-methylphenyl)-6-methyl-8-(4-methylbenzylidene)-5,6,7,8-tetrahydro-1H-pyrido[3,2-*c*]pyridin-2-ones (**30**). The obtained compound was recrystallized from EtOH to give **30** (33%): mp 234–5 °C; ¹H NMR (DMSO-*d*₆) δ 2.17 (s, 3H, NCH₃), 2.33 (s, 3H, ArCH₃), 2.38 (s, 3H, ArCH₃), 2.48–2.49 (m, 4H, CH₂NCH₂), 7.25–7.34 (m, 9H, CH=C & ArH), 7.71 (brs, 1H, NH); MS *m/e* (381, 53%). Anal. (C₂₅H₂₃N₃O) C, H, N.

(E)-3-Cyano-4-(4-methoxyphenyl)-6-methyl-8-(4-methoxybenzylidene)-5,6,7,8-tetrahydro-1H-pyrido[3,2-*c*]pyridin-2-ones (**31**). The synthesized compound was recrystallized from EtOH/H₂O to give **31** (56%): mp 216–7 °C; MS *m/e* (413, 12%). Anal. (C₂₅H₂₃N₃O₃) C, H, N.

(E)-3-Cyano-4-(2-thienyl)-6-methyl-8-(2-thienylidene)-5,6,7,8-tetrahydro-1H-pyrido[3,2-*c*]pyridin-2-ones (**32**). The crude product was recrystallized from BuOH to give **32** (60%): mp 189–90 °C; MS *m/e* (365, 15%). Anal. (C₁₉H₁₅N₃OS₂) C, H, N.

General Procedure for Preparation of (E)-2-Amino-3-cyano-4-aryl-6-methyl-8-arylidene-5,6,7,8-tetrahydro-4H-pyran[3,2-*c*]pyridines 33–35. A mixture of the diarylidene compound **11**, **12**, or **14** (0.01 mol) and malononitrile (0.7 g, 0.01 mol) in butanol (50 mL) was heated under reflux for 5 h. The precipitated product was filtered off and recrystallized.

(E)-2-Amino-3-cyano-4-(4-methylphenyl)-6-methyl-8-(4-methylbenzylidene)-5,6,7,8-tetrahydro-4H-pyran[3,2-*c*]pyridine (**33**). The crude product was recrystallized from EtOH to give **33** (72%): mp 214–5 °C; ¹H NMR (DMSO-*d*₆) δ 2.11 (s, 3H, NCH₃), 2.27 (s, 3H, ArCH₃), 2.29 (s, 3H, ArCH₃), 2.48–2.97 (dd, 2H, *J* = 18 Hz, NCH₂), 3.22–3.47 (dd, 2H, *J* = 15 Hz, CH₂N), 3.97 (s, 1H, pyran-H), 6.77 (brs, 2H, NH₂), 6.86 (s, 1H, CH=C), 7.09–7.20 (m, 8H, ArH); ¹³C NMR δ 21.0, 21.2, 44.9, 54.6, 54.9, 56.5, 113.3, 120.8, 121.7, 127.2, 127.9, 129.3, 129.5, 129.6, 133.5, 136.6, 136.9, 139.5, 140.9, 160.1; MS *m/e* (383, 59%). Anal. (C₂₅H₂₅N₃O) C, H, N.

(E)-2-Amino-3-cyano-4-(4-methoxyphenyl)-6-methyl-8-(4-methoxybenzylidene)-5,6,7,8-tetrahydro-4H-pyran[3,2-*c*]pyridine (**34**). The resultant residue was recrystallized from EtOH to produce **34** (97%): mp 203–4 °C; MS *m/e* (415, 45%). Anal. (C₂₅H₂₅N₃O₃) C, H, N.

(E)-2-Amino-3-cyano-4-(2-thienyl)-6-methyl-8-(2-thienylidene)-5,6,7,8-tetrahydro-4H-pyran[3,2-*c*]pyridine (**35**). The crude product was recrystallized from EtOH/H₂O to give **35** (65%): mp 190–2 °C; MS *m/e* (367, 100%). Anal. (C₁₉H₁₇N₃OS₂) C, H, N.

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References

- (1) Dimmock, J. R.; Arora, V. K.; Duffy, M. J.; Reid, R. S.; Allen, T. M.; Kao, G. Y. Evaluation of Some N-acyl analogues of 3,5-Bis (arylidene)-4-piperidones for Cytotoxic Activity. *Drug Des. Discovery* **1992**, *8*, 291–299.
- (2) Dimmock, J. R.; Raghavan, S. K.; Logan, B. M.; Bigam, G. E. Antileukemic Evaluation of Some Mannich Bases Derived from 2-Arylidene-1,3-diketones. *Eur. J. Med. Chem.* **1983**, *18*, 248–254.
- (3) Baluja, G.; Municio, A. M.; Vega, S. Reactivity of Some α,β -Unsaturated Ketones Towards Sulphydryl Compounds and Their Antifungal Activity. *Chem. Ind.* **1964**, 2053–2054.
- (4) El-Barbary, A. A.; Khodair, A. I.; Pederson, E. B.; Nielsen, C. s-Glucosylated Hydantoins as New Antiviral Agents. *J. Med. Chem.* **1994**, *37*, 73–77.
- (5) Khodair, A. I.; El-Subbagh, H. I.; El-Emam, A. A. Synthesis of Certain 5-Substituted-2-Thiohydantoin Derivatives as Potential Cytotoxic and Antiviral Agents. *Bull. Chim. Farm.* **1997**, *136*, 561–567.
- (6) Al-Obaid, A. M.; El-Subbagh, H. I.; Khodair, A. I.; El-mazar, M. M. A. 5-Substituted-2-thiohydantoin Analogues as a Novel Class of Antitumor Agents. *Anticancer Drugs* **1996**, *7*, 873–880.
- (7) Lorand, T.; Deli, J.; Szabo, D.; Foldesi, A. Potentially Bioactive Pyrimidine Derivatives. *Pharmazie* **1985**, *40*, 53–539.
- (8) Temple, C.; Elliot, R.; Montgomery, J. Pyrido[2,3-*d*]pyrimidines. Synthesis of the 5-Deaza Analogues of Aminopterin, Methotrexate, Folic Acid. *J. Org. Chem.* **1982**, *47*, 761–764.
- (9) Li, L.; Wang, H.; Kuo, S.; Wu, T.; Mauger, A.; Lin, C. Antitumor Agents 155. Synthesis and Biological Evaluation of Substituted 2-Phenyl-4-Quinolones. *J. Med. Chem.* **1994**, *37*, 3400–3407.
- (10) Ambika, D. M.; Das, N. P. In Vitro Effects of Natural Plant Polyphenols on the Proliferation of Normal and Abnormal Human Lymphocytes and Their Secretion of Interleukin-2. *Cancer Lett.* **1993**, *69*, 191–196.
- (11) Cassady, J. M. Natural Products as a Source of Potential Cancer Chemopreventive Agents. *J. Nat. Prod.* **1990**, *53*, 23–30.
- (12) Newmark, H. L. Plant Polyphenolics as Inhibitors of Mutational and Precarcinogenic Events. *Can. J. Physiol. Pharmacol.* **1986**, *65*, 461–466.
- (13) Ramanathan, R.; Tan, C. H.; Das, N. P. Cytotoxic Effects of Polyphenols and Fat Soluble Vitamins on Malignant Human Cultured Cells. *Cancer Lett.* **1992**, *62*, 217–224.
- (14) Narborne, J. B.; Maby, T. J. *The Flavonoids: Advances in Research*; Chapman and Hall: London, 1982.
- (15) Elliott, A. J.; Scheiber, S. A.; Thomas, C.; Pardini, R. S. Inhibition of Glutathione Reductase by Flavonoids: A Structure–activity Study. *Biochem. Pharmacol.* **1992**, *44*, 1603–1616.
- (16) Kuppusamy, U. R.; Das, N. P. Effect of Flavonoids on Cyclic AMP Phosphodiesterase and Lipid Mobilization in Rat Adipocytes. *Biochem. Pharmacol.* **1992**, *44*, 1307–1312.
- (17) Suolinna, E. M.; Buchskaum, R. N.; Racker, E. The Effect of Flavonoids on Aerobic Glycolysis and Growth of Tumor Cells. *Cancer Res.* **1975**, *35*, 1865–1870.
- (18) Haenszel, W.; Locke, R. B.; Segi, M. A Case Control Study of Large Bowel Cancer in Japan. *J. Natl. Cancer Inst.* **1980**, *64*, 17–23.
- (19) Wilpart, M. Dietary Fats and Fiber and Experimental Carcinogenesis: A Critical Review of Published Evidences. In *Causation and Prevention of Colorectal Cancer*; ECP Symposium; Faivre, J., Hill, M. J., Eds.; Excerpta Medica: Amsterdam, 1987.
- (20) Chin, C. Y.; Nagaratnam, P. D. Cytotoxic Effect of Butein on Human Colon Adenocarcinoma Cell Proliferation. *Cancer Lett.* **1994**, *82*, 65–72.
- (21) Aizu, E.; Nakadate, T.; Yamamoto, S.; Kato, R. New Lipoxigenase Inhibitors Lacking Protein Kinase C Inhibitory Effects. *Carcinogenesis* **1986**, *7*, 1809–1812.
- (22) Beutler, J. A.; Cardellina, J. H.; Gray, G. N.; Prather, T. R.; Shaemaker, R. H.; Boyd, M. R. Two New Cytotoxic Chalcones from *Calythropsis Aurea*. *J. Nat. Prod.* **1993**, *56*, 1718–1722.
- (23) Vincenzo, R. De; Scambia, G.; Panici, P.; Ranelletti, F.; Mancuso, S. Effect of Synthetic and Natural Occurring Chalcones on Ovarian Cancer Cell Growth. *Anti-Cancer Drug Des.* **1995**, *10*, 481–490.
- (24) Artico, M.; R. Santo, Di; Costi, R.; Novellino, E.; Greco, G.; Massa, S.; Tramontano, E.; Marongui, M.; De Montis, A.; La Colla, P. Geometrically and Conformationally Restrained Cinnamoyl Compounds as Inhibitors of HIV-1 Integrase. *J. Med. Chem.* **1998**, *41*, 3948–3960.
- (25) Zhao, H.; Neamatic, N.; Hong, H. Coumarine-Based Inhibitors of HIV Integrase. *J. Med. Chem.* **1997**, *40*, 242–249.
- (26) Skulnick, H. I.; Johnson, P. D.; Aristoff, P. A. Structure-Based Design of Nonpeptidic HIV Protease Inhibitors. *J. Med. Chem.* **1997**, *40*, 1149–1164.
- (27) Krapcho, J.; Turk, C. F. Pharmaceutical 7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2H-pyrazolo[4,3-*c*]pyridines. Ger. Offen. 2,411,499, Sept 26, 1974; *Chem. Abstr.* **1975**, 4248c.
- (28) El-Kashef, H. S.; Geies, A. A.; Kamal, El-Dean, A. M.; Abdel-Hafez, A. A. Synthesis of thieno[2,3-*c*]pyridines and related heterocyclic systems. *J. Chem. Technol. Biotechnol.* **1993**, *57*, 15–19.
- (29) Hufford, C. D.; Badria, F. A.; Abou-Karam, M.; Shier, W. T.; Rogers, R. D. Preparation, Characterization and Antiviral Activity of Microbial Metabolites of Stemodin. *J. Nat. Prod.* **1991**, *54*, 1543–1552.
- (30) Weislow, O. S.; Kiser, R.; Fine, D.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New Soluble-formazan Assay for HIV-1 Cytopathic Effects. *J. Natl. Cancer Inst.* **1989**, *81*, 577–586.
- (31) Grever, M.; Schepartz, S.; Chabner, B. The National Cancer Institute Cancer Drug Discovery and Development Program. *Semin Oncol.* **1992**, *19*, 622–638.
- (32) Monks, A.; Scudiero, D.; Skehan, P.; et al. Feasibility of a High-Flux Anticancer Drug Screen Utilizing a Derive Panel of Human Tumor Cell Lines in Culture. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
- (33) Boyd, M.; Paull, K. Some Practical Considerations and Applications of the National Cancer Institute in Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91–109.

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