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Cytotoxicity of 2-aldo- and 2-ketopyridine-N(4)-substituted thiosemicarbazones and mode of action in human Tmolt$_4$ cells

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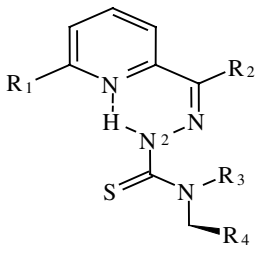
The 2-aldo- and 2-ketopyridine-N(4)-substituted thiosemicarbazones and their copper complexes demonstrated potent cytotoxic activity against a series of murine and human suspended cultured tumor cells. Selected compounds were active against the growth of cultured cells from solid human tumors, i.e. Mck-7 breast effusion, lung A549 and lung MB-9812, bone SOS-2 and clear cell Caki renal tumor. In Tmolt$_4$ T cell leukemia cells the compounds inhibited the syntheses of DNA, RNA and protein over 60 min at 25 to 100 μM. Multiple target sites in nucleic acid metabolism were suppressed by the agents, i.e. DNA polymerase α, ribonucleoside reductase, dihydrofolate reductase, de novo purine synthesis, thymidylate synthetase and nucleoside kinases. The total effects of the agents on DNA metabolism led to the reduction of deoxyribonucleotide pools as well as DNA fragmentation.

1. Introduction

Heterocyclic thiosemicarbazones, thioureas and 2-substituted pyridine N-oxides and their copper(II) complexes have been shown to be potent antineoplastic agents and cytotoxicity has been demonstrated against the growth of a number of murine and human tumor cells [1]. The 2-acetyl-pyridine N(4)-substituted-thiosemicarbazones have demonstrated significant activity in the NCI screens for non-small cell lung cancer, breast cancer, prostate cancer and leukemia [2]. Mode of action studies in L1210 cells have demonstrated that the agents inhibit DNA and RNA syntheses at the sites of IMP dehydrogenase, DNA polymerase α, ribonucleoside reductase, dihydrofolate reductase, nucleoside kinases, DNA topoisomerase II with DNA strand scission without DNA protein linked breaks [1, 6]. The current studies involve a series of 2-formyl-, 6-methyl-2-formyl- and 2-benzoyl- and 2-acetylpyridine N(4)-methyl-(2)-pyridinyl, N(4)-ethyl-(2)-pyridinyl- and N(4)-methyl-N(4)-ethyl-(2)-pyridinylthiosemicarbazones. Their method of synthesis and spectral properties have been reported previously along with their copper(II) complexes [3]. The structure in Table 1 represents the Z form of these novel thiosemicarbazones which is the form found in crystallographic determinations of compound **1** and **6** [4]. The copper(II) complexes are coordinated by the neutral thiosemicarbazone ligands and two chlorines in an approximate square pyramidal sterochemistry [5] as represented by compound **20**, i.e. [Cu(HBz4paeM)Cl$_2$] (Fig. 1). These derivatives have currently been examined for their cytotoxicity and mode of action in human Tmolt$_4$ T leukemia cells.

Table 1: Structures of N(4)-substituted thiosemicarbazones with a pendant pyridine ring



Compd.	Symbol	R_1	R_2	R_3	R_4
1	HFo4pam	H	H	H	2-C_5H_4N
2	HBz4pam	H	C_6H_5	H	2-C_5H_4N
3	HAc4pam	H	CH_3	H	2-C_5H_4N
4	6MFo4pam	CH_3	H	H	2-C_5H_4N
5	HFo4pae	H	H	H	CH_2-2-C_5H_4N
6	HBz4pae	H	C_6H_5	H	CH_2-2-C_5H_4N
7	HAc4pae	H	CH_3	H	CH_2-2-C_5H_4N
8	6MHFo4pae	CH_3	H	H	CH_2-2-C_5H_4N
9	HFopaeM	H	H	CH_3	CH_2-2-C_5H_4N
10	HBz4paeM	H	C_6H_5	CH_3	CH_2-2-C_5H_4N
11	HAcpaeM	H	CH_3	CH_3	CH_2-2-C_5H_4N
12	[Cu(HFo4pam)Cl$_2$]				
13	[Cu(HBz4pam)Cl$_2$]				
14	[Cu(6MHFopam)Cl$_2$]				
15	[Cu(HFo4pae)Cl$_2$]				
16	[Cu(HBz4pae)Cl$_2$]				
17	[Cu(6MHFo4pae)Cl$_2$]				
18	[Cu(HFo4paeM)Cl$_2$]				
19	[Cu(6MHFo4paeM)Cl$_2$]				
20	[Cu(HBz4paeM)Cl$_2$]				

2. Investigations and results

2.1. Cytotoxicity

2-Formyl-, 6-methyl-2-formyl- and 2-benzoyl- and 2-acetylpyridine N(4)-methyl-(2)-pyridinyl, N(4)-ethyl-(2)-pyridinyl- and N(4)-methyl-N(4)-ethyl-(2)-pyridinylthiosemicarbazones and their copper complexes demonstrated potent cytotoxicity against the growth of murine and hu-

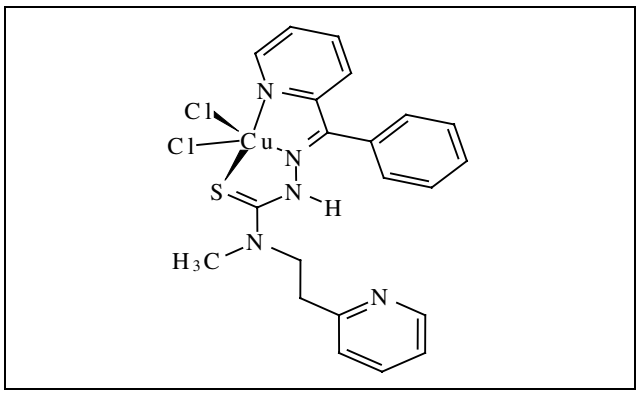


Fig. 1: The bonding in the 5-coordinate[Cu(Hbz4paeM)Cl

man cultured cancer cells (Table 2). In the murine L1210 lymphoid leukemia screen all of the compounds were active except compounds **8** and **20**, i.e. ED_{50} values were greater than 4 μg/ml. Compounds **1**–**4** and **10**–**20** were significantly active in the murine P388 lymphocytic leukemia screen. Human $Tmolt_3$ T cell leukemia growth was reduced by compounds **2**–**15**, **18** and **20** and $Tmolt_4$ T cell growth was inhibited by compounds **1**–**4**, **7**, **9**–**11**, **13** and **16**–**20**. Human Hl-60 leukemia growth was suppressed by compounds **1**, **9** and **19**. Human HuT-8 lymphoma growth was retarded by all of the compounds except compound **13**. THP-1 acute monocytic leukemia growth was inhibited by compounds **2**, **3**, **7**, **9** and **16**. HeLa-S^3 suspended uterine carcinoma growth was effectively reduced by all of the compounds with a number of the agents affording ED_{50} values less than 2 μg/ml. The cytotoxicity in those tumors cultured from human solid tumors demonstrated selectivity for the compounds. KB nasopharynx growth was reduced by compounds **16**–**20**. Lung A549 growth was inhibited by compounds **3**, **5**–**7**, **9** and **12**–**15**. Lung MB-9812 bronchogenic growth was suppressed by all of the compounds except compounds **4** and **8**. In the HeLa solid uterine carcinoma screen only compounds **10**, **13** and **16**–**20** were active. Mck-7 breast effusion growth was retarded by all of the compounds except **1**, **4**, **8** and **17**. In the clear cell Caki renal screen all of the compounds were active except compounds **4**, **8**, **10**, **16** and **17**. Melanoma Sk-2 growth was reduced by compounds **17**–**20** and skin epidermoid A431 growth was suppressed by compounds **1**, **3**, **6** and **9**–**20**. Bone SOS-2 growth was inhibited by compounds **5**, **12**, **13**, **16** and **20**. The compounds were inactive in the adenocarcinoma colon SW480 and ileum HCT-8 screens as well as the brain glioma U-87 MG screen.

2.2. $Tmolt_4$ studies

The mode of action study in $Tmolt_4$ Tcell leukemia cells showed that DNA, RNA and protein syntheses were inhibited over 60 min in a concentration dependent manner from 25 μM to 100 μM (Tables 3–6). DNA polymerase α activity was reduced 35% to 48% over 60 min at 100 μM. m-RNA polymerase activity was not affected by the compounds but r-RNA polymerase activity was reduced marginally 16% to 27% and t-RNA polymerase activity was suppressed 19% to 26% by the four compounds. Ribonucleoside reductase activity was inhibited significantly from 55% to 93% after 60 min at 100 μM. Dihydrofolate reductase activity was suppressed 50% to 70% in a concentration dependent manner. Purine de novo synthesis was significantly inhibited 32% to 42% after 60 min and the regulatory enzyme of the pathway IMP dehydrogenase was also suppressed by 37% to 58%. On the other hand, the other regulatory enzyme of the pathway, PRPP-amido transferase's activity was inhibited 72% to 79% by **18** and **13**, but compounds **3** and **11** actually caused a significant elevation in activity. De novo pyrimidine synthesis was reduced 12% to 37% by the agents. Carbamyl phosphate synthetase activity was suppressed 16% to 43% and aspartate transcarbamylase activity was suppressed 21% by compound **3**. Thymidylate synthetase activity was reduced 12% to 48% after 60 min at 100 μM. Thymidine kinase activity was reduced 27% to 80%; TMP kinase activity was reduced 51% to 73% and TDP was suppressed 38% to 58% by compounds **11**, **13** and **18**. d[ATP] pools were reduced 28% and 23% by compounds **11** and **13**. d[GTP] and d[CTP] pools were reduced 26% and 22% by compound **18**, respectively. d[TTP] was lowered 27% by compound **3** and elevated 24% by compound **18**. $Tmolt_4$ DNA strand scission was evident after 24 h incubation with the agents at 100 μM (Fig. 2). ct-DNA studies demonstrated that after incubation for 24 h at 100 μM the agents caused an hyperchromic shift in the absorption of DNA from 220 to 340 nm to a high (longer) wavelength. Thermal ct-DNA denaturation Tm values for the control was 85 °C, for compound **3** was 67 °C, for compound **11** was 63 °C, for compound **13** was 57 °C and for compound **18** was 60 °C. ct-DNA viscosity for the control was 416.5 s, for compound **3** was 380.6 s, for compound **11** was 398 s, for compound **13** was 402.3 s and for compound **18** was 409 s (time to move through reservoirs).

3. Discussion

The present heterocyclic N(4)-substituted thiosemicarbazones which include a second pyridinyl ring and their respective copper complexes demonstrated potent activity against the growth of suspended leukemia and lymphoma as well as suspended HeLa-S^3 uterine carcinoma cells. This action was more selective but very similar to the action of previously reported 2-acetyl-pyridine N(4) substitued thiosemicarbazones [7]. In these previous derivatives, when $R_3 = H$, and $R_4 =$ substituted phenyl ring they produce significant activity in the KB nasopharynx, glioma,

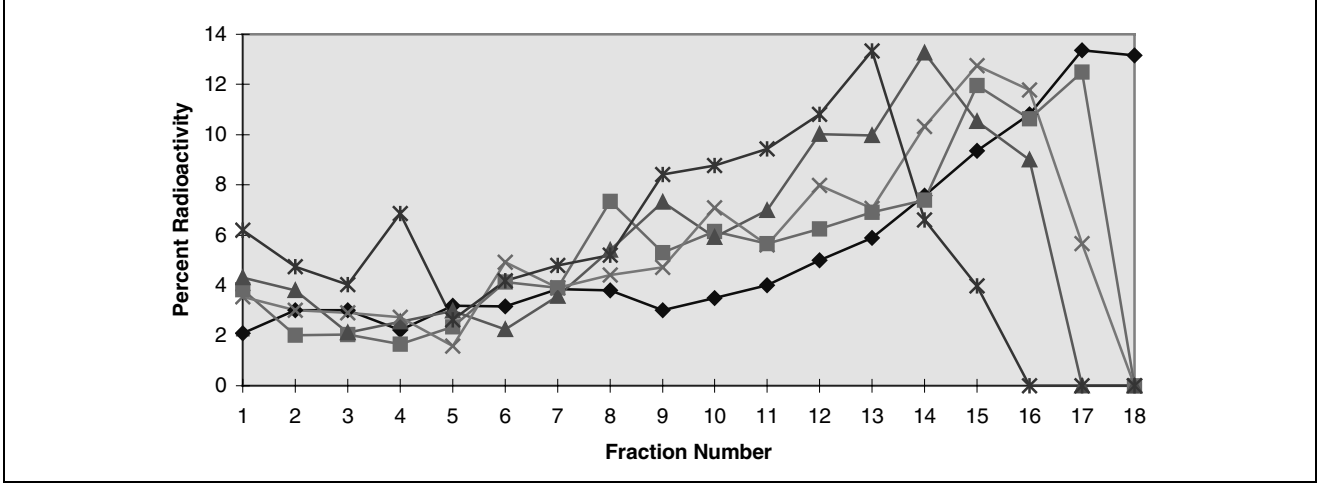


Fig. 2: $Tmolt_4$ T Cell Leukemia DNA strand scission after 24 h at 100 μM. —◆— Control; —■— Cpd 3; —▲— Cpd 11; —×— Cpd 13; —✳— Cpd 18

Table 2: Cytotoxicity of N(4)-substituted-pyridinyl-thiosemicarbazone and their copper complexes in murine and human cultured tumor cells [N = 4] ED_{50} values; μg/ml

Compd.	L1210 Leukemia	P388 Leukemia	$Tmolt_3$ Leukemia	$Tmolt_4$ Leukemia	Hl-60 Leukemia	HuT-78 Lymphoma	THP-1 Acute monocytic	HeLa-S^3 uterine	KB Nasopharynx
1	3.31	3.79	4.03	3.75	2.62	3.34	5.11	2.50	4.71
2	2.65	2.42	2.68	3.10	4.79	2.82	2.98	1.21	5.94
3	2.82	2.66	2.78	3.71	4.32	2.23	3.32	2.72	6.87
4	3.95	3.85	3.08	3.51	4.79	2.99	4.41	2.65	8.34
5	3.23	5.26	3.88	4.27	4.87	3.41	4.01	1.66	5.59
6	2.32	4.67	3.37	4.91	5.10	3.41	4.00	1.82	6.04
7	2.54	4.19	3.27	3.62	4.87	3.81	3.95	2.26	5.47
8	4.23	4.44	2.29	5.00	4.86	3.99	4.13	2.12	7.29
9	2.87	2.44	2.58	2.84	3.94	3.23	2.58	1.51	4.49
10	3.28	3.61	3.41	2.80	4.33	3.64	6.38	1.82	4.23
11	2.43	3.45	1.89	3.31	5.48	3.52	5.08	1.59	6.40
12	2.65	2.63	3.08	4.68	4.71	2.88	4.61	1.59	4.24
13	2.48	2.60	2.98	2.57	5.87	4.46	4.47	2.27	11.23
14	2.46	2.52	1.59	4.42	4.48	3.17	5.69	1.82	5.23
15	2.90	3.85	2.98	4.91	6.11	3.82	6.37	1.06	4.04
16	3.20	3.78	4.48	1.01	6.87	3.34	3.77	1.66	3.94
17	3.84	3.15	4.33	2.86	5.48	3.58	4.68	1.36	3.76
18	2.96	3.63	3.89	1.04	5.02	3.35	5.41	1.82	3.90
19	3.94	3.50	4.07	0.62	3.56	3.70	4.20	1.36	3.89
20	4.12	2.69	2.93	1.16	5.10	2.99	4.56	1.21	3,95
std									
6-MP	2.43	2.04	0.43	2.67	6.36	1.63	3.34	2.12	5.74
Ara-C	2.07	0.79	1.29	2.36	3.90	2.50	2.54	2.13	2.84
HU	2.67	1.30	4.47	6.68	5.22	3.87		1.96	5.27

ED_{50} values < 4 μg/ml are significantly active

SW-480 colon and HCT-8 ileum adenocarcinoma screens [6] where as in the present study only the copper complexes afforded the same activity in the KB screen and none of the compounds were active against the glioma, colon and ileum carcinoma growth. Selective but similar activity was demonstrated by the two groups of compounds in the two lung tumors, and the A431 epidermoid carcinoma. The present compounds did afford good activ-

Table 3: Effects of compound 3 on $Tmolt_4$ leukemia cell metabolism over 60 min (N = 6)

Assay	Percent of control			
	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5[a]	81 + 4*	72 + 5*	61 + 4*
RNA synthesis	100 ± 6[b]	94 + 5	90 + 5	83 + 5
Protein synthesis	100 ± 5[c]	72 + 4*	49 + 4*	44 + 4*
DNA polymerase α	100 ± 6[d]	113 + 5	111 + 6	65 + 5*
mRNA polymerase	100 ± 7[e]	120 + 6	115 + 4	104 + 5
rRNA polymerase	100 ± 4[f]	81 + 4*	77 + 5*	73 + 5*
tRNA polymerase	100 ± 7[g]	104 + 6	87 + 7	74 + 4*
Ribonucleoside reductase	100 ± 5[h]	119 + 6	88 + 5	37 + 3*
Dihydrofolate reductase	100 ± 5[i]	94 + 6	83 + 4	50 + 4*
Purine synthesis	100 ± 5j	90 + 5	76 + 5*	60 + 5*
PRPP amido transferase	100 ± 6[k]	150 + 5*	228 + 7*	316 + 6*
IMP dehydrogenase	100 ± 5[l]	85 + 4	65 + 4*	63 + 4*
Pyrimidine synthesis	100 ± 6[m]	76 + 5*	72 + 4*	69 + 4*
Carbamyl phosphate synthetase	100 ± 7[n]	84 + 5	79 + 3*	78 + 4*
Aspartate transcarbamylase	100 ± 6[o]	90 + 5	86 + 5	79 + 5*
Thymidylate synthetase	100 ± 5[p]	122 + 5	95 + 6	71 + 4*
Thymidine kinase	100 ± 6[q]	88 + 5	79 + 4*	73 + 3*
Thymidine monophosphate kinase	100 ± 7[r]	123 + 5*	106 + 5	96 + 6
Thymidine diphosphate kinase	100 ± 6[s]	111 + 6	94 + 5	88 + 6
d[ATP]	100 + 5[t]			88 + 5
d[GTP]	100 + 6[u]			87 + 4
d[CTP]	100 + 5[v]			93 + 4
d[TTP]	100 + 4[w]			73 + 4*

* P < 0.001; control values based on 10^6 L1210 cells

[a] 26152 dpm
[b] 4851 dpm
[c] 7461 dpm
[d] 47804 dpm
[e] 4239 dpm
[f] 1502 dpm
[g] 6400 dpm
[h] 2744 dpm
[i] 0.868 OD units
[j] 92551 dpm
[k] 0.121 OD units
[l] 76058 dpm
[m] 19758 dpm
[n] 0.392 mol citrulline
[o] 1.064 mol N-carbamyl aspartate
[p] 18463 dpm
[q] 1317 dpm
[r] 1179 dpm
[s] 1891 dpm
[t] 6.17 pmol
[u] 5.27 pmol
[v] 6.87 pmol
[w] 6.94 pmol

Colon SW 480	Lung A549	Lung-MB-9812	HeLa solid uterine	SOS-2 bone	Melanoma Sk Mel-2	HCT-8 Ileum	MCF-7 Breast	Caki Kidney	A431 'skin	Glioma U-87-MG
7.63	5.51	1.93	5.38	9.52	5.38	5.80	4.64	3.93	2.25	5.56
7.97	4.67	1.62	4.91	7.07	6.97	7.50	3.00	2.34	4.45	4.99
8.87	3.69	1.80	5.54	7.52	6.18	5.64	3.71	1.29	2.87	4.81
10.34	4.01	4.12	6.18	6.81	5.89	6.24	7.71	9.21	8.71	4.94
8.59	3.31	1.61	5.99	2.97	6.48	6.23	2.30	1.23	5.95	6.16
7.93	2.71	1.15	4.45	5.25	6.68	6.13	1.27	1.44	2.28	4.41
9.09	3.35	1.78	5.59	7.74	6.26	6.79	2.60	1.84	6.18	6.14
8.13	5.50	5.28	5.93	7.74	6.50	8.40	5.98	8.91	9.72	6.14
8.19	3.12	1.48	4.49	7.14	6.13	7.19	3.14	2.90	2.18	5.53
6.73	5.59	1.97	3.79	6.43	8.51	6.49	2.60	4.31	1.94	6.08
8.54	4.62	1.40	6.25	7.24	5.56	6.34	2.63	3.09	2.61	5.24
4.79	2.18	1.13	4.08	3.15	7.56	5.44	1.14	3.78	2.57	4.66
5.78	2.44	1.09	3.09	2.86	7.82	6.34	2.92	0.90	3.28	5.08
4.37	1.45	1.08	4.37	4.29	7.13	5.44	1.06	0.19	2.12	4.48
7.52	2.29	1.51	5.31	4.44	7.86	6.24	1.00	0.19	3.62	5.82
5.54	5.02	1.81	3.30	3.34	4.22	5.83	2.73	6.23	0.24	5.02
5.45	4.90	1.60	3.75	7.12	2.96	8.45	8.92	4.24	0.27	4.56
5.62	5.55	1.62	3.46	9.11	3.84	9.06	2.78	3.89	0.20	5.65
5..71	4.72	1.74	3.58	4.39	3.48	8.85	2.66	3.66	0.30	5.34
5.59	4.50	2.03	3.52	3.14	3.07	6.85	2.34	3.91	0.28	5.05
3.61	4.71	4.29	5.61	7.16	6.86	1.15	8.83	7.01	9.13	4.46
3.42	5.62	6.16	4.74	8.90	10.53	2.54	12.45	1.38	0.86	1.88
7.33	8.89	7.18	8.12			1.77			2.87	2.27

ity in the breast effusion tumor, and the clear cell Caki renal tumor screens.

The mode of action study of the present compounds in human $Tmolt_4$ Tcell leukemia cells demonstrated a similar pattern as observed for the previous heterocyclic N(4)-acetyl thiosemicarbazones in murine L1210 lymphoid leukemia cells [7]. The magnitude of suppression of DNA synthesis over 60 min at 100 μM was consistent with the ED_{50} values in the cytotoxicity of the four compounds and this pathway appeared to be affected more significantly than RNA and protein syntheses. As observed previously with the N(4) substituted thiosemicarbazones [6, 7] de novo purine synthesis was reduced significantly over 60 min with the activity of its regualtory enzyme IMP dehydrogenase being inhibited by an analogous magnitude while PRPP-amido transferase activity was actually stimulated by the compounds. The de novo pyrimidine pathway was moderatedly reduced by the compounds with the activity of the regulatory enzyme carbamyl phosphate synthetase being affected but the effects on the activity of the other regulatory enzyme, aspartate transcarbamylase were inconsistent. The heterocyclic N(4) acetyl-thiosemi-

Table 4: Effects of compound 11 on $Tmolt_4$ leukemia cell metabolism over 60 min (N = 6)

Assay	Percent of control			
	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5^a	$47 + 4$*	$45 + 4$*	$44 + 3$*
RNA synthesis	100 ± 6^b	$72 + 4$*	$72 + 5$*	$62 + 4$*
Protein synthesis	100 ± 5^c	$68 + 4$*	$64 + 4$*	$43 + 3$*
DNA polymerase α	100 ± 6^d	$90 + 5$	$63 + 4$*	$60 + 2$*
mRNA polymerase	100 ± 7^e	$111 + 6$	$105 + 5$	$96 + 7$
rRNA polymerase	100 ± 4^f	$93 + 6$	$87 + 5$	$84 + 5$
tRNA polymerase	100 ± 7^g	$83 + 4$	$83 + 6$	$81 + 5$
Ribonucleoside reductase	100 ± 5^h	$51 + 4$*	$49 + 5$*	$39 + 4$*
Dihydrofolate reductase	100 ± 5^i	$81 + 5$	$74 + 4$*	$39 + 3$*
Purine synthesis	$100 \pm 5j$	$86 + 5$	$64 + 4$*	$58 + 4$*
PRPP amido transferase	100 ± 6^k	$160 + 5$*	$194 + 5$*	$219 + 7$*
IMP dehydrogenase	100 ± 5^l	$80 + 4$*	$64 + 4$*	$58 + 3$*
Pyrimidine synthesis	100 ± 6^m	$86 + 5$	$77 + 4$*	$64 + 4$*
Carbamyl phosphate synthetase	100 ± 7^n	$63 + 6$*	$63 + 5$*	$57 + 4$*
Aspartate transcarbamylase	100 ± 6^o	$115 + 6$	$95 + 6$	$94 + 5$
Thymidylate synthetase	100 ± 5^p	$94 + 5$	$78 + 5$*	$54 + 4$*
Thymidine kinase	100 ± 6^q	$38 + 4$*	$36 + 4$*	$20 + 3$*
Thymidine monophosphate kinase	100 ± 7^r	$65 + 4$*	$40 + 3$*	$36 + 3$*
Thymidine diphosphate kinase	100 ± 6^s	$61 + 4$*	$52 + 3$*	$42 + 3$*
d[ATP]	$100 + 5^t$			$72 + 4$*
d[GTP]	$100 + 6^u$			$99 + 5$
d[CTP]	$100 + 5^v$			$98 + 6$
d[TTP]	$100 + 4^w$			$88 + 5$

Table 5: Effects of compound 13 on Tmolt$_4$ leukemia cell metabolism over 60 min (N = 6)

Assay	Percent of control			
	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5[a]	34 + 3*	30 + 3*	27 + 2*
RNA synthesis	100 ± 6[b]	58 + 4*	50 + 4*	46 + 3*
Protein synthesis	100 ± 5[c]	79 + 5*	65 + 5*	64 + 4*
DNA polymerase α	100 ± 6[d]	61 + 5*	59 + 4*	52 + 4*
mRNA polymerase	100 ± 7[e]	114 + 5	96 + 6	89 + 5
rRNA polymerase	100 ± 4[f]	84 + 5	82 + 4	78 + 4*
tRNA polymerase	100 ± 7[g]	118 + 6	89 + 5	74 + 5*
Ribonucleoside reductase	100 ± 5[h]	42 + 4*	35 + 3*	7 + 1*
Dihydrofolate reductase	100 ± 5[i]	140 + 5*	93 + 4	36 + 3*
Purine synthesis	100 ± 5j	102 + 5	72 + 4*	68 + 4*
PRPP amido transferase	100 ± 6[k]	93 + 5	65 + 3*	21 + 2*
IMP dehydrogenase	100 ± 5[l]	70 + 4*	69 + 5*	46 + 3*
Pyrimidine synthesis	100 ± 6[m]	100 + 5	96 + 4	88 + 5
Carbamyl phosphate synthetase	100 ± 7[n]	111 + 6	96 + 6	84 + 5
Aspartate transcarbamylase	100 ± 6[o]	109 + 5	101 + 5	93 + 6
Thymidylate synthetase	100 ± 5[p]	123 + 6	53 + 4*	52 + 5*
Thymidine kinase	100 ± 6[q]	64 + 4*	53 + 4*	52 + 4*
Thymidine monophosphate kinase	100 ± 7[r]	52 + 4*	51 + 3*	49 + 4*
Thymidine diphosphate kinase	100 ± 6[s]	70 + 4*	63 + 4*	62 + 4*
d[ATP]	100 + 5[t]			77 + 6
d[GTP]	100 + 6[u]			84 + 4
d[CTP]	100 + 5[v]			94 + 5
d[TTP]	100 + 4[w]			101 + 5

carbazones did not affect the synthesis of pyrimidines in L1210 cells [7] but like the present compounds they did inhibit the activities of DNA polymerase α, ribonucleoside and dihydrofolate reductases, thymidylate synthestase and nucleoside kinases. The targeting of multiple pathways of nucleic acid metabolism is not unusual for metal complexes and the effects appear to be additive to suppress overall DNA synthesis. The ability of these agents to afford DNA strand scission adds to cellular apoptosis and a number of these metal complexes of thiosemicarbazones are known to be DNA topoisomerase II inhibitors which also leads to DNA fragmention. As can be observed for the present compounds ct-DNA viscosity was reduced which is consistent with the observed DNA fragmention. There may be some type of non-specific interaction of the agents with the bases of ct-DNA since the UV absorption was altered in their presence and thermal denaturation Tm values changed. Further analysis is required to establish if this is true.

4. Experimental

4.1. Cytotoxicity

Compounds **1**–**20** (Table 1) were tested for cytotoxic activity by homogenizing the drugs as a 1 mg/ml solution in 0.05% Tween 80/H_2O. These solutions were sterilized by passing them through an acrodisc (0.45 μm). The following cell lines were maintained by literature techniques [8]: mur-

Table 6: Effects of compound 18 on Tmolt$_4$ leukemia cell metabolism over 60 min (N = 6)

Assay	Percent of control			
	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5[a]	33 + 4*	29 + 3*	22 + 2*
RNA synthesis	100 ± 6[b]	74 + 4*	52 + 3*	45 + 4*
Protein synthesis	100 ± 5[c]	83 + 5	58 + 4*	53 + 5*
DNA polymerase α	100 ± 6[d]	89 + 6	70 + 5*	64 + 4*
mRNA polymerase	100 ± 7[e]	111 + 6	100 + 5	96 + 5
rRNA polymerase	100 ± 4[f]	98 + 5	85 + 6	75 + 5*
tRNA polymerase	100 ± 7[g]	94 + 5	79 + 5*	68 + 4*
Ribonucleoside reductase	100 ± 5[h]	73 + 4*	52 + 4*	45 + 3*
Dihydrofolate reductase	100 ± 5[i]	54 + 5*	44 + 4*	30 + 3*
Purine synthesis	100 ± 5[j]	124 + 5	94 + 5	58 + 4*
PRPP amido transferase	100 ± 6[k]	85 + 6	46 + 4*	28 + 3*
IMP dehydrogenase	100 ± 5[l]	52 + 4*	49 + 4*	42 + 3*
Pyrimidine synthesis	100 ± 6[m]	76 + 5*	73 + 5*	71 + 4*
Carbamyl phosphate synthetase	100 ± 7[n]	104 + 7	67 + 5*	66 + 5*
Aspartate transcarbamylase	100 ± 6[o]	118 + 5	121 + 6	126 + 5*
Thymidylate synthetase	100 ± 5[p]	166 + 6*	111 + 5	88 + 5
Thymidine kinase	100 ± 6[q]	107 + 5	52 + 4*	34 + 3*
Thymidine monophosphate kinase	100 ± 7[r]	68 + 5*	48 + 4*	27 + 3*
Thymidine diphosphate kinase	100 ± 6[s]	85 + 5	65 + 4*	50 + 4*
d[ATP]	100 + 5[t]			111 + 6
d[GTP]	100 + 6[u]			74 + 4*
d[CTP]	100 + 5[v]			78 + 4*
d[TTP]	100 + 4[w]			124 + 5

ine L_{1210} lymphoid leukemia and P388 lymphocytic leukemia, human $Tmolt_3$ and $Tmolt_4$ acute lymphoblastic T cell leukemia, Hl-60 leukemia, Hut-78 cutaneous lymphoma, THP-1 acute monocytic leukemia, SW480 colorectal adenocarcinoma, HCT-8 ileocecal adenocarcinoma, MB-9812 lung bronchogenic, A-549 lung carcinoma, SOS-2 osteosarcoma, KB epidermoid nasopharynx, HeLa-S^3 suspended and HeLa solid cervical carcinoma, SK-MEL-2 malignant melanoma, clear cell renal Caki, breast effusion MCF-7 and U-87-MG glioma. The NCI protocol was used to assess the cytotoxicity of the test compounds and standards in each cell line. Values for cytotoxicity were expressed as $ED_{50} = \mu g/ml$, i.e. the concentration of the compound inhibiting 50% of cell growth. ED_{50} values were determined by the trypan blue exclusion technique [17] . A value of less than 4 μg/ml was required for significant activity of growth inhibition. Solid tumor cytotoxicity was determined by means of crystal violet/MeOH and read at 580 nm (Molecular Devices) [9].

4.2. Incorporation studies

Incorporation of labeled precursors into ^{3}H-DNA, ^{3}H-RNA and ^{3}H-protein for 10^6 $Tmolt_4$ leukemia cells was obtained [10] using a concentration range of 25, 50 and 100 μM of the test agents over a 60 min incubation. The incorporation of ^{14}C-glycine (53.0 mCi/mmol) into purines [11] and the incorporation of ^{14}C-formate (53.0 mCi/mmol) into pyrimidines [12] was determined in a similar manner.

4.3. Enzyme assays

Inhibition of various enzyme activities was performed by first preparing the appropriate $Tmolt_4$ leukemia cell homogenates or subcellular fraction, then adding the drug to be tested during the enzyme assay. For the concentration response studies, inhibition of enzyme activity was determined at 25, 50 and 100 μM of compounds **3**, **11**, **13** and **18**, after 60 min incubations. DNA polymerase α activity was determined in cytoplasmic isolated extracts [13]. Nuclear DNA polymerase β was determined by isolating nuclei [14]. The polymerase activity for both α and β was determined with ^{3}H-TTP [15]. Messenger-, ribosomal- and transfer-RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate; individual RNA polymerase activities were determined using ^{3}H-UTP [16, 17]. Ribonucleoside reductase activity was measured using ^{14}C-CDP with dithioerythritol [18]. The deoxyribonucleotides ^{14}C-dCDP were separated from the ribonucleotides by TLC on PEI plates. Thymidine, TMP and TDP kinase activities were determined using ^{3}H-thymidine (58.3 mCi/mmol) [19]. Carbamyl phosphate synthetase activity was determined [20] with citrulline quanitated colorimetrically [21]. Aspartate transcarbamylase activity was measured [20] and carbamyl aspartate was quanitated colorimetrically [22]. Thymidylate synthetase activity was analyzed by the ^3H_2O released which was proportional to the amount of TMP formed from ^{3}H-dUMP [23]. Dihydrofolate reductase activity was determined by a spectrophotometric method [24]. PRPP amidotransferase activity was determined by the method of Spassova et al. [25]. IMP dehydrogenase activity was analyzed with 8-^{14}C-IMP (54 mCi/mmol) (Amersham, Arlington Heights, IL) after separating XMP on PEI plates (Fisher Scientific) by TLC [26]. Protein content was determined for the enzymatic assays by the Lowry et al. technique [27].

4.4. ct-DNA studies

After deoxyribonucleoside triphosphates were extracted [28], levels were determined by the method of Hunting and Henderson [29] with calf thymus DNA, *E. coli* DNA polymerase I, non-limiting amounts of the three deoxyribonucleoside triphosphates not being assayed, and either 0.4 mCi of (^{3}H-methyl)-dTTP or (5-^{3}H)-dCTP. The effects of compounds **3**, **11**, **13** and **18** on DNA strand scission was determined by the methods of Suzuki et al. [31], Pera et al. [30] and Woynarowski et al. [32]. $Tmolt_4$ leukemia cells were incubated with 10 μCi thymidine [methyl-^{3}H, 84.0 Ci/mmol] for 24 h at 37 °C. L1210 cells (10^7) were harvested and then centrifuged at 600 g for 10 min in PBS. They were later washed and suspended in 1 ml of PBS. Lysis buffer (0.5 ml; 0.5 M NaOH, 0.02 M EDTA, 0.01% Triton X-100 and 2.5% sucrose) was layered onto a 5–20% alkaline-sucrose gradient (5 ml; 0.3 M NaOH, 0.7 KCl and 0.01 M EDTA); this was followed by 0.2 ml of the cell preparation. After the gradient was incubated for 2.5 h at room temperature, it was centrifuged at 12,000 RPM at 20 °C for 60 min (Beckman rotor SW60). Fractions (0.2 ml) were collected from the bottom of the gradient, neutralized with 0.2 ml of 0.3 N HCl, and measured for radioactivity. Thermal calf thymus DNA denaturation studies, ct-DNA U.V. absorption studies and DNA viscosity studies were conducted after incubation of compounds **3**, **11**, **13** and **18** at 100 μM at 37 °C for 24 h [33].

4.5. Statistic analysis

Data is displayed in tables and figures as the means ± standard deviations of the mean expressed as a percentage of the control value. N is the number of samples per group. The Student's "t"-test was used to determine the probable level of significance (p) between test samples and control samples.

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