ELSEVIER

www.elsevier.com/locate/farmac

Il Farmaco 56 (2001) 741–748

IL FARMACO

Synthesis and antitumor evaluation of 6-thioxo-, 6-oxo- and 2,4-dioxopyrimidine derivatives

Maria Teresa Cocco *, Cenzo Congiu, Valentina Onnis, Riccardo Piras

Dipartimento di Tossicologia, Università degli Studi di Cagliari, Via ospedale 72, 09124 Cagliari, Italy

Received 28 March 2001; accepted 27 April 2001

Abstract

A series of 6-thioxopyrimidines (**5**, **6**), their 6-oxo- analogs (**11**–**14**), and pyrimidine-2,4-diones (**20**–**26**), were synthesized and evaluated for their antitumoral activity against 60 tumoral cell lines. The activity of propenethioamide (**3**, **4**) and propeneamide (**7**–**10** and **15**–**19**) intermediates is also reported. Among the tested compounds the thioxopyrimidine **5c**, bearing an N^1-benzyl group, showed the best cytostatic activity. Furthermore, high selectivity and cytotoxic activity on the HOP-92 cell line of non-small cell lung cancer was exhibited by 3-amino-2-[(methylamino)thioxamethyl]-3-pyrrolidino-2-propenenitrile (**3a**).

Keywords: Pyrimidine derivatives; Anticancer activity; Synthesis

1. Introduction

In the developed countries tumoral diseases are the second most important cause of death [1]. The estimation for 1999, carried out by WHO, indicates that worldwide malignant neoplasms cause 12.65% of total deaths and 21.40% of deaths are due to non-communicable diseases [2]. Consequently the synthesis and biological evaluation of new pharmacophores as anticancer agents are a continuing interest. In the last two decades uracil and oxopyrimidine derivatives have been investigated extensively in relation to their antiviral and antitumoral properties [3], while the thioxopyrimidines have received less attention.

As a continuation of our program connected with research of new anticancer agents we have now focused our attention on a new series of oxo and thioxopyrimidine derivatives. In this paper we report the results of primary antitumor screening, carried out by the National Cancer Institute (NCI), Bethesda, USA, of N^1-substituted-4-dialkylamino-6-thioxopyrimidine-5-carboxylates (**5**) and -5-carbonitriles (**6**), their oxo-analogs (**11**–**14**), N^3-substituted-6-dialkylaminopyrimidine-2,4-diones (**20**–**26**), as well as their synthetic intermediates (**3**–**4**, **7**–**10**, **15**–**19**).

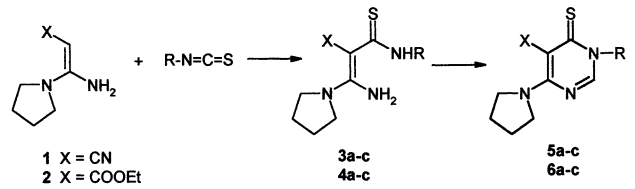
2. Chemistry

The synthetic pathways for the preparation of pyrimidine derivatives are illustrated in Schemes 1–3. The intermediates 3-amino-3-dialkylaminopropenenitriles (**1**) or ethyl 3-amino-3-dialkylaminopropenoates (**2**) were easily obtained with a procedure recently reported by our group [4]. These enamino compounds, possessing two nucleophile centers (N-3 and C-2) could be added to heterocumulenes to yield C- or N-addition products. Thus, the reaction of **1** and **2** with alkyl and aryl isothiocyanates, at room temperature (r.t.) in acetonitrile for a few minutes, afforded 3-amino-2-[(alkyl or arylamino)thioxamethyl]-3-(dialkylamino)-2-propenenitriles (**3**) and ethyl 3-amino-2-[(alkyl or arylamino)thioxamethyl]-3-(dialkylamino)-2-propenoates (**4**) exclusively [5]. The propenethioamides (**3**–**4**) were converted into 6-thioxopyrimidines (**5**–**6**) upon treatment with an excess of dimethylformamide dimethyl acetal (DMF-DMA) in toluene [6] (Scheme 1).

The reactions of propenenitriles (**1**) with alkyl and aryl isocyanates give 3-amino-2-[(alkyl or aryl-

* Corresponding author.
E-mail address: tcocco@vaxcal.unica.it (M.T. Cocco).

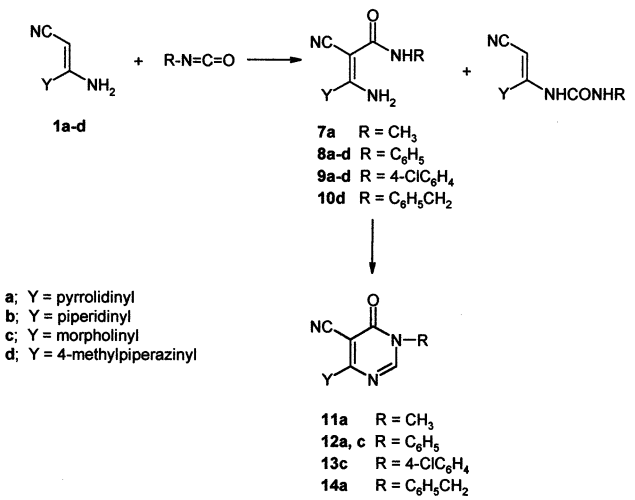
0014-827X/01/$ - see front matter
PII: S0014-827X(01)01123-5



3,5	X	R
a	CN	CH_3
b	CN	C_6H_5
c	CN	$C_6H_5CH_2$

4,6	X	R
a	COOEt	CH_3
b	COOEt	C_6H_5
c	COOEt	$C_6H_5CH_2$

Scheme 1.



Scheme 2.

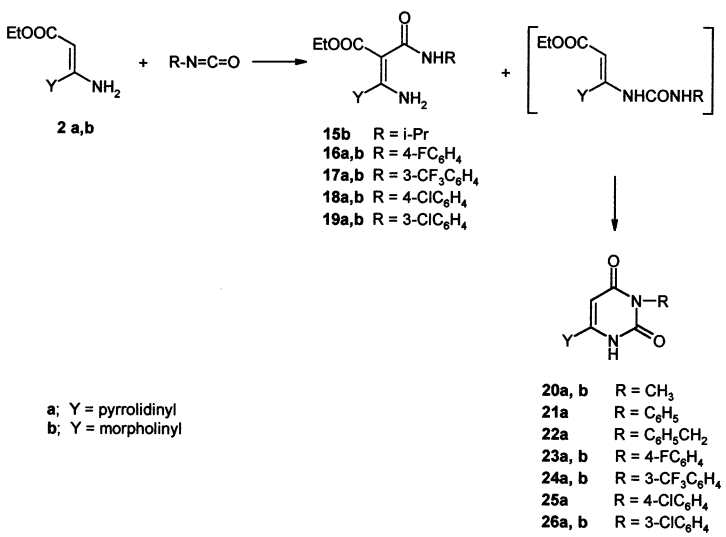
amino)carbonyl]-3-(dialkylamino)-2-propenenitriles (**7**–**10**) together to variable amounts of 3-[(alkyl or arylaminocarbonyl)amino] - 3 - (dialkylamino) - 2 - propenenitriles resulting from attack of isocyanate on the C-2 or the amino group of **1**, respectively. The reaction pathway was remarkably affected by the substitution pattern in both reagents [7]. The following condensation of 2-[(alkyl or arylamino)carbonyl]propenenitrile derivatives (**7**–**10**) with trimethyl orthoformate in the presence of 4-toluensolfonic acid led to 6-oxopyrimidines (**11**–**14**).

The reactions of compounds **2** with methyl, phenyl and benzyl isocyanates led to uracil derivatives (**20**–**22**). When **2b** was reacted with isopropyl isocyanate, ethyl 3-amino-2-[(isopropylamino)carbonyl]-3-morpholino-2-propenoate (**15b**) was exclusively obtained. In all other cases a mixture constituted of variable amounts of ethyl 2-[(alkyl or arylamino)carbonyl]propenoate derivatives and uracils was isolated (Scheme 3). Compounds **20**–**26** presumably originate by intramolecular cyclization of *N*-adducts that in any case we were able to isolate.

The structures of all products were confirmed by elemental and IR and ^{1}H NMR spectral data.

3. Experimental

Melting points (m.p.) were determined on a Kofler hot stage and are uncorrected. IR spectra were recorded on Nujol mulls between salt plates in a Perkin–Elmer 398 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer. Elemental analyses were carried out with a Carlo Erba Model 1106 Elemental Analyzer. Propenethioamides (**3** and **4**), thioxopyrimidines (**5** and **6**), 3-amino-2-[(alkyl or arylamino)carbonyl]-3-(dialkylamino)-2-propenenitriles (**7**–**10**) and 6-oxopyrimidines (**11**–**14**) were prepared following the procedures previously described [5–7].



Scheme 3.

3.1. Reaction of ethyl 3-amino-3-pyrrolidinopropenoate (2a) with isocyanates

To the acetonitrile solution containing **2a** (0.005 mol) the appropriate isocyanate (0.005 mol) was added. The reaction mixture was stirred at r.t. for 0.5 h and the formed precipitate was filtered off, recrystallized from the suitable solvent and identified as ethyl 3-amino-2-[(arylamino)carbonyl]-3-pyrrolidino-2-propenoates (**16a**–**19a**).

Compound **16a**: 40%; m.p. 152–153 °C (benzene); IR: *v* 3340, 3130, 1730, 1630; ^{1}H NMR (DMSO-d_6): δ 1.08 (t, $J = 6.8$, 3H, CH_3), 1.83, 3.32 (m, 8H, pyrrolidinyl), 3.91 (q, $J = 6.8$, 2H, CH_2), 6.95, 7.45 (m, 4H, Ar), 7.56, 8.00 (br s, 2H, NH_2), 10.92 (s, 1H, NH). Compound **17a**: 22%; m.p. 174–175 °C (ethanol); IR: *v* 3470, 3310, 3160, 1650, 1620, 1580; ^{1}H NMR (DMSO-d_6): δ 1.09 (t, $J = 7.2$, 3H, CH_3), 1.84, 3.34 (m, 8H, pyrrolidinyl), 3.93 (q, $J = 7.2$, 2H, CH_2), 7.11, 7.38, 8.20 (m, 4H, Ar), 7.64, 8.07 (br s, 2H, NH_2), 11.20 (s, 1H, NH). Compound **18a**: 30%; m.p. 139–140 °C (benzene); IR: *v* 3320, 3140, 3080, 1660, 1615; ^{1}H NMR (DMSO-d_6): δ 1.09 (t, $J = 6.8$, 3H, CH_3), 1.83, 3.32 (m, 8H, pyrrolidinyl), 3.92 (q, $J = 6.8$, 2H, CH_2), 7.16, 7.49 (m, 4H, Ar), 8.03 (br s, 2H, NH_2), 11.01 (s, 1H, NH). Compound **19a**: 25%; m.p. 164–165 °C (benzene); IR: *v* 3360, 3300, 3150, 1650; ^{1}H NMR (DMSO-d_6): δ 1.08 (t, $J = 6.8$, 3H, CH_3), 1.84, 3.33 (m, 8H, pyrrolidinyl), 3.92 (q, $J = 6.8$, 2H, CH_2), 6.81, 7.10, 7.90 (m, 4H, Ar), 7.76, 8.04 (br s, 2H, NH_2), 11.07 (s, 1H, NH).

Then the acetonitrile solution was evaporated under reduced pressure and the residual solid was crystallized from the appropriate solvent to yield 3-(alkyl or aryl)-6-pyrrolidinopyrimidine-2,4-diones (**20a**–**26a**).

Compound **20a**: 63%; m.p. 263–264 °C (ethanol); IR: *v* 3150, 3040, 1695, 1630; ^{1}H NMR (DMSO-d_6): δ 1.82, 3.21 (m, 8H, pyrrolidinyl), 3.00 (s, 3H, CH_3), 4.41 (s, 1H, H-5), 10.37 (s, 1H, NH). Compound **21a**: 29%; m.p. 243–244 °C (acetonitrile); IR: *v* 3150, 3080, 3060, 1730, 1700, 1620; ^{1}H NMR (DMSO-d_6): δ 1.86, 3.29 (m, 8H, pyrrolidinyl), 4.51 (s, 1H, H-5), 7.09–7.40 (m, 5H, Ar), 10.55 (s, 1H, NH). Compound **22a**: 15%; m.p. 234–235 °C (acetonitrile); IR: *v* 3150, 3070, 1700; ^{1}H NMR (DMSO-d_6): δ 1.84, 3.29 (m, 8H, pyrrolidinyl), 4.47 (s, 1H, H-5), 4.86 (s, 2H, CH_2), 7.17–7.27 (m, 4H, Ar), 10.45 (s, 1H, NH). Compound **23a**: 22%; m.p. 272–273 °C (ethanol); IR: *v* 3150, 3060, 1715, 1690; ^{1}H NMR (DMSO-d_6): δ 1.85, 3.27 (m, 8H, pyrrolidinyl), 4.50 (s, 1H, H-5), 7.16 (m, 4H, Ar), 10.55 (s, 1H, NH). Compound **24a**: 20%; m.p. 276–277 °C (ethanol); IR: *v* 3170, 3120, 3090, 3050, 1720, 1700; ^{1}H NMR (DMSO-d_6): δ 1.86, 3.28 (m, 8H, pyrrolidinyl), 4.53 (s, 1H, H-5), 7.44–7.69 (m, 4H, Ar), 10.64 (s, 1H, NH). Compound **25a**: 12%; m.p. 255–256 °C (ethanol); IR: *v* 3180, 3080, 1720, 1700; ^{1}H NMR (DMSO-d_6): δ 1.85, 3.25 (m, 8H, pyrrolidinyl), 4.50 (s, 1H, H-5), 7.15, 7.41 (m, 4H, Ar), 10.58 (s, 1H, NH). Compound **26a**: 18%; m.p. 275–276 (*n*-PrOH); IR: *v* 3170, 3080, 1725, 1700; ^{1}H NMR (DMSO-d_6): δ 1.85, 3.28 (m, 8H, pyrrolidinyl), 4.51 (s, 1H, H-5), 7.09–7.38 (m, 4H, Ar), 10.60 (s, 1H, NH).

3.2. Reaction of ethyl 3-amino-3-morpholinopropenoate (2b) with isocyanates

To the acetonitrile solution containing **2b** (0.005 mol) the appropriate isocyanate (0.005 mol) was added. The reaction mixture was stirred at r.t. for 0.5 h and the formed precipitate was filtered off, recrystallized from the suitable solvent to give ethyl 3-amino-2-[(alkyl or arylamino)carbonyl]-3-morpholino-2-propenoates (**15b**–**19b**).

Compound **15b**: 25%; m.p. 152–153 °C (benzene); IR: *v* 3240, 3040, 1680, 1640 ; ^{1}H NMR (DMSO-d_6): δ 1.00 (d, $J = 6.7$, 6H, CH_3), 1.11 (t, $J = 6.8$, 3H, CH_3), 3.28, 3.56 (m, 8H, morpholinyl), 3.80 (m, 1H, CH), 3.90 (q, $J = 6.8$, 2H, CH_2), 7.52 (br s, 2H, NH_2), 8.46 (s, 1H, NH). Compound **16b**: 44%; m.p. 155–156 °C (2-PrOH); IR: *v* 3320, 3250, 1670, 1630, 1580; ^{1}H NMR (DMSO-d_6): δ 1.11 (t, $J = 7.2$, 3H, CH_3), 3.41, 3.58 (m, 8H, morpholinyl), 3.93 (q, $J = 7.2$, 2H, CH_2), 6.96, 7.45 (m, 4H, Ar), 7.94, 8.21 (br s, 2H, NH_2), 10.99 (s, 1H, NH). Compound **17b**: 31%; m.p. 154–155 °C (ethanol); IR: *v* 3350, 3310, 3120, 1660, 1610, 1590; ^{1}H NMR (DMSO-d_6): δ 1.11 (t, $J = 6.8$, 3H, CH_3), 3.43, 3.59 (m, 8H, morpholinyl), 3.94 (q, $J = 6.8$, 2H, CH_2), 7.12, 7.37, 8.18 (m, 4H, Ar), 8.02, 8.29 (br s, 2H, NH_2), 11.26 (s, 1H, NH). Compound **18b**: 38%; m.p. 161–162 °C (ethanol); IR: *v* 3380, 3220, 1650, 1620, 1570; ^{1}H NMR (DMSO-d_6): δ 1.11 (t, $J = 6.8$, 3H, CH_3), 3.41, 3.58 (m, 8H, morpholinyl), 3.93 (q, $J = 6.8$, 2H, CH_2), 7.16, 7.45 (m, 4H, Ar), 7.98, 8.26 (br s, 2H, NH_2), 11.08 (s, 1H, NH). Compound **19b**: 44%; m.p. 164–165 °C (MeCN); IR: *v* 3330, 3300, 3100, 1650, 1600; ^{1}H NMR (DMSO-d_6): δ 1.10 (t, $J = 7.0$, 3H, CH_3), 3.42, 3.59 (m, 8H, morpholinyl), 3.93 (q, $J = 7.0$, 2H, CH_2), 6.82, 7.12, 7.87 (m, 4H, Ar), 8.00, 8.27 (br s, 2H, NH_2), 11.13 (s, 1H, NH).

In the reactions with methyl isocyanate, 4-fluorophenyl isocyanate, 3-trifluoromethylphenyl isocyanate and 3-chlorophenyl isocyanate, the mother liquor contained the soluble pyrimidine-2,4-dione derivatives. It was concentrated in vacuo and the residue recrystallized from the appropriate solvent to give the 3-(alkyl or aryl)-6-morpholinopyrimidine-2,4-diones **20b**, **23b**, **24b**, **26b**, respectively. Compound **20b**: 26%; m.p. 260–261 °C (ethanol); IR: *v* 3200, 3150, 3040, 1695, 1630; ^{1}H NMR (DMSO-d_6): δ 3.00 (s, 3H, CH_3), 3.16, 3.57 (m, 8H, morpholinyl), 4.77 (s, 1H, H-5), 10.66 (s, 1H, NH). Compound **23b**: 20%; m.p. 272–273 °C (*n*-PrOH); IR: *v* 3130, 1730, 1710, 1630; ^{1}H NMR (DMSO-d_6): δ 3.32, 3.62 (m, 8H, morpholinyl), 4.88 (s, 1H, H-5), 7.20 (m, 4H, Ar), 10.83 (s, 1H, NH). Compound **24b**: 15%; m.p.

Table 1
GI_{50} values of thioxopyrimidines (**5**, **6**) and oxopyrimidines (**12**, **13**) (10^{-5} M concentrations)

Panel/cell line	Comp.								Panel/cell line	Comp.						
	5a	**5b**	**5c**	**6a**	**6b**	**6c**	**12c**	**13c**		**5a**	**5b**	**5c**	**6a**	**6b**	**6c**	**12c**
Leukemia									*Ovarian cancer*							
HL60(TB)							0.308	1.81	IGROV1			7.71				6.35
K-562						8.75			OVCAR-3			6.34		4.94		
MOLT-4						9.22			OVCAR-4			3.42			9.14	
Non-small cell lung									OVCAR-8			4.92				
EKVX	9.44		6.27						*Renal cancer*							
HOP-62	7.62		4.57						786-0			3.23				
HOP-92			2.25 [d]	9.33		1.94			A498			4.18			4.94	
CNS cancer									ACHN						9.50	
SF-268			2.95		2.85				CAKI-1	3.59		2.31			3.06 [e]	
SF-295			9.79						RXF-393			3.03				
SF-539			3.99						UO-31	2.32 [a]			4.16		5.58	5.94
SNB-19			5.42						*Prostate cancer*							
SNB-75		9.48	2.49			4.77			PC-3			6.49			9.03	
U-251			3.58						DU-145			8.90				
Melanoma									*Breast cancer*							
MALME-3M					5.76				MCF7/ADR-RES			5.77				
SK-MEL-5						6.52			HS 578T			3.00				
UACC-62		1.95 [c]	7.46			6.29			BT-549	1.27 [b]			4.41			
									T-47D						9.38	

[a] TGI 7.03×10^{-5}.
[b] TGI 5.74×10^{-5}.
[c] TGI 6.93×10^{-5}.
[d] TGI 9.85×10^{-5}.
[e] TGI 8.83×10^{-5}.

Table 2
GI_{50} values of uracil derivatives (**20–26**) (10^{-5} M concentrations)

Panel/cell line	Comp.						
	20a	**21a**	**22a**	**23a**	**24a**	**24b**	**26b**
Leukemia							
RPMI-8226					7.64		
Non-small cell lung							
NCI-H226			5.53	2.03 [b]			
NCI-H322							
Colon cancer							
HCC-2998		3.70	8.54				
CNS cancer							
SF-268	7.42		1.54 [a]				
U-251						6.48	
Melanoma							
SK-MEL-2						4.32	
Renal cancer							
A498							3.24
Breast cancer							
MDA-MB-231/ATCC						2.07 [c]	

[a] TGI 7.56×10^{-5}.
[b] TGI 5.08×10^{-5}.
[c] TGI 5.70×10^{-5}.

Table 3
GI_{50} values of ethyl 3-amino-2-[(arylamino)carbonyl]-3-(dialkylamino)-2-propenoates (**16–18**) (10^{-5} M concentrations)

Panel/cell line	Comp.				Panel/cell line	Comp.		
	16a	**17a**	**17b**	**18a**		**16a**	**17a**	**18a**
Leukemia					*Ovarian cancer*			
K-562				6.67	IGROV1			5.41
MOLT-4				7.15	OVCAR-4			6.93
RPMI-8226				9.80	OVCAR-8		3.80	
Non-small cell lung					SK-OV-3		5.23	
HOP-62		4.46			*Renal cancer*			
NCI-H226		2.92 [b]	8.69		786-0		5.26	
NCI-H23	≤0.001 [a]				A498		4.16	6.44
NCI-H322M		7.92			ACHN			4.56
Colon cancer					RXF-393		2.09 [e]	
HCT-116				9.40	SN12C		4.19	
HCT-15	≤0.001				*Breast cancer*			
CNS cancer					MCF7		6.29	
SF-268		5.43			MDA-MB-231/ATCC		4.23	
SF-295		4.22			HS 578T		2.72 [f]	
SF-539		2.01 [c]			MDA-N			
SNB-75		2.35 [d]			BT-549	9.30		
U251		7.69			T-47D		4.77	
Melanoma								
SK-MEL-2				4.93				
UACC-62				4.20				

[a] TGI 5.94×10^{-5}.
[b] TGI 8.37×10^{-5}.
[c] TGI 5.19×10^{-5}.
[d] TGI 8.51×10^{-5}.
[e] TGI 5.36×10^{-5}.
[f] TGI 7.06×10^{-5}.

250–251 °C (*i*-PrOH); IR: *v* 3140, 3070, 1730, 1710, 1630; ^{1}H NMR (DMSO-d_6): δ 3.25, 3.61 (m, 8H, morpholinyl), 4.91 (s, 1H, H-5), 7.48–7.72 (m, 4H, Ar), 10.90 (s, 1H, NH). Compound **26b**: 23%; m.p. 274–275 °C (ethanol); IR: *v* 3120, 3040, 1715, 1690, 1610; ^{1}H NMR (DMSO-d_6): δ 3.26, 3.62 (m, 8H, morpholinyl), 4.90 (s, 1H, H-5), 7.12–7.47 (m, 4H, Ar), 10.87 (s, 1H, NH).

4. Biological evaluation

Evaluation of anticancer activity was performed on the synthesized compounds at the NCI following the known in vitro disease-oriented antitumor screening program which is based upon use of multiple panels of 60 human tumor cell lines [8,9]. Each compound is tested at a minimum of five concentrations at tenfold dilution against every cell line in the panel. A 48-h continuous drug exposure protocol is used, and a sulforhodamine B (SRB) protein assay is used to estimate cell viability or growth [10,11]. The anticancer activity of each compound is deduced from dose–response curves and is presented in Tables 1–5 according to the data provided by NCI [9].

The response parameters GI_{50}, TGI and LC_{50} refer to the drug concentration that produced 50% inhibition, total growth inhibition and 50% cytotoxicity, respectively, and are expressed in 10^{-5} M concentrations. In the tables we report only the activity of those compounds having GI_{50}, TGI and LC_{50} lower than 10×10^{-5} M.

5. Results and discussion

The data presented in Table 1 showed that among the 6-thioxopyrimidines (**5**–**6**) the most active compounds were **5c** and **6c**. Specifically **5c** is active against all CNS cancer lines. It seems plain that the cytostatic activity is associated with presence of a benzyl group on N-3. As a matter of fact, the replacement of benzyl group of **5**,

Table 4
GI_{50} values of 3-amino-2-[(alkyl or arylamino)carbonyl]-3-(dialkylamino)-2-propenenitriles (**8**–**10**) (10^{-5} M concentrations)

Panel/cell line	Comp.							Panel/cell line	Comp.					
	8a	**8b**	**9a**	**9b**	**9c**	**9d**	**10a**		**8a**	**8b**	**9b**	**9c**	**9d**	**10a**
Leukemia								*Melanoma*						
CCRF-CEM						3.00		LOX IMVI				8.33		4.79
HL-60(TB)			0.0635	1.00				M 14		9.10		8.51	8.63	3.06
K-562					6.12	2.30		SK-MEL-2						3.26
MOLT-4					6.40	5.48		SK-MEL-28					7.60	4.66
RPMI-8226						2.74		SK-MEL-5					7.45	2.75
SR						3.51 [b]		UACC-257					4.75	5.90
Non-small cell lung								UACC-62		9.57		4.24	7.78	2.30 [d]
EKVX	7.23	9.83		4.51		7.82		*Ovarian cancer*						
HOP-62				4.13	7.33			IGROV1		9.10	6.65		9.90	
HOP-92							9.76	OVCAR-3				9.68	4.17	
NCI-H226				9.22				OVCAR-8			4.13			
NCI-H23				6.44		6.70		SK-OV-3			7.26			
NCI-H460						7.46		*Renal cancer*						
Colon cancer								786-0					9.56	
COLO-205						1.88 [c]		A498					4.04	4.49
HCC-2998						5.96		CAKi-1					7.99	3.97
HCT-116				9.95		4.16		UO-31	9.80	6.74	2.23	9.09	5.50	4.64
HCT-15						5.80		*Prostate cancer*						
HT-29	8.82							PC-3					3.98	
KM12						5.91		DU-145				9.39	7.93	
CNS cancer								*Breast cancer*						
SF-295				5.39		7.56		MCF7					6.84	
SF-539				7.58				NCI/ADR-RES			7.92	5.69	8.80	
SNB-19				3.68				MDA-MB-435		8.49		6.18	3.75	
SNB-75				2.46 [a]	6.54			MDA-N					5.23	
U251				5.65		9.69		BT-549						1.90 [e]
								T-47D	3.52					

[a] TGI 7.23×10^{-5}.
[b] TGI 9.53×10^{-5}.
[c] TGI 3.52×10^{-5}, LC_{50} 6.61×10^{-5}.
[d] TGI 5.89×10^{-5}.
[e] TGI 6.41×10^{-5}.

Table 5
GI_{50} values of propenethioamides (**3**–**4**) (10^{-5} M concentrations)

Panel/cell line	Comp.				Panel/cell line	Comp.					
	3a	**3b**	**3c**	**4b**		**3a**	**3b**	**3c**	**4a**	**4b**	**4c**
Leukemia					*Renal cancer*						
CCRF-CEM				4.62	A498			3.53			
HL-60 (TB)				4.99	CAKI-1		2.79 [b]		7.71		
K-562				3.70	RXF-393			7.43		6.31	
Non-small cell lung					UO-31		4.67		7.16		5.69
HOP-92	0.0193 [a]	6.25	4.48	0.21 [d]	*Prostate cancer*						
NCI-H460				6.23	PC-3					3.89	
Colon cancer					*Breast cancer*						
HCT-116				6.55	MCF7					5.41	
KM12				6.11	MDA-MB-231/ATCC					2.97	
SW-620				4.88	HS 578T					7.75	
CNS cancer					MDA-MB-435					3.79	
SNB-19				5.03	MDA-N					3.10 [e]	
U251				6.12	BT-549		2.05 [c]	4.69		4.08	
Melanoma					T-47D	5.75		2.51			
M14				7.25							

[a] TGI 8.25×10^{-6}, LC_{50} 9.16×10^{-6}.
[b] TGI 9.25×10^{-5}.
[c] TGI 7.82×10^{-5}.
[d] TGI 5.32×10^{-5}.
[e] TGI 8.49×10^{-5}.

6 with a methyl or phenyl group led to significantly lower cytostatic effects.

The substitution of a sulfur atom on the pyrimidine with an oxygen atom or the introduction of a second oxo substituent in position 2 resulted in the reduction of antitumoral activity. In fact, the 6-oxopyrimidines (**12**–**14**) and uracil derivatives (**20**–**26**) present only moderate random activities (Table 2).

Surprisingly, 2-[(alkyl or arylamino)carbonyl]-propenenitrile derivatives (**7**–**10**), ethyl 2-[(alkyl or arylamino)carbonyl]propenoate derivatives (**15**–**19**) and propenethioamides (**3**–**4**) show a more interesting activity with respect to the pyrimidine series. In ethyl 2-[(alkyl or arylamino)carbonyl]propenoate derivatives (**15**–**19**) the best activity is generally correlated with the presence of a pyrrolidino group and 4-halo-phenyl on N-1 (Table 3). A chlorine atom in the 3-position of the phenyl moiety was ineffective in inducing antiproliferative activity in these compounds, but the 3-CF_3-substituted **17a** shows GI_{50} values in the $2.01–7.92 \times 10^{-5}$ M range on several cell lines of non-small cell lung cancer, CNS cancer, renal cancer and breast cancer and TGI values of the same order on five of these. On the other hand ethyl 3-amino-2-[(4-fluorophenylamino)carbonyl]-3-pyrrolidino-2-propenoate (**16a**) exhibits the most strong antitumoral activity on the NCI-H23 line of non-small cell lung cancer and the HCT-15 line of colon cancer with GI_{50} values less than 1×10^{-8} M. From the analysis of data reported in Table 4 we can deduce that the activity of 3-amino-2-[(alkyl or arylamino)carbonyl]-3-(dialkylamino)-2-propenenitriles (**7**–**10**) appears to be unrelated to the presence of a particular 3-dialkylamino substituent. All active compounds display cytostatic activity on the UO31 line of renal cancer. Moreover, **9d** shows TGI values of 7.23×10^{-5} M on the SNB-75 cell line and **9d** show LC_{50} values of 6.61×10^{-5} M on the COLO 205 line of colon cancer. Among propenethioamides (**3**–**4**) the most active compound was **4b** (19 cell lines over 60) (Table 5), while **3a** is selective on the HOP-92 cell line with GI_{50}, TGI and LC_{50} values of 1.93×10^{-7}, 8.25×10^{-6} and 9.16×10^{-6} M, respectively.

Acknowledgements

We thank the Antitumor Evaluation Branch of the National Cancer Institute for performing the biological evaluation.

References

[1] WHO, Ninth General Program of Work, 1994.
[2] WHO, The World Health Report 2000, 2000 Geneva.
[3] E. Menta, M. Palumbo, Novel antineoplastic agents, Expert Opin.Therap. Patents 7 (1997) 1401–1426.
[4] M.T. Cocco, C. Congiu, A. Maccioni, A. Plumitallo, M.L. Schivo, G. Palmieri, Synthesis and biological activity of some pyrrole derivatives. I, Farmaco Ed. Sci. 43 (1988) 103–112.

[5] M.T. Cocco, C. Congiu, A. Maccioni, V. Onnis, M.L. Schivo, A. De Logu, Synthesis and antimicrobial activity of new 3,5-diaminoisothiazole derivatives, Farmaco 49 (1994) 137–140.
[6] M.T. Cocco, C. Congiu, V. Onnis, M.L. Schivo, A. De Logu, New thioxopyrimidines. Synthesis and evaluation for antimicrobial activity, Farmaco 50 (1995) 73–76.
[7] M.T. Cocco, C. Congiu, A. Maccioni, V. Onnis, Reaction of enaminonitriles with isocyanates. Synthesis of new 2-oxo- and 6-oxopyrimidines, J. Heterocycl. Chem. 31 (1994) 329–334.
[8] M.R. Boyd, Status of the NCI preclinical antitumor drug discovery screen, Principles Practice Oncol. 3 (1989) 1–12.
[9] M.R. Grever, S.A. Schepartz, B.A. Chabner, The National Cancer Institute: cancer drug discovery and development program, Semin. Oncol. 19 (1992) 622–638.
[10] L.V. Rubinstein, R.H. Shoemaker, K.D. Paull, R.M. Simon, S. Tosini, P. Skehan, D.A. Scudiero, A. Monks, M.R. Boyd, Comparison of in vitro anticancer drug screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines, J. Natl. Cancer Inst. 82 (1990) 1113–1118.
[11] P. Skehan, R. Storeng, D.A. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112.