Full Paper

Parallel Synthesis of *O*-Phenoxyethyl and *O*-Adamantyl *N*-acyl Thiocarbamates Endowed with Antiproliferative Activity

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In order to further explore the antiproliferative properties of *O*-phenoxyethyl and *O*-adamantyl acylthiocarbamates (ATCs), a series of 14 derivatives was prepared by a parallel adaptation of a highly convergent one-pot three-step procedure. Ten acylthiocarbamates were selected by the National Cancer Institute drug evaluation program and screened against a panel of 55 to 58 cell lines derived from nine different types of human cancers. In general, the tested compounds showed a widespread micromolar activity with some specificity against leukemia, renal UO-31, central nervous system (CNS) SNB-75, and non-small cell lung HOP-92 cancer cell lines. Bioinformatic COMPARE analyses were carried out to identify possible mechanism(s) of action for acylthiocarbamate antiproliferative activity.

Keywords: **N**-Acylthiocarbamates / Antiproliferative agents / Parallel synthesis

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Introduction

According to the degree of substitution at the thiocarbamic nitrogen atom, thiocarbamates can be distinguished into primary, secondary, and tertiary compounds [1]. Secondary acylthiocarbamates (*i.e.* secondary thiocarbamates bearing an acyl substituent at the thiocarbamic nitrogen, Fig. 1) have been widely studied for their physicochemical properties [1 – 4] and biological activities including elastase inhibition [5], anti-inflammatory [6], antiproliferative [7], antibacterial [7], and fungicidal [7] effects (Fig. 1). Conversely, a limited number of works were focussed on tertiary acylthiocarbamates (hereinafter, ATCs) [8 – 11] which turned out to be novel and potent anti-HIV-1 agents (Fig.1). Despite these data, the biological potential of this chemical class still remains largely unexplored.

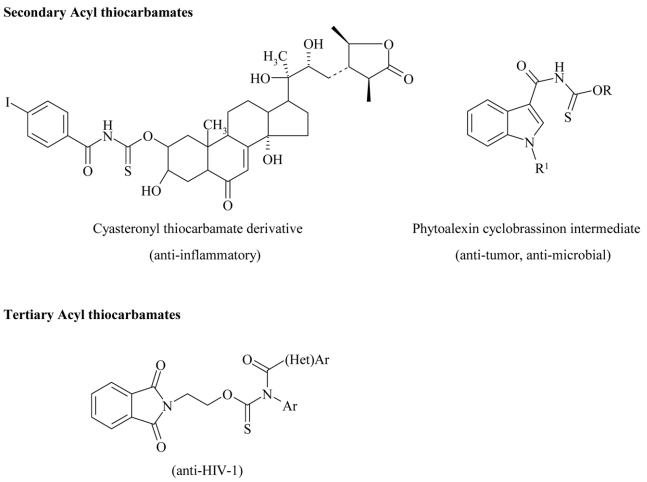

Figure 1. Selected secondary and tertiary acylthiocarbamates (ATCs) endowed with biological activities.

As part of our synthetic program directed toward the development of new HIV-1 non-nucleoside reverse transcriptase inhibitors, the *O*-phenoxyethyl ATCs **I-VII** and their *O*-adamantyl analogue **VIII** (Fig. 2) had revealed moderate cytotoxicity against MT-4 lymphoblastoid T cells [9]. In order to further investigate the antiproliferative properties of ATCs, we planned the synthesis of new

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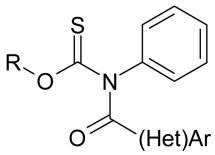
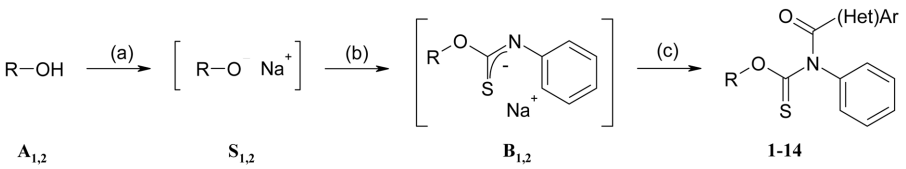
Abbreviation: Pearson correlation coefficient (PCC); tertiary acylthiocarbamates (ATCs)

Table 1. Physical and chemical data of ATC **1** – **14**.



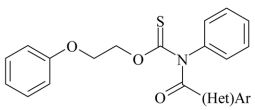
Compound	R	(Het)ArCO	Cryst. solvent(s)[a]	M.p. (°C)	Yield (%)	Formula
1[b]	phenoxyethyl	4-chlorobenzoyl	DE	101 – 103[c]	76[c]	$C_{22}H_{18}ClNO_3S$
2	phenoxyethyl	4-methoxybenzoyl	DE-P	70 – 72	91	$C_{23}H_{21}NO_4S$
3[b]	phenoxyethyl	2-furoyl	DE	98 – 99[d]	84[d]	$C_{20}H_{17}NO_3S$
4[b]	phenoxyethyl	2-thenoyl	DE	91 – 92[e]	87[e]	$C_{20}H_{17}NO_3S_2$
5	adamantyl	2-acetoxybenzoyl	DE-M	144 – 145	53	$C_{26}H_{27}NO_4S$
6	adamantyl	3-nitrobenzoyl	DE-M	157 – 158	55	$C_{24}H_{24}N_2O_4S$
7	adamantyl	4-chlorobenzoyl	DM-M	164 – 166	59	$C_{24}H_{24}ClNO_2S$
8	adamantyl	3,4-dichlorobenzoyl	DE-M	128 – 130	48	$C_{24}H_{23}Cl_2NO_2S$
9	adamantyl	3,5-dichlorobenzoyl	DE-M	154 – 155	54	$C_{24}H_{23}Cl_2NO_2S$
10	adamantyl	3,4,5-trimethoxybenzoyl	DE-M	101 – 102	56	$C_{27}H_{31}NO_5S$
11	adamantyl	1,1'-biphenyl-4-carbonyl	DM-M	182 – 184	52	$C_{30}H_{29}NO_2S$
12	adamantyl	1-naphthoyl	DE-M	167 – 169	62	$C_{28}H_{27}NO_2S$
13[b]	adamantyl	2-furoyl	DE-M	132 – 134[f]	60[f]	$C_{22}H_{23}NO_3S$
14	adamantyl	2-thenoyl	DE-M	163 – 164	56	$C_{22}H_{23}NO_2S_2$

[a] Crystallization solvent(s): DE = diethyl ether, DM = dichloromethane, M = methanol, P = petroleum ether.
[b] Compounds **1**, **3**, **4**, and **13** are identical to **II**, **VI**, **VII**, and **VIII** (Fig: 2), respectively.
[c] Lit. [11]: m.p.: 101 – 103°C, yield: 74%.
[d] Lit. [11]: m.p.: 98 – 99°C, yield: 85%.
[e] Lit. [11]: m.p.: 91 – 92°C, yield: 85%.
[f] Lit. [11]: m.p.: 133-134°C, yield: 58%.

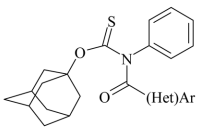


Reaction conditions: (a) NaH (1 eq.), dry DMF, 90°C (for $\mathbf{S}_1$: dry toluene, rt), 30 min; (b) C_6H_5-NCS (1 eq.), 90°C (for $\mathbf{B}_1$: rt), 30 min; (c) dry pyridine (large excess), (Het)ArCOCl (1.2 eq.), rt, 6 h, then 55°C, 1 h (for **1** – **4**: 1.1 eq, rt, 6 h). The structures of alcohols ROH ($\mathbf{A}_{1,2}$) and acyl chlorides (Het)ArCOCl ($\mathbf{C}_{1-11}$) are listed in Fig. 2.

Scheme 1. Synthesis of the title compounds.



I, (Het)Ar = benzoyl: CC_{50} = 122 μM
II, (Het)Ar = 4-Cl-benzoyl: CC_{50} = 133 μM
III, (Het)Ar = 2,4-Cl_2-benzoyl: CC_{50} = 43μM
IV, (Het)Ar = 3,5-Cl_2-benzoyl: CC_{50} = 43μM
V, (Het)Ar = 4-Cl-3-NO_2-benzoyl: CC_{50} = 80 μM
VI, (Het)Ar = 2-furoyl: CC_{50} = 82 μM
VII, (Het)Ar = 2-thenoyl: CC_{50} = 125 μM



VIII, (Het)Ar = 2-furoyl: CC_{50} = 142 μM

Figure 2. ATC-hit compounds [11]: chemical structure and cytotoxicity against MT-4 lymphoblastoid T cells.

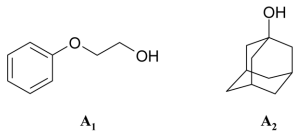
O-phenoxyethyl **2** (Table 1) and *O*-adamantyl derivatives **5** – **12**, **14** (Table 1). The structure-activity relationship (SAR) strategy was focussed on the variation of the acyl moiety (mono-, di-, and tri-substituted benzoyl groups, biphenyl-carbonyl, 1-naphtoyl, 2-furoyl, and 2-thenoyl) whereas the *N*-phenyl group was kept constant. The *O*-adamantyl analogues were privileged according to the well-known antitumor properties of some adamantane derivatives [12 – 17]. To allow rapid analoguing, a parallel synthetic method was set up. Among the prepared compounds, ATCs **1** – **4**, **7** – **10**, **13**, and **14** (Table 1) were selected by the National Cancer Institute (NCI; Bethesda, MD, USA) for antiproliferative testing against a panel of cell lines extracted from nine different human tumors.

Results and discussion

Chemistry

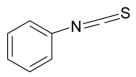
ATCs **1** – **14** were prepared by a parallel adaptation of the previously reported one-pot three-step protocol [9]. As

a) Alcohols $\mathbf{A_{1,2}}$



$\mathbf{A_1}$ $\mathbf{A_2}$

b) Phenyl isothiocyanate



c) Acyl chlorides (Het)ArCOCl ($\mathbf{C_{1\text{-}11}}$)

	(Het)ArCO		(Het)ArCO
$\mathbf{C_1}$	2-acetoxybenzoyl	$\mathbf{C_7}$	3,4,5-trichlorobenzoyl
$\mathbf{C_2}$	3-nitrobenzoyl	$\mathbf{C_8}$	1,1'-biphenyl-4-carbonyl
$\mathbf{C_3}$	4-chlorobenzoyl	$\mathbf{C_9}$	1-naphthoyl
$\mathbf{C_4}$	4-methoxylbenzoyl	$\mathbf{C_{10}}$	2-furoyl
$\mathbf{C_5}$	3,4-dichlorobenzoyl	$\mathbf{C_{11}}$	2-thenoyl
$\mathbf{C_6}$	3,5-dichlorobenzoyl		

Figure 3. Synthetic building blocks.

shown in Scheme 1, the starting alcohols $\mathbf{A_{1,2}}$ (Fig. 3a) were converted into the corresponding alcoholates $\mathbf{S_{1,2}}$ using sodium hydride in anhydrous aprotic solvents (toluene or DMF) and condensed *in situ* with phenylisothiocyanate. The thiocarbamic intermediates $\mathbf{B_{1,2}}$ were acylated by acyl chlorides $\mathbf{C_{1-11}}$ (Fig. 3c) in the presence of pyridine to afford the desired products. To obviate the different reactivity of key-intermediates **S** and **B** towards phenylisothiocyanate and acyl chloride building blocks, respectively, different reaction conditions were adopted for the synthesis of *O*-phenoxyethyl and *O*-adamantyl ATCs (see Experimental, Section 4). The work-up simply required quenching with water, extraction, and filtration; all derivatives were purified by crystallization from the proper solvent or solvent mixtures (Table 1).

To properly evaluate the parallel procedure, **II**, **VI**, **VII**, and **VIII** (Fig. 1) previously prepared by a "one-at-a-time" method [9], were re-synthesized using the parallel variant (compounds **1**, **3**, **4**, and **13** in Table 1). The quality of the products (in terms of yield and purity) was not influenced by the parallelization of the synthetic procedure. The overall yields ranged from 46% to 91% (see Table 1).

Biological data

ATCs **1**–**14** (Table 1) were submitted to the NCI developmental therapeutics program for antiproliferative testing against 55 to 58 cell lines isolated from nine different human tumors; the ten derivatives reported in Tables 2, 3, and 4 were selected and screened by NCI. The results are expressed as GI_{50} (measure of the growth inhibitory power), TGI (measure of the cytostatic activity), and LC_{50} (measure of the cytocidal effect). Table 2 summarizes the number of cell lines against which each compound was screened (55 – 58), the number of lines against which it gave a positive (inferior to 100 μM) GI_{50}, TGI, or LC_{50} value and the corresponding concentration range. The GI_{50} values of the ten molecules are reported in Tables 3 and 4.

Table 2. Anticancer activity of **1**, **2**, **3**, **4**, **7**, **8**, **9**, **10**, **13**, and **14**[a].

Compound	Investigated	Number (No) of human tumor cell lines[b] Giving positive GI_{50}, TGI and LC_{50}					
		GI_{50} (μM)[c]		TGI (μM)[d]		LC_{50} (μM)[e]	
		No	Range	No	Range	No	Range
1	56	56	0.19 – 46.9	52	3.12 – 94.0	32	54.5 – 97.3
2	57	55	0.05 – 88.4	13	29.9 – 98.5	1	98.6
3	56	56	13.8 – 32.2	55	28.2 – 90.2	36	54.5 – 95.9
4	56	56	17.0 – 58.6	45	33.3 – 96.5	13	64.6 – 99.7
7	56	55	16.2 – 99.8	6	31.2 – 89.7	1	58.2
8	55	54	2.8 – 93.2	11	14.4 – 98.5	4	47.9 – 82.2
9	55	55	5.2 – 75.2	9	35.4 – 84.5	2	67.3 – 89.4
10	56	56	2.7 – 66.0	18	21.8 – 92.5	5	67.1 – 98.9
13	58	58	13.7 – 48.5	29	32.4 – 98.7	5	63.7 – 91.0
14	58	58	15.3 – 85.7	14	33.8 – 92.9	3	64.3 – 96.2

[a] Data obtained from NCI's *in-vitro* disease-oriented human tumor cell lines screen.
[b] The table shows the number of cell lines against which each compound was screened, the number of lines against which it gave a positive GI_{50}, or TGI, or LC_{50} value (<100 μM) and the corresponding concentration range.
[c] Compound concentration that produces 50% growth inhibition.
[d] Compound concentration that produces total growth inhibition.
[e] Compound concentration that produces 50% cytocidal effect.

Table 3. Cytotoxicity of **1**, **2**, **3**, **4**, **7**, **8**, **9**, **10**, **13**, and **14** against human cultured cell lines of leukemia, non-small cell lung cancer (NSCLC), colon cancer, CNS cancer, and melanoma.

Panel	Compounds GI_{50} (μM)[a]									
Cell Lines	**1**	**2**	**3**	**4**	**7**	**8**	**9**	**10**	**13**	**14**
Leukemia										
CCRF-CEM	13.7	14.9	20.1	27.6	16.2	**2.8**	**5.2**	**2.9**	16.9	15.3
HL-60(TB)	18.6	20.2	24.3	27.6	17.0	15.8	24.4	**9.7**	19.3	25.0
K-562	30.4	23.8	28.4	36.1	29.8	17.1	–	**2.7**	28.7	26.8
MOLT-4	22.4	19.7	23.8	27.4	26.8	–	–	**2.8**	24.4	26.1
RPMI-8226	18.1	14.6	19.8	20.9	–	–	–	–	–	–
SR	–	–	–	–	–	–	43.0	–	25.7	25.2
NSCLC										
A549/ATCC	16.3	31.1	30.8	28.1	31.9	32.2	42.7	26.0	32.2	32.8
EKVX	20.1	31.7	22.1	29.5	26.6	20.8	21.8	14.8	18.8	17.8
HOP-62	23.0	71.2	16.2	28.3	45.5	44.6	42.7	43.6	28.3	47.7
HOP-92	**3.3**	20.1	14.8	18.8	34.0	31.9	29.0	21.0	19.5	24.8
NCI-H226	–	–	–	–	30.9	31.2	35.5	21.5	27.2	33.3
NCI-H23	18.1	32.4	21.3	20.7	35.7	28.9	36.1	28.0	27.8	30.2
NCI-H322M	21.4	53.4	22.4	36.3	43.0	40.2	45.8	32.6	29.7	35.8
NCI-H460	23.2	34.1	21.9	34.8	30.0	37.8	35.4	27.1	30.8	33.3
NCI-H522	19.1	23.7	17.1	22.9	21.1	13.2	16.0	**4.0**	19.9	17.2
Colon Cancer										
COLO 205	19.9	30.5	21.0	24.8	16.8	15.9	18.6	16.4	16.5	17.8
HCC-2998	19.5	34.5	21.0	24.7	31.6	43.2	33.8	23.4	25.1	32.6
HCT-116	16.2	29.1	17.3	19.4	33.5	24.8	28.1	16.1	19.9	22.2
HCT-15	19.3	44.1	22.3	27.4	34.6	33.3	34.6	26.6	28.2	29.8
HT29	17.6	40.4	19.2	34.1	32.3	29.6	39.4	17.7	33.5	31.8
KM12	19.1	31.3	19.5	26.0	39.1	33.7	40.2	26.5	31.2	31.9
SW-620	19.1	50.0	20.5	25.5	34.4	35.2	39.5	22.7	29.0	40.8
CNS Cancer										
SF-268	25.1	43.9	18.0	34.1	43.3	40.3	52.9	35.7	32.9	37.5
SF-295	27.6	29.2	16.2	26.6	36.8	36.2	34.5	22.1	27.7	27.9
SF-539	18.9	40.2	22.8	34.3	30.7	36.6	40.0	31.3	20.2	34.5
SNB-19	19.2	83.8	26.7	29.4	29.6	33.4	45.6	34.6	34.7	33.7
SNB-75	–	**1.6**	–	24.3	42.8	93.4	61.0	66.0	48.5	85.7
U251	16.2	46.1	16.5	21.4	30.2	34.6	33.0	19.0	23.3	29.1
Melanoma										
LOX IMVI	17.4	52.0	19.3	17.1	–	–	–	–	26.6	22.5
MALME-3M	29.0	28.4	19.9	31.3	54.3	34.9	49.7	29.1	26.8	43.2
M14	16.1	43.2	19.6	17.0	33.9	20.8	22.7	18.9	21.0	19.2
SK-MEL-2	22.1	22.9	19.6	21.3	30.2	20.0	19.3	15.2	21.2	18.2
SK-MEL-28	15.8	> 100	18.2	24.8	> 100	44.5	59.4	30.4	37.9	54.8
SK-MEL-5	–	–	–	–	32.0	19.5	28.3	16.1	17.7	22.1
UACC-257	18.7	34.8	17.9	29.4	46.6	41.3	43.2	26.3	30.9	39.5
UACC-62	16.3	48.0	16.6	18.2	40.6	25.0	32.6	22.1	23.4	25.6

[a] Growth inhibition (GI_{50}); GI_{50} values lower than 10.0 μM are reported in bold.

As shown in Table 2, most of the ATCs showed antiproliferative activity against the majority of the cell lines investigated (54 to 58 out of 55 to 58), but only *O*-phenoxyethyl derivatives **1**, **3**, and **4** demonstrated cytostatic properties at micromolar concentrations in a good number of cell lines (45 to 55) and cytocidal properties in 13 to 36 cell lines.

The average GI_{50} values reported in Fig. 4 indicate that the phenoxyethyl derivatives **1**–**4** showed a widespread activity in the micromolar concentration range against all the considered cancer subpanels. Nevertheless, enhanced potencies were detected against specific cell lines (Tables 3 and 4). Thus, ATC **1** (4-chlorobenzoyl) emerged as particularly effective against HOP-92 non-

Table 4. Cytotoxicity of **1**, **2**, **3**, **4**, **7**, **8**, **9**, **10**, **13**, and **14** against human cultured cell lines of ovarian, renal, prostate, and breast cancers.

Panel	Compounds GI_{50} (μM)[a]									
Cell Lines	**1**	**2**	**3**	**4**	**7**	**8**	**9**	**10**	**13**	**14**
Ovarian Cancer										
IGROV1	16.9	> 100	17.7	25.4	–	–	–	–	–	–
OVAR-3	17.2	38.0	16.5	21.0	44.6	39.5	45.3	28.1	28.0	34.6
OVAR-4	18.6	24.6	20.1	29.8	39.5	38.2	46.7	29.2	36.1	35.0
OVAR-5	17.0	88.4	19.2	28.4	45.6	18.8	46.3	26.5	39.7	29.4
OVAR-8	20.3	52.2	19.8	25.5	39.2	41.5	41.6	27.0	31.2	31.8
SK-OV-3	36.1	52.7	21.8	30.4	53.1	36.7	32.5	25.7	23.4	28.4
Renal Cancer										
786-0	18.7	47.9	19.7	25.2	29.6	30.3	32.8	25.6	31.2	35.4
A498	14.9	18.4	16.0	25.5	64.7	37.1	34.9	25.0	23.3	33.2
ACHN	20.3	44.8	23.0	27.7	34.8	34.8	33.2	29.6	27.1	33.0
CAKI-1	17.4	47.0	18.7	27.2	35.3	29.9	42.1	31.3	33.3	28.3
RXF 393	18.6	26.5	13.8	23.6	99.8	> 100	41.2	32.7	13.7	62.2
SN12C	19.3	43.7	19.5	22.1	45.1	26.8	36.5	28.7	27.8	27.0
TK-10	29.4	60.9	20.9	41.0	37.1	66.9	53.4	42.7	42.3	43.9
UO-31	**0.186**	**0.046**	15.7	–	27.0	21.1	25.1	19.1	24.9	18.7
Prostate Cancer										
PC-3	21.1	35.4	24.1	24.7	34.0	39.9	35.1	22.6	20.5	22.8
DU-145	17.3	37.1	20.3	28.6	76.4	83.1	75.2	58.9	52.9	84.7
Breast Cancer										
MCF7	16.1	41.9	20.7	20.2	33.2	28.0	30.1	29.4	26.3	30.7
NCI/ADR-RES	23.9	37.1	25.7	21.0	36.5	30.1	37.3	22.7	28.6	29.6
MDA-MB-231/ATCC	23.1	86.1	16.4	24.3	29.4	38.2	38.6	33.4	31.8	30.0
HS 578T	46.9	65.1	32.2	58.6	39.9	41.3	48.9	27.4	32.6	32.0
MDA-MB-435	17.0	31.7	16.2	17.0	43.8	30.2	42.2	29.5	28.9	29.8
MDA-N	16.6	35.1	18.9	17.2	49.6	32.7	31.8	25.1	29.4	31.4
BT-549	20.2	49.9	23.0	20.8	43.1	35.9	50.2	32.9	34.5	35.3
T-47D	20.0	19.9	20.5	35.8	54.4	54.6	61.2	43.2	47.3	76.7

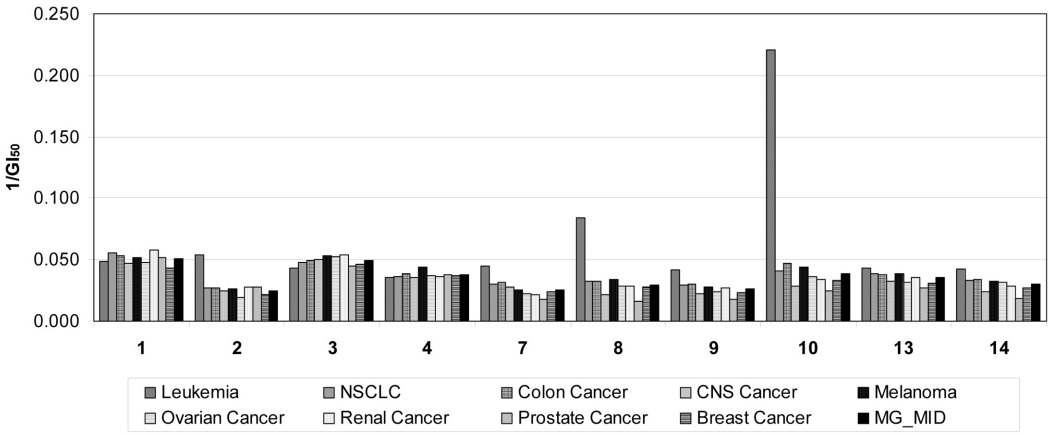
[a] Growth inhibition (GI_{50}); GI_{50} values lower than 10.0 μM are reported in bold.

small cell lung (GI_{50} = 3.3 μM) and UO-31 (GI_{50} = 0.186 μM) renal cancer cell lines; **2** (4-methoxybenzoyl) showed high sensitivity against leukemia (average GI_{50} = 18.6 μM), renal (UO-31 cell line, GI_{50} = 46 nM), and CNS (SNB-75 cell line GI_{50} = 1.6 μM) cancers.

In general, the *O*-adamantyl analogues **7**–**10**, **13**, **14** were endowed with a reduced antiproliferative activity in comparison with their corresponding phenoxyethyl analogues (in particular, compare the MG_MID values for **7** and **1**, **13** and **3**, **14**, and **4**) but they showed a higher degree of specificity against leukemia (Fig. 4). ATC **10** (3,4,5-trimethoxybenzoyl) resulted to be the most active adamantyl congener and emerged effective against all leukemia cell lines in the 2.7 to 9.7 μM concentration range. Furthermore, this compound significantly inhibited the growth of non-small cell lung cancer NCI-H522 cell line (GI_{50} = 4.0 μM, Table 3). ATC **8** (3,4-dichlorobenzoyl) and **9** (3,5-chlorobenzoyl) showed interesting activities against CCRF-CEM leukemia cell line (GI_{50} values: 2.8 μM and 5.9 μM, respectively, Table 3)

The nature of the acyl moiety seems to slightly influence the antiproliferative activity, even though a general structure-activity trend cannot be identified: the most effective ATCs (**1**, **3**, **4**, and **10**) carried acyl moieties with different electronic and steric properties (4-chlorobenzoyl, 3,4,5-trimethoxybenzoyl or heteroaroyl groups).

In order to shed some light on the mechanism of action of the title compounds, a COMPARE analysis [18–24] was performed on ATCs **1**–**4**, **7**–**10**, **13**, and **14**. This bioinformatic tool correlates the antiproliferative profiles of two agents calculating a Pearson correlation coefficient (PCC). A high PCC suggests that the two molecules share a similar antiproliferative mechanism. Matrix COMPARE was used to determine the pair-wise correlation of the cell response pattern (GI_{50} end point) for every combination of two compounds in the set of the ten ATCs



For each compound, the average GI_{50} concentration (in μM) has been calculated for both each tumor subpanel and all cell lines (Mean Graph Midpoint, MG_MID). The so obtained values were converted in the corresponding $1/GI_{50}$ and plotted. The symbol code is the following: MG_MID; Leukemia; NSCLC; Colon Cancer; CNS Cancer; Melanoma; Ovarian Cancer; Renal Cancer; Prostate Cancer; Breast Cancer.

Figure 4. Mean Growth Inhibition (GI_{50}) values of ATCs **1**–**4**, **7**–**10**, **13**, and **14**.

Table 5. Results of a matrix COMPARE analysis of ATCs **1**–**4**, **7**–**10**, **13**, and **14**[a)].

Cpd.	**14**	**13**	**10**	**9**	**8**	**7**	**4**	**3**	**2**
1	0.238 (54)	0.146 (54)	0.041 (53)	0.209 (51)	0.172 (52)	0.129 (53)	**0.543 (55)**	**0.371 (56)**	**0.840 (56)**
2	0.103 (55)	0.096 (55)	0.140 (54)	0.207 (52)	0.107 (53)	0.186 (54)	0.119 (56)	0.187 (56)	
3	−0.042 (54)	0.206 (54)	−0.156 (53)	0.085 (51)	−0.100 (52)	−0.286 (53)	**0.481 (55)**		
4	0.285 (54)	0.318 (54)	0.035 (53)	0.276 (51)	0.157 (52)	−0.004 (53)			
7	**0.708 (56)**	**0.391 (56)**	**0.587 (56)**	**0.688 (54)**	**0.698 (55)**				
8	**0.809 (55)**	**0.497 (55)**	**0.824 (55)**	**0.874 (54)**					
9	**0.791 (55)**	**0.723 (55)**	**0.895 (54)**						
10	**0.651 (56)**	**0.519 (56)**							
13	**0.660 (58)**								

[a)] The Pearson correlation coefficient (PCC) calculated from a matrix COMPARE analysis is given. Numbers in brackets correspond to the number of cell lines that were used for calculation. The data considered significant ($p < 0.01$) are shown in bold. The distinct p values were calculated from the PCCs and the number of cell lines by StaTable vers.1.0.2 (Cytel Software, Cambridge, MA, USA).

screened. Correlations could not be detected when the *O*-phenoxyethyl and *O*-adamantyl subgroups were compared (Table 5). In contrast, within each subgroup a high pattern consistency was found: four out of six possible correlations were found significant ($p < 0.01$) within the phenoxyethyl subgroup, and all 15 correlations in the adamantyl subgroup were considered significant. These observations suggest that both compound classes represent groups of antiproliferative agents with different but characteristic mechanism(s) of action. To further investigate this aspect, the individual **1**–**4**, **7**–**10**, **13**, and **14** screening results at the GI_{50} end point were used as probes to search the NCI standard agent compound set containing 171 agents with confirmed mechanism(s) of action. The only significant ($p < 0.01$) correlations obtained involved the *O*-phenoxyethyl derivatives **1** and **2** with the alkylating agent cisplatin (correlation coefficients: 0.739 and 0.807, respectively) and the *O*-adamantyl ATC **10** with the antimitotic compound *S*-trityl-L-cysteine (PCC = 0.702). These activities may be related to the electrophilic nature of the acylthiocarbamic group and its ability to interact with different cellular substrates according to the steric and electronic properties of the ATC *O*- and *N*-substituents.

Conclusions

The SAR extension studies on the new ATCs confirmed the antiproliferative properties of the series. In the NCI screening, ATCs **1**–**4**, **7**–**10**, **13**, and **14** exhibited a widespread antiproliferative activity at micromolar concen-

trations. The antitumor effect was, on average, higher within the *O*-phenoxyethyl series but the *O*-adamantyl derivatives showed higher selectivity. The nature of the acyl moiety appeared to slightly affect the ATC antiproliferative activity. COMPARE computational analyses provided indications about the possible mechanism(s) of action of the title compounds. The elucidation of the biological target(s) to which the ATC antiproliferative activity is related and the improvement of the anticancer properties will be the objective for future studies.

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The authors have declared no conflict of interest.

Experimental

Chemistry

All building blocks used are commercially available. Alcohols (1-adamantanol, and 2-phenoxyethanol), phenylisothiocyanate, acyl chlorides, and 60% sodium hydride dispersion in mineral oil were purchased by Chiminord and Aldrich Chemical, Milan (Italy). Solvents were reagent grade. DMF was dried on molecular sieves (5 Å 1/16″ inch pellets). Unless otherwise stated, all commercial reagents were used without further purification. Organic solutions were dried over anhydrous sodium sulphate. Thin layer chromatography (TLC) system for routine monitoring the course of reactions and confirming the purity of analytical samples employed aluminium-backed silica gel plates (Merck DC-Alufolien, Kieselgel 60 F_{254}; Merck, Germany): $CHCl_3$ was used as developing solvent and detection of spots was made by UV light and / or by iodine vapours. The parallel solution phase chemistry was performed by using a Carousel-12 Reaction Station™ (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna, Italy). The evaporation of solutions was performed in parallel with an Evaposel™ apparatus (Radleys Discovery Technologies, Italian distributor: StepBio) operating at reduced pressure of about 15 – 20 Torr. Yields were not optimized. Melting points were determined on a Fisher-Johns apparatus (Fischer-Scientific, Pittsburgh, PA, USA) and are uncorrected. IR spectra were recorded on a Perkin Elmer 398 spectrometer (Perkin Elmer, USA) as KBr discs. ^{1}H-NMR spectra (200 MHz) were recorded in $CDCl_3$ on a Varian Gemini 200 instrument (Varian Inc., Palo Alto, CA, USA). Chemical shifts were reported in δ (ppm) units relative to the internal standard tetramethylsilane, and the splitting patterns were described as follows: s (singlet), t (triplet) and m (multiplet). The first order values reported for coupling constants *J* were given in Hz. Elemental analyses were performed by an EA1110 Elemental Analyser (Fison-Instruments, Milan, Italy); all compounds were analyzed for C, H, N and S and the analytical results were within ± 0.4% of the theoretical values.

*Parallel synthesis of O-(2-phenoxyethyl) [(hetero)aroyl](phenyl)thiocarbamates **1 – 4***

A 60% sodium hydride dispersion in mineral oil (0.40 g, 10 mmol) was added in a single portion at 20°C to each numbered reaction tube of a 12-Carousel Reaction Station™, containing a stirred solution of 2-phenoxyethanol (1.254 mL, 10 mmol) in dry toluene (15 mL). After stirring for 30 min, phenyl isothiocyanate (1.194 mL, 10 mmol) was added to each reaction mixture, which was then stirred for 30 min at rt. Then, dry pyridine (5 mL) and the suitable acyl chloride (11 mmol) were added successively, each one in one portion. After stirring at 20°C for 6 h, water (25 mL) was added into each tube. The contents of the tubes were then transferred into a set of separating funnels. More water (125 mL) was added into each funnel. After parallel extraction with diethyl ether (40 mL × 3), the combined extracts of each reaction were washed with water (30 mL × 2), 2 M HCl (30 mL × 2), 1 M $NaHCO_3$ (30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, and filtered in parallel through pads of Florisil1 (diameter 5 × 2 cm) by an in-house device. Parallel evaporating *in vacuo* using an Evaposel™ apparatus gave residues which were purified by crystallization from the suitable solvent (mixture). The IR and ^{1}H-NMR spectra for *O*-(2-Phenoxyethyl) 4-chlorobenzoyl(phenyl)thiocarbamate **1**, *O*-(2-phenoxyethyl) 2-furoyl(phenyl)thiocarbamate **3**, *O*-(2-phenoxyethyl) phenyl(thien-2-ylcarbonyl)thiocarbamate **4** are consistent with literature data [9].

*O-(2-Phenoxyethyl) 4-methoxybenzoyl(phenyl)thiocarbamate **2***

IR (KBr) cm^{-1} 1685; ^{1}H-NMR ($CDCl_3$) δ: 3.77 (s, 3H, methoxy), 3.92 (t, *J* = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.78 (t, *J* = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.60-8.10 (m, 14H, arom H). Anal. Calcd. for $C_{23}H_{21}NO_4S$: C, 67.79; H, 5.19; N, 3.44; S, 7.87. Found: C, 67.59; H, 5.32; N, 3.23; S, 7.98.

*Parallel synthesis of O-1-adamantyl [(hetero)aroyl](phenyl)thiocarbamates **5 – 14***

A 60% sodium hydride dispersion in mineral oil (0.40 g, 10 mmol) was added in a single portion at 20°C to each numbered reaction tube of a 12-Carousel Reaction Station™, containing a stirred solution of 1-adamantanol (1.522 g, 10 mmol) in dry DMF (25 mL). After stirring for 30 min at 90°C, phenyl isothiocyanate (1.194 mL, 10 mmol) was added to each reaction mixture, which was then stirred for 30 min at 90°C. After cooling to 20°C, dry pyridine (5 mL) and the suitable acyl chloride (12 mmol) were added successively, each one in one portion. The resulting mixtures were stirred at 20°C for 6 h, and then at 55°C for 1 h. After cooling to 20°C and addition of water (25 mL) into each vessel, the contents of the tubes were then transferred into a set of separating funnels. More water (125 mL) was added into each funnel. After parallel extraction with dichloromethane (40 mL 6 3), the combined extracts of each reaction were washed

with water (30 mL 6 5), dried over anhydrous Na_2SO_4, and filtered in parallel through pads of Florisil1 (diameter 5 6 2 cm) by an in-house device. Parallel *in-vacuo* evaporation (Evaposel™ apparatus) gave residues which were purified by crystallization from the suitable solvent mixture. The elemental analysis as well as the IR and ^{1}H-NMR spectra for **13** are consistent with literature data [9].

O-1-Adamantyl 2-acetoxybenzoyl(phenyl)thiocarbamate ***5***

IR (KBr) cm^{-1} 1765; ^{1}H-NMR ($CDCl_3$) δ: 1.14 – 1.75 (m, 6H, 3 CH_2), 1.84 – 2.14 (m, 9H, 3 CH_2 and 3 CH), 2.44 (s, 3H, acetyl), 7.01 – 7.94 (m, 9H, arom H). Anal. Calcd. for $C_{26}H_{27}NO_4S$: C, 69.46; H, 6.05; N, 3.12; S, 7.13. Found: C, 69.56; H, 6.13; N, 3.05; S, 7.25.

O-1-Adamantyl 3-nitrobenzoyl(phenyl)thiocarbamate ***6***

IR (KBr) cm^{-1} 1685; ^{1}H-NMR ($CDCl_3$) δ: 1.41 – 1.75 (m, 6H, 3 CH_2), 1.93 – 2.25 (m, 9H, 3 CH_2 and 3 CH), 7.23 – 8.81 (m, 9H, arom H). Anal. Calcd. for $C_{24}H_{24}N_2O_4S$: C, 66.04; H, 5.54; N, 6.42; S, 7.34. Found: C, 66.00; H, 5.54; N, 6.49; S, 7.60.

O-1-Adamantyl 4-chlorobenzoyl(phenyl)thiocarbamate ***7***

IR (KBr) cm^{-1} 1690; ^{1}H-NMR ($CDCl_3$) δ: 1.42 – 1.71 (m, 6H, 3 CH_2), 1.90 – 2.33 (m, 9H, 3 CH_2 and 3 CH), 7.20 – 8.06 (m, 9H, arom H). Anal. Calcd. for $C_{24}H_{24}ClNO_2S$: C, 67.67; H, 5.68; N, 3.29; S, 7.53. Found: C, 67.95; H, 5.70; N, 3.40; S, 7.31.

O-1-Adamantyl 3,4-dichlorobenzoyl(phenyl)thiocarbamate ***8***

IR (KBr) cm^{-1} 1690; ^{1}H-NMR ($CDCl_3$) δ: 1.40 – 1.80 (m, 6H, 3 CH_2), 1.90-2.48 (m, 9H, 3 CH_2 and 3 CH), 7.16 – 8.05 (m, 8H, arom H). Anal. Calcd. for $C_{24}H_{23}Cl_2NO_2S$: C, 62.61; H, 5.04; N, 3.04; S, 6.96. Found: C, 62.43; H, 4.99; N, 3.15; S, 7.07.

O-1-Adamantyl 3,5-dichlorobenzoyl(phenyl)thiocarbamate ***9***

IR (KBr) cm^{-1} 1685; ^{1}H-NMR ($CDCl_3$) δ: 1.32 – 1.78 (m, 6H, 3 CH_2), 1.92 – 2.32 (m, 9H, 3 CH_2 and 3 CH), 7.12 – 7.85 (m, 8H, arom H). Anal. Calcd. for $C_{24}H_{23}Cl_2NO_2S$: C, 62.61; H, 5.04; N, 3.04; S, 6.96. Found: C, 62.52; H, 5.07; N, 3.07; S, 6.72.

O-1-Adamantyl 3,4,5-trimethoxybenzoyl(phenyl)thiocarbamate ***10***

IR (KBr) cm^{-1} 1690; ^{1}H-NMR ($CDCl_3$) δ: 1.45 – 1.80 (m, 6H, 3 CH_2), 1.80 – 2.30 (m, 9H, 3 CH_2 and 3 CH), 3.94 (s, 9H, 3 CH_3O), 7.14 – 7.64 (m, 7H, arom H). Anal. Calcd. for $C_{27}H_{31}NO_5S$: C, 67.34; H, 6.49; N, 2.91; S, 6.66. Found: C, 67.22; H, 6.54; N, 3.07; S, 6.55.

O-1-Adamantyl 1,1'-biphenyl-4-ylcarbonyl(phenyl)thiocarbamate ***11***

IR (KBr) cm^{-1} 1685; ^{1}H-NMR ($CDCl_3$) δ: 1.41 – 1.79 (m, 6H, 3 CH_2), 1.93 – 2.31 (m, 9H, 3 CH_2 and 3 CH), 7.05 – 8.20 (m, 14H, arom H). Anal. Calcd. for $C_{30}H_{29}NO_2S$: C, 77.06; H, 6.25; N, 3.00; S, 6.86. Found: C, 77.30; H, 6.36; N, 3.10; S, 6.51.

O-1-Adamantyl 1-naphthoyl(phenyl)thiocarbamate ***12***

IR (KBr) cm^{-1} 1690; ^{1}H-NMR ($CDCl_3$) δ: 1.42 – 2.13 (m, 15H, 6 CH_2 and 3 CH), 7.03 – 8.60 (m, 12H, arom H). Anal. Calcd. for $C_{28}H_{27}NO_2S$: C, 76.16; H, 6.16; N, 3.17; S, 7.26. Found: C, 76.41; H, 6.18; N, 2.99; S, 7.36.

O-1-Adamantyl 2-furoyl(phenyl)thiocarbamate ***13*** *[9] and O-1-Adamantyl phenyl(2-thenoyl)thiocarbamate* ***14***

IR (KBr) cm^{-1} 1680; ^{1}H-NMR ($CDCl_3$) δ: 1.48 – 1.97 (m, 6H, 3 CH_2), 2.01 – 2.48 (m, 9H, 3 CH_2 and 3 CH), 6.85 – 8.10 (m, 8H, 5 arom H, 3H, thioph). Anal. Calcd. for $C_{22}H_{23}NO_2S_2$: C, 66.47; H, 5.83; N, 3.52; S, 16.13. Found: C, 66.65; H, 6.02; N, 3.45; S, 15.97.

Pharmacology

Evaluation of anticancer activity

The NCI high-flux anticancer drug screen [21, 24, 25] utilized a panel of 60 human tumor cell lines in culture derived from nine cancer types (lung, colon, CNS, ovarian, renal, prostate, and breast cancer, leukemia and melanoma). The compounds were tested at ten-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. According to the NCI protocol, cell lines were exposed to test agents in 96-well plates for the last 48 of a 72 h incubation and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. For each compound, the drug concentration required to produce 50% (GI_{50}) and total (TGI) growth inhibition, and 50% cytocidal effect (LC_{50}) were obtained for 56 to 58 cell lines. Values were calculated for each of these parameters if the level activity was reached; if the effect was not reached or was exceeded, the value is expressed as greater or lesser than the maximum or minimum concentration tested.

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