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New 2-(1-Adamantylcarbonyl)pyridine and 1-Acetyladamantane Thiosemicarbazones–Thiocarbonohydrazones: Cell Growth Inhibitory, Antiviral and Antimicrobial Activity Evaluation

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Abstract—The new thiosemicarbazones and thiocarbonohydrazones **4a–d**, **5a–d** derived from 2-(1-adamantylcarbonyl)pyridine and 1-acetyladamantane were synthesized and evaluated for their inhibitory effect on tumor cell proliferation and their antiviral and antimicrobial activity. Thiosemicarbazone **4a** inhibited tumor cell proliferation (GI50's range: 2.4–100 μM and mean GI50 43.9 μM against various human leukemic cell lines) while thiosemicarbazone **5a** and thiocarbonohydrazone **5d** exhibited significant inhibition of tumor cell proliferation (GI50's range 2.3–23.6 μM and mean GI50 7.2 μM for **5a** and GI50's range 2.4–32.4 μM and mean GI50 12.8 μM for **5d**). These GI50 values are comparable to that of 2-acetylpyridine thiosemicarbazone an important lead in TSC's family. The compounds did not afford specific activity against any of the viruses tested when examined at non-toxic concentrations. A weak activity was found for thiocarbonohydrazones **4d**, **5d** against Gram-(+) bacteria (MIC_{50} 117.3 and 133 μM, respectively). Using a combination of molecular mechanics calculations and NOE spectroscopy it was shown that the parent compounds **4a** and **5a** have opposite configuration around C=N bond. Whether this difference in structure can be correlated with the biological activity will be investigated in future studies.

Introduction

Thiosemicarbazone (TSC) derivatives are a class of compounds that possess a range of biological properties; antitumor, antiviral, antibacterial, antimalarial and antifungal activities have been reported.[1a] Brockman et al. reported for the first time a thiosemicarbazone (i.e., 2-formylpyridine TSC) possessing antitumor activity against L1210 leukemic cells.[1b] Since then intense research has been conducted in this field, mainly focusing on the biological evaluation of heterocyclic TSC's. Heterocyclic TSC's act through the inhibition of ribonucleotide reductase, an enzyme which is directly involved in the synthesis of DNA precursors in mammalian cells.[1a]

2-Acetylpyridine derivatives exhibit potent antiviral, antibacterial and cytotoxic activities; thiosemicarbazones are cytotoxic and thiocarbonohydrazones are inhibitory to HSV-1, HSV-2 and VZV viruses.[2,3]

The replacement of an acetyl group by a longer acyl chain (like butyryl) led to compouds with a better antimicrobial and antitumoral activity profile.[4,5] In recent years we have concentrated on the synthesis of adamantane compounds with antiviral activity.[6] In this context, we report here the synthesis, a preliminary structural study and the cell growth inhibitory, antiviral and antimicrobial activity evaluation of some novel thiosemicarbazones and thiocarbonohydrazones of 1-adamantyl 2-pyridyl ketone, that is compounds **4a–d**. The corresponding derivatives **5a–d** of aliphatic 1-adamantyl methyl ketone were also synthesized and studied.

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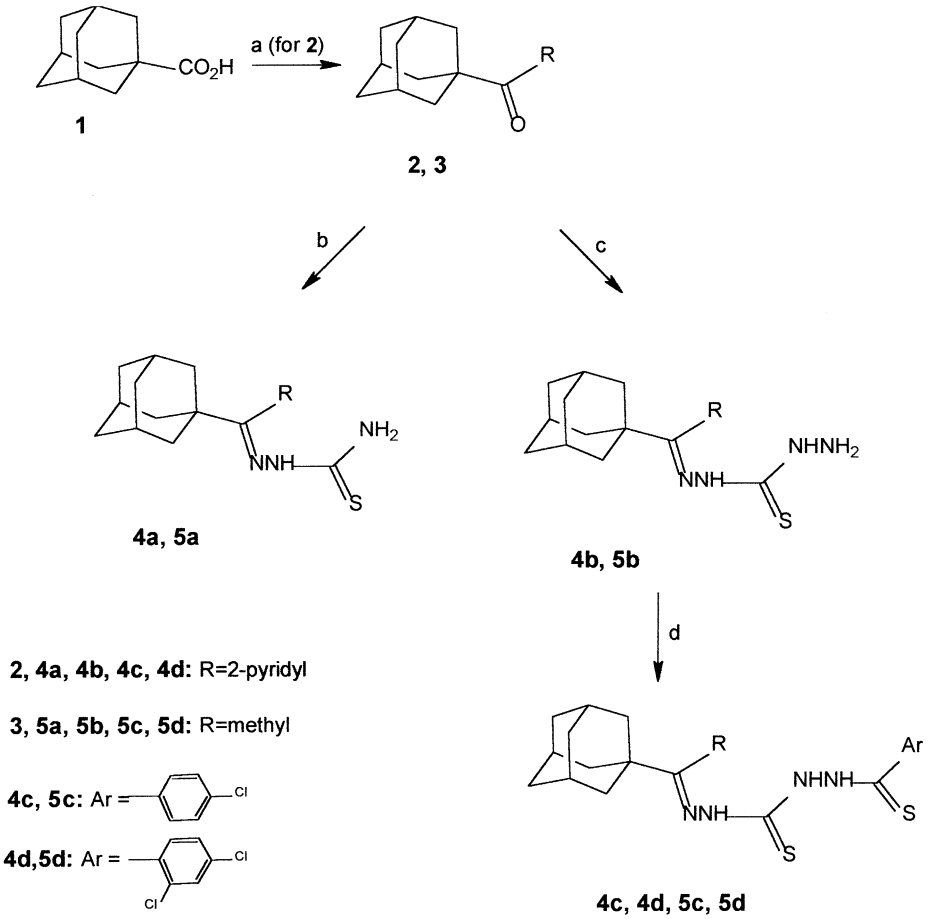
Results and Discussion

Chemistry

The new compounds were synthesized according to Scheme 1. 2-(1-Adamantylcarbonyl)pyridine **2** was prepared by the reaction of 2-pyridinyl lithium with 1-adamantanecarboxylic acid **1**. 1-Acetyladamantane **3** was prepared according to a published procedure based on hydrolysis and decarboxylation of ethyl 1-adamantylcarbonylmalonate.[7] Condensation of ketones **2** and **3** with thiosemicarbazide afforded thiosemicarbazones **4a** and **5a**, respectively. Thiocarbonohydrazones **4b** and **5b**



Scheme 1. Reagents and conditions: (a) 2-pyridinyl lithium, Et_2O/THF, −60 °C and then HCl 10% (82%); (b) $H_2NNHC(=S)NH_2$, MeOH, reflux, 120 h, (77%); (c) $H_2NNHC(=S)NHNH_2$, MeOH, reflux, 15–130 h, (57–59%); (d) Ar–N=C=S, DMF, rt, 2–4 h (75–85%).

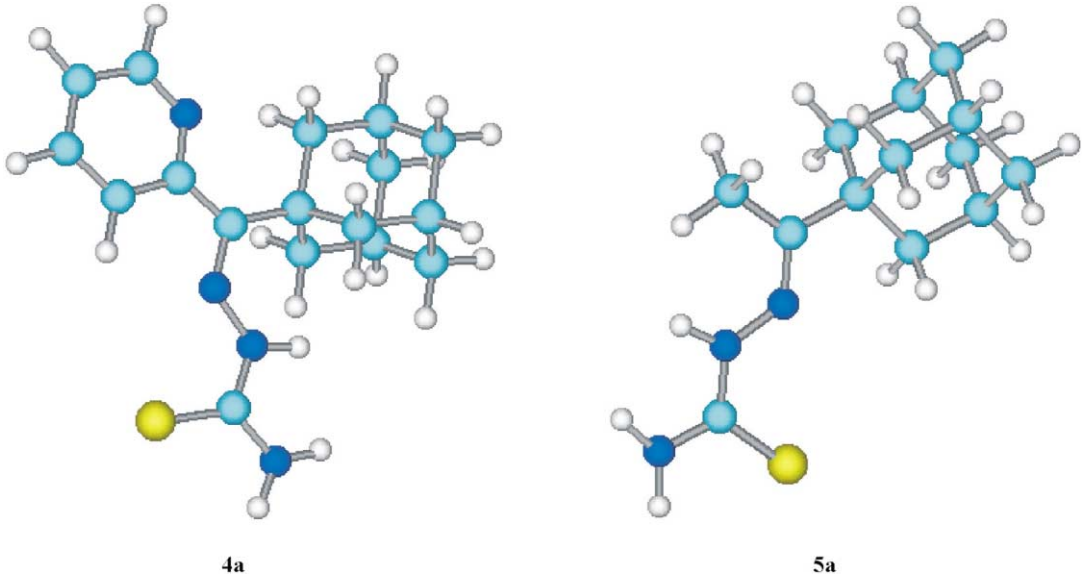


Figure 1. Models for thiosemicarbazones **4a** and **5a** as resulted from the combination of NOE spectroscopy and molecular mechanics calculations.

Table 1. Biological activity of compounds **4a–d**, **5a–d** tested against human leukemic cell lines

	4a	4b	4c	4d	5a	5b	5c	5d	Vinblastine
GI50[a] (μM)									
CCRF-CEM[b]	100.0	100.0	76.0	66.3	23.6	36.8	100.0	5.7	2×10^{-5}
MOLT-4[b]	6.4	100.0	100.0	31.4	2.3	22.2	100.0	32.4	$<2.5\times10^{-4}$
HuT78[b]	2.4	100.0	8.0	23.0	2.3	20.5	100.0	8.1	$<2.5\times10^{-4}$
RPMI8226[b]	97.9	100.0	6.7	4.6	7.1	94.2	100.0	3.6	3×10^{-6}
K562[b]	5.8	41.7	100.0	100.0	2.8	14.7	100.0	24.7	$<2.5\times10^{-4}$
HL60[b]	51.1	100.0	4.1	3.6	5.2	48.7	100.0	2.4	3.6×10^{-5}
TGI[a] (μM)									
CCRF-CEM[b]	100.0	100.0	100.0	100.0	55.2	100.0	100.0	100.0	1.91×10^{-3}
MOLT-4[b]	92.0	100.0	100.0	100.0	9.3	100.0	100.0	100.0	$<2.5\times10^{-4}$
HuT78[b]	26.3	100.0	100.0	81.0	29.1	100.0	100.0	100.0	$<2.5\times10^{-4}$
RPMI8226[b]	100.0	100.0	68.3	29.5	33.3	100.0	100.0	29.3	8×10^{-4}
K562[b]	83.1	100.0	100.0	100.0	10.4	68.4	100.0	100.0	$<2.5\times10^{-4}$
HL60[b]	100.0	100.0	9.5	7.0	24.1	92.0	100.0	6.2	2.5×10^{-4}
LC50[a] (μM)									
CCRF-CEM[b]	100.0	100.0	100.0	100.0	86.7	100.0	100.0	100.0	5.73×10^{-3}
MOLT-4[b]	100.0	100.0	100.0	100.0	59.4	100.0	100.0	100.0	$<2.5\times10^{-4}$
HuT78[b]	100.0	100.0	100.0	100.0	83.1	100.0	100.0	100.0	2.5×10^{-4}
RPMI8226[b]	100.0	100.0	100.0	100.0	69.2	100.0	100.0	100.0	3.28×10^{-3}
K562[b]	100.0	100.0	100.0	100.0	62.6	100.0	100.0	100.0	$<2.5\times10^{-4}$
HL60[b]	100.0	100.0	85.5	31.9	63.6	100.0	100.0	10.0	1.51×10^{-3}

[a]GI50 is the concentration required to inhibit the growth of cells by 50%. TGI is the concentration required to inhibit the growth of cells by 100%. LC50 is the concentration required to reduce the viability of leukemia cells by 50%. Data represent mean values for two separate experiments done in triplicate.
[b]CCRF-CEM and MOLT-4 represent T cell leukemia, HuT78 T lymphoma, RPMI8226 multiple myeloma, K562 chronic myeloid leukaemia and HL60 acute myeloid leukaemia.

were prepared by treating the ketones **2** and **3** with thiocarbohydrazide. Derivatives **4c**,**d** and **5c**,**d** were obtained by treatment of precursors **4b**, **5b** with the suitable aryl isothiocyanates.[2–5,8]

The stereochemistry around the C=N bond of thiosemicarbazone moiety was investigated for the parent compounds **4a**, **5a**, using a combination of molecular mechanics and NOE spectroscopy.[9] For TSC **4a**, molecular mechanics calculations predict an E configuration around the C=N bond (Fig. 1). In agreement with the E stereochemistry is the strong NOE dipolar coupling between C=N–NH and adamantyl protons and the characteristic C=N–NH proton chemical shift of 8.3 ppm in $CDCl_3$ and 8.6 ppm in DMSO-d_6.[1,10] For thiosemicarbazone **5a** the opposite configuration was calculated to be more stable although it is again assigned as E because of the change in the relative priority of the substituents. This configuration is consistent with the strong NOE observed between methyl and C=N–NH protons (Fig. 1).[11]

Biological activity evaluation

Compounds **4a–d** and **5a–d** were tested for their activity against six established cell lines representing different types of human leukemias. Table 1 includes the GI50, TGI and LC50 values determined using the MTT method.[12] Several compounds **4a**,**c**,**d** and **5a**,**b**,**d** proved to be inhibitory to the proliferation of the tumor cells, the thiosemicarbazone **5a** being the most potent. Thiocarbonohydrazone **5d** bearing a 2,4-dichlorophenyl group was the next most active compound. Interestingly, the aliphatic derivatives **5**, as a rule, showed better activity profiles than their heterocyclic counterparts **4**. In comparison with compound **4a**, the corresponding 2-acetylpyridine thiosemicarbazone, an important lead in this series, showed comparable activity against HuT78 cells.[2]

The new compounds **4a–d**, **5a–d** were examined for activity against the replication of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). Cytotoxicity of the compounds was monitored in parallel. Both antiviral activity and cytotoxicity were determined by the MTT method.[12] The TSC's were also evaluated, according to previously reported methods,[13–16] against the following viruses: herpes simplex virus type 1 (HSV-1), thymidine kinase-deficient (TK^-) HSV-1, herpes simplex virus type 2 (HSV-2), varicella-zoster virus (VZV), TK^- VZV, human cytomegalovirus (HCMV), vaccinia virus, vesicular stomatitis virus (VSV), Coxsackie B4 virus, Sindbis virus, Reo-1 virus, parainfluenza-3 virus

Table 2. Anti-HIV-1 and anti-HIV-2 activity and cytotoxicity of compounds **4a–d**, **5a–d** in MT-4 cells[a]

Compd	EC_{50}[b] (μM)		CC_{50}[c] (μM)
	HIV-1 (IIIB)	HIV-2 (ROD)	
AZT	0.0064	0.006	>500
4a	214.0±3.8	>400	>400
4b	>27	>27	27.3±6.2
4c	>3	>3	2.8±0.1
4d	>3	>3	2.6±0.1
5a	>8	>8	7.5±5.6
5b	>7	>7	7.1±3.7
5c	>10	>10	10.1±1.1
5d	>2	>2	2.4±0.1

[a]MT-4 represents a human T-4 lymphocytic cell line.
[b]50% Effective concentration, or concentration required to protect MT-4 cells against the cytopathicity of HIV by 50%.
[c]50% Cytotoxic concentration, or concentration required to reduce the viability of MT-4 cells by 50%.

and Punta Toro virus. The compounds did not afford specific activity against any of the viruses when examined at non-toxic concentrations (Tables 2–4).

Compounds **4a–d**, **5a–d** were also tested for their antimicrobial activity (Table 5). A weak activity was found for thiocarbonohydrazones **4d**, **5d** against the Gram-(+) bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus hominis*. No activity was exhibited against Gram-(−) bacteria.

In conclusion, the results presented in this work point to the potential of some compounds (particularly **5a** and **5d**) in inhibiting tumor cell proliferation with virtually no activity against viruses and only slight activity against gram-positive bacteria (**4d**, **5d**). Thiosemicarbazone **5a** exhibited a GI50's range between 2.3 and 23.6 μM and a mean GI50 7.2 μM and thiocarbonohydrazone **5d** a GI50′s range between 2.4 and 32.4 μM and a mean GI50 12.8 μM against the human leukemic cell lines tested. The GI50 values exhibited by compounds

Table 3. Antiviral activity of various TSC derivatives

Virus[a]	Cell[b]	MIC[c] (μg/mL)												
		4a	**4b**	**4c**	**4d**	**5a**	**5b**	**5c**	**5d**	Acyclovir	Brivudin	Ganciclovir	Cidofovir	Ribavirin
HSV-1 (KOS)	E_6SM	>400	>16	>16	>16	240	>16	>40	>16	0.08	0.005	0.0064	—	—
TK^- HSV-1	E_6SM	>400	>16	>16	>16	>400	>16	>40	9.6	48	400	9.6	—	—
HSV-2 G	E_6SM	>400	>16	>16	>16	240	>16	>40	9.6	0.08	80	0.019	—	—
TK^+ VZV	HEL	>20	>20	>2	>2	>2	10.5	>2	>2	1.1	0.009	—	—	—
TK^- VZV	HEL	>20	>20	>2	>2	>2	>20	>2	>2	12.3	>50	—	—	—
HCMV	HEL	>5	>5	>5	>2	>2	>5	>5	>2	—	—	2	0.27	—
Vaccinia	E_6SM	>400	>16	>16	>16	>400	>16	>40	>16	>400	3.2	>100		240
VSV	HeLa	>400	>80	>3.2	>3.2	>80	>16	>40	>3.2	—	>400	—	—	240
	E_6SM	>400	>16	>16	>16	>400	>16	>40	>16	48	>400	9.6	—	400
Coxsackie B4	HeLa	240	>80	>3.2	>3.2	>80	>16	>40	>3.2	—	>400	—	—	48
	Vero	>3.2	>80	9.6	>3.2	>80	>80	>40	>3.2	—	>400	—	—	240
Sindbis	Vero	>3.2	>80	>16	>3.2	>80	>80	>40	>3.2	—	>400	—	—	80
Reo-1	Vero	>3.2	>80	>16	>3.2	>80	>80	>40	>3.2	—	>400	—	—	80
Parainfluenza-3	Vero	>3.2	>80	>16	>3.2	>80	>80	>40	>3.2	—	>400	—	—	48
Punta Toro	Vero	>3.2	>80	>16	>3.2	>80	>80	>40	>3.2	—	>400	—	—	16

[a]Abbreviations and virus strains: HSV-1, herpes simplex virus type 1 (KOS); TK^- HSV-1, thymidine kinase-deficient HSV-1; HSV-2, herpes simplex virus type 2 (G); VZV, varicella-zoster virus; TK^+ VZV, thymidine kinase wild-type VZV (YS); TK^- VZV, thymidine kinase-deficient VZV (07–1); HCMV, human cytomegalovirus (AD-169) and VSV, vesicular stomatitis virus.
[b]Abbreviations: E_6SM, human embryonic skin-muscle fibroblasts; HEL, human embryonic lung fibroblasts; Vero, African green monkey kidney cells and HeLa, human epithelial cells.
[c]Minimum inhibitory concentration required to reduce virus-induced cytopathicity by 50%.

Table 4. Cytotoxicity of TSC derivatives in cell culture

Cell[a]	MCC[b] (μg/mL)												
	4a	**4b**	**4c**	**4d**	**5a**	**5b**	**5c**	**5d**	ACV	BVDU	GCV	HPMPC	Ribavirin
E_6SM	>400	≥80	80	80	>400	≥80	200	80	>400	>400	>100	—	>400
HEL	≥20	≥20	5	5	5	≥20	≥5	5	>50	>50	>50	>50	—
HeLa	>400	400	≥16	16	400	80	>200	16	—	≥400	—	—	>400
Vero	16	80	80	16	400	80	≥400	16	—	>400	—	—	>400

[a]Abbreviations: see footnote to Table 3.
[b]Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.

Table 5. Antimicrobial activity of compounds **4a–d**, **5a–d** against Gram-(+) bacteria and Gram-(−) bacteria

Compd	MIC_{50}[a] (μM)				
	S. aureus ATCC 6538	*S. epidermidis* ATCC 12228	*S. hominis* ATCC 27844	*Klebsiella pneumoniae* ATCC 13883	*Escherichia coli* ATCC 25922
4a	>3184	>3184	>3184	>3184	>3184
4b	>3040	>3040	>3040	>3040	>3040
4c	1003	1003	1003	>2000	>2000
4d	117.3	117.3	117.3	>1876	>1876
5a	>3984	>3984	>3984	>3984	>3984
5b	>3759	>3759	>3759	>3759	>3759
5c	1148	1148	1148	>2296	>2296
5d	133	133	133	>2128	>2128
Streptomycin	2.1	2.1	2.1	0.6	0.6

[a]50% Minimum inhibitory concentration. All data represent mean values for three separate experiments.

4a, **5a**, **5d** is comparable to that of 2-acetylpyridine thiosemicarbazone considered as a lead in TSC's class of molecules. According to our best knowledge only heterocyclic TSC's have been studied in detail. Thus, the antiproliferative activity of aliphatic compounds **5a–d** can open the avenue for novel studies on TSC derivatives. In addition, since it is known from the literature that only minor modifications in the thiosemicarbazones can lead to significant change in biological activity, this series of compounds merit further investigation.

Using a combination of molecular mechanics calculations and NOE spectroscopy it was shown that the parents compounds **4a** and **5a** have different configuration around C=N bond. This result can be significant for comparison studies only if the two series act to the same biological target. Future studies will be undertaken to fulfill this aim.

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8. The ^{1}H and ^{13}C NMR spectra of the synthesized compounds were assigned using COSY, CHCORR and NOESY spectroscopy. For example for compound **4a**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.58–1.72 (m, 6H, 4,6,10-adamantane H), 1.81 (br s, 6H, 2,8,9-adamantane H), 2.0 (3H, 3,5,7-adamantane H), 6.25 (br s, 1H, NH_2), 7.15 (d, $J=7.6$ Hz, 1H, 3-pyridine H), 7.25 (br s, 1H, $\overline{NH}_2$), 7.35 (dd, $J=7.1$, 5.4 Hz, 5-pyridine H), 7.80 (∼t, $J=7.6$ Hz, 1H, 4-pyridine H), 8.27 (br s, 1H, NH), 8.74 (∼d, $J=4.8$ Hz, 1H, 6-pyridine H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 28.0 (3,5,7-adamantane C), 36.30 (4,6,10-adamantane C), 39.9 (2,8,9-adamantane C), 40.17 (1-adamantane C), 123.63 (3-pyridine C), 124.11 (5-pyridine C), 137.1 (4-pyridine C), 150.6 (6-pyridine C), 159.17 (C=N), 179.03 (2-pyridine C); Spectra and elemental analysis of all the synthesized compounds were in accord with their structure.
9. 2 D NOESY spectra were run in DMSO-d_6 solutions on a Bruker AMX 400 MHz machine with a mixing time of 600 ms. Molecular mechanics were performed on a Pentium PC using the MM+ force field provided by the Hyperchem. This force field is an extension of MM2 force field. Energy minimization was run using conjugate gradient and Newton–Raphson algorithms and an energy gradient tolerance of 0.001 kcal mol^{-1} $Å^{-1}$.
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