Synthesis and Antitumour activity of some aryl semicarbazones

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Summary

Various 4-substituted phenyl semicarbazone derivatives were synthesized and evaluated *in vitro* by NCI in the 3-cell line, one dose primary anticancer assay. Three compounds showed significant activity against breast MCF7 cell line and were further evaluated for potential anticancer activity in an *in vitro* human disease-oriented tumour cell line screening panel that consisted of 60 human tumour cell lines arranged in nine subpanels, representing diverse histologies. Leukemia, colon, ovarian and breast cancer cell lines were relatively more sensitive to these compounds than the other cell lines. The 4-carboxy substituted p-nitrobenzylidene phenyl semicarbazone (**1c**) emerged as the most active compound with average GI_{50} value (the molar drug concentration required for the 50% growth inhibition) of 28.6µM. This compound showed greater activity than methotrexate against NCI-H226(Lung), BT-549 and T-47D(Breast) cancer cell lines.

Keywords: Aryl semicarbazones, Antitumour, Methotrexate.

1. Introduction

Semicarbazones are of considerable interest due to the many bioactivities, which they possess and the medicinal potential of toluyl semicarbazide was first recorded by Cotti[1]. Since then the semicarbazones have revealed a broad spectrum of therapeutic activity especially as anticonvulsants[2-4], tuberculostatics[5], sodium channel blockers[6] etc. A number of antineoplastic agents derived from thiosemicarbazones have been developed[7,8], but so far not many semicarbazones have been studied. Dimmock et al.[9] reported the antileukemic activity of thiosemicarbazone and semicarbazone derivatives and the semicarbazones were found to be less toxic. Alkyl and cycloalkylnitroso semicarbazones have displayed excellent activity against leukemia L5222 in rats[10]. Attachment of the semicarbazone group to a substituted partially hydrogenated naphthopyran and fluorene rings resulted with antileukemic activity in mice[11] and against various tumours in different experimental animals[12]. Some 4-[[4-chloro-5-methyl-2(methylthio)phenyl]sulfonyl]-1-aryl semi-carbazides exhibited weak or moderate activity against some human tumour cell lines and 4-chloro-phenyl substituted compounds showed relatively high activity[13]. Several alkylamino substituted semicarbazones have proved active on CNS and breast cancer cell lines at 10^{-4}M concentration[14]. The present study was aimed to explore the antitumoral activity of some 4-substituted phenyl semicarbazones. This paper reports the synthesis and *in vitro* anticancer drug screening of these new compounds utilizing a panel of 60 diverse human tumour cell lines. Sensitive cell lines show GI_{50} (molar drug concentration required to cause 50% growth inhibition) values $<10^{-8}$M and insensitive cell lines show $>10^{-4}$M.

2. Investigation and Results

The 4-substituted phenyl semicarbazones were prepared starting from 4-substituted anilines as depicted in the Scheme. All compounds were evaluated *in vitro* by NCI in the 3-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung) and SF-268 (CNS) at 11×10^{-4}M concentration and reported in Table 1.

Table 1. Primary anticancer evaluation of the semicarbazone derivatives in 3-cell lines at 11×10^{-4} M concentration

Compounds	Growth percentages		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
1a	110	48	93
1b	118	72	115
1c	12	11	32
2a	82	28	43
2b	86	22	55
2c	117	95	111
2d	115	101	111
2e	85	71	93
2f	118	80	112
2g	130	95	114
2h	131	91	107
2I	95	79	76
2j	98	79	76

Three compounds **1c**, **2b** and **2c** were further evaluated in the *in vitro* human disease-oriented tumour cell line screening panel developed at the NCI. The GI_{50} values against each cell line are presented in Table 2.

Table 2. *In vitro* cytotoxicity of the compounds **1c**, **2b** and **2c** against 60 human cancer cell lines.

Cell line	1c	GI$_{50}$ (μM) 2b	2c	MTX	Cell line	1c	GI$_{50}$ (μM) 2b	2c	MTX
1. Leukemia					M14	37.4	36.8	25.5	0.032
CCRF-CEM	30.6	36.2	25.9	0.029	SK-MEL-2	25.0	42.0	58.7	0.087
HL-60(TB)	37.2	27.2	46.1	0.039	SK-MEL-28	41.7	85.1	61.2	>1
K-562	13.3	40.8	28.7	0.026	SK-MEL-5	16.2	46.3	65.5	0.087
MOLT-4	22.9	37.6	39.8	0.028	UACC-257	28.4	58.6	79.5	0.790
RPMI8226	18.7	>100	30.3	0.033	UACC-62	26.3	60.4	75.4	0.028
SR	43.4	22.3	17.9	0.033	**6. Ovarian Cancer**				
2. Non Small Cell Lung Cancer					IGROVI	33.1	58.8	31.2	0.069
A549/ATCC	35.3	>100	>100	0.033	OVCAR-3	4.54	41.9	30.1	0.400
EKVX	23.1	>100	61.0	8.700	OVCAR-4	21.2	>100	59.9	>1
HOP-62	21.6	50.8	40.8	X	OVCAR-5	40.6	>100	>100	0.980
HOP-92	16.9	94.3	65.4	>1	OVCAR-8	28.6	45.1	37.6	0.031
NCI-H226	22.2	66.0	42.8	23.0	SK-OV-3	21.3	81.4	45.5	X
NCI-H23	23.2	80.6	41.6	0.043	**7. Renal Cancer**				
NCI-H322M	34.1	90.2	53.7	X	786-O	39.1	73.7	45.6	0.033
NCI-H460	26.5	68.0	>100	0.028	A498	20.2	>100	>100	1.900
NCI-H522	7.85	22.4	41.8	0.450	ACHN	26.6	>100	66.7	0.040
3. Colon Cancer					CAKI-1	28.2	94.4	44.8	X
COLO205	23.1	35.4	38.4	0.870	RXF393	26.1	>100	>100	>1
HCC-2998	20.2	40.8	33.9	0.110	SN12C	29.9	85.4	32.7	0.031
HCC-116	22.3	48.2	30.1	X	TK-10	34.4	>100	>100	>1
HCT-15	25.5	48.0	35.0	0.030	UO-31	22.1	60.7	41.0	X
HT29	23.8	45.5	30.1	X	**8. Prostate Cancer**				
KM12	27.7	33.7	36.0	0.033	PC-3	32.0	>100	35.7	0.027
SW620	40.9	74.3	36.0	0.033	DU-145	29.9	>100	72.3	0.045
4. CNS Cancer					**9. Breast Cancer**				
SF-268	44.0	49.0	56.6	0.052					
SF-295	17.4	67.8	61.6	0.036	MCF7	27.4	58.1	29.7	0.036
SF-539	49.3	41.8	38.4	0.100	NCI/ADR-RES	27.0	68.3	33.3	0.078
SNB-19	38.6	38.6	43.9	X	MDA-MB231	28.6	>100	44.7	X
SNB-75	46.4	56.8	>100	X	HS578T	45.4	60.6	46.6	>1
U251	28.2	50.1	43.1	0.063	MDA-MB-435	29.0	16.4	19.0	>1
5. Melanoma					MDA-N	30.6	22.1	19.4	0.030
LOXIMVI	31.3	47.4	44.7	0.026	BT-54	35.2	43.7	45.4	66.0
MALME-3M	53.0	74.4	78.1	>1	T-47D	11.0	88.7	57.4	22.0

The X mark indicates not tested and the values >100 indicates absence of activity.
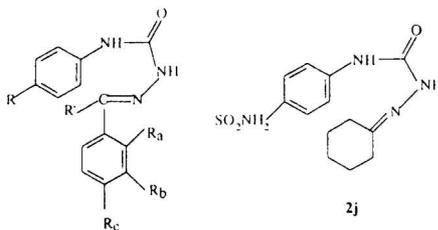
3. Discussion

In the preliminary testing of the aryl semicarbazones against 3 human tumour cell lines (Table 1) at a single dose of 11×10^{-4}M, compound **1c** of the 4-carboxyphenyl series was active against all

the 3-cell lines and two compounds of the 4-sulfamoylphenyl series (**2b** and **2c**) were active against the breast MCF7 cell line. These three compounds were selected by NCI for further evaluation for antitumour activity in an *in vitro* human disease-oriented tumour cell line arranged in nine subpanels, representing diverse histologies. Leukemia, colon, ovarian and breast cancer cell lines were relatively more sensitive than were the other cell lines. The GI_{50} values for these compounds along with the literature data of methotrexate $(MTX)^{15,16}$ are listed in Table 2. For these compounds, no TGI (total growth inhibition) or LC_{50} (50% cell kill) level was reached in all the cell lines (log TGI and log $LC_{50} > -4.00$, 100µM being the highest concentration tested). This indicated that the mechanism of antitumoral activity was cytostatic rather than cytotoxic. The nitrobenzylidene derivatives (**1c, 2c**) showed greater activity than the hydroxybenzylidene derivative (**2b**) against most of the cell lines. And the p-nitrobenzylidene deivative (**1c**) was found to be better than the o-nitro derivative (**2c**). The compound **1c** showed GI_{50} values of 7.85µM, 4.54µM, 11µM and 13.3µM against NCI-H522 (Lung), OVCAR-3 (Ovarian), T-470 (Breast) and K-562 (Leukemia) cell lines respectively. Compounds **1c, 2b** and **2c** showed greater activity than methotrexate (MTX) against BT-54 breast cancer cell line with GI_{50} of 35.2µM, 43.7µM, 45.4µM and 66µM respectively. The compound **1c** emerged as the most promising antitumour agent with more potency than methotrexate against NCI-H226 (Lung), BT-549 and T-47D (Breast) cell lines. Hence it can be concluded that some aryl semicarbazones could also serve as prototypic molecules for future developments in the anticancer field.

4. Experimental

4.1. Synthesis of compounds

Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. Elemental analyses were undertaken for all the compounds and were within 0.4% of the calculated values. Spectroscopic data were recorded on the following

Table 3. Physical constants of the compounds

Compound	R	R'	R_a	R_b	R_c	Yield	M.P.	Mol. For.	R_f^a
1a	COOH	H	OH	H	H	52	201	$C_{15}H_{13}N_3O_4$	0.88
1b	COOH	H	Cl	H	H	54	280	$C_{15}H_{12}N_3O_3Cl$	0.41
1c	COOH	H	H	H	NO_2	56	299	$C_{15}H_{12}N_4O_5$	0.65
2a	SO_2NH_2	H	H	H	H	53	143	$C_{14}H_{14}N_4O_3S$	0.55
2b	SO_2NH_2	H	OH	H	H	66	207	$C_{14}H_{14}N_4O_4S$	0.65
2c	SO_2NH_2	H	NO_2	H	H	75	163	$C_{14}H_{13}N_5O_5S$	0.55
2d	SO_2NH_2	H	H	OCH_3	OH	76	137	$C_{15}H_{16}N_4O_5S$	0.58
2e	SO_2NH_2	H	H	H	$N(Me)_2$	74	191	$C_{16}H_{19}N_5O_3S$	0.50
2f	SO_2NH_2	CH_3	H	H	H	66	147	$C_{15}H_{16}N_4O_3S$	0.58
2g	SO_2NH_2	CH_3	H	H	OH	64	149	$C_{15}H_{16}N_4O_4S$	0.57
2h	SO_2NH_2	CH_3	H	H	NH_2	54	122	$C_{15}H_{17}N_5O_3S$	0.71
2l	SO_2NH_2	H	H	-OCH_2O-		71	183	$C_{15}H_{14}N_4O_5S$	0.67
2j	SO_2NH_2	-	-	-	-	59	152	$C_{13}H_{18}N_4O_3S$	0.46

[a] In TLC eluant for compounds **1a-c** and **2a** was benzene and for other compounds **2b-j** benzene : ethanol (9.8:0.2).

The spectral data of a representative compound (**1b**) was as follows:
IR (KBr): 3450 (NH), 3440-3370 (OH of COOH), 3300-3240 (CONH), 1690 (C=O of COOH),

1640 (C=O), 1590 (C=N), 840 cm^{-1}. ^{1}H-NMR (CDCl$_3$) δppm: 5.8 (s, 1H, ArNH, D$_2$O

Scheme. Synthetic protocol of the compounds
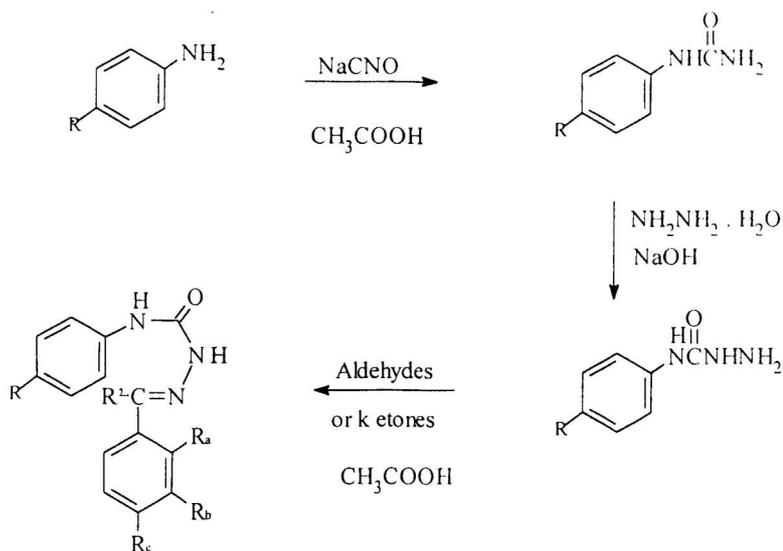
instruments: IR, Jasco infrared spectrometer; ^{1}H-NMR, Jeol Fx 90Q FT-NMR spectrometer (90MHz) and were consistent with the proposed structures. TLC was carried on silica gel chromatograms.

4.1.1. Preparation of 4-carboxyphenyl semicarbazones (1a-c)

The 4-carboxyphenyl semicarbazide was prepared as reported by Pandeya et al.[3] Equimolar quantities (0.003mol) of 4-carboxyphenyl semicarbazide and appropriate aldehyde were refluxed in ethanol in presence of glacial acetic acid for 1-2h. On cooling, the precipitate was obtained and recrystallized with 95% ethanol to give **1a-c**.

exchangeable), 6.9 (s, 1H, Carbimino H), 7.2-7.6 (m, 8H, ArH), 8.84 (s, 1H, COOH), 9.01 (s,

1H, CONH, D_2O exchangeable).

4.1.2. Preparation of 4-sulfamoylphenyl semicarbazones (2a-j)

Equimolar quantities (0.005mol) of 4-sulfamoylphenyl semicarbazide and the appropriate

aldehyde or ketone were refluxed in ethanol in presence of glacial acetic acid for 1-3h. The

product obtained after cooling was filtered and recrystallized with 95% ethanol.

The spectral data of a representative compound **2b** was as:

IR (KBr): 3450, 3300-3240 (NH), 1640 (C=O), 1590 (C=N), 1499, 1314, 1160 (S=O) and 840

cm^{-1}; ^{1}H-NMR (CDCl$_3$) δppm: 5.89 (s, 1H, ArNH, D_2O exchangeable), 6.83 (s, 1H, Carbimino

H), 7.2-7.7 (m, 9H, aromatic OH), 7.65 (bs, 2H, SO_2NH_2), 9.81 (s, 1H, CONH, D_2O

exchangeable).

4.2. Biological Evaluation

4.2.1. NCI *in vitro* Cytotoxicity Assay

The NCI uses the sulforhodamine B assay for assessing the cytotoxicity of test agents in their

panel of 60 cell lines[17]. Briefly, cell lines were inoculated into a series of 96-well microtitre

plates, with varied seeding densities depending on the growth characteristics of particular cell

lines. Following a 24-h drug-free incubation, test agents were added routinely at five 10-fold

dilutions with a maximum concentration of $10^{-4}M$. After 2 or 6 days of drug exposure, the

change in protein stain optical density allowed the inhibition of cell growth to be analyzed.

Acknowledgement

Reference

1. Cotti L., (1940) Biochem. Terap. Sper. 27, 366; (1943) C.A. 37, 4421.

2. Dimmock J. R., Baker G. B., (1994) Epilepsia 35(3), 645.

3. Pandeya S. N., Aggarwal N., Jain J. S., (1999) Pharmazie 54, 300.

4. Pandeya S. N., Mishra V., Singh P. N., Rupainwar D. C., (1998) Pharmacol. Res. 37, 17.

5. Fedorova O. V., Mordovskoi G. G., Rusinov G. L., Ovchinnikova I. G., Zueva M. N., Kravchenko M. A., Chupakhin O. N., (1998) Khim.-Farm. Zh. 32(2), 11.

6. Wang Y., Cai S. X., Lan N. C., Keena J. F. W., Illyin V. I., Weber E., WO 98 47,869 (1998) C. A. 26, P343416v.

7. Schulze W., Jungstand W., Gutsche W., Wohlrabe K., Horn G., (1978) Pharmazie 33, 111.

8. Fichtner J., Arnold W., (1983) Pharmazie 38, 30.

9. Dimmock J. R., Kirkpatrick D. L., Negrave L. E., Russel K. L., Pannekock W. J., (1991) Can. J. Pharm. Sci. 16, 1.

10. Gugova R., Raikov Z., Zakhariev S., Demirov G., Todorov D., Harionova M., (1989) Drugs Exp. Clin. Res. 15, 497.

11. Schaffner K., Traxler P., Zimmermann M., Wehrli W., Scmidtappin K. H., EP 183649, (1986) C. A. 105, 152929j.

12. Vancurova J., Krepelka J., Rezabez K., Melka M., CS212670, (1985) C. A. 102, P2037042.

13. Brzozowski Z., (1998) Acta Pol. Pharm. 55(3), 233.

14. Conti G. M., Gaozza C. H., (1996) Farmaco 51(6), 419.

15. Rosowsky A., Wright J. E., Vaidya C. M., Bader H., Forsch R. A., Mota C. E., Pardo J., Chen C. S., Chen Y. N., (1998) J. Med. Chem. 41, 5310.

16. Rosowsky A., Papouli A.T., Forsch R. A., Queener S. F., (1999) J. Med. Chem. 42, 1007.

17. Boyd M. R., Paull K. D., (1995) Drug Development Res. 34, 91.