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Synthesis and cytotoxic activity of silacycloalkyl-substituted heterocyclic aldehydes and their thiosemicarbazones

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A series of 5-[1-methylsilacyclo-pentyl/-hexyl]-2-furfural, 5-[1-methylsilacyclo-pentyl/-hexyl]-2-thiophene carbaldehyde and 1,1-bis(5-formyl-2-furyl)silacyclo-pentane/-hexane and their thiosemicarbazones has been synthesized and subjected to antitumour assay. The effects of the substituents and the heterocycle were examined to establish structure–activity relationships. Thiosemicarbazones of 5-(1-methylsilacyclohexyl)furfural and 5-(1-methylsilacyclopentyl)furfural were very active (1.0–4.0 μg ml^{-1}) *in vitro* against human fibrosarcoma HT-1080 and mouse hepatoma MG-22A cells. At the same time, they were less toxic for normal fibroblasts 3T3. All compounds synthesized exhibited low or moderate toxicity (LD_{50} 152–1904 mg kg^{-1}).

KEYWORDS: 5-[1-methylsilacyclo-pentyl/-hexyl]-2-furfural; 5-[1-methylsilacyclo-pentyl/-hexyl]-2-thiophene carbaldehyde; 1,1-bis(5-formyl-2-furyl)-1-silacyclo-pentane/-hexane; thiosemicarbazones; cytotoxicity; toxicity

INTRODUCTION

The biological activity of organic compounds can be changed or improved by the introduction of an organosilicon substituent, which increases the lipophilicity and may also change the metabolism of the compound.[1] This influence depends on the structure of the organic substituents bound to the silicon atom. In some cases the inclusion of the silicon into the carbocyclic ring increases the cytotoxicity of the compound.[2,3] In addition, a series of thiosemicarbazones has been shown to possess cytotoxic activity against the cancer cells.[4,5]

Therefore, we have synthesized new heterocyclic aldehydes and their thiosemicarbazones containing silacyclopentyl and silacyclohexyl substituents and determined their cytotoxic activity *in vitro* on HT-1080, MG-22A and NIH 3T3 cell lines.

MATERIALS AND METHODS

Chemistry

^{1}H NMR spectra were recorded on a Varian 200 Mercury instrument (200 MHz) using $CDCl_3$ as a solvent and hexamethyldisiloxane ($\delta = 0.055$ ppm) as internal standard. Gas chromatography–mass spectrometry (GC–MS) was undertaken using HP 6890 (70 eV) apparatus. GC analysis was performed on a Varian instrument equipped with flame-ionization detector using column packed with 5% OV-17 Chromosorb W-HP (80–100 mesh). Thiophene-2-carbaldehyde and furan-2-carbaldehyde were distilled prior use; *N*-methylpiperazine was dried on CaH_2 and distilled prior use; 1-chloro-1-methylsilacyclo-pentane and -hexane were synthesized by a known method.[6]

Cytotoxicity *in vitro*

Monolayer tumour cell lines MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), NIH 3T3 (normal mouse fibroblasts) were cultivated for 72 h in standard Dulbecco's modified Eagle's medium (Sigma) without an indicator and antibiotics.[7] After the ampoule was defrosted, not more than four passages were performed. The control cells and cells with test substances in the range of $(2–5) \times 10^4$ cells/ml concentration (depending on line nature) were placed on separate 96-well plates. Solutions containing test compounds were diluted and added to the wells to give final concentrations of 50, 25, 12.5 and 6.25 μg ml^{-1}. The control cells were treated in the same manner, but with the absence of the test compounds. Plates were cultivated for 72 h. The quantity of cells surviving was determined using crystal violet

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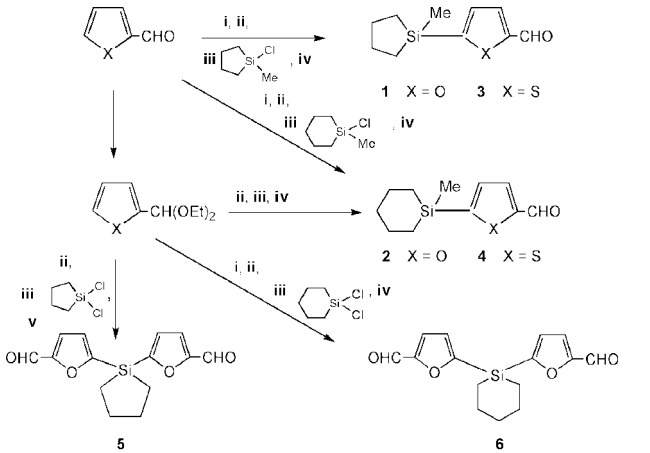
(CV), neutral red (NR) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) coloration, which was assayed by multiscan spectrophotometer. The quantity of living cells on the control plate was taken in calculations as 100%.[7,8] The concentration of NO was determined according to Freshney.[8] Mean lethal dose (LD_{50}) was determined on 3T3 cells (alternative to LD_{50} *in vivo* test) according to the protocols of Committee on the Validation of Alternative Methods (ICCVAM) and National Toxicology Program (NTP) of the Interagency Center for the Evaluation of Alternative Methods (NICEATM).

RESULTS AND DISCUSSION

Silicon- and germanium-containing heterocyclic aldehydes have been regioselectively prepared by a one-pot procedure[9] from the corresponding furan- and thiophene-carbaldehydes using lithium *N*-methylpiperazide (LNMP)–butyllithium–chlorocyclosilane–water as the sequence of reagents (Scheme 1). After blocking with a suitable aminolithium compound, the aldehyde function is regenerated by hydrolysis in neutral or weakly acid conditions. Mild conditions for the hydrolysis are required to preserve the silacyclo group bound to the heterocycle. In the case of 2-furaldehyde and 2-thiophenecarbaldehyde, this procedure gives the 5-metallated derivatives regiospecifically in good yield.

The second synthetic route to silyl-substituted aldehydes was a carbonyl blocking by conversion to diethylacetal, metallation by *n*-BuLi and substitution of lithium atoms with the corresponding electrophile. The deprotection of the aldehyde function was carried out using a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA).[10]

Yields, boiling points, mass spectra and ^{1}H NMR data for the new compounds obtained are summarized in Table 1. All



i LNMP/THF, -78°C; ii n-BuLi, -20°C, 5h; iii cyclochlorosilane, -78°C → r.t., 5h; iv H_2O (H^+)
v H_2O/*p*-TSA

Scheme 1.

these aldehydes **1**–**6** were involved in a condensation reaction with thiosemicarbazide. Yields, melting points, element analysis and ^{1}H NMR data for the new thiosemicarbazones **7**–**12** obtained are summarized in Table 2.

The new organosilicon compounds **1**–**12** were evaluated for their cytotoxic activity *in vitro* against two monolayer tumour cell lines, i.e. HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma), and mouse normal fibroblasts NIH 3T3. The experimental results are presented in Tables 3 and 4.

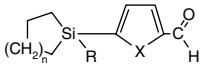
Most of the thiosemicarbazones (**7, 8**, and **10**–**12**) exhibited higher cytotoxicity on both cancer cell lines than the corresponding aldehydes (**1, 2**, and **4**–**6**). The exceptions were the thienyl derivative **9** and the bisfuryl derivative **11** (on MG-22A cells) containing a silacyclopentyl group. At the same time, thiosemicarbazones **7**–**11** were also less toxic for normal fibroblasts NIH 3T3. Only in the case of the bisfuryl derivative with a 6-membered silacycle was the aldehyde **6** less toxic than the corresponding thiosemicarbazone **12**.

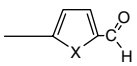
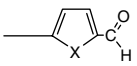
In both series of compounds the cytotoxicity depended on the size of the silicon-containing ring, on the type of heterocycle and on their number in the molecule.

The furan aldehydes containing a 5-membered silacycle inhibited both cancer cell lines more effectively than the compounds containing a 6-membered ring. Some cell selectivity was observed in the thiosemicarbazone series: 5-membered ring compounds were more active against MG-22A cells, but 6-membered ring compounds were more active against HT-1080. In contrast, in the bisfuryl series the highest cytotoxicity against both cancer cell lines was exhibited by 6-membered ring compounds, both for aldehydes and thiosemicarbazones. In the thienyl series, thiosemicarbazones containing a 6-membered silacycle were also more active against HT-1080 cells, but there was difference in the mode of action on MG-22A cells: 6-membered ring compounds influenced the activity of mitochondrial enzymes in the cell more, whereas the 5-membered ring compounds more effectively attacked the cell membranes.

In most cases the furan aldehydes and their thiosemicarbazones exhibited higher cytotoxicity than the corresponding thiophene derivatives. The exception was aldehyde **4**, containing a 6-membered silacycle. The 6-membered ring derivative of bisfuryl aldehyde **6** being more active than the corresponding monofuryl aldehyde **2** was also the single exception in this series of compounds. In all other cases (aldehydes and thiosemicarbazones) the bis-derivatives were less cytotoxic.

The highest cytotoxic activity on HT-1080 cells was recorded for thiosemicarbazones of furan aldehydes containing the 6-membered silacycle **8** (IC_{50}1 μg ml^{-1}) and the 5-membered silacycle **7** (IC_{50}2.7 μg ml^{-1}), the latter also being the most active compound against MG-22A cells. This compound readily increased the NO concentration in the cultural medium of the HT-1080 line (700%); but, in general, there was no direct correlation between the cytotoxicity and the NO-inducing ability of these types of compound. It must be noted that compound **7**, although being highly cytotoxic on tumour

Table 1. Physical and analytical data for the aldehydes 

R	*n*	X	Synthesis method	B.p. (°C)/mmHg	Yield (%)	^{1}H NMR, δ (ppm); J (Hz)	MS–GC m/z (%)
Me (**1**)	1	O	A B	100–105/5	45 85	0.56 (3H, s, Me), 0.80–1.38 (4H, m, CH_2–Si), 1.66–1.92 (4H, m, CH_2–C), 6.89 (1H, d, H^3), 7.33 (1H, d, H^4), 9.78 (1H, s, CHO); $J_{3,4}$ 3.9	194 (M^+, 31), 179 ($M^+ - Me$, 40), 166 (40), 151 (100), 138 (45), 123 (65), 110 (25), 95 (67), 85 (25), 79 (36), 67 (31), 53 (40), 43 (58)
Me (**2**)	2	O	A	110–115/5	57	0.33 (3H, s, Me), 0.55–1.11 (4H, m, CH_2–Si), 1.33–2.00 (6H, m, CH_2–C), 6.82 (1H, d, H^3), 7.22 (1H, d, H^4), 9.73 (1H, s, CHO); $J_{3,4}$ 3.8	208 (M^+, 49), 193 ($M^+ - Me$, 53), 179 (22), 165 ($M^+ - C_2H_4 - Me$, 100), 152 ($M^+ - 2C_2H_4$, 51), 139 ($M^+ - 2C_2H_4 - Me$, 83), 123 (57), 112 (83), 97 (56), 85 (69), 77 (45), 69 (51), 55 (48)
Me (**3**)	1	S	A B	126–130/7	61 90	0.47 (3H, s, Me), 0.62–1.0 (4H, m, CH_2–Si), 1.42–1.96 (4H, m, CH_2–C), 7.35 (1H, d, H^3), 7.78 (1H, d, H^4), 9.91 (1H, s, CHO); $J_{3,4}$ 3.9	210 (M^+, 45), 195 ($M^+ - Me$, 66), 182 ($M^+ - C_2H_4$, 38), 167 ($M^+ - C_2H_4 - Me$, 43), 154 ($M^+ - 2C_2H_4$, 100), 139 ($M^+ - 2C_2H_4 - Me$, 72), 126 (10), 111 (13), 97 (47), 85 (27), 75 (21), 69 (38), 43 (46)
Me (**4**)	2	S	A	125–130/5	71	0.31 (3H, s, Me), 0.71–1.11 (4H, m, CH_2–Si), 1.33–1.89 (6H, m, CH_2–C), 7.33 (1H, d, H^3), 7.80 (1H, d, H^4), 10.0 (1H, s, CHO); $J_{3,4}$ 4.0	224 (M^+, 58), 209 ($M^+ - Me$, 42), 181 ($M^+ - C_3H_7$, 86), 168 ($M^+ - 2C_2H_4$, 20), 155 (100), 139 (69), 112 (38), 97 (75), 85 (38), 75 (31), 69 (30), 53 (42)
 (**5**)[a]	1	O	B	m.p., 94–95	90	0.96–1.36 (4H, m, CH_2–Si), 1.58–1.93 (4H, m, CH_2–C), 6.93 (2H, d, H^3), 7.27 (2H, d, H^4), 9.78 (2H, s, CHO); $J_{3,4}$ 3.9	274 (M^+, 100), 246 ($M^+ - C_2H_4$, 30), 218 ($M^+ - 2C_2H_4$, 73), 203 (12), 189 ($M^+ - C_4H_9Si$, 65), 175 (16), 162 (37), 145 (33), 123 (100), 110 (38), 95 (64), 79 (100), 66 (63), 53 (80), 45 (85)
 (**6**)[b]	2	O	B	m.p., 99–100	85	1.07–1.33 (4H, m, CH_2–Si), 1.33–2.00 (6H, m, CH_2–C), 6.93 (2H, d, H^3), 7.29 (2H, d, H^4), 9.76 (2H, s, CHO); $J_{3,4}$ 3.8	288 (M^+, 100), 260 ($M^+ - C_2H_4$, 22), 231 (41), 219 (16), 203 (15), 192 (44), 176 (18), 164 (58), 147 (31), 136 (48), 123 (100), 115 (30), 103 (32), 91 (63), 79 (86), 65 (48), 53 (60), 45 (60)

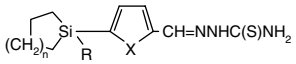
[a] Anal. (**5**) Found/calc. (%): C, 55.02/54.86; H, 4.60/4.61; $C_{14}H_{14}O_4Si$.
[b] Anal. (**6**) Found/calc. (%): C, 56.87/56.21; H, 5.15/5.03; $C_{15}H_{16}O_4Si$.

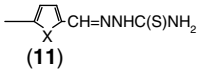
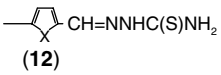
cell lines, was also of low toxicity for normal fibroblasts NIH 3T3 (IC_{50}604 μg ml^{-1}, LD_{50}1600 mg kg^{-1}).

EXPERIMENTAL

5-(1-Methylsilacyclohexyl)-2-furfural (2): method A

To a suspension of lithium *N*-methylpiperazide, prepared from *N*-methylpiperazine (20 mmol) in 40 ml of dry tetrahydrofuran (THF) and *n*-BuLi (20 mmol) in hexane at −78 °C, was added 2-furaldehyde (18 mmol) at −78 °C. The mixture was stirred for 15 min and a hexane solution of *n*-BuLi (20 mmol) was added and the reaction mixture stirred at −20 °C for 4 h. A solution of 1-methyl-1-chlorosilacyclohexane (20 mmol) in 10 ml absolute THF was added dropwise at −78 °C and the mixture was allowed to warm to room temperature and stirred for 10 h. The mixture was hydrolysed by stirring with 1 M HCl (120 ml) at 0 °C for 10 min and neutralized with aqueous Na_2CO_3 solution. The resulting mixture was extracted with Et_2O, and the organic layer was dried with $MgSO_4$ and concentrated. The mixture was filtered through Al_2O_3; after evaporation of Et_2O the residue

Table 2. Physical and analytical data for the thiosemicarbazones 

R	*n*	X	M.p. (°C)	Yield (%)	Anal. Found/calc. (%) C	H	N	S	^{1}H NMR, δ (ppm), J (Hz)
Me (**7**)	1	O	141–142	55	49.60 49.40	6.32 6.41	15.75 15.71	12.05 11.97	0.31 (3H, s, Me), 0.51–0.95 (4H, m, CH_2–Si), 1.35–1.75 (4H, m, CH_2–C), 6.82 (1H, d, H^3), 7.05 (1H, d, H^4), 7.55 (1H, s, NH_2), 7.91 (1H, s, CH = N), 8.17 (1H, s, NH_2), 11.22 (1H, s, NH); $J_{3,4}$ 3.7
Me (**8**)	2	O	135–137	70	51.18 51.21	6.61 6.80	15.01 14.93	11.35 11.39	0.27 (3H, s, Me), 0.57–1.11 (4H, m, CH_2–Si), 1.24–1.93 (6H, m, CH_2–C), 6.82 (1H, d, H^3), 7.09 (1H, d, H^4), 7.60 (1H, s, NH_2), 7.96 (1H, s, CH = N), 8.22 (1H, s, NH_2), 11.44 (1H, s, NH); $J_{3,4}$ 3.9
Me (**9**)	1	S	173–175	58	47.06 46.57	6.00 6.04	14.45 14.82	22.69 22.62	0.35 (3H, s, Me), 0.48–0.88 (4H, m, CH_2–Si), 1.41–1.81 (4H, m, CH_2–C), 7.28 (1H, d, H^3), 7.48 (1H, d, H^4), 7.55 (1H, s, NH_2), 8.17 (1H, s, NH_2), 8.26 (1H, s, CH = N), 11.46 (1H, s, NH); $J_{3,4}$ 3.8
Me (**10**)	2	S	174–176	77	48.56 48.44	6.21 6.43	14.17 14.12	21.84 21.55	0.27 (3H, s, Me), 0.67–1.07 (4H, m, CH_2–Si), 1.27–1.84 (6H, m, CH_2–C), 7.24 (1H, d, H^3), 7.42 (1H, d, H^4), 7.51 (1H, s, NH_2), 8.11 (1H, s, NH_2), 8.22 (1H, s, CH = N), 11.42 (1H, s, NH); $J_{3,4}$ 3.9
 (**11**)	1	O	204–206	45	45.69 45.50	4.79 4.75	19.98 19.02	15.25 15.02	0.82–1.17 (4H, m, CH_2–Si), 1.56–1.84 (4H, m, CH_2–C), 6.91 (2H, d, H^3), 7.06 (2H, d, H^4), 7.59 (2H, s, NH_2), 7.96 (2H, s, CH = N), 8.12 (2H, s, NH_2), 11.42 (2H, s, NH); $J_{3,4}$ 4.0
 (**12**)	2	O	209–210	67	47.03 46.98	4.97 5.10	18.64 19.34	14.23 14.76	0.86–1.25 (4H, m, CH_2–Si), 1.28–1.95 (6H, m, CH_2–C), 6.93 (4H, m, H^3, H^4), 7.57 (2H, s, NH_2), 7.95 (2H, s, CH = N), 8.15 (2H, s, NH_2), 11.37 (2H, s, NH); $J_{3,4}$ 3.9

was distilled *in vacuo* to yield 2.12 g (57%) of **2**, b.p. 110–115 °C/5 mmHg.

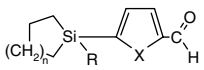
5-(1-Methylsilacyclopentyl)-2-furfural (1): method B

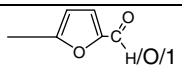
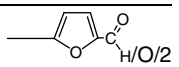
A suspension of 2-furfuraldiethylacetal[10] (20 mmol) in 70 ml of anhydrous diethyl ether was cooled to −25 °C. To the cold, stirred mixture was added (20 mmol) of a hexane solution of *n*-BuLi at such a rate so as not to exceed a reaction temperature of −20 °C. After addition, the mixture was allowed to warm to −10 °C and stirred for 4 h. A solution of 1-methyl-1-chlorosilacyclopentane (20 mmol) in 10 ml anhydrous diethyl ether was added dropwise at −25 °C and the mixture was allowed to warm to room temperature and stirred for 10–12 h. After that the mixture was refluxed for 5 h. The mixture was cooled, the precipitated lithium chloride was removed by filtration through Al_2O_3, and the filtrate was concentrated by evaporation of solvents to yield 3.97 g (75%) of yellow oil. MS *m/z* (%): 268 (M^+, 5), 223 (M^+ − OEt, 100), 195 (40), 139 (10), 127 (12), 99 (8), 71 (8) 45 (15). The ethereal solution (10 ml) of 5-(1-methylsilacyclopentyl)-2-furfuraldiethylacetal (3.97 g) was allowed to reflux with *p*-TSA and 7 ml of water for 6 h. The layers were separated and the aqueous phase extracted with diethyl ether. The ether layer was combined with the ethereal extracts and washed with 5% Na_2CO_3 solution until the washings remained basic. The extracts were dried over $MgSO_4$, filtered through Al_2O_3, concentrated and fractionated *in vacuo* at 100–105 °C/5 mmHg to give 2.63 g (90.7%) of aldehyde **1**.

Aldehydes **3**–**6** were prepared analogously using methods A and B (Table 1). Yields, b.p., ^{1}H NMR and mass spectral data of compounds **1**–**6** are presented in Table 1.

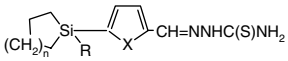
Synthesis of thiosemicarbazones 7–12: general procedure

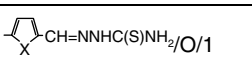
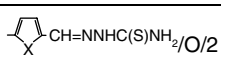
Thiosemicarbazide (2.4 mmol) in 10 ml of water was added to a solution of 2.4 mmol of aldehyde in 5 ml of ethanol. The mixture was heated 3 h at 60–70 °C. The reaction mixture was

Table 3. Cytotoxicity (IC_{50} μg ml^{-1})[a] of 

Cell line	Method	R/X/*n* Me/O/1 (**1**)	Me/O/2 (**2**)	Me/S/1 (**3**)	Me/S/2 (**4**)	 (**5**)	 (**6**)
HT-1080	CV	3	29	5	4	59	15
	MTT	2.6	32	11	6	44	11
	$NO^{\bullet}$[b]	450	100	1450	250	27	300
MG-22A	CV	3.4	47	11	15	29	24
	MTT	1.4	42	20	20	18	10
	$NO^{\bullet}$	83	37	300	350	113	300
3T3	NR	10.2	94	19	26	127	14
3T3	LD_{50}(mg kg^{-1})	227	625	319	370	889	352

[a] IC_{50} (μg ml^{-1}) providing 50% cell killing effect (CV: coloration; MTT: coloration).
[b] $NO^{\bullet}$: concentration (%) (CV: coloration).

Table 4. Cytotoxicity (IC_{50} μg ml^{-1}) of 

Cell line	Method	R/X/*n* Me/O/1 (**7**)	Me/O/2 (**8**)	Me/S/1 (**9**)	Me/S/2 (**10**)	 (**11**)	 (**12**)
HT-1080	CV	2.7	1	100	17	34	5.7
	MTT	2.8	1	100	1	34	4.5
	$NO^{\bullet}$	700	350	31	1	225	160
MG-22A	CV	1.1	2	1	nce[a]	>100	6.4
	MTT	2.5	4	65	1	61	5.2
	$NO^{\bullet}$	325	200	64	3	17	150
3T3	NR	604	67	119	770	135	1.4
3T3	LD_{50}(mg kg^{-1})	1600	647	822	1904	1094	152

[a] nce: no cytotoxic effect.

cooled and filtered, the resulting precipitate was washed with water and recrystallized from a water/ethanol mixture (1 : 1). The reaction yields, melting points, analysis and ^1H NMR are summarized in Table 2.

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