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NOVEL CYANOGUANIDINES WITH POTENT ORAL ANTITUMOUR ACTIVITY

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Abstract: 4-Pyridyl cyanoguanidines with hydrophobic aromatic side chains showed potent antiproliferative activity in the human breast and lung cancer cell lines MCF-7, NYH and H460. *In vivo*, treatment with N-(6-chlorophenoxyhexyl)-N'-cyano-N''-4-pyridylguanidine (**18**, 20 mg/kg/day po.), gave a complete remission of tumours in a model of NYH inoculated nude mice. © 1997 Elsevier Science Ltd.

In our search for novel antitumour compounds, activity was found in our *in vivo* screen, with N-(5-phenoxypentyl)-N'-cyano-N''-4-pyridylguanidine (**4**) (Figure 1) in a rat model with Yoshida ascites sarcoma cell tumours.

This finding prompted us to search for more potent antitumour compounds, exploring the structure-activity relationship for this substance class. Our primary selection was made *in vitro*, determining antiproliferative activity in a series of cancer cell lines, among which MCF-7, a human breast cancer, NYH, a human small cell lung cancer and NCI-H460, a human non-small cell lung cancer. These cell lines represent important targets for cancer therapy since breast cancer and lung cancer are among the most common of all types of cancer in man. The treatment of these types of cancer is most often based on a combination of surgery and chemotherapy. However, in cases where surgery is not curative and chemotherapy is the only choice, most tumours develop resistance to the treatment over a short period of time. Thus, there is today no efficient chemotherapeutic treatment available for resistant cancers, such as the non-small cell lung cancer.

Structure-activity relationships (SAR)

Pyridyl cyanoguanidines are known as potassium channel openers.[1] Among them, Pinacidil (N-1,2,2-trimethylpropyl-N'-cyano-N''-4-pyridylguanidine) is a structural prototype and as such a very potent hypotensive compound. Replacement of the side chain of Pinacidil by longer aryl containing side chains as in N-(5-phenoxypentyl)-N'-cyano-N''-4-pyridylguanidine (**4**) causes a loss of activity in this respect. However, compound **4** with no potassium channel opening activity was able to increase life span in an *in vivo* rat model carrying Yoshida ascites tumours.

To explore this finding further and to study the structure-activity relationships for this compound class *in vitro* the following modifications were made. Based on the first lead compound, variations of the chain length, pyridyl substitution pattern and substitution in the end aryl group were made. Also, bioisosteric replacements of the cyanoguanidine group were tried.

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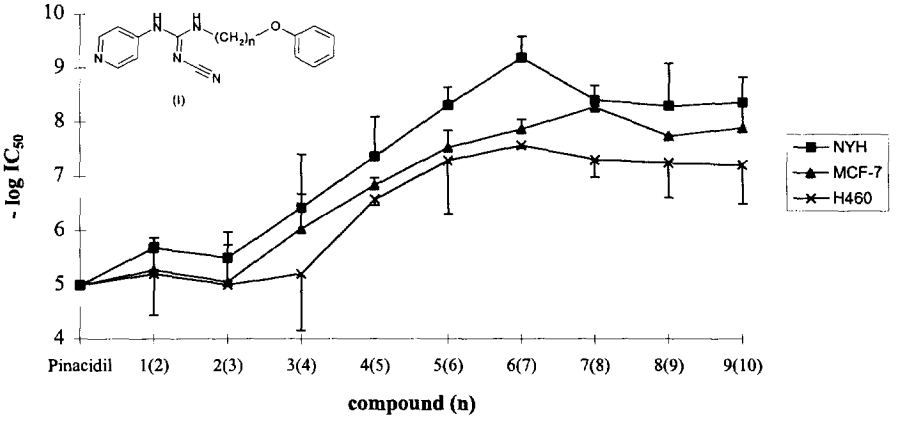
The cyanoguanidine moiety was found crucial for the antiproliferative effect of these compounds. Replacement of the cyanoguanidine group by e.g. urea and thiourea groups or 1,1-diamino-2-nitroethene, as well as 3,4-diamino-1,2,5-thiadizole-1-oxide in the basic structure (I) (Table 1) with an "optimal" side chain did not give compounds with any significant activity (data not shown).

On the other hand the chain length was found to be of considerable importance for the activity of the compounds, as seen for the homologous compounds **1-9** (Figure 1). An optimal inhibitory effect on cell proliferation in the three human cell lines NYH, MCF-7 and H460 was found for homologues of compound **4** with a chain length of 7 (compound **6**) and 8 (compound **7**) carbons, with an IC_{50}, respectively, of 0.64 nM and 3.8 nM in NYH cells. The shorter chain compounds **1-3**, were found less active (Figure 1).

Figure 1. Antiproliferative activity *in vitro* of chain length homologous cyanoguanidines

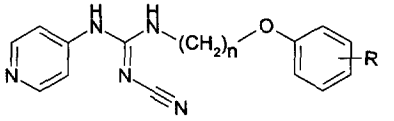


Compounds **10** and **11**, the 3-pyridyl analogues of **5** and **6**, were 10-100 times less potent in this assay (Table1). This was generally true for 3-pyridyl analogues as compared with active 4-pyridyl derivatives. Substitution of the pyridyl group by other simple aromatic groups e.g. phenyl, substituted phenyl, benzyl gave less active compounds in our *in vitro* assays (data not shown).

Monosubstitution with chloro, nitro or methoxy groups in the 2-, 3- or 4-position of the terminal phenoxy group in the cyanoguanidine **5**, with a 6 carbon alkyl chain was then investigated. The most potent compounds *in vitro* were the 2-substituted derivatives with IC_{50}'s in the nM range (Table 1). No clear preference was found for different substituents (Cl, NO_2, OMe) in identical positions.

Following positive *in vivo* results (*vide infra)* chain length homologues of compound **18** (compound code CHS828) were prepared. However, no significant improvement of the *in vitro* activity was found for the close homologues **21-23** (Table 1).

Table 1. Antiproliferative activity *in vitro* of cyanoguanidine analogues (IC_{50} ± SD (nM)).



(I)

Compound	n	R	NYH	MCF-7	H460
4 vs 3 pyridyl					
5	6	H	4.9 ± 2.2	30.0 ± 14.0	52.0 ± 5.4
6	7	H	0.6 ± 0.3	14.0 ± 21.0	27.0 ± 24.0
10 (3-pyridyl)	6	H	52.0 ± 16.0	230.0 ± 120.0	530.0 ± 100.0
11 (3-pyridyl)	7	H	22.0 ± 29.0	42.0 ± 4.6	360.0 ± 260.0
monosubstitution					
12	6	2-Cl	0.6 ± 0.1	1.9 ± 1.6	5.8 ± 0.8
13	6	2-OMe	0.5 ± 0.1	1.1 ± 1.2	21.0 ± 18.0
14	6	2-NO_2	0.6 ± 0.1	6.3 ± 1.3	41.0 ± 4.0
15	6	3-Cl	6.1 ± 1.8	8.8 ± 6.5	59.0 ± 6.2
16	6	3-OMe	1.9 ± 3.0	3.8 ± 1.8	38.0 ± 22.0
17	6	3-NO_2	5.7 ± 1.0	31.0 ± 17.0	74.0 ± 14.0
18 (CHS828)	6	4-Cl	2.7 ± 2.0	31.0 ± 24.0	50.0 ± 20.0
19	6	4-OMe	12.0 ± 17.0	37.0 ± 19.0	37.0 ± 24.0
20	6	4-NO_2	52.0 ± 12.0	120.0 ± 72.0	590.0 ± 33.0
chain length phenoxy					
21	5	4-Cl	6.3 ± 1.0	53.0 ± 7.9	590.0 ± 150.0
22	7	4-Cl	6.2 ± 0.8	56.0 ± 22.0	69.0 ± 11.0
23	8	4-Cl	15.0 ± 17.0	54.0 ± 21.0	480.0 ± 65.0
tri-substituted					
24	6	2,4,5-Cl_3	2.1 ± 2.4	4.4 ± 0.8	13.0 ± 12.0

In vitro.

The *in vitro* activity of the compounds was determined in a selected set of human cancer cell lines: the MCF-7, a model of hormone dependent breast cancer[2] and the NCI-H460, derived from a large cell carcinoma of the lung, both relatively chemosensitive,[3,4] and the NYH derived from small cell lung cancer, a cell line characterized by resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea[5]. In addition, the antiproliferative activity of compounds **18** (CHS828) and **4** was determined in a few additional cancer cell lines and was compared to our reference compounds Daunomycin and Paclitaxel (Table 2). The data obtained showed that compound **18** had a potency similar to that of the reference compounds in the MCF-7 and NYH cell lines, but it showed a lower activity in H460, B16, Lewis, Yoshida, and Walker cell lines. Thus, Daunomycin and Paclitaxel have a more broad cytotoxic effect in the tumour types tested than the cyanoguanidines. The antiproliferative activity was further tested in human lung fibroblasts to determine the extent of cytotoxic effects on normal cells. In these cells no cytotoxic effects were observed with compound **18** at concentrations < 40 nM, but strong cytotoxic effects were found with Paclitaxel, which was active at concentrations from 0.9 nM.

Table 2. Antiproliferative and cytotoxic effect of compounds **4** and **18** in comparison with Daunomycin and Paclitaxel (IC_{50} ± SD (nM)).

Cell line/Comp.	4	18 (CHS828)	Daunomycin	Paclitaxel
NYH	44,0 ± 8.1	2.7 ± 2,0	3,9 ± 0,9	4,3 ± 3,3
MCF-7	150,0 ± 110,0	31,0 ± 24,0	39,0 ± 4,9	4,9 ± 0,8
H460	270,0 ± 350,0	50,0 ± 20,0	3,5 ± 3,7	7,0 ± 0,2
B16	530,0 ± 17,0	280,0 ± 300,0	9,2 ± 1,3	8,0 ± 1,4
Lewis	300,0 ± 360,0	560,0 ± 64,0	61,0 ± 5,7	61,0 ± 23,0
Yoshida	660,0 ± 200,0	140,0 ± 86,0	7,4 ± 1,8	7,9 ± 0,0
Walker	610,0 ± 110	500,0 ± 180,0	47,0 ± 2,8	150,0 ± 0,7
Fibroblasts	ND	40,0 ± 42.4	ND	0,9 ± 0,2

In vivo

All compounds were tested in our primary *in vivo* screen, a rat model with Yoshida ascites tumours.[6,7] This assay was intended to be used in the selection of compounds for a further evaluation. However, only a few cyanoguanidines were found significantly active e.g. compound **18** with a 100 % increase of life span (data not shown). Even though the active compounds increased life span they did not seem to decrease the tumour burden in the animals. This may be due to the relatively low (μM) antiproliferative activity found *in vitro* in Yoshida tumour cells. Also, the bioavailability of the compounds may differ when administered orally.

To supplement this test, the most active compounds *in vitro* in the NYH assay were selected for *in vivo* testing in nude mice carrying this same tumour as xenotransplants.[8,9] When treatment was initiated on day 14, the median tumour size was 50 mm^2 and on day 28, when treatment was stopped, the control tumour areas had increased 4 to 5 fold. In this assay, compound **18** (CHS828) was the most active, causing not only a stagnation of tumour growth, but also a complete remission of the tumours (Table 3).

Table 3. Antitumour activity of **18** (CHS828) and analogues *in vivo* in a NYH treatment model in nude mice.

Compound	6	16	24	18	18	18	Daunomycin	Paclitaxel
Amount (mg/kg)	20[a]	20[a]	20[a]	20[a]	25[a]	50[a]	0.5[b]	10[c]
No of tumours	11	8	9	20	9	13	8	7
T/C (%)[d]	35[e]	42[e]	0[e]	0[e]	0[f]	0[f]	105[f]	48[e]

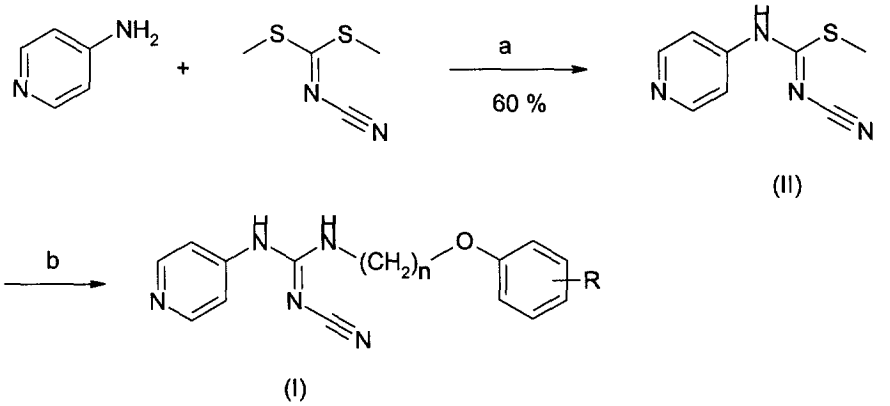
a) po., b) ip. and c) sc. administration once daily. d) Tumour size calculated as area from two perpendicular diameters. $p < 0.05$ Mann-Whitneys U-test. T/C (%) = (Median treated tumour size divided by median control tumour size) x 100. Vehicle controls tumour area median (95 % confidence interval) : e) 242 mm^2 (185-300), n = 14; f) 311 mm^2 (106-353), n = 9.

The animals in this assay were treated once daily orally with compound **18** at doses of 20 or 50 mg/kg starting from day 14 and continuing to day 28. In a 4 week follow-up observation period, after the end of treatment on day 28, none in the high but two mice in the low dosage group had tumour recurrence. The mice did not seem to be adversely affected by the medication, which was indicated by a normal increase in body weight. The reference compound Paclitaxel significantly, but not totally, reversed tumour growth, while Daunomycin had virtually no effect in this assay. Both reference compounds were tested at a dose close to the maximum tolerated dose in mice (Table 3).

These exciting results prompted us to test some selected cyanoguanidines from the *in vitro* optimized series (Table1). Thus, a significant antitumour effect was found with the 7-carbon phenoxy analogue **6**, the mono 2-methoxy analogue **16** and the trichloro analogue **24** (Table 3). Compound **24** was found as active as compound **18**, displaying however toxic side effects in mice and rats.

Chemistry

The cyanoguanidines (I) were prepared by coupling the starting amino pyridine with S,S'-dimethyl N-cyano-dithio-iminocarbonate.[10,11] The obtained key intermediate, S-methyl-N-cyano-N'-4-pridylisothiourea (II), was then coupled with a selection of primary amines to give (I) in excellent yield (Scheme 1). All amines were prepared by conventional methods in good yield. Alternatively, the title compounds (I) were obtained by reacting the corresponding pyridylthioureas with an excess of cyanamide and N-N'-dicyclohexylcarbdiimide.[1,10,11]



Scheme 1. a) NaH, DMAP, DMF, 16h; b) amine, DMAP, Et_3N, pyridine, 5h, 55 °C.

Conclusion

A novel series of cyanoguanidines with potent antitumour activity was prepared *via* a short convergent synthesis. The most active compounds found in our SAR studies were 4-pyridyl cyanoguanidines with a 6-8 carbon long side chain and a terminal, substituted phenoxy function. These compounds, in particular compound **18**, CHS828, showed a high antiproliferative activity *in vitro* in human cancer cell lines. Furthermore, they exhibited a very potent antitumour activity in a therapeutic treatment model in nude mice, inoculated with the human lung cancer NYH cells. Thus, compound **18** caused a complete remission of tumours, when administered orally once daily for two weeks.

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11. Preparations: S-Methyl N-cyano-N'-4-pyridylisothiourea (II): To 4-Aminopyridine (7.6 g, 81 mmol) and S,S'-dimethyl N-cyano-dithio-iminocarbonate (14.0 g, 96 mmol) in dimethylformamide (60 ml) NaH (4.60 g, 50% dispersion in mineral oil, 0 °C) was slowly added. The mixture was stirred at 0°C for 6 hours, then at room temperature overnight. Ether (250 ml) and petroleum ether (50 ml) were added. After decanting of the supernatant phase the oily residue was stirred twice with ether:petroleum ether (5:1) (250 ml). After decanting the resulting semi-solid was treated with ice-water (150 ml) and filtered. The stirred, ice-cooled filtrate was treated with glacial acetic acid (5.6 ml) and the precipitate was collected by filtration and washed with water and small portions of ether. It was further purified by stirring with a 1:5 mixture of acetone and ether. Yield 9.3 g, 60 %. IR (KBr): 3430 cm^{-1} (-NH-), 2170 cm^{-1} (-C≡N). ^1H NMR (DMSO) δ: 2.62 (s, 3H), 7.46 (d, 2H), 8.41 (d, 2H), 11.75 (bs, 1H). Anal.($C_8H_8N_4S$, ½ H_2O) Calcd. C: 47.74, H: 4.51, N: 27.84, S: 15.93, H_2O: 4.4. Found C: 48.02, H: 4.49, N: 27.69, S: 16.41, H_2O: 3.1.
Procedures for preparing the title compounds (I) exemplified by
N-(6-(4-Chlorophenoxy)hexyl)-*N'*-cyano-*N''*-4-pyridylguanidine (**18**): 6-(4-Chlorophenoxy)-hexylamine (12.3 g, 10 mmol), *S*-methyl *N*-cyano-*N'*-4-pyridylisothiourea (1.75 g, 9.1 mmol), triethylamine (1.3 ml, 9.1 mmol) and 4-dimethylaminopyridine (10 mg) were dissolved in dry pyridine (9 ml). The reaction mixture was stirred at 55°C for 5 hours and then cooled to room temperature. The product was precipitated from ether (10 ml) as white crystals. Yield 3.02 g, (89 %). Mp. 147-148 °C.. IR (KBr, 0.3 %): 2170 cm^{-1} (-C≡N). ^1H NMR (DMSO) δ: 1.37 (m, 2H, CH_2), 1.42 (m, 2H, CH_2), 1.55 (m, 2H, H-2, CH_2), 1.73 (m, 2H, H-5, CH_2), 3.28 (q, 2H, H-1, NH-CH_2), 3.95 (t, 2H, H-6, O-CH_2), 6.94 (d, 2H, H-2, H-6, phenyl), 7.22 (bd, 2H, H-3, H-5, pyridyl), 7.30 (d, 2H, H-3, H-5, phenyl), 7.86 (t, 1H, NH), 8.38 (d, 2H, H-2, H-6, pyridyl), 9.45 (bs, 1H, NH). ^{13}C NMR (DMSO) δ: 25.05 (C-4, $C$$H_2$), 25.83 (C-3, $C$$H_2$), 28.40 (C-2, $C$$H_2$), (C-5, $C$$H_2$), 41.69 (C-1, NH-$C$$H_2$), 67.66 ((C-6, O-$C$$H_2$), 114.48 (C-3,C-5, pyridyl), 116.08 (C-2, C-6, phenyl), 116.37 (-C≡N), 123.96 (C-4, phenyl), 129.10 (C-3, C-5, phenyl), 146.04 (C-4, pyridyl), 149.80 (C-2, C-6, pyridyl), 157.23 ((NH)$_2$$C$=N), 157.44 (C-1, phenyl). Anal.($C_{19}H_{22}ClN_5O$) Calcd. C: 61.34, H: 5.96; N: 18.83. Found C: 61.20, H: 6.00, N: 18.92.
Alternative procedure: *N*-(6-(4-Chlorophenoxy)hexyl)-*N'*-4-pyridylthiourea (4.0 g, 11 mmol) was suspended in acetonitrile (23 ml) and dicyclohexylcarbodiimide (4.5 g, 22 mmol), cyanamide (0.92 g, 22 mmol) and triethylamine (0.15 ml, 1.1 mmol) were added. The mixture was stirred at room temperature for 9 days, and the reaction mixture was filtered and washed with acetonitrile. The white solid containing the product and dicyclohexylthiourea was triturated with chloroform (20 ml) overnight and filtered to give the product as white crystals. Yield 2.36 g (57 %). Spectroscopic data as above.

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