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EB1627: a soluble prodrug of the potent anticancer cyanoguanidine CHS828

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Abstract—To overcome pharmacokinetic and solubility problems observed in early clinical trials with the potent anticancer compound CHS828, we synthesised a series of prodrugs with improved properties. The best compound obtained was EB1627, with a tetraethyleneglycol moiety attached to the parent drug via a carbonate linkage. This compound was found soluble enough to be given i.v. and the drug was rapidly released in vivo exerting a very potent inhibitory activity alone and in combination with known cytostatics (etoposide) in animal models in vivo.

Our recent drug candidate, the 4-pyridyl-cyanoguanidine CHS828 (**1**), has demonstrated a potent anticancer activity in preclinical in vivo models.[1,2] However, during our early clinical investigations with this compound, (**1**), we found a variable absorption, and some undesired toxicity at high concentrations in humans, when administered orally.[3] To address this problem, we decided to look for a soluble prodrug of the parent compound to be able to dose the drug intravenously, and thereby achieve proper control of dosing.

Furthermore, in view of the recently suggested mode of action of this compound class (discussed below), and due to the well-established practice to use drug combinations in cancer therapy, we wanted to investigate the combined effects of a cyanoguanidine and a commonly used cytostatic, such as etoposide, in tumour-bearing animals.

Early attempts to solubilise the parent drug CHS828 (**1**) as its salt, for example, hydrochloride, hydrobromide, mesylate etc. did not provide a compound with a sufficient increase in solubility. We therefore decided to combine a quaternisation of the pyridine nitrogen with a solubilising group in form of a prodrug. Our goal was to obtain a high solubility and chemical stability of the prodrug and a rapid release of the drug in vivo.

The pyridine nitrogen was selected as the point of attachment of the prodrug group, preparing a so-called soft quaternary salt.[4] This type of prodrug is generally easily biodegradable and it served as starting point for our fine tuning of compound properties such as solubility and stability.

The prodrugs of formula **I** were prepared by reacting the 4-pyridyl-cyanoguanidine CHS828 (**1**) with an iodomethyl ester **III** followed by transformation of the formed quaternary iodide to the corresponding chloride (Scheme 1). The iodomethyl esters **III** were prepared from the corresponding chloromethyl esters **II** by the well-known Finkelstein reaction (Scheme 1).

The starting chloromethyl esters **II** were synthesised by reacting the solubilising group with chloromethyl chloroformate or chloromethyl chlorosulfate to obtain carbonate, carbamate or ester prodrugs, respectively

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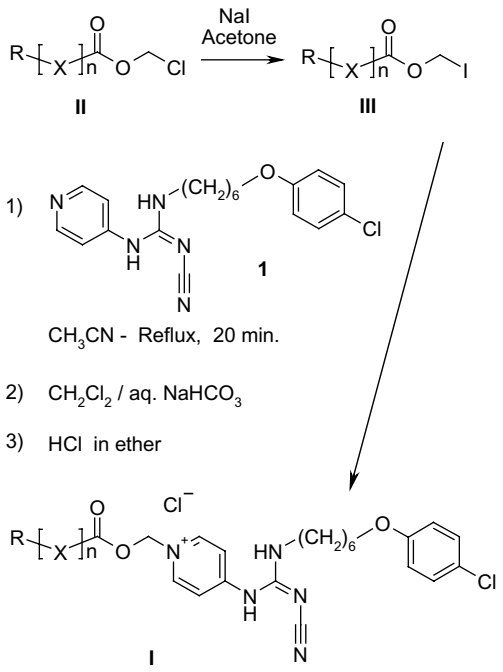
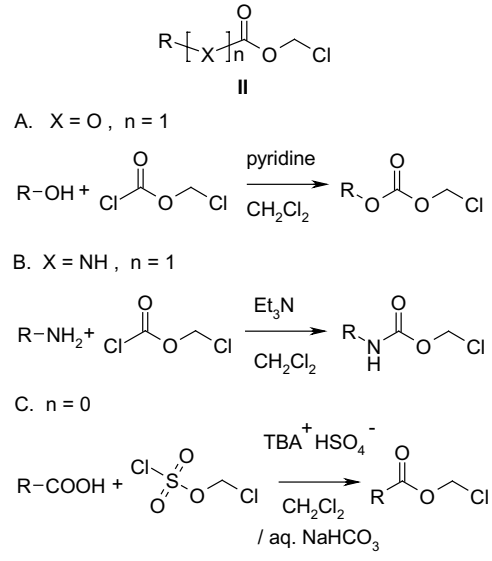


Scheme 1.



Scheme 2.

(Scheme 2).[5,6] Any amino group in the R side chain was BOC-protected during synthesis and deprotected with HCl in ether in the final step.

The stability of esters, carbonates and carbamates, as the hydrolysable part of the prodrugs, was investigated. We also tested a selection of polar groups to improve solubility (representative examples are given in Table 1).

The best compound was EB1627 (**2**), with a tetraethyleneglycol group in combination with a quaternary pyridyl nitrogen which gave a sufficient solubility, and a carbonate linkage which gave a good chemical stability and a rapid release of the active drug in vivo.

In comparison with EB1627 (**2**) compounds with a carbamate linkage (e.g., compound **5**, Table 1) were stable in both buffer and serum. Thus, even though such compounds were nicely soluble, they could not be used as prodrugs. Furthermore we found that the α-amino acid ester, as in compound **6**, was not stable enough, but rapidly cleaved chemically in the buffer.

The use of a primary aliphatic amine as the solubilising group in our prodrugs was also investigated. However, even though compound **7** showed a high solubility as its double salt this compound could not be further used due to toxicological effects in vivo. This effect was probably due to the surfactant properties of the prodrug as such. Our preferred polar group became ethylene glycol and, surprisingly, only a short chain of glycol units was necessary to obtain a sufficient solubility. Increased solubility was found going from 2 to 4 ethylene glycol units as in compounds **3**, **4** to our final choice EB1627 (**2**) (Table 1). Ethylene glycol has been used as a solubilising group, for example, in prodrugs of paclitaxel, however, a good solubility has only been obtained using long chain polyethylene glycols.[7] Interestingly, in our case the combination of a quaternary nitrogen and a small well-defined polar group was found effective.

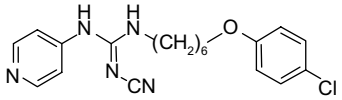
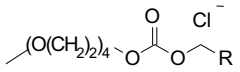
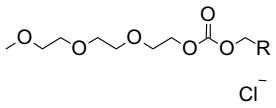
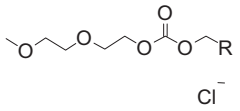
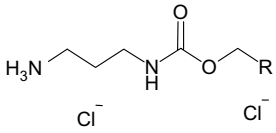
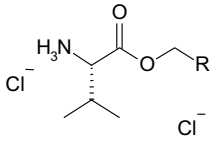
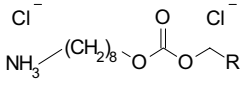
The parent anticancer agent CHS828 has been recently found to inhibit the IKK activity, via inhibition of the IκB kinase complex.[8] This led to suppression of the Nuclear Factor-κB activity in cancer cells. NF-κB is known to induce expression of antiapoptotic proteins, protecting the cell from apoptosis. It was found that inhibition of NF-κB by CHS828 in NYH small cell lung cancer correlated well with inhibition of cell proliferation in vitro and in vivo. Thus, the inhibition of the IκB kinase complex is suggested to be one important target for the activity of this compound class. Furthermore, many cancer cells exhibit a high constitutive activity of NF-κB, and upon treatment with cytostatics such as etoposide, a topoisomerase II inhibitor, the NF-κB activity is further increased. This increase will protect the cells from apoptosis; thus, etoposide may induce resistance in cancer cells via this mechanism.

In vitro, CHS828 is known to potently inhibit cell growth in a broad spectrum of cancer cells such as NYH (small cell lung cancer), H460 (non-small cell lung cancer), MCF-7 (breast) PC-3 (prostate), HT1080 (fibrosarcoma) and HT29 (colorectal) cells.[2]

EB1627 (**2**) and the other prodrugs which were tested in vitro in NYH cells efficiently inhibited cell proliferation, and thus all prodrug constructs, except the stable compound **5**, were rapidly cleaved during this assay to give the parent compound (Table 1).

In vivo tests were performed in nude mice inoculated with NYH small cell lung cancer cells.[2] Previous experiments had shown that CHS828 (**1**) totally suppressed tumour growth at 20 mg/kg/day p.o. when given

Table 1. Properties of cyanoguanidine prodrugs

Compound[9]	Structure	Solubility (μg/mL) pH 7.4; pH 5.5	*T*1/2 (h) buffer pH 7.4	*T*1/2 (min) Human serum	Inhibition (pIC50) NYH cells
CHS828 (**1**)[a]		0.5; 2	nd	nd	9.3
EB1627 (**2**)[b]		120; >1200	>4	25	9.1
3[c]		83; 1200	>4	30	9.3
4[d]		14; 34	>4	27	9.2
5[e]		250; >1200	>4	>240	7.8
6[f]		nd; nd	Unstable	nd	8.3
7[g]		38; >1200	>4	15	9.4

R = CHS828 (**1**).
[a] Parent drug.
[b] Best prodrug.
[c] Slightly less soluble.
[d] Less soluble.
[e] Stable in serum, no prodrug.
[f] Unstable in buffer.
[g] Toxic as prodrug.

therapeutically from day 14.[2] Similarly, EB1627 (**2**), at 36 mg/kg/day (20 mg/kg/day CHS828) p.o., inhibited tumour growth as efficiently as CHS828.

In mice and rats, both p.o. and i.p. administration gave a similar and reliable biological response. Furthermore, the absorption of CHS828 as compared with EB1627 after p.o. administration was assessed in minipigs. Qualitatively, both compounds were absorbed equally well when measured as CHS828, however, with very large intra-individual variation. Due to the rapid cleavage of EB1627 to CHS828, no EB1627 was observed in the minipigs when administered p.o. Furthermore, EB1627 was administered i.v. in minipigs, thus the bioavailability of CHS828 when given as EB1627 could be estimated to 36% (range 13–91% $n = 6$). However, the large variation in absorption observed for EB1627 in minipigs suggested that this prodrug cannot be used to improve the p.o. absorption properties of CHS828 in humans. In our further animal experiments EB1627 was administered i.p. as compared with i.v. for convenience.

Combination of EB1627 and etoposide at suboptimal doses resulted in additive effects on tumour suppression (Fig. 1). In addition, using etoposide resistant cells (NYH/VM-26res), we were able to restore sensitivity to etoposide by pretreatment with EB1627 (**2**) and etoposide using suboptimal doses of both compounds (Fig. 2). Thus, one weekly treatment with EB1627 (50 mg/kg, as CHS828, i.p.) in combination with a daily administration of etoposide (10 mg/kg, p.o.) gave a total suppression of tumour growth even in this etoposide resistant cell line. We suggest that this impressive effect using a very low dose of EB1627 was due to the mechanism of action described above.

In conclusion, a soluble prodrug, EB1627 (**2**), was synthesised from the parent drug CHS828. This compound could be administered i.v., thus allowing a controlled dosing to the patients. Also, to overcome toxicity problems in the clinic and to potentiate the effects of other cytostatics by suppression of NF-κB, the compound may be used in very low doses in combination with

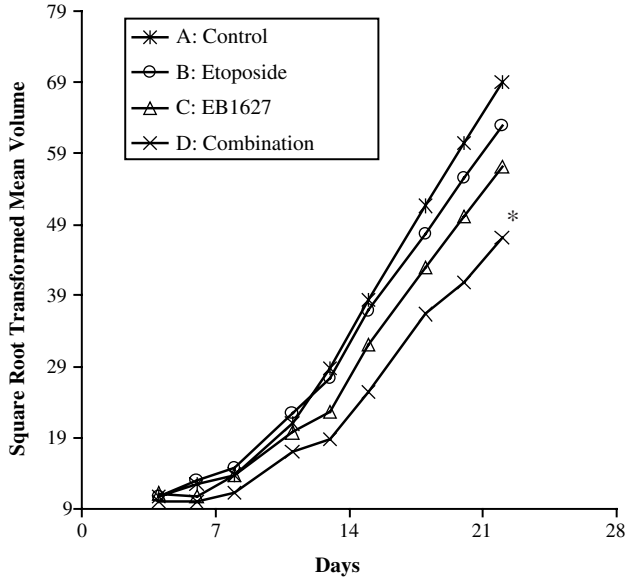


Figure 1. Effects on tumour growth with EB1627 (**2**) and etoposide alone and in combination in nude mice with NYH SCLC tumour cells. Female mice were inoculated with 1×10^7 NYH cells in both flanks. A. Control, B. etoposide, 10 mg/kg/day, p.o. C. EB1627 (2), 15 mg/kg/2 × weekly as CHS828, i.p. D. etoposide, 10 mg/kg/day p.o. and EB1627 (2) 15 mg/kg/2 × weekly as CHS828, i.p. $*p < 0.5$.

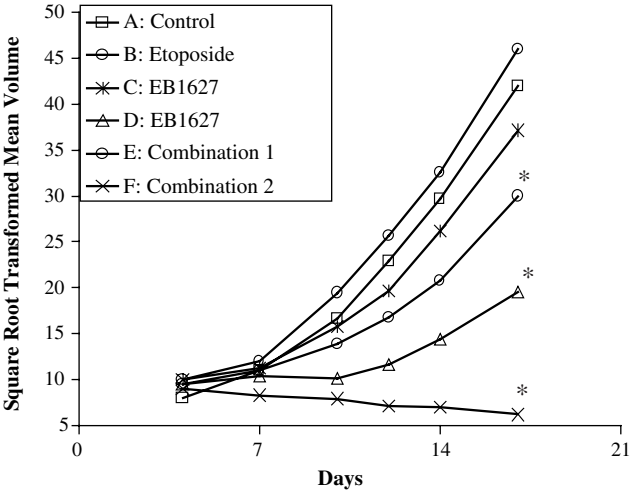


Figure 2. Effects on tumour growth with EB1627 (**2**) and etoposide alone and in combination in nude mice with NYH/VM-26res SCLC tumour cells. Female mice were inoculated with 1×10^7 NYH cells in both flanks. A. Control, B. etoposide, 10 mg/kg/day, p.o. C. EB1627 (2), 30 mg/kg/week, as CHS828, i.p. D. EB1627 (2) 50 mg/kg/week, as CHS828, i.p. E. etoposide, 10 mg/kg/day p.o. and EB1627 (2) 30 mg/kg/week as CHS828, i.p. starting 24 h prior administration of etoposide. F. etoposide, 10 mg/kg/day p.o. and EB1627 (2) 50 mg/kg/week as CHS828, i.p. starting 24 h prior to administration of etoposide. $*p < 0.1$.

known cytostatic agents. Thus, a combination of EB1627 with etoposide (Vepesid®) was found to give impressive synergistic antitumour activities in an animal model of etoposide resistant tumours.

Acknowledgements

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9. All new compounds were spectroscopically characterised. Selected data. **2** (EB1627): ^{1}H NMR (DMSO) δ 11.98 (br, 1H), 9.09 (br, 1H), 8.74 (d, 2H), 7.61 (br, 2H), 7.30 (d, 2H), 6.95 (d, 2H), 6.22 (s, 2H), 4.26 (m, 2H), 3.96 (t, 2H), 3.63 (m, 2H), 3.55–3.40 (m, 14H), 3.23 (s, 3H), 1.71 (m, 2H), 1.59 (m, 2H), 1.41 (m, 4H). Mp 158–159 °C Anal. ($C_{30}H_{43}Cl_2N_5O_8$) C, H, N, Cl. **3**: ^{1}H NMR (DMSO) δ 11.89 (br, 1H), 9.04 (br, 1H), 8.73 (d, 2H), 7.60 (br, 2H), 7.30 (d, 2H), 6.94 (d, 2H), 6.22 (s, 2H), 4.26 (m, 2H), 3.96 (t, 2H), 3.62 (m, 2H), 3.55–3.40 (m, 10H), 3.23 (s, 3H), 1.71 (m, 2H), 1.59 (m, 2H), 1.41 (m, 4H). Mp 162–163 °C. Anal. ($C_{28}H_{39}Cl_2N_5O_7$) C, H, N, Cl. **4**: ^{1}H NMR (DMSO) δ 11.87 (br, 1H), 9.06 (br, 1H), 8.74 (d, 2H), 7.58 (br, 2H), 7.30 (d, 2H), 6.94 (d, 2H), 6.22 (s, 2H), 4.26 (m, 2H), 3.96 (t, 2H), 3.61 (m, 2H), 3.52 (m, 2H), 3.40 (m, 4H), 3.22 (s, 3H), 1.71 (m, 2H), 1.58 (m, 2H), 1.41 (m, 4H). Mp 166–167 °C Anal. ($C_{26}H_{35}Cl_2N_5O_6$) C, N, H, Cl. **5**: ^{1}H NMR (DMSO) δ 11.9 (br, 1H), 9.1 (br, 1H), 8.71 (d, 2H), 8.03 (br, 3H), 7.96 (t, 1H), 7.60 (br, 2H), 7.30 (d, 2H), 6.95 (d, 2H), 6.13 (s, 2H), 3.95 (t, 2H), 3.36 (q, 2H), 3.06 (q, 2H), 2.75 (m, 2H), 1.69 (m, 4H), 1.58 (m, 2H), 1.40 (m, 4H). **6**: ^{1}H NMR (DMSO) δ 8.84 (2H, d), 7.7 (2H, br), 7.41 (2H, d), 7.05 (2H, d), 6.55 (2H, dd), 4.34 (1H, d), 4.15 (2H, t), 3.62 (2H, t), 2.53 (1H, m), 2.0–1.7 (4H, m), 1.65–1.45 (4H, m), 1.13 (6H, t). Anal. ($C_{25}H_{35}Cl_3N_6O_3$) C, N, H, Cl. **7**: ^{1}H NMR (DMSO) δ 12.08 (br, 1H), 9.16 (br, 1H), 8.76 (d, 2H), 8.06 (br, 3H), 7.62 (br, 2H), 7.30 (d, 2H), 6.95 (d, 2H), 6.23 (s, 2H), 4.14 (t, 2H), 3.96 (t, 2H), 3.4 (br, 2H), 2.72 (m, 2H), 1.8–1.15 (m, 20H). Mp 159–161 °C.