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Synthesis, *in vitro* and *in vivo* Evaluation of a Delivery System for Targeting Anticancer Drugs to the Brain

A 1,4-dihydropyridine ⇌ pyridinium salt type redox system is described as a general and flexible method for site-specific and sustained delivery of drugs to the brain. This concept was used in the present investigation as a model to deliver an alkylating antitumor agent into the brain. A bis-(chloroethyl)amine drug was hooked to 1,4-dihydropyridine chemical delivery system (CDS) through an amide linkage. Five new target compounds (**23–27**) of the 1,4-dihydropyridine CDS type were synthesized through the reduction of five new pyridinium quaternary intermediates (**18–22**). The synthesized 1,4-dihydropyridines were subjected to various chemical and biological investigations to evaluate their ability to cross the blood-brain barrier (BBB), and to be oxidized biologically into their corresponding quaternary compounds. The *in vitro* oxidation studies showed that 1-benzyl-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}-1,4-dihydropyridine (**23**) and 1-(4-nitrobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-1,4-dihydropyridine (**27**) could be oxidized into their corresponding quaternary compounds **18** and **22**, respectively, at an adequate rate, which ensure the release of the carried anticancer drug. The *in vivo* studies showed that compound **23** was able to cross the BBB at detectable concentrations. On the other hand, the *in vitro* alkylation activity studies revealed that 1-(4-nitrobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}pyridinium bromide (**22**) is an alkylating agent with activity comparable to the known drug chlorambucil.

Keywords: Anticancer drug; Brain delivery system; Alkylating activity

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Introduction

Optimization of drug delivery into the site of activity by means of chemical modifications of known drugs or their analogs has gained increasing interests in the past decades [1]. The concept of developing methods for site-specific delivery of biologically active agents is highly desirable to improve the efficacy, decrease the toxicity, and consequently, improve their therapeutic indices [2, 3]. The delivery of drugs to the brain is often seriously limited by the blood brain barrier (BBB) [4] which could be a major impediment for the treatment of CNS diseases. Many drugs are unable to reach the active sites in the brain at therapeutic concentrations due to the BBB. Various attempts have been made to overcome the limited access of drugs into the brain and consequently, reduce the systemic side effects [5–10]. One of these attempts was the linking of the active drug to a brain-specific carrier, which delivers the drug specifically into the brain, where it is cleaved enzymatically from the carrier [11–16]. The dihydropyridine redox-chemical delivery systems (CDSs), have successfully been utilized by Bodor et al.[17, 18] to deliver different alkylating agents to the brain. The use of the dihydropyridine system as a carrier affects the bidirectional transport of the drug species into and out of the brain. *In vivo* enzymatic oxidation of the dihydropyridine moiety to the ionic pyridinium salt inside the brain prevents its elimination "locked-in", while elimination from the general circulation is accelerated (Figure 1). The main disadvantage observed with 1-methyl-1,4-dihydronicotinate carrier was its instability against air oxidation and the hydration of the C_5–C_6 double bond. This instability makes the final drug-carrier complex unstable as well [16].

The main objective of the present study is focused on the synthesis of the target compounds **23–27**, as brain specific antitumor agents, that incorporate into their structure a 1-(benzyl or substituted benzyl)-1,4-dihydropyridine moiety to provide brain targeting in a manner similar to that of the mentioned CDSs (Figure 1). The substituents on the benzyl moiety of the carrier were selected to be electron withdrawing to decrease the electron density on the ring nitrogen of the 1,4-dihydropyridine moiety. According to the literature [19], the rates of oxidation and

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Full Paper

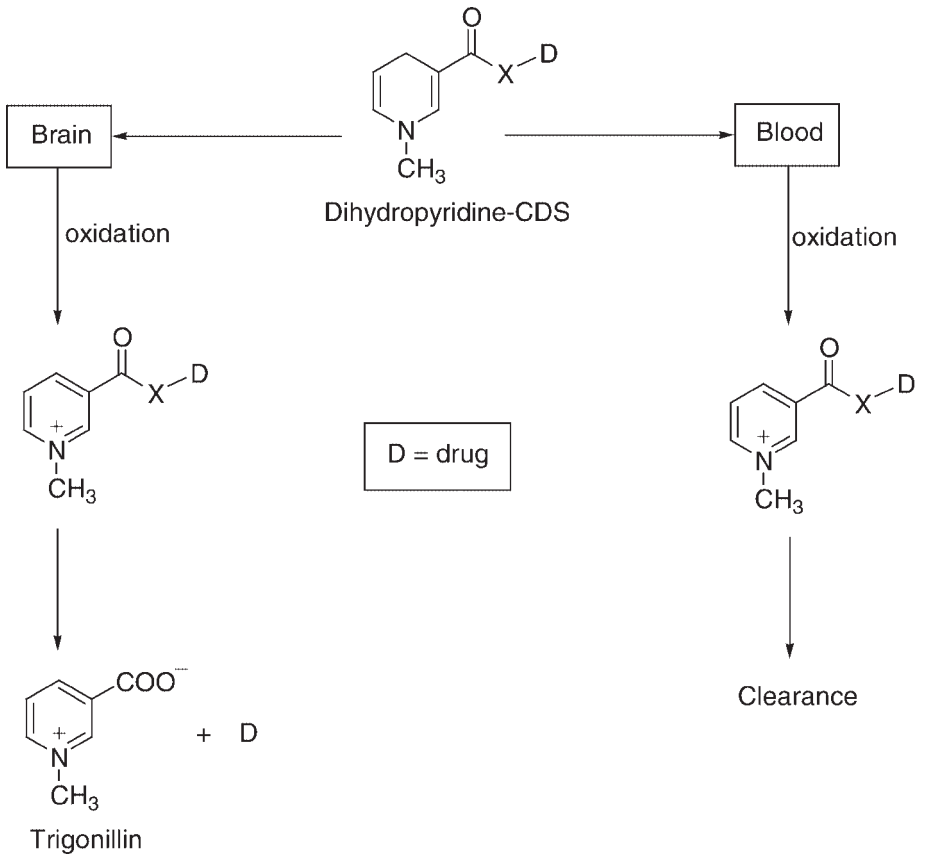


Figure 1. Distribution, systemic clearance, and brain "lock-in" pathways of brain-specific CDSs.

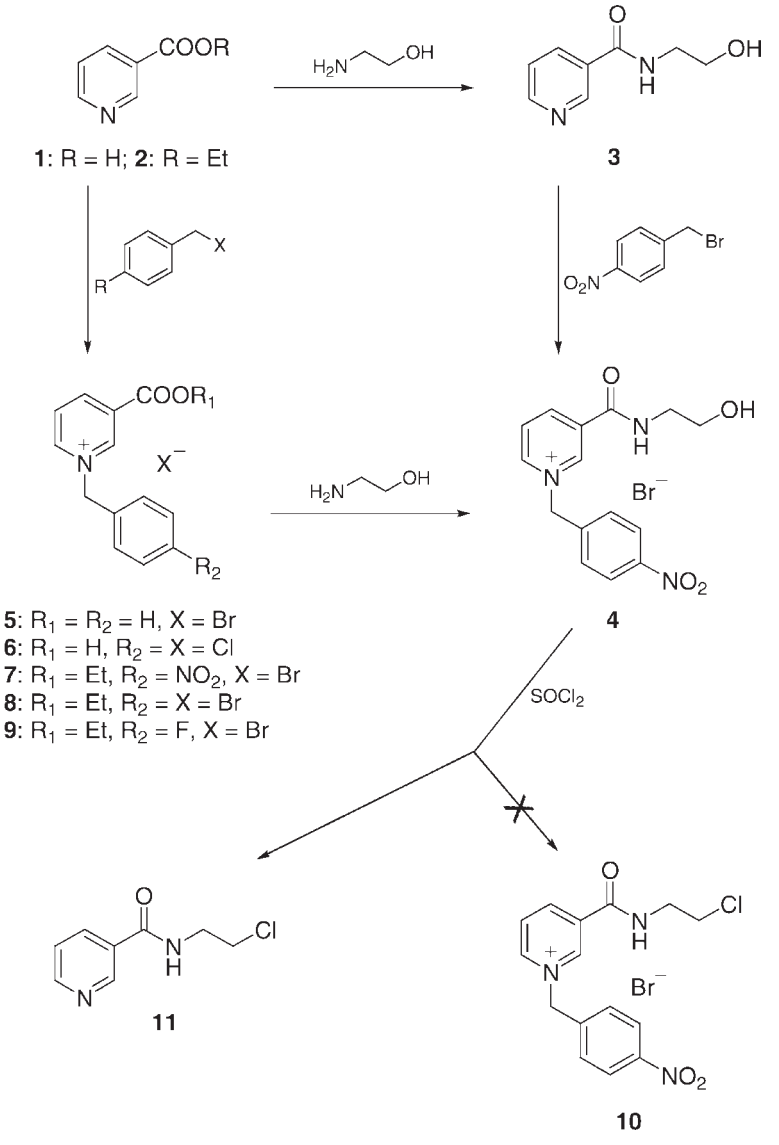
hydration of the 1,4-dihydropyridines should be reduced by the decrease of the electron density on the nuclear nitrogen atom.

Results and discussion

Chemistry

The synthesis of the target compounds 1-(benzyl or 4-substituted benzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-1,4-dihydropyridines (**23–27**) necessitates the use of more than one route, in order to optimize the yield and the purity of the prepared intermediates and final products. The intermediate 3-[N-(2-hydroxyethyl)carbamoyl]pyridine (**3**) was prepared using three different routes. Nicotinic acid (**1**) was reacted with thionyl chloride, then treated with ethanolamine to give **3** (56 % yield). The other two routes involved the reaction of **1** with ethanolamine in the presence of dicyclohexylcarbodiimide (DCC) or the direct condensation of ethyl nicotinate (**2**) and ethanolamine to afford **3** in 20 % and 35 % yield, respectively (Scheme 1). 1-(4-Nitrobenzyl)-3-[N-(2-hydroxyethyl)carbamoyl]pyridinium bromide (**4**) was prepared by the direct quaternarization of **3** using 4-nitrobenzyl bromide (42 % yield). The quaternarization of nicotinic acid (**1**) or ethyl nicotinate (**2**) using a variety of 4-substituted benzyl halides afforded the intermediates **5–9** (Scheme 1). Compound **4** was then prepared adopting an alternate route, by reacting the ester **7** with ethanolamine in boiling toluene with overall yield of 60 %, based on ethyl nicotinate. Conversion of the 2-hydroxyethyl function in **4** into the 2-chloroethyl derivative **10** via its reaction with thionyl chloride either neat or in variety of nonpolar solvents was unsuccessful. 3-[N-(2-chloroethyl)carbamoyl]pyridine (**11**), with the cleavage of the benzyl function, was isolated from the reaction mixture rather than **10**. This quaternary salt cleavage may be attributed to the instability of **4** in acidic conditions (Scheme 1).

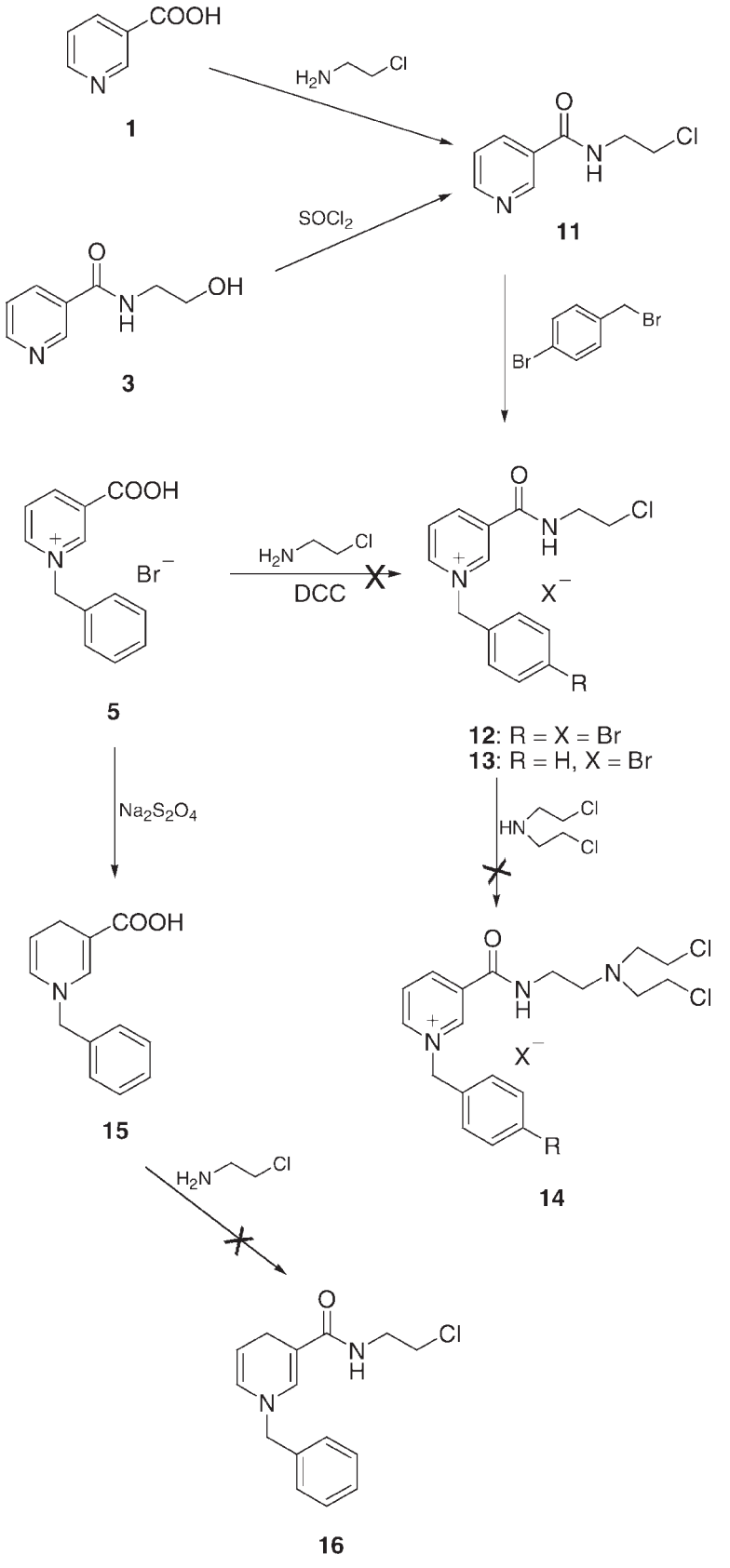
Compound **11** was synthesized later, using two different synthetic routes. Nicotinic acid (**1**) was reacted with 2-chloroethylamine in presence of dicyclohexylcarbodiimide (DCC) to give **11** (30 % yield), or through the reaction of **3** with thionyl chloride (25 % yield). Stirring of **11** with



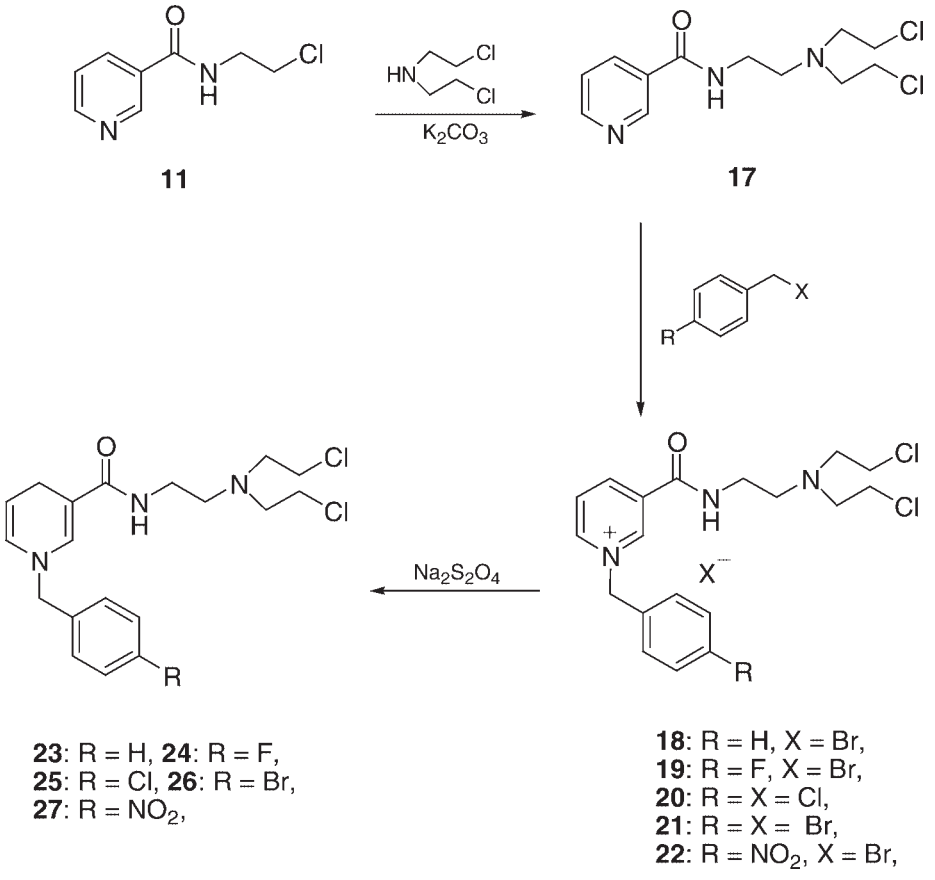
Scheme 1.

4-bromobenzyl bromide in acetonitrile at room temperature afforded the corresponding quaternary salt **12**. On the other hand, attempts to synthesize **13** via an alternate route through the condensation of the nicotinic acid quaternary salt **5** and 2-chloroethylamine in presence of DCC was unsuccessful due to the insolubility of **5** in the solvents used. As a trial to reach the final targets, represented by compound **14**, the quaternary salt **12** was reacted with bis-(2-chloroethyl)amine in toluene. Compound **12** did not dissolve in organic solvents at room temperature, and did decompose upon heating. For these reasons the synthetic route was modified to reach the final targets through the reduction of the quaternary **5** into the 1,4-dihydropyridine analog **15**, with expected improvement in the solubility properties. Compound **5** was reduced into its corresponding 1,4-dihydropyridine **15** using sodium dithionite in alkaline medium. Compound **15** proved to be extremely unstable in the used reaction conditions and consequently, its reaction with 2-chloroethylamine to yield **16** was not possible (Scheme 2).

An alternative synthetic strategy was adopted as described, in Scheme 3, to obtain the final targets. 3-[N-(2-chloroethyl)carbamoyl]pyridine (**11**) was treated with bis-(2-chloroethyl)amine and potassium carbonate in refluxing toluene to afford 3-{N-[2-bis(2-chloroethyl)-aminoethyl]carbamoyl}pyridine (**17**). Compound **17** was



Scheme 2.



Scheme 3.

then quaternarized using variety of 4-substituted benzyl halides in acetonitrile to give the quaternary salts 1-(4-substituted benzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}pyridinium halides (**18–22**). It is worth mentioning that the quaternarization process did not take place on the other nitrogen atoms existing in compound **17**, the carbamoyl nitrogen atom is too weak as a nucleophile, and the tertiary nitrogen of bis(2-chloroethyl)aminoethyl moiety is sterically hindered to be alkylated. The obtained quaternary salts **18**–**22** were then subjected to reduction process using sodium dithionite in alkaline medium, to give the corresponding final targets 1-(4-substituted benzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-1,4-dihydropyridines **23**–**27**, (Scheme 3).

Biological investigations

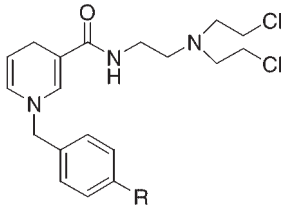
The prepared 1,4-dihydropyridines **23** and **27** were subjected to various chemical and biological investigations to evaluate the ability of these compounds to cross the BBB, and to be oxidized biologically into their corresponding quaternary compounds. This oxidation process is very crucial to predict the ability of the 1,4-dihydropyridines CDSs to release the anticancer drug at the site of action, i.e. the brain. In this study high performance liquid chromatography (HPLC) was used to detect and monitor the oxidation of the tested 1,4-dihydropyridines into their corresponding quaternaries either chemically or in biological fluids. The mobile phase was chosen after several trials using various proportions of acetonitrile and water at different pHs. Experimental parameters including mobile phase, flow rate, type of column, and the linearity range were studied in order to determine the optimal conditions for the assay procedure. The HPLC analysis showed that the 1,4-dihydropyridines were separated from blood and brain homogenate at retention time of 5.5–6.0 min, while the quaternaries were separated at retention time of 5.0–5.4 min. The chromatographic system used allowed a complete base line separation with good resolution of the peaks. The mean calibration curve was plotted, the mean best-fit linear regression equation was derived, and used to estimate the concentrations of the quaternary salts.

Hydrogen peroxide oxidizes the 1,4-dihydropyridines by a free radical mechanism. This oxidation could give an idea about the different behavior of various derivatives toward *in vivo* oxidation into the corresponding quaternaries which facilitates the release of the carried anticancer drug [20]. A freshly prepared solution of the tested 1,4-dihydropyridine derivatives **23** and **27**, with specific concentrations, was mixed with 30 % hydrogen peroxide solution. The increased concentrations of the oxidation products (the corresponding quaternary salts **18** and **22**) were monitored by HPLC using UV detector at λ_{max} 262 nm. The data obtained indicated the facile oxidative conversion of the N-benzyl-1,4-dihydropyridine analog **23** into the corresponding quaternary **18** with high oxidation rate (K_{app} = 29.14 × 10^{-2} min^{-1}, $t_{½}$ = 2.4 min). The N-(4-nitrobenzyl)-1,4-dihydropyridine derivative **27** converted into the corresponding quaternary salt **22** with oxidation rate almost twofold less than **23**, (K_{app} = 12.6 × 10^{-2} min^{-1}, $t_{½}$ = 5.5 min), (Table 1). These results indicated that the type of substituent on the benzyl moiety could manipulate the stability of such compounds toward oxidation. The 4-nitro electron withdrawing group of **27** decreases the electron density on the ring nitrogen of 1,4-dihydropyridine and hence lowers the rate of oxidation. The rate of oxidation needed to be neither too fast nor too slow. Fast oxidation process will convert the drug into the corresponding quaternary salt in the blood before reaching the target organ, the brain, while slow oxidation process will allow the crossing of the CDSs into the brain, but it will delay the release of the carried drug. Accordingly, the moderate rate of oxidation will ensure the survival of the 1,4-dihydropyridine species in the blood till reaching the brain.

The *in vivo* oxidation of the prepared 1,4-dihydropyridine derivatives in the brain could be predicted by spiking the test compounds in brain homogenate. Such study could inform about the rate of their conversion into the corresponding quaternaries and hence the release of the carried drug [20]. The data obtained indicated the facile oxidative conversion of the N-benzyl-1,4-dihydropyridine analog **23** into the corresponding quaternary **18** with high oxidation rate (K_{app} = 14.2 × 10^{-2} min^{-1}, $t_{½}$ = 4.9 min). The N-(4-nitrobenzyl)-1,4-dihydropyridine derivative **27** converted into the corresponding quaternary **22** with oxidation rate fifteen times less than **23**, (K_{app} = 0.9 × 10^{-2} min^{-1}, $t_{½}$ = 77.0 min), (Table 1). These results are in consistency with those obtained from the chemical oxidation by the use of hydrogen peroxide.

Compound **23** was selected for *in vivo* study due to its relative ease of conversion to the quaternary **18**. Compound **23**, at a dose of 40 mg/kg, was injected to rats. At selected time intervals, blood samples and the brains were collected. The concentrations of the quaternary salt **18**, which were produced in blood after administration of compound **23**, were measured in both blood and brain homogenate using HPLC assay. The mean concentration – time profiles of the quaternary salt **18** in blood and brain homogenate samples are shown in Figure 2 and Table 2. The concentration of compound **18** declined rapidly in blood (K_{app} = 111.6 × 10^{-2} min^{-1}, $t_{½}$ = 37.2 min), and was not detected after 120 min. In the meantime, the concentration of **18** increased steadily in the brain, reaching its maximum 90 min after administration, followed by a steady decline indicating the sustained release of the anticancer drug.

Table 1. Rates of oxidative conversion of the 1,4-dihydropyridines **23** and **27** into their corresponding quaternary salts **18** and **22**.



Compound	R	Hydrogen peroxide				Brain homogenate			
		Regression data		K_{app} min^{-1} × 10^{-2}	$t_{½}$ (min)	Regression data		K_{app} min^{-1} × 10^{-2}	$t_{½}$ (min)
		n	r			n	r		
23	H	5	0.99	29.14 ± 0.07	2.4	7	0.98	14.2 ± 0.2	4.9
27	NO_2	8	0.99	12.60 ± 0.03	5.5	8	0.99	0.9 ± 0.3	77.0

n = no. of determinations, r = correlation coefficient.

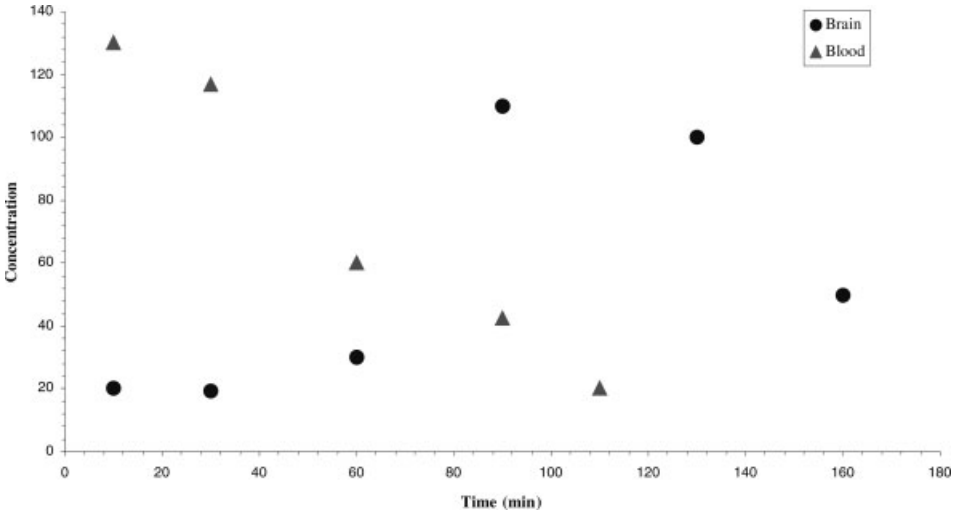


Figure 2. The concentration of compound **18** in brain (μg/g) and blood (μg/mL) of rats after administration of compound **23**.

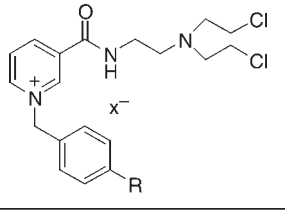
Table 2. Mean concentration of the quaternary salt **18** in brain homogenate (μg/g) and blood (μg/mL) of rats after administration of compound **23** (40 mg/kg).

Time (min)	Mean concentration ± SD	
	Blood	Brain homogenate
10	129.9 ± 21.3	20.3 ± 6.2
30	117.2 ± 12.6	19.3 ± 4.2
60	59.9 ± 5.1	30.1 ± 8.6
90	42.3 ± 5.9	110.3 ± 12.8
110	20.4 ± 5.3	100.3 ± 15.5
130	0.0	–
160	0.0	50.3 ± 8.7

no. of determinations (n) = 3

The prepared quaternaries **18–22** were evaluated for their alkylating activity using 4-(4-nitro-benzyl)pyridine as an analytical reagent [21]. 4-(4-Nitro-benzyl)pyridine reacts with alkylating agents and gives a purple color upon basification. The intensity of the produced color is directly proportional to the degree of alkylation. This method differentiates between the reactivities of the tested compounds by constructing a calibration curve for each compound under the specified conditions. The alkylating potency of the 1,4-dihydropyridines were examined in the form of their oxidized analogs (the quaternaries **18–22**), using chlorambucil as positive control. All of the test compounds proved to be active alkylating agents (Table 3). The 4-nitro derivative **22** is the most active member of this series, with alkylating activity (K_{app} =

Table 3. The alkylating activity of the quaternary salts **18–22**.



Compound	R	X	λ_{max}	Temp.	Regression data		K_{app} $min^{-1} \times 10^{-2}$	$t_{½}$ (min)
					n	r		
18	H	Br	546	55	7	0.93	3.16 ± 0.116	21.9
19	F	Br	542	55	6	0.95	6.54 ± 0.128	10.6
20	Cl	Cl	548	55	5	0.97	3.28 ± 0.091	21.1
21	Br	Br	545	55	6	0.98	5.73 ± 0.231	12.1
22	NO_2	Br	535	55	4	0.97	13.5 ± 0.032	5.1
Chlorambucil	–	–	550	83	8	0.99	10.19 ± 0.051	6.8

n = no. of determinations, r = correlation coefficient.

13.5×10^{-2} min^{-1}, $t_{½}$ = 5.1 min) comparable to that of chlorambucil, ($K_{app} = 10.19 \times 10^{-2}$ min^{-1}, $t_{½}$ = 6.8 min).

As can be concluded from the obtained results, the *in vitro* oxidation studies showed the ability of the tested 1,4-dihydropyridines (**23** and **27**) to be oxidized into the corresponding quaternary salts. The rate of oxidation proved to be manipulated by the type of substituent on the N-benzyl group of the CDSs. The electron withdrawing 4-nitro group optimized the rate of oxidation to ensure the delivery of the drug across BBB into the brain and the sustained release of the anticancer drug. The *in vivo* studies showed that the 1,4-dihydropyridines, represented by compound **23**, could cross the BBB at detectable concentrations. Also, the *in vitro* alkylation activity studies showed that compound **22** is an alkylating agent with activity comparable to the known drug chlorambucil.

Acknowledgements

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Experimental

Melting points were determined on a Mettler FP 80 melting point apparatus (Mettler, Manchester, UK) and are uncorrected. Microanalyses were performed on a Perkin-Elmer 240 elemental analyzer (Perkin-Elmer, Shelton, CT, USA) at the Central Research Laboratory, College of Pharmacy, King Saud University. All of the new compounds were analyzed for C, H, and N and agreed with the proposed structures within 0.4 % of the theoretical values. ^{1}H NMR spectra were recorded on a Varian XL 500 MHz FT spectrometer (Varian, Palo Alto, CA, USA); chemical shifts are expressed in δ ppm with reference to TMS. Thin-layer chromatography was performed on precoated (0.25-mm) silica gel plates (E. Merck, Darmstadt, Germany); compounds were detected with a 254-nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. KUBOTA 6800 compact high speed refrigerated centrifuge 20,000 rpm (Heraeus Instruments, Hanau, Germany) was used for the centrifugation of the samples for 10 min at 4 °C. High performance JASCO liquid chromatograph 600E equipped with PU-980 pump and connected to PU-750 UV/Vis. absorbance detector (Waters, Milford, MA, USA); a μBondapak reverse-phase C_{18} column (Waters), 10 μM (4.6 mm id × 250 mm), operated at ambient temperature, with injection volume of 20 μL, and flow rate of 1.5 mL/min was used for the detection of the 1,4-dihydropyridines and the corresponding quaternary compounds in both chemical and biological investigations. The peak area integrations were performed using a chromatographic data module.

3-[N-(2-Hydroxyethyl)carbamoyl]pyridine (**3**)

Method A

A mixture of nicotinic acid (**1**, 1.2 g, 0.01 mol) and thionyl chloride (10 mL) was stirred and heated under reflux (neat) for 1 h. Excess thionyl chloride was removed under reduced pressure, and the obtained residue was dissolved in dry toluene (20 mL). The solution was then washed with Na_2CO_3 solution (25 mL, 20 %), and dried over anhydrous Na_2SO_4. Ethanolamine (0.6 mL, 0.01 mol) was added and the mixture was heated under reflux for 5 h. Solvent was evaporated under reduced pressure, and the obtained residue was extracted with diethyl ether (3 × 10 mL). The ethereal extract was dried and evaporated in vacuo to afford **3** as a yellowish oil (56 %). ^{1}H NMR ($CDCl_3$), δ 3.48–3.56 (m, 2 H, NHC*H*$_2$-), 3.82–3.94 (m, 2 H, C*H*$_2$OH), 7.53–7.58 (m, 1 H, pyridine-H), 8.17 (d, 1 H, *J* = 7 Hz, pyridine-H), 8.63 (d, 1 H, *J* = 7 Hz, pyridine-H), 8.96 (s, 1 H, pyridine-H), 9.59 (brs, 1 H, NH), 10.50 (s, 1 H, OH). Anal. ($C_8H_{10}N_2O_2$) C, H, N.

Method B

To a stirred solution of nicotinic acid (**1**, 1.2 g, 0.01 mol) and ethanolamine (0.6 mL, 0.01 mol) in pyridine, dicyclohexylcarbodiimide (DCC, 2.1 g, 0.01 mol) was added portionwise. The reaction mixture was stirred at room temperature for 24 h. The separated solid was filtered, and the filtrate was evaporated under reduced pressure. The obtained residue was extracted with diethyl ether (3 × 10 mL). The combined ethereal extract was dried and evaporated in vacuo to give **3** as yellowish oil (20 %).

Method C: A mixture of ethyl nicotinate (**2**, 1.4 g, 0.01 mol) and ethanolamine (0.6 mL, 0.01 mol) in dioxane (30 mL) was heated under reflux for 10 h. The reaction mixture was evaporated under reduced pressure, and the obtained residue was extracted with diethyl ether (3 × 10 mL). The combined ethereal extract was dried and evaporated *in vacuo* to give **3** as yellowish oil (35 %).

1-(4-Nitrobenzyl)-3-[N-(2-hydroxyethyl)carbamoyl]pyridinium bromide (**4**).

Method A

A mixture of **3** (1.7 g, 0.01 mol) and 4-nitrobenzyl bromide (2.2 g, 0.01 mol) in acetonitrile (30 mL) was stirred at room temperature for 24 h. Solvent was removed under reduced pressure and the obtained residue was triturated with petroleum ether 60–80 °C till solidification. The obtained solid was recrystallized from EtOH/Hexane to give **4** (42 %): mp 123–5 °C; H NMR (DMSO-d_6), δ 3.45–3.52 (m, 2 H, NHC*H*$_2$-), 3.85–3.96 (m, 2 H, C*H*$_2$OH), 6.78 (s, 2 H, PhC*H*$_2$-), 8.20–8.25 (dd, 4 H, *J* = 7 Hz, ArH), 8.78–8.81 (m, 1 H, pyridine-H), 9.40 (d, 1 H, *J* = 7.5 Hz, pyridine-H), 9.98 (d, 1 H, *J* = 7.5 Hz, pyridine-H), 10.56 (s, 1 H, pyridine-H), 10.72 (brs, 1 H, NH), 11.2 (s, 1 H, OH). Anal. ($C_{15}H_{16}BrN_3O_4$) C, H, N.

Method B

A mixture of **7** (3.7 g, 0.01 mol) and ethanolamine (0.6 mL, 0.01 mol) in toluene (30 mL) was heated under reflux for 10 h. The reaction mixture was evaporated under reduced pressure, and the obtained residue was triturated with petroleum ether 60–80 °C till solidification. The obtained solid was recrystallized from EtOH/hexane to give **4** (60 %).

General Procedure for Preparation of 1-(4-substituted benzyl)pyridinium halide-3-carboxylic acid **5** *and* **6** *or Ethyl 1-(4-substituted benzyl)pyridinium halide-3-carboxylate* **7**–**9**

A mixture of nicotinic acid (**1**, 1.2 g, 0.01 mol) or ethyl nicotinate (**2**, 1.4 g, 0.01 mol) and the appropriate 4-substituted benzyl halide (0.01 mol) in acetonitrile (25 mL) was stirred at room temperature for 24 h. Solvent was removed under reduced pressure and the remaining residue was triturated with petroleum ether 60–80 °C to afford **5–9**.

1-Benzyl-pyridinium bromide-3-carboxylic acid (**5**)

The crude product was recrystallized from AcOH/EtOH to give **5** (70 %): mp 171–3 °C; ^{1}H NMR (DMSO-d_6) δ 5.89 (s, 2 H, PhCH_2-), 7.48–7.59 (m, 5 H, *J* = 15 Hz, ArH), 8.20–8.25 (m, 1 H, pyridine-H), 8.83 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.25 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.50 (s, 1 H, pyridine-H), 11.25 (s, 1 H, COOH). Anal. ($C_{13}H_{12}BrNO_2$) C, H, N.

1-(4-Chlorobenzyl)pyridinium chloride-3-carboxylic acid (**6**)

The crude product was recrystallized from ACOH/EtOH to give **6** (40 %): mp 213–5 °C; ^{1}H NMR(DMSO-d_6) δ 5.94 ((s, 2 H, PhCH_2-), 7.51–7.64 (dd, 4 H, *J* = 15 Hz, ArH), 8.15–8.28 (m, 1 H, pyridine-H), 8.84 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.22 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.55 (s, 1 H, pyridine-H), 11.52 (s, 1 H, COOH). Anal. ($C_{13}H_{11}Cl_2NO_2$) C, H, N.

Ethyl 1-(4-nitrobenzyl)pyridinium bromide-3-carboxylate (**7**)

The crude product was recrystallized from AcOH to give **7** (82 %): mp 107–9 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (t, 3 H, *J* = 7 Hz, CH_3CH$_2$-), 4.37–4.57 (q, 2 H, *J* = 7 Hz, CH$_3$CH_2-), 6.79 (s, 2 H, PhCH_2-), 8.21–8.26 (dd, 4 H, *J* = 7 Hz, ArH), 8.78–8.81 (m, 1 H, pyridine-H), 9.39 (d, 1 H, *J* = 8 Hz, pyridine-H), 9.98 (d, 1 H, *J* = 8 Hz, pyridine-H), 10.24 (s, 1 H, pyridine-H). Anal. ($C_{15}H_{15}BrN_2O_4$) C, H, N.

Ethyl 1-(4-bromobenzyl)pyridinium bromide-3-carboxylate (**8**)

The crude product was recrystallized from AcOH to give **8** (45 %): mp 93–5 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, 3 H, *J* = 7 Hz, CH_3CH$_2$), 4.35–4.47 (q, 2 H, *J* = 7 Hz, CH$_3$CH_2), 5.98 (s, 2 H, PhCH_2-), 7.57–7.69 (dd, 4 H, *J* = 9 Hz, ArH), 8.29–8.32 (m, 1 H, pyridine-H), 9.00 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.36 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.87 (s, 1 H, pyridine-H). Anal. ($C_{15}H_{15}Br_2NO_2$) C, H, N.

Ethyl 1-(4-flourobenzyl)pyridinium bromide-3-carboxylate (**9**)

The crude product was chromatographed on C_{18} silica (20 % MeOH, H_2O) to give **9** as yellowish brown gum (59 %): ^{1}H NMR (DMSO-d_6) δ 1.37 (t, 3 H, *J* = 7 Hz, CH_3CH$_2$-), 4.35–4.47 (q, 2 H, *J* = 7 Hz, CH$_3$CH_2-), 6.07 (s, 2 H, PhCH_2-), 7.26–7.32 (m, 2 H, ArH), 7.72–7.75 (m, 2 H, ArH), 8.31–8.35 (m, 1 H, pyridine-H), 9.01 (d, 1 H, *J* = 8 Hz, pyridine-H), 9.49 (d, 1 H, *J* = 8 Hz, pyridine-H), 9.83 (s, 1 H, pyridine-H). Anal. ($C_{15}H_{15}FBrNO_2$) C, H, N.

3-[N-(2-Chloroethyl)carbamoyl]pyridine (**11**)

Method A

2-Chloroethylamine HCl (2.32 g, 0.02 mol) was dissolved in water (25 mL) and basified using 30 % NaOH solution (10 mL). The aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was separated, dried, and evaporated to give the free base of 2-chloroethylamine which dissolved in pyridine (30 mL). Nicotinic acid (**1**, 1.2 g, 0.01 mol) was added to the pyridine solution followed by dicyclohexylcarbodiimide (DCC, 2.1 g, 0.01 mol) portionwise. The reaction mixture was stirred at room temperature for 20 h. The precipitated product was then filtered and dissolved in CH_2Cl_2. The organic layer was washed with water, dried, and evaporated under reduced pressure. The obtained residue was then recrystallized from EtOH/Hexane to give **11** (30 %): mp 228–30 °C, ^{1}H NMR (DMSO-d_6) δ 3.59–3.63 (q, 2 H, *J* = 6 Hz, NHCH_2-), 3.76 (t, 2 H, *J* = 6 Hz, -CH$_2$Cl), 7.52–7.54 (m, 1H, pyridine-H), 8.18–8.21 (m, 1 H, pyridine-H), 8.72–8.73 (m, 1 H, pyridine-H), 8.96 (brs, 1 H, NH), 9.02 (s, 1 H, pyridine-H). Anal. ($C_8H_9ClN_2O$) C, H, N.

Method B

A mixture of **3** (1.7 g, 0.01 mol) and thionyl chloride (10 mL) was heated under reflux for 2 h. Excess thionyl chloride was removed under reduced pressure, and the obtained residue was triturated with petroleum ether 60–80 °C filtered, dried and recrystallized from EtOH/Hexane (25 %).

1-(4-Bromobenzyl)-3-[N-(2-chloroethyl)carbamoyl]pyridinium bromide (**12**)

4-Bromobenzyl bromide (2.5 g, 0.01 mol) was added to a stirred solution of **11** (1.9 g, 0.01 mol) in acetonitrile (20 mL). The reaction mixture was stirred at room temperature for 18 h. Excess solvent was evaporated under reduced pressure, and the obtained residue was triturated with petroleum ether 60–80 °C. The solid product was then recrystallized from MeOH/Hexane to give **12** (20 %): mp 162–5 °C, ^{1}H NMR (DMSO-d_6), δ 3.55–3.65 (q, 2 H, *J* = 6.5 Hz, NHCH_2-), 3.78 (t, 2 H, *J* = 6 Hz, -CH$_2$Cl), 5.98 (s, 2 H, PhCH_2), 7.56–7.70 (dd, 4 H, *J* = 9 Hz, ArH), 8.30–8.33 (m, 1 H, pyridine-H), 8.98 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.40 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.76 (s, 1 H, pyridine-H), 10.23 (brs, 1 H, NH). Anal. ($C_{15}H_{15}ClBr_2N_2O$) C, H, N.

1-Benzyl-1,4-dihydropyridine-3-carboxylic acid (**15**)

A suspension of 1-benzylpyridinium bromide-3-carboxylic acid (**5**, 2.9 g, 0.01 mol), in deaereated water (200 mL) and CH_2Cl_2 (100 mL) was cooled to 0 °C and stirred, under nitrogen stream. Na_2CO_3 (6.4 g, 0.06 mol) was added portionwise over a period of 10 min. $Na_2S_2O_4$ (7.0 g, 0.04 mol) was then added portionwise over a period of 15 min. Stirring was continued, under nitrogen stream at 0 °C, for another 1 h. The organic layer was separated, washed with cold deaereated water, dried, and evaporated in vacuo to afford **15** as a yellowish oil (20 %). ^{1}H NMR ($CDCl_3$) δ 2.59–2.68 (m, 2 H, C_4-H), 4.73–4.79 (m, 1 H, C_5-H), 5.59–5.83 (m, 3 H, PhCH_2- & C_6-H), 7.21 (s, 1 H, C_2-H), 7.34–7.52 (m, 5 H, ArH), 10.32 (s, 1 H, COOH). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

3-{N-[2-Bis(2-chloroethyl)aminoethyl]carbamoyl}pyridine (**17**)

A solution of bis-(2-chloroethyl)amine HCl (2.2 g, 0.013 mol) in aqueous NaOH (30 %, 10 mL) was extracted with toluene (20 mL). The organic layer was dried using anhydrous Na_2SO_4, filtered, and added to a solution of **11** (1.9 g, 0.01 mol) and K_2CO_3 (2.8 g, 0.02 mol) in dry toluene (20 mL). The reaction mixture was heated under reflux for 18 h. Solvents were then evaporated in vacuo and the obtained residue was dissolved in water (30 mL), extracted with CH_2Cl_2 (2 × 30 mL). The combined extract was evaporated in vacuo to give **17** as a yellowish oil (32 %). ^{1}H NMR ($CDCl_3$), δ 3.62–3.99 (m, 8 H, -NCH$_2$-), 4.09 (t, 2 H, *J* = 6 Hz, -CH$_2$Cl), 4.55 (t, 2 H, *J* = 6 Hz, -CH$_2$Cl), 7.53–7.70 (m, 1 H, pyridine-H), 8.13–8.35 (m, 2 H, pyridine-H), 9.83–9.98 (m, 2 H, pyridine-H & NH). Anal. ($C_{12}H_{17}Cl_2N_3O$) C, H, N.

General Procedure for Preparation of 1-(benzyl or 4-substituted benzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-pyridinium halides (**18–22**)

The appropriate benzyl halide (0.015 mol) was added to a stirred solution of 3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}pyridine (**17**, 2.9 g, 0.01 mol) in acetonitrile (30 mL). The reaction mixture was stirred at room temperature for 18 h. Solvent was removed under reduced pressure to afford the crude residues of **18–22**.

1-Benzyl-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-pyridinium bromide (**18**)

The crude product was recrystallized from EtOH/Hexane to give **18** (45 %): mp 48–9 °C; ^{1}H NMR (DMSO-d_6) δ 2.68–2.93 (m, 8 H, -CH_2N-), 4.15 (t, 2 H, *J* = 8 Hz, -CH_2Cl), 4.61 (t, 2 H, *J* = 8 Hz, -CH_2Cl), 6.42 (s, 2 H, PhCH_2-), 7.43–7.52 (m, 5 H, ArH), 7.83–7.92 (m, 1 H, pyridine-H), 8.34 (d, 1 H, *J* = 6 Hz, pyridine-H), 8.88 (d, 1 H, *J* = 6 Hz, pyridine-H), 9.53 (s, 1 H, pyridine-H), 10.53 (brs, 1 H, NH). Anal. ($C_{19}H_{24}Cl_2BrN_3O$) C, H, N.

1-(4-Flourobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}pyridinium bromide (**19**)

The crude product was recrystallized from MeOH/Hexane to give **19** (65 %): mp 78–9 °C; ^{1}H NMR (DMSO-d_6) δ 3.62–3.77 (m, 8 H, -CH_2N-), 4.09 (t, 2 H, *J* = 9.5, -CH_2Cl), 4.56 (t, 2 H, *J* = 9.5 Hz, -CH_2Cl), 5.96 (s, 2 H, PhCH_2-), 7.28–7.69 (m, 4 H, ArH), 8.26–8.29 (m, 1 H, pyridine-H), 8.92 (d, 1 H, *J* = 8 Hz, pyridine-H), 9.31 (d, 1 H, *J* = 6 Hz, pyridine-H), 9.68 (s, 1 H, pyridine-H), 10.08 (brs, 1 H, NH). Anal. ($C_{19}H_{23}FCl_2BrN_3O$) C, H, N.

1-(4-Chlorobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}pyridinium chloride (**20**)

The crude product was recrystallized from EtOH/Hexane to give **20** (50 %): mp 65–7 °C; ^{1}H NMR (DMSO-d_6) δ 3.30–3.65 (m, 8 H, -CH_2N-), 4.12 (t, 2 H, *J* = 10 Hz, -CH_2Cl), 4.62 (t, 2 H, *J* = 10 Hz, -CH_2Cl), 5.78 (s, 2 H, PhCH_2-), 7.25–7.70 (m, 4 H, ArH), 8.24–8.26 (m, 1 H, pyridine-H), 8.94 (d, 1 H, *J* = 7 Hz, pyridine-H), 9.25 (d, 1 H, *J* = 6 Hz, pyridine-H), 9.72 (s, 1 H, pyridine-H), 10.52 (brs, 1 H, NH). Anal. ($C_{19}H_{23}Cl_4N_3O$) C, H, N.

1-(4-Bromobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}pyridinium bromide (**21**)

The crude product was recrystallized from EtOH/Hexane to give **21** (52 %): mp 59–60 °C; ^{1}H NMR (DMSO-d_6) δ 3.34–3.68 (m, 8 H, -NCH_2-), 4.12 (t, 2 H, *J* = 8 H, -CH_2Cl), 4.57 (t, 2 H, *J* = 8 H, -CH_2Cl), 5.85 (s, 2 H, PhCH_2-), 7.45–7.76 (dd, 4 H, *J* = 9 Hz, ArH), 8.21 (m, 1 H, pyridine-H), 8.94–9.16 (m, 2 H, pyridine-H), 9.48 (s, 1 H, pyridine-H), 10.34 (brs, 1 H, NH). Anal. ($C_{19}H_{23}Cl_2Br_2N_3O$) C, H, N.

1-(4-Nitrobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}pyridinium bromide (**22**)

The crude product was recrystallized from MeOH/Hexane to give **22** (72 %): mp 91–3 °C; ^{1}H NMR (DMSO-d_6) δ 3.35–3.56 (m, 8 H, -NCH_2-), 4.19–4.61 (m, 4H, -CH_2Cl), 6.05 (s, 2 H, PhCH_2-), 7.72–7.75 (m, 5 H, ArH & pyridine-H), 8.26–8.34 (m, 2 H, pyridine-H), 9.19–9.35 (m, 2 H, pyridine-H & NH). Anal. ($C_{19}H_{23}Cl_2BrN_4O_3$) C, H, N.

General Procedure for Preparation of 1-(benzyl or 4-substituted benzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-1,4-dihydropyridines (**23–27**)

A suspension of 1-(benzyl or 4-substituted benzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}pyridinium halides (**18–22**, 0.01 mol), in deaereated water (200 mL) and CH_2Cl_2 (100 mL) was cooled to 0 °C and stirred under nitrogen stream. Na_2CO_3 (6.4 g, 0.06 mol) was added portionwise over a period of 15 min. $Na_2S_2O_4$ (7.0 g, 0.04 mol) was then added portionwise over a period of 15 min. Stirring was continued, under nitrogen stream at 0 °C, for another 1 h. The organic layer was separated, washed with cold deaereated water, dried and evaporated *in vacuo* to give the crude **23–27**.

1-Benzyl-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-1,4-dihydropyridine (**23**)

The obtained crude product was chromatographed on silica gel (5 % EtOAc, $CHCl_3$) to give **23** as a sticky gum (60 %); ^{1}H NMR ($CDCl_3$) δ 2.64–2.72 (m, 2 H, C_4-H), 3.62–3.86 (m, 8 H, -NCH_2-), 4.18–4.33 (m, 4 H, -CH_2Cl), 4.71–4.79 (m, 1 H, C_5-H), 5.57–5.74 (m, 3 H, C_6-H & PHCH_2-), 6.78 (s, 1 H, C_2-H), 7.14 (m, 5 H, ArH), 10.27 (brs, 1 H, NH). Anal. ($C_{19}H_{25}Cl_2N_3O$) C, H, N.

1-(4-Flourobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamolyl}-1,4-dihydropyridine (**24**)

The obtained crude product was chromatographed on silica gel (5 % EtOAc, $CHCl_3$) to give **24** as a sticky gum (75 %); ^{1}H NMR ($CDCl_3$) δ 2.50–2.54 (m, 2 H, C_4-H), 3.38–3.67 (m, 8 H, -NCH_2-), 4.04–4.29 (m, 4 H, -CH_2Cl), 4.53–4.58 (m, 1 H, C_5-H), 5.52–5.73 (d, 1 H, *J* = 7 Hz, C_6-H), 5.93 (s, 2 H, PhCH_2-), 6.98–7.67 (m, 5 H, ArH & C_2-H), 9.65 (brs, 1 H, NH). Anal. ($C_{19}H_{24}FCl_2N_3O$) C, H, N.

1-(4-Chlorobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}-1,4-dihydropyridine (**25**)

The obtained crude product was chromatographed on silica gel (5 % EtOAc, $CHCl_3$) to give **25** as a sticky gum (80 %); ^{1}H NMR ($CDCl_3$) δ 2.93–3.01 (m, 2 H, C_4-H), 3.49–3.65 (m, 8 H, -NCH_2-), 3.75–4.23 (m, 4 H, -CH_2Cl), 4.53–4.62 (m, 1 H, C_5-H), 4.75 (s, 2 H, PhCH_2-), 5.73–5.85 (m, 1 H, C_6-H), 6.83–7.35 (m, 5 H, ArH & C_2-H), 9.49 (brs, 1 H, NH). Anal. ($C_{19}H_{24}Cl_3N_3O$) C, H, N.

1-(4-Bromobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]-carbamoyl}-1,4-dihydropyridine (**26**)

The obtained crude product was chromatographed on silica gel (5 % EtOAc, $CHCl_3$) to give **26** as a sticky gum (75 %); ^{1}H NMR ($CDCl_3$) 2.48–2.73 (m, 2 H, C_4-H), 3.41–3.59 (m, 8 H, -NCH_2-), 3.74–3.90 (t, 2 H, *J* = 8 Hz, -CH_2Cl), 4.25–4.28 (t, 2 H, *J* = 8 Hz, -CH_2Cl), 4.72–4.83 (m, 1 H, C_5-H), 5.88–5.91 (m, 3 H, PhCH_2- & C_6-H), 7.23–7.54 (m, 5 H, ArH & C_2-H), 10.33 (brs, 1 H, NH). Anal. ($C_{19}H_{24}Cl_2BrN_3O$) C, H, N.

1-(4-Nitrobenzyl)-3-{N-[2-bis(2-chloroethyl)aminoethyl]carbamoyl}-1,4-dihydropyridine (**27**)

The obtained crude product was chromatographed on silica gel (5 % EtOAc, $CHCl_3$) to give **27** as a sticky gum (64 %); ^{1}H NMR ($CDCl_3$) δ 2.89–3.01 (m, 2 H, C_4-H), 3.47–3.76 (m, 8 H, -NCH_2-), 4.15–4.39 (m, 4 H, -CH_2Cl), 4.62–4.72 (m, 1 H, C_5-H), 4.93–5.15 (m, 3 H, PhCH_2- & C_6-H), 7.23–7.68 (m, 5 H, ArH & C_2-H), 9.63 (brs, 1 H, NH). Anal. ($C_{19}H_{24}Cl_2N_4O_3$) C, H, N.

Chemical oxidation of the 1,4-dihydropyridine analogs **23** *and* **27** *by hydrogen peroxide*

The 1,4-dihydropyridine analogs **23** and **27** (0.1 mg) were added to 30 % hydrogen peroxide (2 mL). The mixture was stirred and samples were monitored by HPLC for the concentration of the corresponding quaternaries **18** and **22**. Acetonitrile (10 %) in 0.2 % acetic acid solution was used as the mobile phase at a flow rate of 1.5 mL/min and UV detector was used to follow the formation of the products at λ_{max} 262 nm.

Kinetics of oxidation of the 1,4-dihydropyridine analogs **23** *and* **27** *in brain homogenate*

In an ice bath, the rat brain tissue (about 1.4 g) was homogenized in 20 mL of phosphate buffer (pH 7.4). Aliquot of the brain homogenate (4 mL) was kept in 37 °C water bath for 5 min. The tested 1,4-dihydropyridine, 0.2 mL of a 6.25×10^{-4} mol in

DMSO, was added to the brain homogenate. At each time interval, 0.5 mL of the brain homogenate-drug mixture was mixed with 0.5 mL acetonitrile and kept in the freezer (4 °C), until assayed. After the collection of all samples, they were centrifuged and supernatants were analyzed by HPLC for their quaternary salts contents, using the same mobile phase and conditions mentioned under hydrogen peroxide chemical oxidation.

In vivo study of the 1,4-dihydropyridine analog **23**

Eighteen male Wistar rats (150 ± 50 g) were used in this study. Rats were randomly divided into 6 groups for different sampling time, and each group was housed in one cage. The animals were anesthetized with urethane solution (0.7 mL, 25 % in H_2O). After 10 min, each animal was injected with compound **23** solution in a mixture of DMSO: phosphate buffer (pH 7.4), 2:1 at concentration of 25 mg/mL through the tail vein at a dose of 40 mg/kg. At appropriate time interval (10, 30, 60, 90, 110, 130 and 160 min), the animal was decapitated and 1 mL blood was collected from the trunk and stored in heparinized tube. Meanwhile, brain was removed, weighed, and covered with aluminum foil. The blood samples and the brains were kept in a deep freezer (–86 °C) until assayed. Each brain was homogenized with 1 mL of water, 4 mL of acetonitrile was added and the mixture was homogenized again. Blood samples were mixed with 4 mL of acetonitrile, for protein precipitation, and vortexed at high speed for 1 min. Both brain homogenate and blood samples were centrifuged at 20,000 rpm for 10 min, and the supernatants were evaporated under nitrogen. The residue were reconstituted with the mobile phase and analyzed using the same HPLC conditions mentioned above, under hydrogen peroxide chemical oxidation. The mean calibration curve of the quaternary salt was plotted, the mean best-fit linear regression equation was derived and used to estimate the concentration of the quaternary salt at different time intervals.

Evaluation of the alkylating activity of the prepared quaternary compounds **18–22**

To a solution of the quaternary alkylating agents **18–22** (10 mg) in methyl ethyl ketone (5 mL), 4-(4-nitrobenzyl)pyridine reagent (5 mL) and water (1 mL) were added. The mixture was then either heated on a boiling water bath or kept at 55 °C. 0.2 mL were pipetted from the reaction mixture at different time intervals; 5, 10, 15, 25, 30 and 35 min. The reaction mixture was cooled for 1 min in an ice bath, triethylamine reagent (3 mL) was added and the solution was mixed [21]. The intensity of the purple color immediately developed was measured at the appropriate λ_{max} within 2 min against a reagent blank.

References

[1] P. Gwilt, *Drug Delivery Systems* in *Modern Pharmacology*, 2nd ed (Ed.: C. Craig; R. Stitzel), Little, Brown, and Company, Boston, Toronto, USA, **1986**, pp. 96–109.

[2] M. Mort, *Modern Drug Dis.*, **2000**, *3*, 30–34.

[3] Y. Chien, *Controlled Drug Release from Polymeric Delivery Systems* in *Drug Delivery Systems* (Ed.: R. Juliano), Oxford Univ. Press, Oxford, UK. **1980**, pp. 11–83.

[4] S. Rapoport, Sites and Functions of the Blood-Brain Barrier in: *Blood-Brain Barrier in Physiology and Medicine* (Ed.: S. Rapoport), Raven Press. New York, **1976**, pp. 43–86.

[5] P. Chem, N. Bodor, W. Wu, L. Prokai, *J. Med. Chem.*, **1998**, *41*, 3773–3781.

[6] K. Rana, N. Bodor, A. Elkoussi, I. Raad, A. Miyake, H. Houck, N. Gilder-Sleeve, *J. Med. Virol.*, **1986**, *20*, 1–8.

[7] W. Anderson, J. Simpkins, P. Woodard, D. Winood, W. Stern, N. Bodor, *Psychopharmacology (Berl.)*, **1987**, *92*, 157–163.

[8] N. Greig, E. Daly, D. Sweeney, S. Rapoport, *Cancer Chemother. Pharmacol.*, **1990**, *25*, 320–325.

[9] P. Krogsgaard-Larsen, A. Christiansen, *Eur. J. Med. Chem.*, **1979**, *14*, 157–164.

[10] M. Brewster, K. Raghavan, E. Pop, N. Bodor, *Antimicrob. Agents Chemother.*, **1994**, *38*, 817–823.

[11] E. Pop, N. Bodor, *Review – Epilepsy Res.*, **1992**, *13*, 1–16.

[12] K. Raghavan, T. Loftsson, M. Brewster, N. Bodor, *Pharm. Res.*, **1992**, *9*, 743–749.

[13] M. Brewster, E. Pop, A. Braunstein, A. Pop, A. Druzgala, A. Drinculescu, W. Anderson, A. Elkoussi, N. Bodor, *Pharm. Res.*, **1993**, *10*, 1356–1362.

[14] M. Brewster, M. Bartruff, W. Anderson, P. Druzgala, N. Bodor, E. Pop, *J. Med. Chem.*, **1994**, *37*, 4237–4244.

[15] U. Eisner, J. Kuthan, *Chem. Rev.*, **1972**, *72*, 1.

[16] N. Bodor, H. Farag, F. Omar, *Pharm. Sci.*, 1995, *1*, 301–306.

[17] K. Raghavan, E. Shek, N. Bodor, *Anti-Cancer Drug Des.*, **1987**, *2*, 25–36.

[18] N. Bodor, V. Venkatraghavan, D. Winwood, K. Estes, M. Brewster, *Int. J. Pharm.*, **1989**, *53*, 195–208.

[19] M. Brewster, A. Simay, K. Czako, D. Winwood, H. Farag, N Bodor, *J. Org. Chem.*, **1989**, *54*, 3721–3725.

[20] N. Bodor, J. Simpkins, *Science*, **1983**, *221*, 65–67.

[21] N. A. El-Koussi, Ph.D. Thesis, Faculty of Pharmacy, Assiut University, **1989**.