



Quantitative Structure–Activity Relationships for a Series of Symmetrical Bisquaternary Anticancer Compounds

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Abstract—56 biscationic dibromides with distinct polar heads [bis(4-substituted)pyridinium, bis(4-aminoquinolinium), bisquinolinium, and bisisoquinolinium moieties] and several spacers between the two charged nitrogen atoms were synthesised. This oriented synthesis produced 45 inhibitors of choline kinase with antitumour activity against the HT-29 cell line. In an attempt to understand the antiproliferative activity, a quantitative structure–activity relationship was developed. The unknown σ_R and σ_R^+ descriptors for the diallylamino, pyrrolidino, piperidino and perhydroazepino groups and σ_R for the *N*-methylanilino moiety, were estimated by ¹³C NMR spectroscopy in a simple, fast and reproducible manner. The electron characteristic of the substituent at position 4 of the heterocycle and the theoretical lipophilic character of the whole molecule were found to significantly affect the antitumour activity. 1,1'-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(*N*-methylanilino)pyridinium] dibromide is the most active compound of the series so far described and shows a reasonable agreement between predicted and observed antiproliferative data (predicted pIC₅₀ = 6.50, experimental pIC₅₀ = 6.46). © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

The need to selectively target any drug is not only desirable but of the utmost necessity, especially in cancer chemotherapy. All the anti-neoplastic drugs known so far are limited to their selectivity and therefore tend to destroy all actively proliferating cells, including the normal cells. Medicinal chemists are eager to develop drug-targeted systems that would lower or eliminate the side effects of drugs, and thus increase their therapeutic index.

The advances in quantitative structure–activity relationship (QSAR) studies have widened the scope of rationalising drug design and the search for the mechanisms of drug actions. Bisquaternary salts have been among the favourite classes of chemical compounds for QSAR studies.^{1,2} Factors contributing to such popularity have included the facility of synthesis and the wide variations in potency induced by changes in structure. With the aim to rationalize the SAR of biscationic derivatives in terms of physicochemical

properties, we have applied the classical Hansch analysis to the series of compounds under study looking for correlations between the variation of anti-proliferative activity and the variation of parameters describing the electronic and hydrophobic properties of the molecules.³

Lipid metabolic pathways are frequently altered during carcinogenesis. Some of them play an important role in mitogenic signalling such as diacylglycerol and phosphatidylinositol. Phosphorylcholine (*PCho*) is generated by choline kinase (ChoK) after mitogenic stimulation by growth factors, and is found increased in human tumours.^{4–6} In vivo evidence that ChoK is a novel target for the design of antitumour drugs has been reported,^{7,8} pointing out that ChoK might play a role in growth promotion or signal transduction in carcinogenesis. We have correlated the inhibitory effect on proliferation of symmetrical bisquaternary compounds⁶ with their ability to inhibit the production of *PCho* in whole cells. When the 1,2-ethylene(bisbenzyl) moiety was used as a linker between the two 4-substituted pyridinium cationic heads,⁹ the structures were screened for their activity inhibiting isolated ChoK (under ex vivo conditions). The 4-NR₂ group made a substantial contribution and was suggested⁹ that the role of the

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4-NR₂ group was electronic, via delocalisation of the positive charge. The importance of frontier orbital energies (LUMO) of model compounds has been emphasised and interpreted.¹⁰

There is a need for group substituent constants that are experimental unavailable. Herein, we describe a method which gives an estimation of several unknown σ_R and σ_R^+ descriptors. Although estimated constants are not as dependable as reliable experimental constants, they are nevertheless of considerable use. We have tried to correlate $p(\text{IC}_{50})_{\text{ChoK}}$ of the whole set of compounds with the electronic and lipophilic parameters. The attempts have been fruitless but when the set of compounds was smaller, it was suggested the occurrence of either charge transfer or bipolar interactions.¹⁰ The activity against the HT-29 cell line is normally higher than the corresponding activity against ChoK and it might be possible that the bisquaternary salts also act on another unknown target (see Tables 4–6). The substitution pattern of the bis(4-substitutedpyridinium) moiety and the modification of the spacer connecting the two cationic heads have led to a more potent anti-proliferative symmetrical bisquaternary compound that arises from a QSAR study.

Results and Discussion

Chemistry

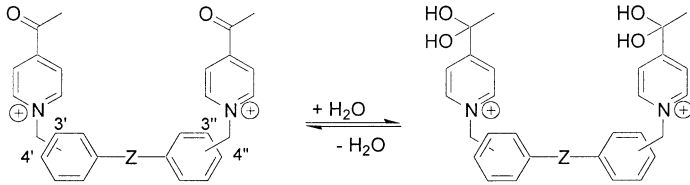
Compounds **1–63** were prepared by quaternisation of the heterocycle nitrogen by the appropriate dibromide in butanone as described previously (Scheme 1).^{9,10} The species comprising the transition state of the reaction are more polar than the reagents and the lone pair of the endocyclic nitrogen of the aromatic system will have to be free to attack the electrophile. Therefore the solvent must be polar to stabilise the transition state and aprotic to not interact with the *N*-lone pair by hydrogen-bonding. Butanone was then selected.

Compound 4-(*N*-methylanilino)pyridine **64** was obtained by reacting the 4-chloropyridine hydrochloride and *N*-methylaniline in refluxing pentanol as shown in

Scheme 2. Compound **64** was previously described by heating a mixture of 4-chloropyridine and *N*-methylaniline under reflux on a boiling water bath.¹¹ Protonation of the pyridine ring, however, brings about an increase in the lability of the halogen and, moreover, the use of a high-boiling-point alcohol led us to an increase in the yield of **64**.

Although the NMR of compounds **1–63** looked clean, in general there were problems with their elemental analyses. When the necessary molecules of water were added, the elemental analyses fitted perfectly. The C, H and N values did not improve significantly when the drying of the samples was carried out in high vacuum at higher temperatures. Nevertheless, the situation was different for compound **23** (Table 1): in the ¹H and ¹³C NMR spectra, each signal appeared in duplicate, showing, therefore, the presence of a second very similar structure (**23b**) to the bis[4-(acetyl)pyridinium] compound (**23a**). The ¹H NMR spectrum showed that compound **23a** is in 69% in the mixture. Keeping in mind that water is present in **23a**, it is plausible that it could be chemically added to the carbonyl groups to give the bis(*gem*diol) form **23b**. This might be the only possibility for the compatibility of the elemental analysis for the two structures **23a** and **23b**. The π -deficient heterocyclic system exerts a significant electron-withdrawing effect on the carbonyl group and consequently nucleophilic attack at that centre takes place readily, in spite of the poor nucleophilicity of water. The situation is similar to highly halogenated ketones and aldehydes, which form stable hydrates due to the electronegativity of the groups attached to the carbon carbon.¹² Scott's group reported a direct application of NMR spectroscopy to the structure of well-designed tetrahedral inhibitor complexes. In the case of trypsin,¹³ a ¹³C NMR study of the complex between the enzyme and *p*-amidino-phenylpyruvic acid revealed characteristic resonances (δ 202.5 ppm for the keto group of the substrate and δ 95.4 ppm for the hydrated keto form). In our case, the resonance of the carbonyl group of **23a** was at δ 195.56 ppm and that of the *gem*diol carbon of **23b** was at δ 98.14 ppm. The same hydration of the carbonyl moieties was found for the rest of the bis(acetyl)pyridinium structures (**5a,b**, **30a,b**, **10a,b** and **16a,b**). Table 1 shows

Table 1. Ratio of the bis[4-(acetyl)pyridinium] **5a**, **30a**, **10a**, **16a** and **23a** and the corresponding bis(*gem*diol) compounds **5b**, **30b**, **10b**, **16b** and **23b**



Space isomer	Z	Bisacetyl form/ (%)	Bis(<i>gem</i> diol) form/(%)	Bis[4-(acetyl)] form (%)	Bis(<i>gem</i> diol) form (%)
3',3''	—	5a	5b	43	57
4',4''	—	10a	10b	73	27
4',4''	CH ₂	16a	16b	44	56
4',4''	(CH ₂) ₂	23a	23b	69	31
4',4''	(CH ₂) ₃	30a	30b	69	31

the ratio of the bis[4-(acetyl)pyridinium] **5a**, **30a**, **10a**, **16a** and **23a** and the corresponding bis(*gem*diol) compounds **5b**, **30b**, **10b**, **16b** and **23b**.

Spectroscopic behaviour of compounds 57–59

This paper reports on an NMR study of **57–59** (Table 5), including the full assigned ^1H and ^{13}C NMR spectra. One of the most characteristic features of the ^1H NMR CD_3OD spectra of **57–59** is that the signals at $\approx\delta$ 8.3 ppm (H-2_{pyr}) and $\approx\delta$ 6.9 ppm (H-3_{pyr}) are very broad singlets at room temperature and they sharpen and change to doublets on warming (see Fig. 1 for details).¹⁴ Regarding the ^{13}C NMR CD_3OD spectrum, it must be pointed out that the signals, at δ 143.63 ppm (C-2_{pyr}) and at δ 110.26 ppm (C-3_{pyr}) do not appear in the DEPT experiment, but they appear at higher temperatures (ca. 77 °C in $\text{DMSO-}d_6$).

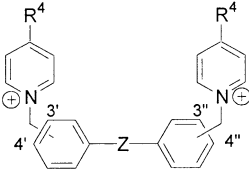
T_1 of pyridinium protons were determined for structures **57–59** (see Experimental for details). These protons relax faster than the rest of the aromatic protons on the

same structure. On the other hand, different compounds with pyridinium protons that show two doublets on the ^1H NMR spectra at room temperature, relax similarly to the rest of the aromatic protons on the same structure (data not shown).

For viscous liquids, molecular orientations are not random, and transfer of energy spin–lattice relaxation is efficient and longitudinal relaxation time (T_1) is small,¹⁵ and this is why broad signals were obtained for H-2 and H-3 of the pyridium moiety of **57–59** in the NMR spectra. In the cases in which the relaxation of ^{13}C nuclei is produced by a spin–spin relaxation, it is common to notice the broadening of lines in ^{13}C NMR spectra. For instance, ^{13}C nuclei linked to ^{14}N nuclei appear frequently as very broad signals.¹⁴

The extension of the conjugation through the 4-amino substituent on the pyridinium ring must result in an overall reduced tumbling of the molecule, and the consequent reduction of T_1 .¹⁶ ^1H and ^{13}C NMR spectra were studied in $\text{DMSO-}d_6$ gradually increasing the tem-

Table 2. CSD^a of several R^4 moieties of symmetrical bispyridinium structures and bibliographical values of descriptors σ_P , σ_R and σ_R^+



Compd	R^4	Z	Spacer isomer	CSD ^a	σ_P^b	σ_R^b	σ_R^{+b}
1	–NMe ₂	—	3',3''	–3.92	–0.71	–0.88	–1.22
2	–NH ₂	—	3',3''	–3.62	–0.63	–0.80	–1.10
3	–CH ₂ OH	—	3',3''	–0.71	0.04	–0.07	–0.15
4	–Me	—	3',3''	–0.82	–0.17	–0.16	–0.25 ^c
5	–COMe	—	3',3''	0.22	0.50	0.20	0.06
6	–NMe ₂	—	4',4''	–3.96	–0.71	–0.88	–1.22
7	–NH ₂	—	4',4''	–3.67	–0.63	–0.80	–1.10
8	–CH ₂ OH	—	4',4''	–0.66	0.04	–0.07	–0.15
9	–Me	—	4',4''	–0.89	–0.17	–0.16	–0.25 ^c
10	–COMe	—	4',4''	0.22	0.50	0.20	0.06
11	–CH=NOH	—	4',4''	–0.56	–0.10 ^c	—	–0.12
12	–NMe ₂	CH ₂	4',4''	–3.97	–0.71	–0.88	–1.22
13	–NH ₂	CH ₂	4',4''	–3.71	–0.63	–0.80	–1.10
14	–CH ₂ OH	CH ₂	4',4''	–0.72	0.04	–0.07	–0.15
15	–Me	CH ₂	4',4''	–0.88	–0.17	–0.16	–0.25 ^c
16	–COMe	CH ₂	4',4''	0.22	0.50	0.20	0.06
17	–CN	CH ₂	4',4''	0.96	0.65	0.13	0.13
18	–CH=NOH	CH ₂	4',4''	–0.57	–0.10 ^c	—	–0.12
19	–NMe ₂	(CH ₂) ₂	4',4''	–3.98	–0.71	–0.88	–1.22
20	–NH ₂	(CH ₂) ₂	4',4''	–3.72	–0.63	–0.80	–1.10
21	–CH ₂ OH	(CH ₂) ₂	4',4''	–0.73	0.04	–0.07	–0.15
22	–Me	(CH ₂) ₂	4',4''	–0.89	–0.17	–0.16	–0.25 ^c
23	–COMe	(CH ₂) ₂	4',4''	0.19	0.50	0.20	0.06
24	–CN	(CH ₂) ₂	4',4''	1.01	0.65	0.13	0.13
25	–COOH	(CH ₂) ₂	4',4''	0.31	0.41	0.11	—
26	–NMe ₂	(CH ₂) ₃	4',4''	–4.03	–0.71	–0.88	–1.22
27	–NH ₂	(CH ₂) ₃	4',4''	–3.77	–0.63	–0.80	–1.10
28	–CH ₂ OH	(CH ₂) ₃	4',4''	–0.71	0.04	–0.07	–0.15
29	–Me	(CH ₂) ₃	4',4''	–0.88	–0.17	–0.16	–0.25 ^c
30	–COMe	(CH ₂) ₃	4',4''	0.22	0.50	0.20	0.06
31	–CN	(CH ₂) ₃	4',4''	0.97	0.65	0.13	0.13

^aCSD = $(\delta^{13}\text{C}_{\text{R}^4} - \delta^{13}\text{C}_{\text{H}})_{\text{CD}_3\text{OD}}$; positive values indicate decreased shielding; CSD values are the average of three measurements.

^b σ_P : Hammett constant for *para* substitution; σ_R : electronic parameter for resonance effect; σ_R^+ : electronic parameter defined for systems where a + charge is delocalised between substituent and reaction centre via 'through resonance'; otherwise stated, the σ_P , σ_R and σ_R^+ values have been taken from ref 24.

^cRef 25.

perature (see Fig. 1) to diminish the viscosity and to increase molecular tumbling, making T_1 larger.

The ^1H NMR spectrum of **58** was run at 57°C and showed a doublet at δ 8.45 ppm ($J_{2,3}=7.5$ Hz) and another one, not so well resolved at δ 6.91 ppm. When the same spectrum was run at 97°C , the doublet corresponding to H-2_{pyr} appeared slightly shifted at δ 8.42 ppm ($J_{2,3}=7.5$ Hz) and the signal corresponding to H-3_{pyr} appeared perfectly defined as a doublet centred at δ 6.91 ppm ($J_{2,3}=7.4$ Hz). The spectrum run at 77°C showed an intermediate behaviour to that shown at 57 and 97°C .

Pharmacology

Compounds **1–63** were tested using purified ChoK from yeast. Next, the bisquaternary compounds **1–63** were screened on anti-proliferative assays, using the human tumour-derived cell line HT-29 mainly resistant to chemotherapy.¹⁷ Recently, the function of the tyrosine kinase Src has been identified as an essential step for the tumorigenic activity of HT-29 cells.¹⁸ Given that ChoK inhibitors drastically impair proliferation of *src*-transformed marine fibroblasts,⁶ HT-29 cells seemed to be a good system to use in the screening for ChoK inhibitors as new anticancer drugs. The Hill equation was fitted to the data to obtain estimates of the IC_{50} . Table 4 shows the structures and biological results for compounds **1–56** arranged in decreasing order of their inhibitory potencies of isolated ChoK.

Application of the ^{13}C chemical shift differences to the determination of the σ_R and σ_R^+ constants of several dialkylamino groups

Over the years, considerable interest has been shown in the application and use of Hammett parameters for correlation with electronic properties associated with various substituents. Since the Hammett constants

represent a measure of the interaction between the substituent of the aromatic ring and the reaction centre, attempts are often made to use a variety of physical methods to obtain linear correlations between the sigma values and the given set of physical parameters.¹⁹ Methods of estimation of substituent constants experimental as yet unavailable are required.

The NMR chemical shift serves as a sensitive probe for the estimation and calculation of the transmission of the electronic effects of substituents. The Hammett relationship has also been extended to pyridine as an empirical method for correlating reactivity with aromatic structure.^{20,21} In the pyridine ring, unlike the benzene ring, the nuclear nitrogen atom acts as a functional centre. The nitrogen ring polarizes the ring towards itself, both inductively and mesomerically. Such an effect is considerably accentuated in the pyridinium ion, where the nitrogen carries a positive charge.

The chemical shifts of the *N*-methyl protons of substituted pyridinium halides have been correlated with Hammett constants.²² As part of a programme for the development of new ChoK inhibitors as anti-proliferative drugs, our efforts were focused on the synthesis of hemicholinium-3 derivatives.⁶ Theoretical and observed substituent induced chemical shifts of the methylene protons in *N*-benzylpyridinium salts were reported in substantial agreement with those expected from the overall electronic effects of the substituents.²³ The ^{13}C NMR spectra have been determined for a series of 31 symmetrical bisquaternary compounds, and the existence of any correlation between such chemical shift and the Hammett and Hammett-type constants was investigated. The 2-substituted pyridinium salts were omitted owing to steric hindrance effects caused by the presence of bulky groups close to the reactive site. Hence, only 4-substituted bis-pyridinium compounds were used in this investigation.

It should be noted that all the substituents (R^4) of **1–31** are electron-releasing, electron-neutral or electron-withdrawing (Table 2). Throughout the data there is a spread of values without clustering at either end, which gives validity to the correlations. Donor substituents caused an upfield displacement of the methylene resonance according to their donating ability, while electron-withdrawing groups produced the opposite effect. Table 2 shows that 4-dimethylamino (**1**, **6**, **12**, **19** and **26**) and 4-amino (**2**, **7**, **13**, **20** and **27**) groups produce the maximum upfield shift relative to the unsubstituted parent compounds. The chemical shift difference (CSD) is measured as the difference between the ^{13}C chemical shift of the methylene group bearing the N^+ of the 4-substituted bispyridinium and the same CH_2 group of the 4-unsubstituted one in CD_3OD . The correlation equation that results using the Hammett constant for *para* substitution σ_P is eq 1:[†]

Table 3. Calculated σ_R and σ_R^+ values and errors of several amino, dialkylamino and cyclic dialkylamino groups from correlations 2 and 3

R^4	CSD ^a	σ_R^b	$\sigma_R^+^c$
–NH ₂	-3.97 ± 0.04	-0.80^d	-1.10 ± 0.050^d
–NMe ₂	-3.70 ± 0.05	-0.88 ± 0.028^d	-1.22 ± 0.053^d
–N(allyl) ₂	-3.74 ± 0.02	-0.80 ± 0.065	-1.09 ± 0.033
–NC ₄ H ₉ ^e	-3.97 ± 0.03	-0.85 ± 0.063	-1.16 ± 0.033
–NC ₅ H ₁₀ ^f	-4.14 ± 0.02	-0.89 ± 0.057	-1.21 ± 0.029
–NC ₆ H ₁₂ ^g	-4.02 ± 0.01	-0.86 ± 0.057	-1.18 ± 0.028

^aCSD = $(\delta^{13}\text{C}_{\text{R}^4} - \delta^{13}\text{C}_{\text{H}})_{\text{CD}_3\text{OD}}$; positive values indicate decreased shielding; CSD values are the average of three measurements.

^b σ_P : Hammett constant for *para* substitution; σ_R : electronic parameter for resonance effect; σ_R^+ : electronic parameter defined for systems where a + charge is delocalised between substituent and reaction centre via ‘through resonance’; otherwise stated, the σ_P , σ_R and σ_R^+ values have been taken from ref 24.

^cRef 25.

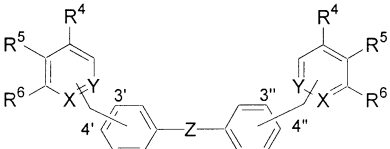
^dRef 24.

^ePyrrolidino.

^fPiperidino.

^gPerhydroazepino.

[†]These compounds group into seven different chemical behaviours. The equations that describe each one are similar to eq 1, and hence the conclusions. The same reasoning applies to eqs 2 and 3.

Table 4. Structure, parameter and calculated log *P* values, and biological results for the compounds


Compd.	X	Y	R ⁴	R ⁵ + R ⁶	Spacer isomer	Z	σ _R ^a	σ _R ^{±b}	clog <i>P</i> ^c	CA ^d	AA ^e
32	N ⁺	CH	–NH ₂	(CH=CH) ₂	4',4''	(CH ₂) ₂	–0.80	–1.10	–2.13	5	2
33	N ⁺	CH	–NH ₂	2H	4',4''	(CH=CH) _t	–0.80	–1.10	–5.00 ^f	6	20
34	N ⁺	CH	–H	(CH=CH) ₂	4',4''	(CH ₂) ₃	0.00	0.00	0.08	9	2.5
35	N ⁺	CH	–NC ₅ H ₁₀ ^g	2H	4',4''	(CH ₂) ₂	–0.89	–1.21	–0.93	9.6	0.4
29	N ⁺	CH	–Me	2H	4',4''	(CH ₂) ₃	–0.16	–0.25	–1.02	12.5	6
36	N ⁺	CH	–NC ₆ H ₁₂ ^h	2H	4',4''	(CH ₂) ₂	–0.86	–1.18	0.09	15	0.4
37	N ⁺	CH	–N(allyl) ₂	2H	4',4''	(CH ₂) ₂	–0.80	–1.09	–0.44	17	0.55
19	N ⁺	CH	–NMe ₂	2H	4',4''	(CH ₂) ₂	–0.88	–1.22	–2.83	17	2
38	N ⁺	CH	–H	2H	3',3''	—	0.00	0.00	–3.10	17	30
39	N ⁺	CH	–NC ₄ H ₈ ⁱ	2H	4',4''	(CH ₂) ₂	–0.85	–1.16	–1.94	0	1
40	CH	N ⁺	–H	(CH=CH) ₂	4',4''	(CH ₂) ₃	0.00	0.00	–0.06	20	2
41	N ⁺	CH	–NMe ₂	2H	4',4''	(CH ₂) ₄	–0.88	–1.22	–1.53	22	0.6
26	N ⁺	CH	–NMe ₂	2H	4',4''	(CH ₂) ₃	–0.88	–1.22	–2.04	22	2.5
20	N ⁺	CH	–NH ₂	2H	4',4''	(CH ₂) ₂	–0.80	–1.10	–4.67	23	4
42	N ⁺	CH	–NMe ₂	2H	4',4''	(CH=CH) _c	–0.88	–1.22	–3.16 ^f	23	9
43	N ⁺	CH	–H	2H	4',4''	—	0.00	0.00	–3.25	23	30
44	N ⁺	CH	–NC ₄ H ₈ ⁱ	2H	4',4''	(CH ₂) ₃	–0.85	–1.16	–1.15	25	0.5
45	N ⁺	CH	–H	2H	4',4''	(CH ₂) ₃	0.00	0.00	–2.18	25	15
46	N ⁺	CH	–NMe ₂	2H	4',4''	(CH=CH) _t	–0.88	–1.22	–3.16 ^f	25	15
47	N ⁺	CH	–NH ₂	2H	4',4''	(CH=CH) _c	–0.80	–1.10	–5.00 ^f	30	15
2	N ⁺	CH	–NH ₂	2H	3',3''	—	–0.80	–1.10	–4.80	31	ND ^j
48	N ⁺	CH	–H	2H	4',4''	(CH ₂) ₂	0.00	0.00	–2.97	31	60
49	N ⁺	CH	–H	2H	4',4''	CH ₂	0.00	0.00	–3.4	31	70
15	N ⁺	CH	–Me	2H	4',4''	CH ₂	–0.16	–0.25	–2.25	33	40
50	N ⁺	CH	–H	(CH=CH) ₂	4',4''	(CH ₂) ₂	0.00	0.00	–0.43	34	4
5	N ⁺	CH	–COMe ^j	2H	3',3''	—	—	—	–4.21 ^k	35	100
28	N ⁺	CH	–CH ₂ OH	2H	4',4''	(CH ₂) ₃	–0.07	–0.15	–3.46	35	> 50
4	N ⁺	CH	–Me	2H	3',3''	—	–0.16	–0.25	–1.95	40	ND ^j
51	N ⁺	CH	–H	(CH=CH) ₂	4',4''	—	0.00	0.00	–0.71	50	10
8	N ⁺	CH	–CH ₂ OH	2H	4',4''	—	–0.07	–0.15	–4.53	60	ND ^j
52	N ⁺	CH	–NC ₄ H ₈ ⁱ	2H	4',4''	CH ₂	–0.85	–1.16	–2.37	60	1
6	N ⁺	CH	–NMe ₂	2H	4',4''	—	–0.88	–1.22	–3.12	60	20
53	CH	N ⁺	–H	(CH=CH) ₂	4',4''	(CH ₂) ₂	0.00	0.00	–0.57	60	20
54	CH	N ⁺	–H	(CH=CH) ₂	4',4''	CH ₂	0.00	0.00	–1.00	60	20
30	N ⁺	CH	–COMe ^j	2H	4',4''	(CH ₂) ₃	—	—	–3.49 ^k	65	ND ^j
12	N ⁺	CH	–NMe ₂	2H	4',4''	CH ₂	–0.88	–1.22	–3.27	70	5
31	N ⁺	CH	–CN	2H	4',4''	(CH ₂) ₃	0.13	0.13	–2.36	75	ND ^j
14	N ⁺	CH	–CH ₂ OH	2H	4',4''	CH ₂	–0.07	–0.15	–4.68	90	ND ^j
7	N ⁺	CH	–NH ₂	2H	4',4''	—	–0.80	–1.10	–4.95	90	40
55	N ⁺	CH	–H	(CH=CH) ₂	4',4''	CH ₂	0.00	0.00	–0.86	100	6.02
27	N ⁺	CH	–NH ₂	2H	4',4''	(CH ₂) ₃	–0.80	–1.10	–3.88	100	7
22	N ⁺	CH	–Me	2H	4',4''	(CH ₂) ₂	–0.16	–0.25	–1.82	100	20
9	N ⁺	CH	–Me	2H	4',4''	—	–0.16	–0.25	–2.10	100	50
3	N ⁺	CH	–CH ₂ OH	2H	3',3''	—	–0.07	–0.15	–4.38	100	100
21	N ⁺	CH	–CH ₂ OH	2H	4',4''	(CH ₂) ₂	–0.07	–0.15	–4.25	100	100
1	N ⁺	CH	–NMe ₂	2H	3',3''	—	–0.88	–1.22	–2.96	> 100	20
10	N ⁺	CH	–COMe ^j	2H	4',4''	—	—	—	–4.27 ^k	> 100	ND ^j
11	N ⁺	CH	–CH=NOH	2H	4',4''	—	—	–0.12	–3.54	> 100	ND ^j
56	CH	N ⁺	–H	(CH=CH) ₂	4',4''	—	0.00	0.00	–0.85	> 100	ND ^j
13	N ⁺	CH	–NH ₂	2H	4',4''	CH ₂	–0.80	–1.10	–5.10	> 100	ND ^j
16	N ⁺	CH	–COMe ^j	2H	4',4''	CH ₂	—	—	–4.51 ^k	> 100	ND ^j
17	N ⁺	CH	–CN	2H	4',4''	CH ₂	0.13	0.13	–3.58	> 100	ND ^j
18	N ⁺	CH	–CH=NOH	2H	4',4''	CH ₂	—	–0.12	–3.69	> 100	ND ^j
23	N ⁺	CH	–COMe ^j	2H	4',4''	(CH ₂) ₂	—	—	–4.02 ^k	> 100	70
24	N ⁺	CH	–CN	2H	4',4''	(CH ₂) ₂	0.13	0.13	–3.15	> 100	ND ^j
25	N ⁺	CH	–COOH	2H	4',4''	(CH ₂) ₂	0.11	—	–3.50	> 100	ND ^j

^aCSD = (δ¹³C_{R4} – δ¹³C_H)_{CD₃OD}; positive values indicate decreased shielding; CSD values are the average of three measurements.^bσ_P: Hammett constant for *para* substitution; σ_R: electronic parameter for resonance effect; σ_R[±]: electronic parameter defined for systems where a + charge is delocalised between substituent and reaction centre via 'through resonance'; otherwise stated, the σ_P, σ_R and σ_R[±] values have been taken from ref 24.^cPredicted by using the Ghose–Crippen modified atomic contribution system²⁶ (ATOMIC5 option) of the PALLAS 2.0 programme.²⁷^dCA: (IC₅₀)_{ChoK} (μM): Choline kinase activity was analysed by measuring the conversion of labelled Cho into *P*Cho in the presence of different concentrations of compounds.^eAA: (IC₅₀)_{HT-29} (μM): The anti-proliferative activity was analysed on HT-29 cultured cells by measuring the number of cells remaining after 6 days of incubation with the compounds, relative to control, non-treated cells. IC₅₀ refers to the concentration at which 50% inhibition of choline kinase or proliferation activities are reached.^fThe PALLAS programme does not differentiate between the *cis*- and *trans*-isomers.^gPiperidino.^hPerhydroazepino.ⁱPyrrolidino.^jNot included in the derivation of eqs 4 and 5 as it does not exist purely in the bis(acetyl) form but as a mixture of bis(acetyl) and bis(1,1-dihydroxyethyl) forms.^kIt is calculated as a weighted average of the bis(acetyl) and bis(1,1-dihydroxyethyl) forms.^lND, not determined.

$$\text{CSD} = -1.08 (\pm 0.10) + 3.55 (\pm 0.20) \sigma_P \quad (1)$$

$$n = 31, r = 0.919, s = 0.521, F_{1,29} = 331.00, p < 0.001$$

where n is the number of compounds, r is the correlation coefficient, and s is the standard deviation between estimated and actual chemical shifts. The Fisher test is highly significant here ($p < 0.001$). The numbers in parentheses account for the standard error of the regression coefficients.

Although the nomenclature *para* is not correct for the pyridine derivatives, it is used to keep the homogeneity regarding the Hammett parameter σ_R because the substituent is formally *para* in relation to N^+ . There is a

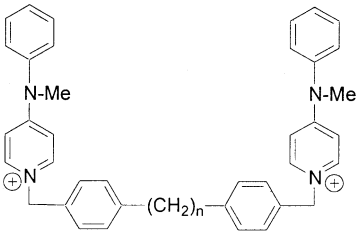
reasonable correlation between the ^{13}C chemical shift and σ_P ($r^2 = 0.920$), which is enhanced when the resonance effects σ_R and σ_R^+ ($r^2 = 0.976$ and $r^2 = 0.986$, respectively) are used. When only the resonance component is considered ($\sigma_P = \sigma_I + \sigma_R$), an excellent correlation results (eq 2):

$$\text{CSD} = -0.27 (\pm 0.06) + 4.23 (\pm 0.26) \sigma_R \quad (2)$$

$$n = 29, r = 0.988, s = 0.292, F_{1,27} = 1,100.35, p < 0.001$$

The resonance effects operate primarily through the electrons of the π -bond system. Since the π -bond system of the aromatic ring of the pyridinium compounds also

Table 5. Experimental, and theoretical $\text{p}(\text{IC}_{50})_{\text{HT-29}}$ values for compounds **57**, **58** and **59** calculated with correlation 5



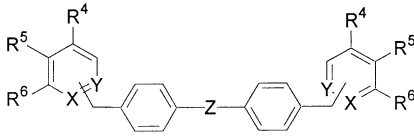
Compound	n	$\text{p}(\text{IC}_{50})_{\text{ChoK}}^a$	$\text{p}(\text{IC}_{50})_{\text{HT-29}}^a$ experim.	$\text{p}(\text{IC}_{50})_{\text{HT-29}}^b$ theoreti.	Δ (deviation) ^c
57	1	5.62	6.16	6.35	0.19
58	2	5.19	6.46	6.50	0.04
59	3	5.82	5.89	6.68	0.79

^a $\text{pIC}_{50} = -\log \text{IC}_{50}$, bearing in mind that the higher the value of pIC_{50} the more potent is the compound.

^bCalculated from correlation 5.

^c $\Delta = \text{p}(\text{IC}_{50})_{\text{HT-29}}^{\text{theoret.}} - \text{p}(\text{IC}_{50})_{\text{HT-29}}^{\text{experim.}}$.

Table 6. Structure, parameter and calculated log P values, and biological results for the compounds



Compd.	X	Y	R^4	$\text{R}^5 + \text{R}^6$	Z	σ_R^a	cLog P^b	$\text{p}(\text{IC}_{50})^c$	$\text{p}(\text{IC}_{50})^d$
60	N^+	CH	$-\text{NC}_6\text{H}_{12}^e$	2H	$(\text{CH}_2)_3$	-0.86	0.59	5.82	6.52
61	N^+	CH	$-\text{N}(\text{Allyl})_2$	2H	$(\text{CH}_2)_3$	-0.80	0.35	5.82	6.40
62	N^+	CH	$-\text{NC}_6\text{H}_{12}^e$	2H	CH_2	-0.86	-0.35	6.00	5.89
57	N^+	CH	$-\text{N}(\text{Me})\text{Ph}$	2H	CH_2	-0.78	-0.06	5.40	6.15
58	N^+	CH	$-\text{N}(\text{Me})\text{Ph}$	2H	$(\text{CH}_2)_2$	-0.78	0.37	5.14	6.46
40	CH	N^+	-H	$(\text{CH}=\text{CH})_2$	$(\text{CH}_2)_3$	0.00	-0.06	4.70	5.70
59	N^+	CH	$-\text{N}(\text{Me})\text{Ph}$	2H	$(\text{CH}_2)_3$	-0.78	0.88	5.17	5.89
34	N^+	CH	-H	$(\text{CH}=\text{CH})_2$	$(\text{CH}_2)_3$	0.00	0.08	5.04	5.60
36	N^+	CH	$-\text{NC}_6\text{H}_{12}^e$	2H	$(\text{CH}_2)_2$	-0.86	0.09	4.82	6.40
51	N^+	CH	-H	$(\text{CH}=\text{CH})_2$	$(\text{CH}_2)_2$	0.00	-0.43	4.47	5.40
52	N^+	CH	-H	$(\text{CH}=\text{CH})_2$	—	0.00	-0.71	4.30	5.00
54	N^+	CH	-H	$(\text{CH}=\text{CH})_2$	$(\text{CH}_2)_2$	0.00	-0.57	4.22	4.70
63	N^+	CH	$-\text{NC}_5\text{H}_{10}^f$	2H	$(\text{CH}_2)_3$	-0.89	-0.42	5.74	6.52
37	N^+	CH	$-\text{N}(\text{Allyl})_2$	2H	$(\text{CH}_2)_2$	-0.80	-0.44	4.77	6.26

^a σ_R : electronic parameter for resonance effect. It was calculated from eq 5.

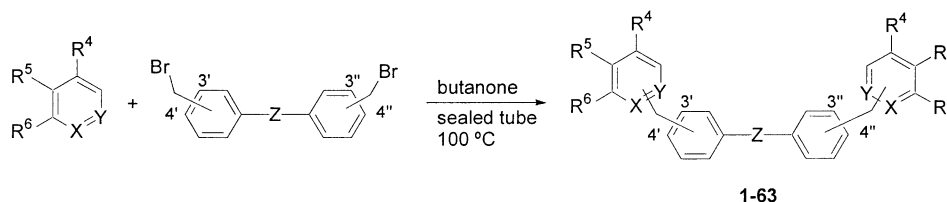
^bPredicted by using the Ghose–Crippen modified atomic contribution system²⁶ (ATOMIC5 option) of the PALLAS 2.0 programme.²⁷

^cCholine kinase activity.

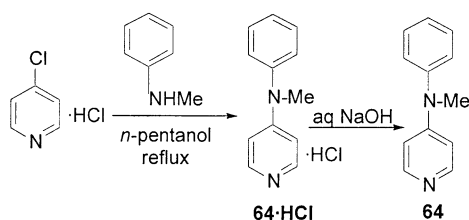
^dThe anti-proliferative activity was analyzed on HT-29 cultured cells. IC_{50} refers to the concentration at which 50% inhibition of choline kinase or proliferation activities are reached.

^ePerhydroazepino.

^fPiperidino.



Scheme 1.



Scheme 2.

contains a positively charged nitrogen, the transmission of the resonance effect to the methylene carbons is expected to be through the σ_R^+ descriptor^{24,25} (eq 3):

$$\text{CSD} = 0.03 (\pm 0.05) + 3.36 (\pm 0.07) \sigma_R^+ \quad (3)$$

$$n = 30, r = 0.993, s = 0.210, F_{1,28} = 2,121.51, p < 0.001$$

The better correlation is eq 3 and this is consistent with conventional chemical concepts. R^4 is *para* to the positively charged ring nitrogen and is in direct conjugation with it. The greater resonance effect of R^4 would obviously cause better delocalisation of the positive charge and, hence, the CSD would become more negative (i.e., negative values indicate increased shielding).

Values for σ_R and σ_R^+ parameters are available for only a relatively small number of substituents. In this paper, the σ_R and σ_R^+ values are reported for an acyclic dialkylamino group such as the dialkylamino moiety and four cyclic dialkylamino groups, such as the pyrrolidino, piperidino, and perhydroazepino moieties (see Table 3).

QSAR of the antiproliferative activity against the HT-29 cell line

The octanol–water partition coefficient, used in its logarithmic form ($\log P$), is the most widely accepted measure of lipophilicity. Reproducibility and accuracy of experimental $\log P$ determinations are compromised for extremely lipophilic and/or hydrophilic compounds such as the biscationic structures **1–63**. Fragmental methods make it possible to create data banks and to perform $\log P$ calculations by computer.

Correlations 4 and 5 show the anti-proliferative activities of bisquaternary compounds, the Hammett-type constants σ_R and σ_R^+ of the substituents at position 4 of the heterocycle and the $\log P$ values of the bisalts. Such $\log P$ values reported in Tables 4 and 6 were calculated by using the Ghose–Crippen modified atomic

contribution system²⁶ (ATOMIC5 option) of the PAL-LAS 2.0 programme.²⁷

$$\begin{aligned} \text{p(IC}_{50})_{\text{HT-29}} = & 5.36 - 0.96 (\pm 0.10) \sigma_R^+ \\ & + 0.35 (\pm 0.04) \log P \end{aligned} \quad (4)$$

$$n = 37, r = 0.907, s = 0.309, F_{2,34} = 78.55, p < 0.001$$

$$\begin{aligned} \text{p(IC}_{50})_{\text{HT-29}} = & 5.36 - 1.31 (\pm 0.13) \sigma_R^+ \\ & 0.35 (\pm 0.04) \log P \end{aligned} \quad (5)$$

$$n = 37, r = 0.910, s = 0.304, F_{2,34} = 81.68, p < 0.001$$

where $\text{p(IC}_{50})_{\text{HT-29}} = -\log (\text{IC}_{50})_{\text{HT-29}}$, bearing in mind that the higher the value of $\text{p(IC}_{50})_{\text{HT-29}}$ the more potent is the compound. Both eqs 4 and 5 give good cross-validated r_{cv}^2 values (q^2) of 0.82 and 0.833, respectively. The quality of the two eqs 4 and 5 is almost identical. This is largely due to the high collinearity between σ_R and σ_R^+ [$r = 0.997$ for the groups shown in Tables 2 and 3 ($n = 11$)]. Eq 5 is preferred since it has a slightly better standard deviation (s) than eq 4. A most significant aspect of this study is that every data point was included in the formulation of eqs 4 and 5. Such results are rarely found and merit special consideration. We find this to be quite unusual since one usually finds some outliers in QSAR work which must be omitted to obtain a high correlation.

Based on eq 5, one could predict which type of compound would give better activity. At this stage the knowledge of the parameter σ_R of a group such as the *N*-methylanilino one acquires a great significance. The *N*-methylanilino moiety is both a strong electron-releasing and a highly lipophilic group and, according to eq 5, should be an excellent R^4 substituent for a bispyridinium dibromide as a promising antitumour compound. However, the σ_R value of the *N*-methylanilino group is not available and, accordingly, it has been estimated by using correlation eq 2, obtaining the following values: $\text{CSD} = -3.65 \pm 0.037$; $\sigma_R = -0.78 \pm 0.073$. From eq 3, $\sigma_R^+ = -1.07 \pm 0.038$ for the *N*-methylanilino group. This Hammett-type parameter for such a group has been already obtained by Popova et al. ($\sigma_R^+ = -1.26$) by the rate of amine acylation with *p*-nitrobenzenesulfonyl bromide in a nitrobenzene solution at 25 °C.²⁸

If the $\text{p(IC}_{50})_{\text{HT-29}}$ values were calculated with correlation (eq 5) for compounds **57**, **58** and **59** and compare

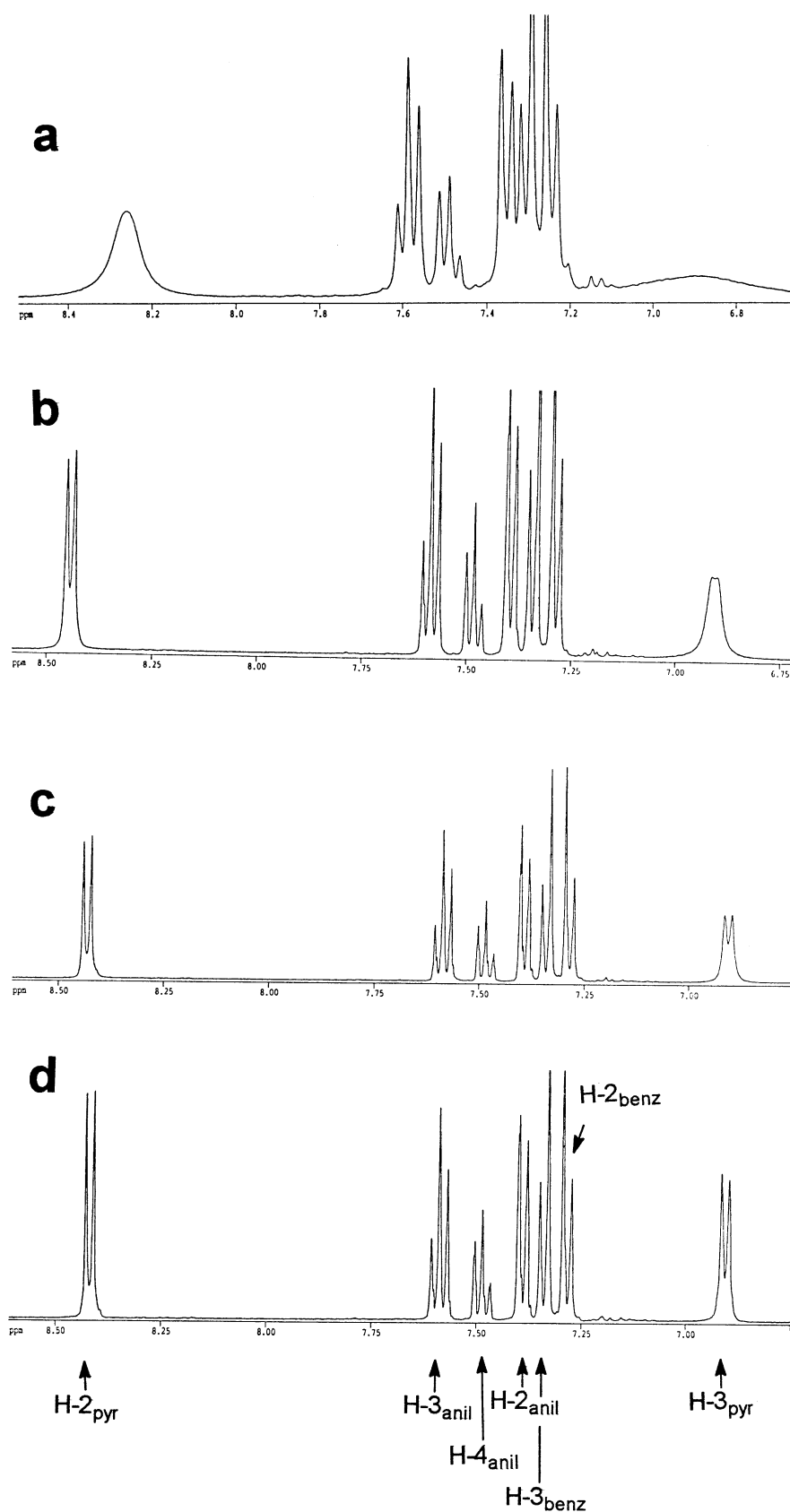


Figure 1. Expansion of the 400 ^1H MHz spectrum of **58** (26.7 mg mL $^{-1}$) showing the signals for aromatic protons: (a) CD_3OD at 25°C; (b) $\text{DMSO}-d_6$ at 57°C; (c) $\text{DMSO}-d_6$ at 77°C; (d) $\text{DMSO}-d_6$ at 97°C.

them with the experimental ones, compound **59** deviates (Table 5) more than the standard deviation (s) of eq 5. It is possible that, between a more ample range of $\text{clog } P$, there exists a parabolic relationship between $\text{p(IC}_{50})_{\text{HT-29}}$ and $\text{clog } P$ and, hence, the maximum activity is reached with compound **58**, a decrease of activity taking place as the number of methylene units increases from 3 onwards [$Z = (\text{CH}_2)_m$, general formula of Table 5]. The linear equations should be considered as limited segments of the more general parabolic equation and the former may be interpreted as indicating a situation where the maximum activity had not been reached. Whenever a small range of $\text{clog } P$ values is available and the addition of $(\text{clog } P)^2$ term is not statistically justifiable, a linear equation may give the best correlation and this could be our case.

In order to check this hypothesis compounds, with their $\text{clog } P$ taken in between the values -0.71 and 0.88 were selected and prepared (Table 6) and correlation eq 6 was obtained:

$$\text{p(IC}_{50})_{\text{HT-29}} = 5.58 - 0.91(\pm 0.31)(\text{clog } P)^2 + 0.34(\pm 0.16)\text{clog } P - 1.04(\pm 0.18) \sigma_R \quad (6)$$

$$n = 14, r = 0.934, s = 0.238, F_{3,10} = 23.84, p < 0.001$$

Eq 6 is a significant parabolic equation obtained for the biscationic compounds [$F_{3,10} = 23.84$; $(F_{3,10})_{0.001} = 12.55$, and satisfied the F test at the level of 99.9%] which gives an ideal lipophilic character ($\text{clog } P_0$) of 0.18 for maximum activity.

Regarding the molecules **57**, **58** and **59**, the deviations found between the theoretical values, calculated from correlation eq 6, and the experimental ones of $\text{p(IC}_{50})_{\text{HT-29}}$ were: 0.21 (**57**), -0.07 (**58**) and 0.09 (**59**).

Conclusions

The electronic and lipophilic parameters were found to play important roles in the antitumour activity of bis-quaternary dibromides. A ^{13}C NMR method was included which can complement the *classic* procedure for determination of these constants for several dialkyl-amino groups. It has been shown that there is a remarkable consistency between the results obtained with this method using proper assumptions. Within the range of the data, reasonable prediction should be possible by eq 5, which predicts approximately 82.6% of the variance of the $\text{p(IC}_{50})_{\text{HT-29}}$. The present study sheds light on the influence of the electronic properties of groups placed at position 4 of the heterocycle and on the global lipophilicity of compounds. Strongly electron-donating and highly lipophilic groups provide analogues with interesting antiproliferative properties. The 4-(*N*-methylanilino) group appears to be particularly effective in providing a highly active compound. The discovery of compound **58**, as a novel anticancer

compound, is expected to provide impetus for developing a new treatment in the field of cancer therapy. Further studies with this compounds are underway and will be published elsewhere.

Experimental

Chemistry

Melting points (mp) were taken in open capillaries on an electrothermal melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a 400.13 MHz ^1H and 100.03 MHz ^{13}C NMR Bruker ARX 400 or 300.13 MHz ^1H and 75.78 MHz ^{13}C NMR Bruker AMX-300 spectrometers, and chemical shifts (ppm) are reported relative to the solvent peak (CD_2HOD in CD_3OD at δ 3.31 and 49.9 ppm; DMSO in $\text{DMSO}-d_6$ at δ 2.50 and 39.5 ppm). CD_3OD is used as a NMR solvent unless otherwise stated. Signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublet; ddd, double doublet of doublet; t, triplet; dt, double triplet; q, quadruplet; m, multiplet. Longitudinal relaxation times (T_1) were determined using an inversion recovery experiment on an Inova 400 MHz NMR instrument. T_1 experiments were conducted in undegassed CD_3OD solvent. All final products had satisfactory (within $\pm 0.4\%$) C, H, and N analyses. High resolution liquid secondary ion mass spectra (HR LSIMS) were carried out on a VG Auto-Spec Q high resolution mass spectrometer (Fisons Instruments). All compounds were dried at 40°C and 0.1 mmHg for 15 h, but many held on tenaciously to solvent molecules, especially water which appears to be a solvate. 3,3'-Bis(bromomethyl)biphenyl,²⁹ 4,4'-bis(bromomethyl)biphenyl,^{30,31} bis[*p*-(bromomethyl)diphenyl]methane,³² bis-*p*-(bromomethyl)bibenzyl,³² 1,3-bis[4-(bromomethyl)phenyl]propane,³² 4-bis[4-(bromomethyl)phenyl]butane,³² *trans*- and *cis*-4,4'-bis(bromomethyl)stilbene,³³ 4-(diallylamino)pyridine,¹⁰ 4-piperidinopyridine,¹⁰ 4-(perhydroazepino)pyridine,¹⁰ **19**,¹⁰ **20**,¹⁰ **21**,¹⁰ **22**,¹⁰ **24**,¹⁰ **24**,¹⁰ **25**,¹⁰ **32**,^{33,34} **36**,¹⁰ **37**,¹⁰ **39**,¹⁰ and **48**,¹⁰ were synthesized according to literature procedures. Quinoline, isoquinoline, pyridine, 4-aminopyridine, 4-(dimethylamino)pyridine, 4-pyrrolidinopyridine, 4-(hydroxymethyl)pyridine, 4-methylpyridine and 4-acetylpyridine were obtained from Aldrich.

4-(*N*-Methylanilino)pyridine hydrochloride **64**·HCl.

Three grams (19.9 mmol) of 4-chloropyridine-HCl and 6.43 mL (59.9 mmol) of *N*-methylaniline were dissolved in *n*-pentanol (40 mL). Such a solution was kept at reflux for 24 h under argon and then was rotaevaporated off. A pale yellow oil was isolated by flash chromatography using a gradient elution ($\text{CH}_2\text{Cl}_2 \rightarrow \text{CH}_2\text{Cl}_2/\text{MeOH}$: 100/5 $\rightarrow$ $\text{CH}_2\text{Cl}_2/\text{MeOH}$: 100/10), which after dissolving in diethyl ether and adding some drops of a saturated solution of hydrogen chloride in diethyl ether precipitated. This white solid was identified as the title compound (2.89 g, 79%). Mp $189\text{--}191^\circ\text{C}$. TLC (top layer of *n*-BuOH/HOAc/ H_2O : 5/1/4): 0.38. ^1H NMR (400.13 MHz): δ 8.16 (d, $J_{2,3} = 7.5$ Hz, 2H, pyridinium- H_4), 7.60 (t, $J_{3,4} = 7.6$ Hz, 2H, anilino- H_3), 7.49 (t, $J = 7.4$

Hz, 1H, anilino- H_4), 7.37 (d, $J_{2,3} = 7.4$ Hz, 2H, anilino- H_2), 6.90 (d, $J_{2,3} = 7.5$ Hz, 2H, pyridinium- H_3), 3.54 (s, 6H, CH_3N). ^{13}C NMR (100.03 MHz): δ 159.49 (C-4_{pyr}), 144.97 (C-1_{anilino}), 140.74 (C-2_{pyr}), 131.99 (C-3_{anilino}), 129.98 (C-4_{anilino}), 127.60 (C-2_{anilino}), 109.42 (C-3_{pyr}), 41.24 (CH_3N). HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{12}H_{13}N_2NaCl$ ($M + Na$)⁺ 243.0665. Found m/z : 243.0664. Anal. for $C_{12}H_{13}N_2Cl \cdot H_2O$: calcd: C, 57.21; H, 4.26; N, 3.70. Found: C, 57.07; H, 4.44; N, 3.76. The free base **64** was liberated from 4-(*N*-methylanilino)-pyridine·HCl **64**·HCl after treatment with aqueous sodium hydroxide and subsequent extraction with diethyl ether.

General experimental procedure for the preparation of bisquaternary compounds

The heterocyclic structure (1.10 mmol) and the corresponding bis(bromomethyl) compound $BrCH_2ZCH_2Br$ (0.54 mmol) in dry butanone (50 mL) were heated in a sealed tube at 100 °C for 24 h. After filtration and washing thoroughly with butanone and $CHCl_3$, the solid product was purified by recrystallisation from EtOH or EtOH/MeOH, after adding Et_2O to turbidity.

1,1'-[Biphenyl-3,3'-diylbis(methylene)]bis[(4-dimethylamino)pyridinium] dibromide (1). Yield: 66.3%. Mp 245–247 °C. 1H NMR (300.13 MHz): δ 8.36 (d, $J = 7.9$ Hz, 4H, H-2_{pyr}), 7.84 (s, 2H, Ph), 7.69 (d, $J = 7.7$ Hz, 2H, Ph), 7.52 (t, $J = 7.7$ Hz, 2H, Ph), 7.41 (d, $J = 7.7$ Hz, 2H, Ph), 7.02 (d, $J = 7.9$ Hz, 4H, H-3_{pyr}), 5.48 (s, 4H, CH_2N^+), 3.24 (s, 12H, NMe_2). ^{13}C NMR (75.78 MHz): δ 157.99 (C-4_{pyr}), 143.17 (C-2_{pyr}), 142.58 (C-1_{Ph}), 137.04 (C-3_{Ph}), 131.10 (C-5_{Ph}), 128.89, 128.74, 128.56 (C-4,6,2_{Ph}), 109.21 (C-3_{pyr}), 61.61 (CH_2N^+), 40.42 (NMe_2). HR LSIMS (thioglycerol), calcd m/z for $C_{28}H_{32}N_4Br$ ($M-Br$)⁺ 503.1810. Found m/z : 503.1809. Anal. for $C_{28}H_{32}N_4Br_2 \cdot 2.81H_2O$: calcd C, 52.96; H, 5.97; N, 8.82. Found: C, 53.35; H, 5.64; N, 8.43.

1,1'-[Biphenyl-3,3'-diylbis(methylene)]bis(4-aminopyridinium) dibromide (2). Yield: 70%. Mp: 310–312 °C dec. 1H NMR (300.13 MHz): δ 8.26 (d, $J = 7.6$ Hz, 4H, H-2_{pyr}), 7.78 (s, 2H, Ph), 7.69 (d, $J = 7.8$ Hz, 2H, Ph), 7.53 (t, $J = 7.8$ Hz, 2H, Ph), 7.38 (d, $J = 7.8$ Hz, 2H, Ph), 6.87 (d, $J = 7.6$ Hz, 4H, H-3_{pyr}), 5.42 (s, 4H, CH_2N^+). ^{13}C NMR (75.78 MHz): δ 160.90 (C-4_{pyr}), 144.13 (C-2_{pyr}), 142.68 (C-1_{Ph}), 136.97 (C-3_{Ph}), 131.14 (C-5_{Ph}), 128.95, 128.62, 128.35 (C-4,6,2_{Ph}), 111.03 (C-3_{pyr}), 61.91 (CH_2N^+). Anal. for $C_{24}H_{24}N_4Br_2 \cdot 0.1H_2O$: calcd C, 54.38; H, 4.60; N, 10.57. Found: C, 54.38; H, 4.56; N, 10.75.

1,1'-[Biphenyl-3,3'-diylbis(methylene)]bis[(4-hydroxymethyl)pyridinium] dibromide (3). Yield: 69%. Mp 76–78 °C. 1H NMR (400.13 MHz): δ 9.11 (d, $J = 6.6$ Hz, 4H, H-2_{pyr}), 8.08 (d, $J = 6.6$ Hz, 4H, H-3_{pyr}), 8.02 (s, 2H, Ph), 7.77 (d, $J = 7.1$ Hz, 2H, Ph), 7.55 (m, 4H, Ph), 5.92 (s, 4H, CH_2N^+), 4.91 (s, 4H, CH_2OH). ^{13}C NMR (100.13 MHz): δ 165.35 (C-4_{pyr}), 145.35 (C-2_{pyr}), 142.59 (C-1_{Ph}), 135.70 (C-3_{Ph}), 131.37 (C-5_{Ph}), 129.54, 129.49, 129.20 (C-4,6,2_{Ph}), 126.13 (C-3_{pyr}), 64.82 (CH_2N^+), 62.87 (CH_2OH). Anal. for $C_{26}H_{26}N_2O_2Br_2 \cdot 0.4H_2O$:

calcd C, 55.22; H, 4.78; N, 4.95. Found: C, 55.28; H, 5.09; N, 4.83.

1,1'-[Biphenyl-3,3'-diylbis(methylene)]bis(4-methylpyridinium) dibromide (4). Yield: 33.3%. Mp 142–144 °C dec. 1H NMR (400.13 MHz): δ 9.02 (d, $J = 6.6$ Hz, 4H, H-2_{pyr}), 8.01 (s, 2H, Ph), 7.95 (d, $J = 6.6$ Hz, 4H, H-3_{pyr}), 7.76 (d, $J = 7.5$ Hz, 2H, Ph), 7.54 (m, 4H, Ph), 5.88 (s, 4H, CH_2N^+), 2.67 (s, 6H, CH_3). ^{13}C NMR (100.13 MHz): δ 161.90 (C-4_{pyr}), 144.95 (C-2_{pyr}), 142.61 (C-1_{Ph}), 135.68 (C-3_{Ph}), 131.36 (C-5_{Ph}), 129.54, 129.45, 129.19 (C-4,6,2_{Ph}), 126.14 (C-3_{pyr}), 64.71 (CH_2N^+), 22.06 (Me). Anal. for $C_{26}H_{26}N_2Br_2 \cdot H_2O$: calcd C, 57.37; H, 5.18; N, 5.15. Found: C, 57.30; H, 5.12; N, 4.79.

1,1'-[Biphenyl-3,3'-diylbis(methylene)]bis(4-acetylpyridinium) dibromide (5a). Yield: 76% (mixture of **5a** and **5b**). Mp 166–168 °C, 148–150 °C dec (mixture of **5a** and **5b**); 166–168 °C liquifies. 1H NMR (400.13 MHz): δ 9.42 (d, $J = 6.6$ Hz, 4H, H-2_{pyr}), 8.51 (d, $J = 6.6$ Hz, 4H, H-3_{pyr}), 8.11 (s, 2H, Ph), 7.79 (m, 6H, Ph), 6.01 (s, 4H, CH_2N^+), 2.75 (s, 6H, CH_3CO). ^{13}C NMR (100.13 MHz, selected data): δ 195.50 (CH_3CO), 65.75 (CH_2N^+), 29.06 (CH_3CO).

1,1'-[Biphenyl-3,3'-diylbis(methylene)]bis{[4-(1,1-dihydroxy-ethyl)acetylpyridinium] dibromide (5b). 1H NMR (400.13 MHz): δ 9.20 (dd, $J = 6.8$ and 1.7 Hz, 4H, H-2_{pyr}), 8.20 (d, $J = 6.6$ Hz, 4H, H-3_{pyr}), 8.11 (d, $J = 1.4$ Hz, 2H, Ph), 7.78 (m, 2H, Ph), 7.58 (m, 4H, Ph), 5.96 (s, 4H, CH_2N^+), 1.60 (s, 6H, $CH_3C(OH)_2$). ^{13}C NMR (100.13 MHz, selected data): δ 165.74 ($CH_3C(OH)_2$), 65.06 (CH_2N^+), 27.11 ($CH_3C(OH)_2$). Anal. for $C_{28}H_{26}N_2O_2Br_2$ (mixture of **5a** and **5b**): calcd C, 57.74; H, 4.50; N, 4.81. Found: C, 58.06; H, 4.85; N, 4.47.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis[(4-dimethylamino)pyridinium] dibromide (6). Yield: 79%. Mp 313–315 °C. 1H NMR (300.13 MHz): δ 8.28 (d, $J = 7.9$ Hz, 4H, H-2_{pyr}), 7.69 (d, $J = 8.4$ Hz, 4H, Ph), 7.49 (d, $J = 8.4$ Hz, 4H, Ph), 7.02 (d, $J = 7.9$ Hz, 4H, H-3_{pyr}), 5.43 (s, 4H, CH_2N^+), 3.25 (s, 12H, NMe_2). ^{13}C NMR (75.78 MHz): δ 158.02 (C-4_{pyr}), 143.15 (C-2_{pyr}), 142.00 (C-1_{Ph}), 135.70 (C-4_{Ph}), 130.07 (C-3_{Ph}), 128.93 (C-2_{Ph}), 109.19 (C-3_{pyr}), 61.36 (CH_2N^+), 40.41 (NMe_2). HR LSIMS (thioglycerol), calcd m/z for $C_{28}H_{32}N_4Br$ ($M-Br$)⁺ 503.1810. Found m/z : 503.1811. Anal. for $C_{28}H_{32}N_4Br_2 \cdot 2.2H_2O$: calcd C, 53.89; H, 5.88; N, 8.98. Found: C, 54.17; H, 5.71; N, 8.63.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis(4-aminopyridinium) dibromide (7). Yield: 63.3%. Mp > 310 °C. 1H NMR (400.13 MHz): δ 8.20 (d, $J = 7.4$ Hz, 4H, H-2_{pyr}), 7.71 (d, $J = 8.2$ Hz, 4H, Ph), 7.47 (d, $J = 8.2$ Hz, 4H, Ph), 6.87 (d, $J = 7.4$ Hz, 4H, H-3_{pyr}), 5.39 (s, 4H, CH_2N^+). ^{13}C NMR (100.13 MHz): δ 160.92 (C-4_{pyr}), 144.11 (C-2_{pyr}), 142.30 (C-1_{Ph}), 135.68 (C-4_{Ph}), 129.96 (C-3_{Ph}), 128.98 (C-2_{Ph}), 111.00 (C-3_{pyr}), 61.62 (CH_2N^+). HR LSIMS (thioglycerol), calcd m/z for $C_{24}H_{24}NBr$ ($M-Br$)⁺ 447.1184. Found m/z : 447.1185. Anal. for $C_{24}H_{24}N_4Br_2 \cdot 0.5H_2O$: calcd C, 53.65; H, 4.69; N, 10.63. Found: C, 53.77; H, 4.55; N, 10.18.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis[(4-hydroxymethyl)pyridinium] dibromide (8). Yield: 62%. Mp $>315^{\circ}\text{C}$. ^1H NMR (400.13 MHz): δ 9.02 (d, $J=6.5$ Hz, 4H, H-2_{pyr}), 8.09 (d, $J=6.5$ Hz, 4H, H-3_{pyr}), 7.75 (d, $J=8.2$ Hz, 4H, Ph), 7.62 (d, $J=8.2$ Hz, 4H, Ph), 5.88 (s, 4H, CH₂N⁺), 4.93 (s, 4H, CH₂OH). ^{13}C NMR (100.13 MHz): δ 165.45 (C-4_{pyr}), 145.33 (C-2_{pyr}), 142.76 (C-1_{Ph}), 134.43 (C-4_{Ph}), 130.77 (C-3_{Ph}), 129.32 (C-2_{Ph}), 126.15 (C-3_{pyr}), 64.65 (CH₂N⁺), 62.88 (CH₂OH). HR LSIMS (thioglycerol), calcd m/z for C₂₆H₂₅N₂O₂ (M–Br–BrH)⁺ 397.1916. Found m/z : 397.1916. Anal. for C₂₆H₂₆N₂O₂Br₂: calcd C, 55.93; H, 4.69; N, 5.02. Found: C, 55.73; H, 4.72; N, 5.39.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis(4-methylpyridinium) dibromide (9). Yield: 87%. Mp 303–305 $^{\circ}\text{C}$. ^1H NMR (300.13 MHz): δ 8.94 (d, $J=6.6$ Hz, 4H, H-2_{pyr}), 7.96 (d, $J=6.6$ Hz, 4H, H-3_{pyr}), 7.73 (d, $J=8.4$ Hz, 4H, Ph), 7.61 (d, $J=8.4$ Hz, 4H, Ph), 5.85 (s, 4H, CH₂N⁺), 2.68 (s, 6H, CH₃). ^{13}C NMR (75.78 MHz): δ 161.89 (C-4_{pyr}), 144.93 (C-2_{pyr}), 142.68 (C-1_{Ph}), 134.46 (C-4_{Ph}), 130.78 (C-3_{Ph}), 130.17 (C-2_{pyr}), 129.18 (C-3_{Ph}), 64.43 (CH₂N⁺), 22.06 (Me). HR LSIMS (thioglycerol), calcd m/z for C₂₆H₂₆N₂Br (M–Br)⁺ 445.1279. Found m/z : 445.1279. Anal. for C₂₆H₂₆N₂Br₂·0.8H₂O: calcd C, 57.75; H, 5.15; N, 5.18. Found: C, 57.57; H, 4.84; N, 4.91.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis(4-acetylpyridinium) dibromide (10a). Yield: 63% (mixture of **10a** and **10b**). Mp $>315^{\circ}\text{C}$ dec (mixture of **10a** and **10b**). ^1H NMR (400.13 MHz): δ 9.33 (d, $J=6.6$ Hz, 4H, H-2_{pyr}), 8.52 (d, $J=6.6$ Hz, 4H, H-3_{pyr}), 7.76 (d, $J=8.3$ Hz, 4H, Ph), 7.69 (d, $J=8.3$ Hz, 4H, Ph), 6.02 (s, 4H, CH₂N⁺), 2.76 (s, 6H, CH₃CO). ^{13}C NMR (100.13 MHz, selected data): δ 195.55 (CH₃CO), 65.49 (CH₂N⁺), 29.15 (CH₃).

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis{[4-(1,1-dihydroxy-ethyl)]acetylpyridinium} dibromide (10b). ^1H NMR (400.13 MHz): δ 9.10 (d, $J=6.6$ Hz, 4H, H-2_{pyr}), 8.21 (d, $J=6.6$ Hz, 4H, H-3_{pyr}), 7.76 (d, $J=8.3$ Hz, 4H, Ph), 7.68 (d, $J=8.3$ Hz, 4H, Ph), 5.93 (s, 4H, CH₂N⁺), 1.62 (s, 6H, CH₃C(OH)₂). ^{13}C NMR (100.13 MHz, selected data): δ 98.14 (CH₃C(OH)₂), 64.81 (CH₂N⁺), 27.12 (CH₃C(OH)₂). HR LSIMS (thioglycerol) for the mixture of **10a** and **10b**, calcd m/z for C₂₈H₂₆N₂O₂Br (M–Br)⁺ 501.1178. Found m/z : 501.1178. Anal. for C₂₈H₂₆N₂O₂Br₂·1.5H₂O (mixture of **10a** and **10b**): calcd C, 55.19; H, 4.80; N, 4.60. Found: C, 55.28; H, 4.89; N, 4.87.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bis[(4-hydroxyiminoethyl)pyridinium] dibromide (11). Yield: 59%. Mp 244–246 $^{\circ}\text{C}$. ^1H NMR (400.13 MHz): δ 9.04 (d, $J=6.8$ Hz, 4H, H-2_{pyr}), 8.32 (s, 2H, CH), 8.24 (d, $J=6.8$ Hz, 4H, H-3_{pyr}), 7.75 (d, $J=8.3$ Hz, 4H, Ph), 7.64 (d, $J=8.3$ Hz, 4H, Ph), 5.88 (s, 4H, CH₂N⁺). ^{13}C NMR (100.13 MHz): δ 151.65 (C-4_{pyr}), 146.01 (C-2_{pyr} or C-5_{Ph}), 145.59 (C-5_{Ph} or C-2_{pyr}), 142.75 (C-1_{Ph}), 134.26 (C-4_{Ph}), 130.88 (C-3_{Ph}), 129.24 (C-2_{Ph}), 125.70 (C-3_{pyr}), 64.76 (CH₂N⁺). Anal. for C₂₆H₂₄N₄O₂Br₂·0.4H₂O: calcd C, 52.79; H, 4.23; N, 9.47. Found: C, 52.72; H, 4.01; N, 9.18.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis[(4-dimethylamino)pyridinium] dibromide (12). Yield: 72%. Mp 190–192 $^{\circ}\text{C}$. ^1H NMR (300.13 MHz): δ 8.21 (d, $J=7.9$ Hz, 4H, H-2_{pyr}), 7.31 (d, $J=8.3$ Hz, 4H, Ph), 7.25 (d, $J=8.3$ Hz, 4H, Ph), 6.99 (d, $J=7.9$ Hz, 4H, H-3_{pyr}), 5.33 (s, 4H, CH₂N⁺), 3.98 (s, 2H, CH₂ Ph), 3.24 (s, 12H, Me). ^{13}C NMR (75.78 MHz): δ 157.96 (C-4_{pyr}), 143.58 (C-1_{Ph}), 143.03 (C-2_{pyr}), 134.06 (C-4_{Ph}), 130.84 (C-3_{Ph}), 129.63 (C-2_{Ph}), 109.11 (C-3_{pyr}), 61.44 (CH₂N⁺), 41.97 (CH₂Ph), 40.38 (Me). HR LSIMS (thioglycerol), calcd m/z for C₂₉H₃₄N₄Br (M–Br)⁺ 517.1967. Found m/z : 517.1966. Anal. for C₂₉H₃₄N₄Br₂·2.5H₂O: calcd C, 54.13; H, 6.11; N, 8.71. Found: C, 54.10; H, 5.96; N, 8.40.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis(4-aminopyridinium) dibromide (13). Yield: 79%. Mp 254–256 $^{\circ}\text{C}$. ^1H NMR (300.13 MHz): δ 8.14 (d, $J=7.6$ Hz, 4H, H-2_{pyr}), 7.30 (d, $J=8.3$ Hz, 4H, Ph), 7.25 (d, $J=8.3$ Hz, 4H, Ph), 6.85 (d, $J=7.6$ Hz, 4H, H-3_{pyr}), 5.30 (s, 4H, CH₂N⁺), 3.97 (s, 2H, CH₂Ph). ^{13}C NMR (75.78 MHz): δ 160.78 (C-4_{pyr}), 143.99 (C-2_{pyr}), 143.56 (C-1_{Ph}), 134.04 (C-4_{Ph}), 130.88 (C-3_{Ph}), 129.61 (C-2_{Ph}), 110.96 (C-3_{pyr}), 61.70 (CH₂N⁺), 41.98 (CH₂Ph). HR LSIMS (thioglycerol), calcd m/z for C₂₅H₂₆N₄Br (M–Br)⁺ 461.1341. Found m/z : 461.1339. Anal. for C₂₅H₂₆N₄Br₂·H₂O: calcd C, 53.59; H, 5.04; N, 10.00. Found: C, 53.49; H, 4.96; N, 9.76.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis[(4-hydroxymethyl)pyridinium] dibromide (14). Yield: 71%. Mp 199–200 $^{\circ}\text{C}$, 153–155 $^{\circ}\text{C}$ dec. ^1H NMR (300.13 MHz): δ 8.97 (d, $J=6.7$ Hz, 4H, H-2_{pyr}), 8.05 (d, $J=6.7$ Hz, 4H, H-3_{pyr}), 7.45 (d, $J=8.1$ Hz, 4H, Ph), 7.30 (d, $J=8.1$ Hz, 4H, Ph), 5.79 (s, 4H, CH₂N⁺), 4.90 (s, 4H, CH₂OH), 4.00 (s, 2H, CH₂ Ph). ^{13}C NMR (75.78 MHz): δ 165.21 (C-4_{pyr}), 145.17 (C-2_{pyr}), 144.21 (C-1_{Ph}), 132.80 (C-4_{Ph}), 131.10 (C-3_{Ph}), 130.40 (C-2_{Ph}), 126.07 (C-3_{pyr}), 64.69 (CH₂N⁺), 62.86 (CH₂OH), 41.99 (CH₂Ph). HR LSIMS (thioglycerol), calcd m/z for C₂₇H₂₈N₂O₂Br (M–Br)⁺ 491.1334. Found m/z : 491.1335. Anal. for C₂₇H₂₈N₂O₂Br₂·0.2H₂O: calcd C, 56.31; H, 4.97; N, 4.86. Found: C, 56.20; H, 4.99; N, 5.08.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis(4-methylpyridinium) dibromide (15). 95 $^{\circ}\text{C}$. Mp 232–235 $^{\circ}\text{C}$. ^1H NMR (300.13 MHz): δ 8.87 (d, $J=6.6$ Hz, 4H, H-2_{pyr}), 7.93 (d, $J=6.6$ Hz, 4H, H-3_{pyr}), 7.43 (d, $J=8.2$ Hz, 4H, Ph), 7.30 (d, $J=8.2$ Hz, 4H, Ph), 5.75 (s, 4H, CH₂N⁺), 4.00 (s, 2H, CH₂ Ph), 2.66 (s, 6H, Me). ^{13}C NMR (75.78 MHz): δ 161.75 (C-4_{pyr}), 144.79 (C-2_{pyr}), 144.19 (C-1_{Ph}), 132.82 (C-4_{Ph}), 131.09 (C-3_{Ph}), 130.35 (C-3_{pyr} or C-2_{Ph}), 130.10 (C-2_{Ph} or C-3_{pyr}), 64.53 (CH₂N⁺), 41.98 (CH₂Ph), 22.03 (Me). HR LSIMS (thioglycerol), calcd m/z for C₂₇H₂₇N₂ (M–Br–BrH)⁺ 379.2174. Found m/z : 379.2173. Anal. for C₂₇H₂₈N₂Br₂·0.3H₂O: calcd C, 59.42; H, 5.28; N, 5.13. Found: C, 59.36; H, 5.55; N, 4.99.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis(4-acetylpyridinium) dibromide (16a). Yield: 38% (mixture of **16a** and **16b**). Mp 185–187 $^{\circ}\text{C}$ dec (mixture of **16a** and **16b**). ^1H NMR (400.13 MHz): δ 9.26 (d, $J=6.9$ Hz, 4H,

H-2_{pyr}), 8.48 (d, $J=6.9$ Hz, 4H, H-3_{pyr}), 7.50 (d, $J=8.1$ Hz, 4H, Ph), 7.32 (d, $J=8.1$ Hz, 4H, Ph), 5.91 (s, 4H, CH₂N⁺), 4.02 (s, 2H, CH₂Ph), 2.74 (s, 6H, CH₃CO). ¹³C NMR (100.623 MHz, selected data): δ 195.55 (CH₃CO), 65.62 (CH₂N⁺), 29.11 (CH₃).

1,1' - [Methylenebis(benzene - 1,4 - diylmethylene)]bis[4 - (1,1-dihydroxy-ethyl)acetylpyridinium] dibromide (16b). ¹H NMR (400.13 MHz): δ 9.03 (d, $J=6.9$ Hz, 4H, pyridine-H₂), 8.17 (d, $J=6.9$ Hz, 4H, pyridine-H₃), 7.47 (d, $J=8.1$ Hz, 4H, Ph), 7.32 (d, $J=8.1$ Hz, 4H, Ph), 5.82 (s, 4H, CH₂N⁺), 4.02 (s, 4H, CH₂Ph), 1.60 (s, 6H, CH₃C(OH)₂). ¹³C NMR (100.03 MHz, selected data): δ 98.13 (CH₃C(OH)₂), 64.92 (CH₂N⁺), 27.13 (CH₃C(OH)₂). HR LSIMS (thioglycerol) for the mixture of **16a** and **16b**, calcd m/z for C₂₉H₂₈N₂O₂Br (M-Br)⁺ 515.1334. Found m/z : 515.1335. Anal. for C₂₉H₂₈N₂O₂Br₂·1.3H₂O (mixture of **16a** and **16b**): calcd C, 56.20; H, 4.98; N, 4.52. Found: C, 56.18; H, 5.06; N, 4.56.

1,1' - [Methylenebis(benzene-1,4-diylmethylene)]bis(4-cyanopyridinium) dibromide (17). Yield: 41%. Mp 255–257 °C. ¹H NMR (400.13 MHz): δ 9.33 (d, $J=6.6$ Hz, 4H, H-2_{pyr}), 8.51 (d, $J=6.6$ Hz, 4H, H-3_{pyr}), 7.51 (d, $J=8.1$ Hz, 4H, Ph), 7.34 (d, $J=8.1$ Hz, 4H, Ph), 5.94 (s, 4H, CH₂N⁺), 4.07 (s, 2H, CH₂Ph). ¹³C NMR (100.03 MHz, selected data): δ 144.64 (C-2_{pyr}), 132.47 (C-3_{pyr}), 115.18 (CN), 66.37 (CH₂N⁺), 42.02 (CH₂Ph). HR LSIMS (thioglycerol), calcd m/z for C₂₇H₂₂N₄Br (M-Br)⁺ 481.1028. Found m/z : 481.1029. Anal. for C₂₇H₂₂N₄Br₂·H₂O: calcd C, 55.88; H, 4.17; N, 9.65. Found: C, 56.12; H, 3.93; N, 9.28.

1,1' - [Methylenebis(benzene-1,4-diylmethylene)]bis[4-hydroxyiminomethylpyridinium] dibromide (18). Yield: 48%. Mp 175–177 °C. ¹H NMR (300.13 MHz): δ 8.97 (d, $J=7.0$ Hz, 4H, H-2_{pyr}), 8.30 (s, 2H, CH), 8.20 (d, $J=7.0$ Hz, 4H, H-3_{pyr}), 7.45 (d, $J=8.3$ Hz, 4H, Ph), 7.31 (d, $J=8.3$ Hz, 4H, Ph), 5.78 (s, 4H, CH₂N⁺), 4.02 (s, 2H, CH₂Ph). ¹³C NMR (75.78 MHz): δ 151.50 (C-4_{pyr}), 145.87 (C-2_{pyr} or CH), 145.58 (CH or C-2_{pyr}), 144.26 (C-1_{Ph}), 132.62 (C-4_{Ph}), 131.15 (C-3_{Ph}), 130.48 (C-2_{Ph}), 125.64 (C-3_{pyr}), 64.84 (CH₂N⁺), 42.00 (CH₂Ph). HR LSIMS (thioglycerol), calcd m/z for C₂₇H₂₇N₄O₂ (M-2Br+H)⁺ 439.2134. Found m/z : 439.2134. Anal. for C₂₇H₂₆N₄Br₂·H₂O: calcd C, 52.62; H, 4.58; N, 9.09. Found: C, 52.96; H, 4.56; N, 8.66.

1,1' - [Ethylenebis(benzene-1,4-diylmethylene)]bis(4-acetylpyridinium) dibromide (23a). Yield: 57% (mixture of **23a** and **23b**). Mp 222–224 °C dec (mixture of **23a** and **23b**). ¹H NMR (300.13 MHz): δ 9.26 (d, $J=6.9$ Hz, 4H, H-2_{pyr}), 8.49 (d, $J=6.9$ Hz, 4H, H-3_{pyr}), 7.45 (d, $J=8.1$ Hz, 4H, Ph), 7.30 (d, $J=8.1$ Hz, 4H, Ph), 5.90 (s, 4H, CH₂N⁺), 2.94 (s, 2H, CH₂Ph), 2.75 (s, 6H, CH₃CO). ¹³C NMR (75.479 MHz, selected data): δ 195.56 (CH₃CO), 65.71 (CH₂N⁺), 29.11 (CH₃).

1,1' - [Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(1,1-dihydroxyethyl)pyridinium] dibromide (23b). ¹H NMR (300.13 MHz): δ 9.03 (d, $J=6.9$ Hz, 4H, H-2_{pyr}), 8.18 (d, $J=6.9$ Hz, 4H, H-3_{pyr}), 7.44 (d, $J=8.3$ Hz, 4H, Ph),

7.30 (d, $J=8.3$ Hz, 4H, Ph), 5.82 (s, 4H, CH₂N⁺), 2.94 (s, 4H, CH₂Ph), 1.52 (s, 6H, CH₃C(OH)₂). ¹³C NMR (75.78 MHz): δ 98.14 (CH₃C(OH)₂), 65.02 (CH₂N⁺), 27.13 (CH₃C(OH)₂). HR LSIMS (thioglycerol) for the mixture of **23a** and **23b**, calcd m/z for C₃₀H₃₀N₂O₂Br (M-Br)⁺ 529.1491. Found m/z : 529.1593. Anal. for C₃₀H₃₀N₂O₂Br₂·2H₂O (mixture of **23a** and **23b**): calcd C, 55.74; H, 5.30; N, 4.33. Found: C, 55.87; H, 5.61; N, 4.39.

1,1' - [Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis[4-dimethylaminopyridinium] dibromide (26). Yield: 92%. Mp 133–135 °C. ¹H NMR (300.13 MHz): δ 8.24 (d, $J=7.8$ Hz, 4H, H-2_{pyr}), 7.32 (d, $J=8.1$ Hz, 4H, H-3_{pyr}), 7.24 (d, $J=8.1$ Hz, 4H, Ph), 7.00 (d, $J=7.8$ Hz, 4H, H-3_{pyr}), 5.34 (s, 4H, CH₂N⁺), 3.24 (s, 12H, Me), 2.63 (t, $J=7.6$ Hz, 4H, CH₂Ph), 1.89 (q, $J=7.6$ Hz, 2H, C-CH₂-C). ¹³C NMR (75.78 MHz): δ 157.97 (C-4_{pyr}), 144.80 (C-1_{Ph}), 143.06 (C-2_{pyr}), 133.65 (C-4_{Ph}), 130.46 (C-3_{Ph}), 129.52 (C-2_{Ph}), 109.11 (C-3_{pyr}), 61.54 (CH₂N⁺), 40.39 (Me), 35.97 (CH₂Ph), 34.15 (C-CH₂-C). HR LSIMS (thioglycerol+Na⁺), calcd m/z for C₃₁H₃₈N₄Br (M-Br)⁺ 545.2280. Found m/z : 545.2281. Anal. for C₃₁H₃₈N₄Br₂·H₂O: calcd C, 57.77; H, 6.26; N, 8.69. Found: C, 57.48; H, 6.41; N, 8.76.

1,1' - [Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis[4-aminopyridinium] dibromide (27). Yield: 47%. Mp 207–209 °C. ¹H NMR (300.13 MHz): δ 8.17 (d, $J=7.6$ Hz, 4H, H-2_{pyr}), 7.31 (d, $J=8.3$ Hz, 4H, Ph), 7.24 (d, $J=8.3$ Hz, 4H, Ph), 6.86 (d, $J=7.6$ Hz, 4H, H-3_{pyr}), 5.31 (s, 4H, CH₂N⁺), 2.63 (t, $J=7.6$ Hz, 4H, CH₂Ph), 1.90 (q, $J=7.6$ Hz, 2H, C-CH₂-C). ¹³C NMR (75.78 MHz): δ 160.79 (C-4_{pyr}), 144.80 (C-1_{Ph}), 144.00 (C-2_{pyr}), 133.59 (C-4_{Ph}), 130.47 (C-3_{Ph}), 129.46 (C-2_{Ph}), 110.95 (C-3_{pyr}), 61.81 (CH₂N⁺), 35.98 (CH₂Ph), 34.08 (C-CH₂-C). HR LSIMS (thioglycerol), calcd m/z for C₂₇H₃₀N₄Br (M-Br)⁺ 489.1654. Found m/z : 489.1654. Anal. for C₂₇H₃₀N₄Br₂·1.3H₂O: calcd C, 54.61; H, 5.53; N, 9.43. Found: C, 54.76; H, 5.65; N, 9.25.

1,1' - [Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis[4-hydroxymethylpyridinium] dibromide (28). Yield: 70%. 132–134 °C, 78–80 °C dec. ¹H NMR (300.13 MHz): δ 8.98 (d, $J=6.8$ Hz, 4H, H-2_{pyr}), 8.06 (d, $J=6.8$ Hz, 4H, H-3_{pyr}), 7.44 (d, $J=8.1$ Hz, 4H, Ph), 7.28 (d, $J=8.1$ Hz, 4H, Ph), 5.80 (s, 4H, CH₂N⁺), 4.91 (s, 4H, CH₂OH), 2.65 (t, $J=7.7$ Hz, 4H, CH₂Ph), 1.90 (q, $J=7.7$ Hz, 2H, C-CH₂-C). ¹³C NMR (75.78 MHz): δ 165.21 (C-4_{pyr}), 145.58 (C-1_{Ph}), 145.19 (C-2_{pyr}), 132.34 (C-4_{Ph}), 130.73 (C-3_{Ph}), 130.24 (C-2_{Ph}), 126.07 (C-3_{pyr}), 64.85 (CH₂N⁺), 62.88 (CH₂OH), 36.01 (CH₂Ph), 34.04 (C-CH₂-C). Anal. for C₂₉H₃₂N₂O₂Br₂·1.4H₂O: calcd C, 55.68; H, 5.61; N, 4.48. Found: C, 55.69; H, 5.68; N, 4.63.

1,1' - [Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis[4-methylpyridinium] dibromide (29). Yield: 75%. Very hygroscopic to determine its mp. ¹H NMR (300.13 MHz): δ 8.90 (d, $J=6.6$ Hz, 4H, H-2_{pyr}), 7.94 (d, $J=6.6$ Hz, 4H, H-3_{pyr}), 7.44 (d, $J=8.1$ Hz, 4H, Ph), 7.28 (d, $J=8.1$ Hz, 4H, Ph), 5.76 (s, 4H, CH₂N⁺), 2.67 (s, 6H, Me), 2.65 (t, $J=7.6$ Hz, 4H, CH₂Ph), 1.90 (q,

$J = 7.6$ Hz, 2H, C–CH₂–C). ¹³C NMR (75.78 MHz): δ 161.69 (C-4_{pyr}), 145.78 (C-1_{Ph}), 145.19 (C-2_{pyr}), 132.34 (C-4_{Ph}), 130.68 (C-2_{Ph}), 130.20–130.08 (C-3_{pyr} or C-3_{Ph}), 64.64 (CH₂N⁺), 35.97 (CH₂Ph), 34.00 (C–CH₂–C), 22.03 (Me). Anal. for C₂₉H₃₂N₂Br₂·1.5H₂O: calcd C, 58.50; H, 5.93; N, 4.70. Found: C, 58.28; H, 5.92; N, 4.84.

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis(4-acetylpyridinium) dibromide (30a). Yield: 75% (mixture of **30a** and **30b**). Mp 232–233 °C dec (mixture of **30a** and **30b**). ¹H NMR (400.13 MHz): δ 9.25 (d, $J = 6.6$ Hz, 4H, H-2_{pyr}), 8.48 (d, $J = 6.6$ Hz, 4H, H-3_{pyr}), 7.48 (d, $J = 8.1$ Hz, 4H, Ph), 7.30 (d, $J = 8.1$ Hz, 4H, Ph), 5.91 (s, 4H, CH₂N⁺), 2.74 (s, 6H, CH₃CO), 2.66 (t, 4H, $J = 7.5$ Hz, CH₂ Ph), 1.91 (q, $J = 7.5$ Hz, C–CH₂–C). ¹³C NMR (100.03 MHz, selected data): δ 195.55 (CH₃CO), 65.79 (CH₂N⁺), 29.08 (CH₃).

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis[(4-(1,1-dihydroxyethyl)pyridinium) dibromide (30b). ¹H NMR (400.13 MHz): δ 9.04 (d, $J = 6.7$ Hz, 4H, H-2_{pyr}), 8.18 (d, $J = 6.7$ Hz, 4H, H-3_{pyr}), 7.47 (d, $J = 8.2$ Hz, 4H, Ph), 7.31 (d, $J = 8.2$ Hz, 4H, Ph), 5.83 (s, 4H, CH₂N⁺), 1.62 (s, 6H, CH₃C(OH)₂), 2.66 (t, 4H, $J = 7.7$ Hz, CH₂ Ph), 1.90 (q, $J = 7.6$ Hz, C–CH₂–C). ¹³C NMR (100.03 MHz, selected data): δ 98.14 (CH₃C(OH)₂), 65.09 (CH₂N⁺), 27.08 (CH₃C(OH)₂). Anal. for C₃₁H₃₂N₂O₂Br₂·3.3H₂O (mixture of **30a** and **30b**): calcd C, 54.45; H, 5.69; N, 4.10. Found: C, 54.28; H, 5.34; N, 4.05.

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis(4-cyanopyridinium) dibromide (31). Yield: 29%. Mp 240–241 °C. ¹H NMR (400.13 MHz): δ 9.33 (d, $J = 6.5$ Hz, 4H, H-2_{pyr}), 8.51 (d, $J = 6.5$ Hz, 4H, H-3_{pyr}), 7.50 (d, $J = 8.1$ Hz, 4H, Ph), 7.32 (d, $J = 8.1$ Hz, 4H, Ph), 5.93 (s, 4H, CH₂N⁺), 2.67 (t, $J = 7.7$ Hz, 4H, CH₂Ph), 1.91 (q, $J = 7.7$ Hz, 2H, C–CH₂–C). ¹³C NMR (100.03 MHz): δ 147.34 (C-2_{pyr}), 146.10 (C-1_{Ph}), 132.47 (C-3_{pyr}), 131.18 (C-4_{Ph}), 130.88 (C-3_{Ph}), 130.80 (C-2_{Ph}), 130.12 (C-4_{pyr}), 115.22 (CN), 66.54 (CH₂N⁺), 36.02 (CH₂Ph), 33.94 (C–CH₂–C). Anal. for C₂₉H₂₆N₄Br₂·0.3H₂O: calcd C, 58.46; H, 4.50; N, 9.40. Found: C, 58.41; H, 4.40; N, 9.15.

trans-1,1' - [Stilbene - 4,4' - diylbis(methylene)]bis(4-amino-pyridinium) dibromide (33). Yield: 43%. Mp 329–331 °C. ¹H NMR (300.13 MHz): δ 8.17 (d, $J = 7.6$ Hz, 4H, H-2_{pyr}), 7.63 (d, $J = 8.4$, 4H, Ph), 7.37 (d, $J = 8.4$, 4H, Ph), 7.24 (s, 2H, CH), 6.86 (d, $J = 7.6$, 4H, H-3_{pyr}), 5.33 (s, 4H, CH₂N⁺). ¹³C NMR (75.78 MHz): δ 160.90 (C-4_{pyr}), 144.06 (C-2_{pyr}), 139.55 (C-1_{Ph}), 135.48 (C-4_{Ph}), 129.91 (CH), 129.74 (C-3_{Ph}), 128.55 (C-2_{Ph}), 110.99 (C-3_{pyr}), 61.73 (CH₂N⁺). Anal. for C₂₉H₃₂N₂Br₂·1.5H₂O: calcd C, 58.50; H, 5.93; N, 4.70. Found: C, 58.28; H, 5.92; N, 4.84. Anal. for C₂₆H₂₆N₄Br₂: calcd C, 56.33; H, 4.73; N, 10.11. Found: C, 56.05; H, 4.48; N, 10.15.

1,1' - [Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis-quinolinium dibromide (34). Yield: 36%. Mp 158–160 °C dec. ¹H NMR (400.13 MHz): δ 9.52 (d, $J = 5.9$ Hz, 2H, H-2_{quin}), 9.28 (d, $J = 8.3$ Hz, 2H, H-4_{quin}), 8.52 (d, 2H, H-5_{quin} or H-8_{quin}), 8.45 (d, 2H, H-8_{quin} or H-5_{quin}),

8.18 (m, 4H, H-3_{quin} and H-6_{quin} or H-7_{quin}), 8.02 (t, 2H, H-7_{quin} or H-6_{quin}), 7.29 (d, $J = 8.2$ Hz, 4H, Ph), 7.23 (d, $J = 8.2$ Hz, 4H, Ph), 6.31 (s, 4H, CH₂N⁺), 2.60 (t, $J = 7.7$ Hz, 4H, CH₂ Ph), 1.90 (q, $J = 7.7$ Hz, 2H, C–CH₂–C). Anal. for C₃₅H₃₂N₂Br₂·0.6H₂O: calcd C, 64.55; H, 5.14; N, 4.30. Found: C, 64.70; H, 5.42; N, 4.45.

1,1' - [Methylenebis(benzene-1,4-diylmethylene)]bis[(4-diallylamino)pyridinium] dibromide (37). Yield: 47%. Mp 112–114 °C. ¹H NMR (400.13 MHz): δ 8.25 (d, $J_{2,3} = 7.9$ Hz, 4H, H-2_{pyr}), 7.32 (d, $J_{2,3} = 8.2$ Hz, 4H, H-2_{benz}), 7.26 (d, $J_{2,3} = 8.2$ Hz, 4H, H-3_{benz}), 7.01 (d, $J_{2,3} = 7.9$ Hz, 4H, H-3_{pyr}), 5.89 (ddt, $J_{2,3'} = 17.2$, $J_{2,3} = 10.2$, $J_{1,2} = 4.8$ Hz, 4H, H-2_{allyl}), 5.35 (s, 4H, CH₂N⁺), 5.27 (ddt, $J_{2,3} = 10.2$, $J_{3,3'} = 1.3$, $J_{1,3} = 1.5$ Hz, 4H, H-3_{allyl}), 5.21 (ddt, $J_{2,3'} = 17.2$, $J_{3,3'} = 1.3$, $J_{1,3'} = 1.5$ Hz, 4H, H-3'_{allyl}), 4.23 (dt, $J_{1,2} = 4.8$, $J_{1,3} = 1.5$, $J_{1,3'} = 1.5$ Hz, 4H, H-1_{allyl}), 3.99 (s, 2H, CH₂Ph). ¹³C NMR (100.03 MHz): δ 157.89 (C-4_{pyr}), 143.66 (C-1_{Ph}), 143.49 (C-2_{pyr}), 133.85 (C-4_{Ph}), 131.47 (C-2_{allyl}), 130.91 (C-3_{Ph}), 129.74 (C-2_{Ph}), 118.38 (C-3_{allyl}), 109.92 (C-3_{pyr}), 61.70 (CH₂N⁺), 54.23 (C-1_{allyl}), 41.98 (CH₂Ph). HR LSIMS (thioglycerol + Na⁺), calcd m/z for C₃₇H₄₂N₄Br (M–Br)⁺ 621.2593. Found m/z : 621.2593. Anal. for C₃₇H₄₂N₄Br₂·3.2H₂O: calcd C, 58.46; H, 6.42; N, 7.37. Found: C, 58.30; H, 6.20; N, 7.51.

1,1' - [Biphenyl-3,3' - diylbis(methylene)]bispiridinium (38). Yield: 57.3%. Mp 74–75 °C. ¹H NMR (400.13 MHz): δ 9.25 (d, $J = 6.6$ Hz, 4H, H-2_{pyr}), 8.62 (dt, $J = 6.6$ and 1.2 Hz, 2H, H-4_{pyr}), 8.15 (m, 4H, H-3_{pyr}), 8.06 (s, 2H, Ph), 7.78 (m, 2H, Ph), 7.57 (m, 4H, Ph), 5.98 (s, 4H, CH₂N⁺). ¹³C NMR (100.03 MHz): δ 147.34 (C-2_{pyr}), 146.08 (C-4_{pyr}), 142.57 (C-1_{Ph}), 135.48 (C-3_{Ph}), 131.41 (C-5_{Ph}), 129.80 (C-3_{pyr}), 129.64, 129.62, 129.35 (C-4,6,2_{Ph}), 65.53 (CH₂N⁺). Anal. for C₂₄H₂₂N₂Br₂·2H₂O: calcd C, 53.95; H, 4.91; N, 5.24. Found: C, 54.00; H, 4.55; N, 5.12.

1,1' - [Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis-isoquinolinium dibromide (40). Yield: 90%. Mp 80–82 °C. ¹H NMR (400.13 MHz): δ 10.11 (s, 2H, H-1_{isoq}), 8.67 (dd, $J = 6.8$ and 1.2 Hz, 2H, H-3_{isoq}), 8.51 (d, $J = 8.3$ Hz, 2H, H-5_{isoq} or H-8_{isoq}), 8.28 (d, $J = 8.2$ Hz, 2H, H-8_{isoq} or H-5_{isoq}), 8.23 (ddd, $J = 8.2$, 7.0 and 0.8 Hz, 2H, H-6_{isoq} or H-7_{isoq}), 8.05 (ddd, $J = 8.2$, 7.0 and 0.9 Hz, 2H, H-7_{isoq} or H-6_{isoq}), 7.50 (d, $J = 8.0$ Hz, 4H, Ph), 7.27 (d, $J = 8.0$ Hz, 4H, Ph), 6.10 (s, 4H, CH₂N⁺), 2.63 (t, $J = 7.7$ Hz, 4H, CH₂Ph), 1.88 (q, $J = 7.7$ Hz, 2H, C–CH₂–C). Anal. for C₃₅H₃₂N₂Br₂·2H₂O: calcd C, 62.14; H, 5.36; N, 4.14. Found: C, 62.04; H, 5.34; N, 4.17.

1,1' - [Butane - 1,4 - diylbis(benzene - 1,4 - diylmethylene)]-bis[(4-dimethylamino)pyridinium] dibromide (41). Yield: 89%. Mp 100–103 °C. ¹H NMR (300.13 MHz): δ 8.32 (d, $J = 7.9$ Hz, 4H, H-2_{pyr}), 7.39 (d, $J = 8.2$ Hz, 4H, Ph), 7.31 (d, $J = 8.2$ Hz, 4H, Ph), 7.09 (d, $J = 7.9$ Hz, 4H, H-3_{pyr}), 5.42 (s, 4H, CH₂N⁺), 3.33 (s, 12H, NMe₂), 2.72 (t, 4H, CH₂ Ph), 1.70 (q, 4H, C–CH₂–C). ¹³C NMR (75.78 MHz): δ 157.98 (C-4_{pyr}), 145.13 (C-1_{Ph}), 143.05 (C-2_{pyr}), 133.51 (C-4_{Ph}), 130.42 (C-3_{Ph}), 129.44 (C-2_{Ph}), 109.11 (C-3_{pyr}), 61.57 (CH₂N⁺), 40.39 (NMe₂), 36.28

(CH₂Ph), 32.05 (C–CH₂–C). HR LSIMS (thioglycerol + Na⁺), calcd *m/z* for C₃₂H₄₀N₄Br (M–Br)⁺ 559.2436. Found *m/z*: 559.2436. Anal. for C₃₂H₄₀N₄Br₂·1.2H₂O: calcd C, 58.05; H, 6.46; N, 8.46. Found: C, 57.86; H, 6.35; N, 8.26.

cis-1,1'-[Stilbene-4,4'-diylbis(methylene)]bis[(4-dimethylamino)pyridinium] dibromide (42). Yield: 61%. Mp 133–135 °C. ¹H NMR (300.13 MHz): δ 8.23 (d, *J* = 7.9 Hz, 4H, H-2_{pyr}), 7.26 (s, 8H, Ph), 7.01 (d, *J* = 7.9 Hz, 4H, H-3_{pyr}), 6.66 (s, 2H, CH), 5.34 (s, 4H, CH₂N⁺), 3.25 (s, 12H, CH₃). ¹³C NMR (75.78 MHz): δ 158.00 (C-4_{pyr}), 143.11 (C-2_{pyr}), 139.38 (C-1_{ph}), 135.16 (C-4_{ph}), 131.32 (CH), 130.79 (C-3_{ph}), 129.40 (C-2_{ph}), 109.18 (C-3_{pyr}), 61.36 (CH₂N⁺), 40.42 (Me). HR LSIMS (thioglycerol), calcd *m/z* for C₃₀H₃₄N₄Br (M–Br)⁺ 529.1967. Found *m/z*: 529.1968. Anal. for C₃₀H₃₄N₄Br₂·H₂O: calcd C, 57.34; H, 5.77; N, 8.92. Found: C, 57.62; H, 5.92; N, 8.81.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bispyridinium dibromide (43). Yield: 98%. Mp 275–277 °C. ¹H NMR (300.13 MHz): δ 9.15 (dd, *J* = 6.6 and 1.2 Hz, 4H, H-2_{pyr}), 8.64 (dt, *J* = 9.1 and 1.3 Hz 2H, H-4_{pyr}), 8.16 (m, 4H, H-3_{pyr}), 7.75 (d, *J* = 8.5 Hz, 4H, Ph), 7.64 (d, *J* = 8.5 Hz, 4H, Ph), 5.94 (s, 4H, CH₂N⁺). ¹³C NMR (75.78 MHz): δ 147.39 (C-2_{pyr}), 146.05 (C-4_{pyr}), 142.79 (C-1_{ph}), 134.24 (C-4_{ph}), 130.91 (C-3_{ph}), 129.78 (C-3_{pyr}), 129.26 (C-2_{ph}), 65.32 (CH₂N⁺). HR LSIMS (thioglycerol), calcd *m/z* for C₂₄H₂₃N₂ (M–2Br + H)⁺ 339.1861. Found *m/z*: 339.1860. Anal. for C₂₄H₂₂N₂Br₂·0.8H₂O: calcd C, 56.23; H, 4.64; N, 5.46. Found: C, 56.11; H, 4.36; N, 5.24.

1,1'-[Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis[(4-pyrrolidino)pyridinium] dibromide (44). Yield: 49%. Mp 182–186 °C, dec at 158 °C. ¹H NMR (400.13 MHz): δ 8.21 (d, *J* = 7.5 Hz, 4H, H-2_{pyr}), 7.31 (d, *J* = 8.0 Hz, 4H, Ph), 7.24 (d, *J* = 8.0 Hz, 4H, Ph), 6.85 (d, *J* = 7.5 Hz, 4H, H-3_{pyr}), 5.32 (s, 4H, CH₂N⁺), 3.54 (t, *J* = 6.7 Hz, 8H, H-2_{pyrrolid}), 2.63 (t, *J* = 7.6 Hz, 4H, CH₂Ph), 2.11 (q, *J* = 6.7 Hz, 8H, H-3_{pyrrolid}), 1.89 (q, *J* = 7.6 Hz, 2H, C–CH₂–C). ¹³C NMR (100.03 MHz): δ 155.14 (C-4_{pyr}), 144.77 (C-1_{ph}), 142.98 (C-2_{pyr}), 133.77 (C-4_{ph}), 130.45 (C-3_{ph}), 129.46 (C-2_{ph}), 109.68 (C-3_{pyr}), 61.57 (CH₂N⁺), 49.72 (C-2_{pyrrolid}), 35.99 (CH₂Ph), 34.20 (C–CH₂–C), 26.13 (C-3_{pyrrolid}). HR LSIMS (thioglycerol + Na⁺), calcd *m/z* for C₃₅H₄₂N₄Br (M–Br)⁺ 597.2593. Found *m/z*: 597.2593. Anal. for C₃₅H₄₂N₄Br₂·3H₂O: calcd C, 57.38; H, 6.60; N, 7.65. Found: C, 57.19; H, 6.47; N, 7.75.

1,1'-[Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bispyridinium dibromide (45). Yield: 76%. Mp 62–64 °C. ¹H NMR (300.13 MHz): δ 9.10 (dd, *J* = 6.6 and 1.2 Hz, 4H, H-2_{pyr}), 8.61 (dt, *J* = 9.1 and 1.3 Hz 2H, H-4_{pyr}), 8.13 (m, 4H, H-3_{pyr}), 7.47 (d, *J* = 8.2 Hz, 4H, Ph), 7.29 (d, *J* = 8.2 Hz, 4H, Ph), 5.85 (s, 4H, CH₂N⁺), 2.65 (t, *J* = 7.6 Hz, 4H, CH₂Ph), 1.92 (q, *J* = 7.6 Hz, 2H, C–CH₂–C). ¹³C NMR (75.78 MHz): δ 147.21 (C-2_{pyr}), 145.87 (C-4_{pyr}), 145.65 (C-1_{ph}), 132.09 (C-4_{ph}), 130.73 (C-3_{ph}), 130.33 (C-2_{ph}), 129.68 (C-3_{pyr}), 65.52 (CH₂N⁺), 35.98 (CH₂Ph), 33.97 (C–CH₂–C). Anal. for C₂₇H₂₈N₂Br₂·0.8H₂O: calcd

C, 58.46; H, 5.38; N, 5.05. Found: C, 58.54; H, 5.47; N, 5.11.

trans-1,1'-[Stilbene-4,4'-diylbis(methylene)]bis[(4-dimethylamino)pyridinium] dibromide (46). Yield: 81%. Mp >315 °C, 190–192 °C dec. ¹H NMR (400.13 MHz): δ 8.25 (d, *J* = 6.5 Hz, 4H, H-2_{pyr}), 7.63 (d, *J* = 8.3 Hz, 4H, Ph), 7.39 (d, *J* = 8.3 Hz, 4H, Ph), 7.23 (s, 2H, CH), 7.00 (d, *J* = 6.5 Hz, 4H, H-3_{pyr}), 5.36 (s, 4H, CH₂N⁺), 3.24 (s, 12H, CH₃). ¹³C NMR (100.03 MHz): δ 158.01 (C-4_{pyr}), 143.09 (C-2_{pyr}), 139.52 (C-1_{ph}), 135.49 (C-4_{ph}), 129.91 (CH), 129.83 (C-3_{ph}), 128.53 (C-2_{ph}), 109.16 (C-3_{pyr}), 61.47 (CH₂N⁺), 40.38 (Me). HR LSIMS (thioglycerol + Na⁺), calcd *m/z* for C₃₀H₃₄N₄Br (M–Br)⁺ 529.1934. Found *m/z*: 529.1934. Anal. for C₃₀H₃₄N₄Br₂·1.3H₂O·0.5CH₃OH: calcd C, 56.37; H, 5.99; N, 8.62. Found: C, 56.28; H, 5.85; N, 8.27.

cis-1,1'-[Stilbene-4,4'-diylbis(methylene)]bis(4-aminopyridinium) dibromide (47). Yield: 40%. Mp 273–275 °C. ¹H NMR (300.13 MHz): δ 8.17 (d, *J* = 7.5 Hz, 4H, H-2_{pyr}), 7.25 (s, 8H, Ph), 6.87 (d, *J* = 7.5 Hz, 4H, H-3_{pyr}), 6.66 (s, 2H, CH), 5.31 (s, 4H, CH₂N⁺). ¹³C NMR (75.78 MHz): δ 160.84 (C-4_{pyr}), 144.06 (C-2_{pyr}), 139.37 (C-1_{ph}), 135.13 (C-4_{ph}), 131.34 (CH), 130.80 (C-3_{ph}), 129.35 (C-2_{ph}), 111.01 (C-3_{pyr}), 61.63 (CH₂N⁺). Anal. for C₂₆H₂₆N₄Br₂·0.5H₂O: calcd C, 55.81; H, 4.95; N, 9.82. Found: C, 55.67; H, 4.57; N, 9.44.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bispyridinium dibromide (49). Yield: 82%. Mp 243–245 °C. ¹H NMR (300.13 MHz): δ 9.09 (dd, *J* = 6.6 and 1.2 Hz, 4H, H-2_{pyr}), 8.61 (dt, *J* = 9.1 and 1.3 Hz, 2H, H-4_{pyr}), 8.12 (m, 4H, H-3_{pyr}), 7.47 (d, *J* = 8.2 Hz, 4H, Ph), 7.32 (d, *J* = 8.2 Hz, 4H, Ph), 5.84 (s, 4H, CH₂N⁺), 4.02 (s, 2H, CH₂Ph). ¹³C NMR (75.78 MHz): δ 147.26 (C-2_{pyr}), 145.90 (C-4_{pyr}), 144.31 (C-1_{ph}), 132.59 (C-4_{ph}), 131.15 (C-3_{ph}), 130.50 (C-2_{ph}), 129.71 (C-3_{pyr}), 65.41 (CH₂N⁺), 41.99 (CH₂Ph). HR LSIMS (thioglycerol), calcd *m/z* for C₂₅H₂₅N₂ (M–2Br + H)⁺ 353.2018. Found *m/z*: 353.2018. Anal. for C₂₅H₂₄N₂Br₂·1.3H₂O: calcd C, 56.05; H, 5.00; N, 5.23. Found: C, 56.26; H, 4.87; N, 5.24.

1,1'-[Ethylenebis(benzene-1,4-diylmethylene)]bisquinolinium dibromide (50). Yield: 34%. Mp 180–182 °C, 154–156 °C dec. ¹H NMR (300.13 MHz): δ 9.54 (d, 2H, H-2_{quin}), 9.30 (d, 2H, H-4_{quin}), 8.51 (d, 2H, H-5_{quin} or H-8_{quin}), 8.45 (d, 2H, H-8_{quin} or H-5_{quin}), 8.19 (m, 4H, H-3_{quin} and H-6_{quin} or H-7_{quin}), 8.02 (t, 2H, H-7_{quin} or H-6_{quin}), 7.26 (d, *J* = 8.4 Hz, 4H, Ph), 7.21 (d, *J* = 8.4 Hz, 4H, Ph), 6.32 (s, 4H, CH₂N⁺), 2.87 (s, 4H, CH₂Ph). HR LSIMS (thioglycerol), calcd *m/z* for C₃₄H₃₁N₂ (M–2Br + H)⁺ 467.2487. Found *m/z*: 467.2489. Anal. for C₃₄H₃₀N₂Br₂·1.8H₂O: calcd C, 61.98; H, 5.14; N, 4.25. Found: C, 61.18; H, 5.17; N, 4.07.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bisquinolinium dibromide (51). Yield: 71%. Mp 247–249 °C, 178–181 °C dec. ¹H NMR (300.13 MHz): δ 9.61 (d, 2H, H-2_{quin}), 9.31 (d, 2H, H-4_{quin}), 8.53 (d, 2H, H-5_{quin} or H-8_{quin}), 8.46 (d, 2H, H-8_{quin} or H-5_{quin}), 8.21 (m, 4H, H-3_{quin} and H-6_{quin} or H-7_{quin}), 8.02 (t, 2H, H-7_{quin} or H-6_{quin}),

7.66 (d, $J=8.4$ Hz, 4H, Ph), 7.46 (d, $J=8.4$ Hz, 4H, Ph), 6.41 (s, 4H, CH_2N^+). Anal. for $\text{C}_{32}\text{H}_{26}\text{N}_2\text{Br}_2 \cdot 1.9\text{H}_2\text{O}$: calcd C, 60.76; H, 4.75; N, 4.43. Found: C, 61.00; H, 4.77; N, 4.12.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis[(4-pyrrolidino)pyridinium] dibromide (52). Yield: 72%. Mp 139–141 °C. ^1H NMR (400.13 MHz): δ 8.19 (d, $J=7.6$ Hz, 4H, H-2_{pyr}), 7.31 (d, $J=8.1$ Hz, 4H, Ph), 7.25 (d, $J=8.1$ Hz, 4H, Ph), 6.84 (d, $J=7.6$ Hz, 4H, H-3_{pyr}), 5.32 (s, 4H, CH_2N^+), 3.97 (s, 2H, CH_2Ph), 3.54 (t, $J=6.8$ Hz, 8H, H-2_{pyrrolid}), 2.11 (q, $J=6.8$ Hz, 8H, H-3_{pyrrolid}). ^{13}C NMR (100.03 MHz): δ 155.13 (C-4_{pyr}), 143.58 (C-1_{Ph}), 142.97 (C-2_{pyr}), 134.22 (C-4_{Ph}), 130.85 (C-3_{Ph}), 129.62 (C-2_{Ph}), 109.71 (C-3_{pyr}), 61.47 (CH_2N^+), 49.74 (C-2_{pyrrolid}), 41.99 (CH_2Ph), 26.14 (C-3_{pyrrolid}). HR LSIMS (thioglycerol + Na^+), calcd m/z for $\text{C}_{33}\text{H}_{38}\text{N}_4\text{Br}$ ($\text{M}-\text{Br}$)⁺ 569.2280. Found m/z : 569.2279. Anal. for $\text{C}_{33}\text{H}_{38}\text{N}_4\text{Br}_2 \cdot 3\text{H}_2\text{O}$: calcd C, 56.26; H, 6.30; N, 7.95. Found: C, 56.22; H, 6.35; N, 7.91.

1,1'-[Ethylenebis(benzene-1,4-diylmethylene)]bisisoquinolinium dibromide (53). Yield: 91%. Mp 258–260 °C. ^1H NMR (300.13 MHz): δ 10.09 (s, 2H, H-1_{isoquin}), 8.66 (dd, $J=6.9$ and 1.5 Hz, 2H, H-3_{isoquin}), 8.48 (d, $J=6.9$ Hz, 2H, H-4_{isoquin}), 8.52 (dd, $J=8.3$ and 0.9 Hz, 2H, H-5_{isoquin} or H-8_{isoquin}), 8.30 (d, $J=8.0$ Hz, 2H, H-8_{isoquin} or H-5_{isoquin}), 8.25 (dt, $J=6.6$ and 1.2 Hz, 2H, H-6_{isoquin} or H-7_{isoquin}), 8.07 (dt, $J=6.6$ and 1.2 Hz, 2H, H-7_{isoquin} or H-6_{isoquin}), 7.48 (d, $J=8.1$ Hz, 4H, Ph), 7.29 (d, $J=8.1$ Hz, 4H, Ph), 5.94 (s, 4H, CH_2N^+), 2.93 (s, 4H, CH_2Ph). HR LSIMS (thioglycerol), calcd m/z for $\text{C}_{34}\text{H}_{30}\text{N}_2\text{Br}$ ($\text{M}-\text{Br}$)⁺ 545.1592. Found m/z : 545.1593. Anal. for $\text{C}_{34}\text{H}_{30}\text{N}_2\text{Br}_2 \cdot \text{H}_2\text{O}$: calcd C, 63.37; H, 5.00; N, 4.35. Found: C, 63.29; H, 4.84; N, 4.29.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bisisoquinolinium dibromide (54). Yield: 83%. Mp 277–279 °C. ^1H NMR (300.13 MHz): δ 10.09 (s, 2H, H-1_{isoquin}), 8.65 (dd, $J=6.8$ and 1.5 Hz, 2H, H-3_{isoquin}), 8.50 (dd, $J=8.3$ and 1.0 Hz, 2H, H-5_{isoquin} or H-8_{isoquin}), 8.44 (d, $J=6.8$ Hz, 2H, H-4_{isoquin}), 8.27 (d, $J=7.7$ Hz, 2H, H-8_{isoquin} or H-5_{isoquin}), 8.22 (ddd, $J=7.6$, 6.7 and 1.0 Hz, 2H, H-6_{isoquin} or H-7_{isoquin}), 8.04 (ddd, $J=8.3$, 6.7 and 1.5 Hz, 2H, H-7_{isoquin} or H-6_{isoquin}), 7.50 (d, $J=8.2$ Hz, 4H, Ph), 7.30 (d, $J=8.2$ Hz, 4H, Ph), 5.94 (s, 4H, CH_2N^+), 3.99 (s, 2H, CH_2Ph). HR LSIMS (thioglycerol), calcd m/z for $\text{C}_{33}\text{H}_{28}\text{N}_2\text{Br}$ ($\text{M}-\text{Br}$)⁺ 531.1436. Found m/z : 531.1435. Anal. for $\text{C}_{33}\text{H}_{28}\text{N}_2\text{Br}_2 \cdot \text{H}_2\text{O}$: calcd C, 62.87; H, 4.80; N, 4.44. Found: C, 62.87; H, 4.66; N, 4.34.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bisquinolinium dibromide (55). Yield: 30%. Mp 167–169 °C. ^1H NMR (300.13 MHz): δ 9.53 (d, 2H, H-2_{quin}), 9.28 (d, 2H, H-4_{quin}), 8.50 (d, 2H, H-5_{quin} or H-8_{quin}), 8.44 (d, 2H, H-8_{quin} or H-5_{quin}), 8.18 (m, 4H, H-3_{quin} and H-6_{quin} or H-7_{quin}), 8.01 (t, 2H, H-7_{quin} or H-6_{quin}), 7.30 (d, $J=8.3$ Hz, 4H, Ph), 7.24 (d, $J=8.3$, 4H, Ph), 6.31 (s, 4H, CH_2N^+), 3.95 (s, 2H, CH_2Ph). HR LSIMS (thioglycerol), calcd m/z for $\text{C}_{33}\text{H}_{28}\text{N}_2\text{Br}$ ($\text{M}-\text{Br}$)⁺ 531.1436. Found m/z : 531.1436. Anal. for $\text{C}_{33}\text{H}_{28}\text{N}_2\text{Br}_2 \cdot 2.3\text{H}_2\text{O}$: calcd C, 60.62; H, 5.03; N, 4.28. Found: C, 60.50; H, 4.77; N, 4.05.

1,1'-[Biphenyl-4,4'-diylbis(methylene)]bisisoquinolinium dibromide (56). Yield: 80%. Mp 247–249 °C, 179–181 °C dec. ^1H NMR (300.13 MHz): δ 10.17 (s, 2H, H-1_{isoquin}), 8.73 (d, $J=6.8$ Hz, 2H, H-3_{isoquin}), 8.54 (d, 2H, H-5_{isoquin} or H-8_{isoquin}), 8.50 (d, $J=6.8$ Hz, 2H, H-4_{isoquin}), 8.31 (d, 2H, H-8_{isoquin} or H-5_{isoquin}), 8.25 (t, 2H, H-6_{isoquin} or H-7_{isoquin}), 8.07 (t, 2H, H-7_{isoquin} or H-6_{isoquin}), 7.73 (d, $J=8.5$ Hz, 4H, Ph), 7.69 (d, $J=8.5$ Hz, 4H, Ph), 6.05 (s, 4H, CH_2N^+). HR LSIMS (thioglycerol), calcd m/z for $\text{C}_{32}\text{H}_{26}\text{N}_2\text{Br}$ ($\text{M}-\text{Br}$)⁺ 517.1279. Found m/z : 517.1278. Anal. for $\text{C}_{32}\text{H}_{26}\text{N}_2\text{Br}_2 \cdot 0.5\text{H}_2\text{O}$: calcd C, 63.28; H, 4.48; N, 4.61. Found: C, 63.36; H, 4.75; N, 4.55.

1,1'-[Methylenebis(benzene-1,4-diylmethylene)]bis[(4-N-methylanilino)pyridinium] dibromide (57). Yield: 70%. Mp 116–117 °C. ^1H NMR (300.13 MHz): δ 8.28 (s, 4H, H-2_{pyr}, $T_1=1.7$ s), 7.58 (t, $J_{2,3}=6.7$ Hz, 4H, H-3_{anil}), 7.48 (t, $J_{3,4}=7.4$ Hz, 2H, H-4_{anil}), 7.34 (d, $J_{2,3}=7.7$ Hz, 4H, H-2_{anil}), H_{anil} $T_1=2.5$ s, and 2.7 s, 7.33 (d, $J_{2,3}=8.1$ Hz, 4H, H-2_{benz}), 7.25 (d, $J_{2,3}=8.1$ Hz, 4H, H-3_{benz}), 6.90 (s, 4H, H-3_{pyr}, $T_1=1.6$ s), 5.37 (s, 4H, CH_2N^+), 3.97 (s, 4H, CH_2Ph), 3.52 (s, 6H, CH_3N). ^{13}C NMR (75.57 MHz): δ 158.37 (C-4_{pyr}), 144.79 (C-1_{anil}), 144.22 (C-1_{Ph}), 143.65 (C-2_{pyr}), 133.90 (C-4_{Ph}), 132.00 (C-3_{Ph}), 130.87, 130.08, 129.78 (C-3_{anil}, C-2_{anil}, C-4_{anilino} or C-2_{Ph}), 127.51 (C-4_{anil}), 110.25 (C-3_{pyr}), 61.76 (CH_2N^+), 41.97 (Ph- CH_2 -Ph), 41.47 (CH_3N). HR LSIMS (thioglycerol + Na^+), calcd m/z for $\text{C}_{39}\text{H}_{38}\text{N}_4\text{Br}$ ($\text{M}-\text{Br}$)⁺ 641.2280. Found m/z : 641.2279. Anal. for $\text{C}_{39}\text{H}_{38}\text{N}_4\text{Br}_2 \cdot 0.8\text{H}_2\text{O}$: calcd C, 63.52; H, 5.77; N, 7.50. Found: C, 63.56; H, 5.41; N, 7.60.

1,1'-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(N-methylanilino)pyridinium] dibromide (58). Yield: 22%. Mp 115–116 °C. ^1H NMR (400.13 MHz, room temperature): δ 8.26 (s, 4H, H-2_{pyr}, $T_1=1.6$ s), 7.58 (t, $J_{2,3}=7.6$ Hz, 4H, H-3_{anil}), 7.49 (t, $J_{3,4}=7.4$ Hz, 2H, H-4_{anil}), 7.35 (d, $J_{2,3}=7.7$ Hz, 4H, H-2_{anil}), H_{anil} $T_1=2.8$ s, 2.9 s, and 3.0 s, 7.31 (d, $J_{2,3}=8.2$ Hz, 4H, H-2_{benz}), 7.24 (d, $J_{2,3}=8.2$ Hz, 4H, H-3_{benz}), 6.89 (s, 4H, H-3_{pyr}, $T_1=1.6$ s), 5.36 (s, 4H, CH_2N^+), 3.53 (s, 6H, CH_3N), 2.91 (s, 4H, CH_2Ph). ^{13}C NMR (100.13 MHz, room temperature): δ 158.45 (C-4_{pyr}), 144.80 (C-1_{anil}), 144.21 (C-1_{Ph}), 143.63 (C-2_{pyr}), 133.52 (C-4_{Ph}), 132.01 (C-3_{Ph}), 130.57, 130.11, 129.53 (C-3_{anil}, C-2_{anil}, C-2_{Ph}), 127.48 (C-4_{anil}), 110.26 (C-3_{pyr}), 61.92 (CH_2N^+), 41.43 (CH_3N), 38.23 (CH_2Ph).

^1H NMR (400.13 MHz, DMSO- d_6 , 57 °C): δ 8.45 (d, $J_{2,3}=7.5$ Hz, 4H, H-2_{pyr}), 7.59 (t, $J_{2,3}=7.7$ Hz, 4H, H-3_{anil}), 7.48 (d, $J_{3,4}=7.4$ Hz, 4H, H-4_{anil}), 7.40 (d, $J_{2,3}=7.4$ Hz, 4H, H-2_{anil}), 7.34 (d, $J_{2,3}=8.1$ Hz, 4H, H-2_{benz}), 7.29 (d, $J_{2,3}=8.1$ Hz, 4H, H-3_{benz}), 6.91 (d, 4H, H-3_{pyr}), 5.36 (s, 4H, CH_2N^+), 3.53 (s, 6H, CH_3N^+), 2.91 (s, 4H, CH_2Ph). ^{13}C NMR (100.13 MHz, DMSO- d_6 , 57 °C): δ 156.16 (C-4_{pyr}), 143.18 (C-1_{anil}), 142.59 (C-2_{pyr}), 142.08 (C-1_{Ph}), 132.92 (C-4_{Ph}), 130.53 (C-3_{anil}), 128.88 (C-3_{Ph}), 128.47 (C-4_{anil}), 128.09 (C-2_{Ph}), 126.25 (C-2_{anil}), 108.92 (C-3_{pyr}), 59.41 (CH_2N^+), 40.76 (CH_3N), 36.21 (CH_2Ph). HR LSIMS (thioglycerol + Na^+), calcd m/z for $\text{C}_{40}\text{H}_{40}\text{N}_4\text{Br}$ ($\text{M}-\text{Br}$)⁺ 655.2436. Found m/z : 655.2435. Anal. for

$C_{40}H_{40}N_4Br_2 \cdot 1.5H_2O$: calcd C, 63.09; H, 5.95; N, 7.48. Found: C, 62.92; H, 5.67; N, 7.34.

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis[(4-*N*-methylanilino)pyridinium] dibromide (59). Yield: 30%. Mp 120–121 °C. 1H NMR (300.13 MHz): δ 8.27 (s, 4H, H-2_{pyr}, T_1 = 1.5 s), 7.59 (t, $J_{2,3}$ = 7.5 Hz, 4H, H-3_{anil}), 7.51 (t, $J_{3,4}$ = 7.3 Hz, 2H, H-4_{anil}), 7.32 (d, $J_{2,3}$ = 7.5 Hz, 4H, H-2_{anil}), H_{anil} T_1 = 2.5 s, and 2.5 s, 7.28 (d, $J_{2,3}$ = 8.1 Hz, 4H, H-2_{benz}), 7.21 (d, $J_{2,3}$ = 8.0 Hz, 4H, H-3_{benz}), 6.88 (s, 4H, H-3_{pyr}, T_1 = 1.4 s), 5.38 (s, 4H, CH_2N^+), 3.51 (s, 6H, CH_3N), 3.47 (t, J = 7.1 Hz, 4H, Ph- CH_2 -C), 1.17 (q, J = 7.1 Hz, 2H, C- CH_2 -C). ^{13}C NMR (75.78 MHz): δ 158.37 (C-4_{pyr}), 144.78 (C-1_{anil}), 144.21 (C-1_{Ph}), 143.76 (C-2_{pyr}), 133.60 (C-4_{Ph}), 132.00 (C-3_{Ph}), 130.48, 130.08, 129.60 (C-3_{anil}, C-2_{anil}, C-2_{Ph} or C-2_{anil}), 127.50 (C-2_{anil}), 110.29 (C-3_{pyr}), 61.89 (CH_2N^+), 41.44 (CH_3N), 35.97 (Ph- CH_2 -C), 15.46 (C- CH_2 -C). HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{41}H_{42}N_4Br$ (M-Br)⁺ 669.2593. Found m/z : 669.2593. Anal. for $C_{41}H_{42}N_4Br_2 \cdot 4H_2O$: calcd C, 60.15; H, 6.02; N, 6.99. Found: C, 59.86; H, 6.13; N, 6.81.

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis[(4-perhydroazepino)pyridinium] dibromide (60). Yield: 61%. Very hygroscopic to determine its mp. 1H NMR (400.13 MHz): δ 8.20 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-2_{pyr}), 7.32 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-2_{benz}), 7.25 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-3_{benz}), 7.04 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-3_{pyr}), 5.32 (s, 4H, CH_2N^+), 3.72 (t, 8H, H-2_{perhydroazep}), 2.64 (t, J = 7.7 Hz, 4H, CH_2 Ph), 1.90 (q, J = 7.7 Hz, 2H, C- CH_2 -C), 1.84 (m, 8H, H-3_{perhydroazep}), 1.58 (m, 8H, H-4_{perhydroazep}). ^{13}C NMR (100.13 MHz): δ 157.22 (C-4_{pyr}), 144.86 (C-1_{Ph}), 143.33 (C-2_{pyr}), 133.55 (C-4_{Ph}), 130.48 (C-3_{Ph}), 129.55 (C-2_{Ph}), 109.02 (C-3_{pyr}), 61.55 (CH_2N^+), 51.33 (C-2_{perhydroazep}), 35.99 (C-2_{Ph}), 34.05 (C- CH_2 -C), 27.35 (C-3_{perhydroazep}), 24.93 (C-4_{perhydroazep}). HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{35}H_{42}N_4Br$ (M-Br)⁺ 597.2593. Found m/z : 597.2593. HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{39}H_{50}N_4Br$ (M-Br)⁺ 653.3219. Found m/z : 653.3221. Anal. for $C_{39}H_{50}N_4Br_2 \cdot 4H_2O$: calcd C, 58.07; H, 7.25; N, 6.94. Found: C, 57.80; H, 7.03; N, 7.18.

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis[(4-diallylamino)pyridinium] dibromide (61). Yield: 67%. Very hygroscopic to determine its mp. 1H NMR (400.13 MHz): δ 8.26 (d, J = 7.9 Hz, 4H, H-2_{pyr}), 7.32 (d, J = 8.2 Hz, 4H, H-2_{benz}), 7.25 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-3_{benz}), 7.02 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-3_{pyr}), 5.89 (ddt, $J_{2,3'} = 17.2$, $J_{2,3} = 10.5$, $J_{1,2} = 4.9$ Hz, 4H, H-2_{allyl}), 5.35 (s, 4H, CH_2N^+), 5.27 (ddt, $J_{2,3} = 10.5$, $J_{3,3'} = 1.0$, $J_{1,3} = 1.0$ Hz, 4H, H-3_{allyl}), 5.21 (ddt, $J_{2,3'} = 17.2$, $J_{3,3'} = 1.0$, $J_{1,3'} = 1.0$ Hz, 4H, H-3'_{allyl}), 4.23 (dt, $J_{1,2} = 4.9$, $J_{1,3} = 1.8$, $J_{1,3'} = 1.8$ Hz, 4H, H-1_{allyl}), 2.64 (t, J = 7.7 Hz, 4H, CH_2 Ph), 1.90 (q, J = 7.7 Hz, 2H, C- CH_2 -C). ^{13}C NMR (100.13 MHz): δ 157.88 (C-4_{pyr}), 144.94 (C-1_{Ph}), 143.49 (C-2_{pyr}), 133.41 (C-4_{Ph}), 131.48 (C-2_{allyl}), 130.50 (C-3_{Ph}), 129.60 (C-2_{Ph}), 118.35 (C-3_{allyl}), 109.90 (C-3_{pyr}), 61.80 (CH_2N^+), 54.23 (C-1_{allyl}), 35.99 (CH_2 Ph), 34.05 (C- CH_2 -C). HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{39}H_{46}N_4Br$ (M-Br)⁺ 649.2906. Found m/z : 649.2905. Anal. for

$C_{39}H_{46}N_4Br_2 \cdot 4H_2O$: calcd C, 58.36; H, 6.78; N, 6.98. Found: C, 58.27; H, 6.40; N, 6.93.

1,1' - [Methylenebis(benzene-1,4-diylmethylene)]bis[(4-perhydroazepino)pyridinium] dibromide (62). Yield: 58%. Mp 168–170 °C. 1H NMR (300.13 MHz): δ 8.19 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-2_{pyr}), 7.32 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-2_{benz}), 7.26 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-3_{benz}), 7.04 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-3_{pyr}), 5.31 (s, 4H, CH_2N^+), 3.99 (s, 2H, CH_2 Ph), 3.71 (t, 8H, H-2_{perhydroazep}), 1.84 (m, 8H, H-3_{perhydroazep}), 1.59 (m, 8H, H-4_{perhydroazep}). ^{13}C NMR (75.78 MHz): δ 157.18 (C-4_{pyr}), 143.61 (C-1_{Ph}), 143.33 (C-2_{pyr}), 134.05 (C-4_{Ph}), 130.89 (C-3_{Ph}), 129.70 (C-2_{Ph}), 109.03 (C-3_{pyr}), 61.40 (CH_2N^+), 51.32 (C-2_{perhydroazep}), 41.98 (Ph- CH_2 -Ph), 27.34 (C-3_{perhydroazep}), 24.93 (C-4_{perhydroazep}). HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{37}H_{46}N_4Br$ (M-Br)⁺ 625.2906. Found m/z : 625.2904. Anal. for $C_{37}H_{46}N_4Br_2 \cdot 3.5H_2O$: calcd C, 57.74; H, 6.94; N, 7.28. Found: C, 57.58; H, 6.81; N, 7.32.

1,1' - [Propane - 1,3 - diylbis(benzene - 1,4 - diylmethylene)]-bis[(4-piperidino)pyridinium] dibromide (63). Yield: 58%. Mp 86–88 °C. 1H NMR (300.13 MHz): δ 8.19 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-2_{pyr}), 7.31 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-2_{benz}), 7.25 (d, $J_{2,3}$ = 8.2 Hz, 4H, H-3_{benz}), 7.12 (d, $J_{2,3}$ = 7.9 Hz, 4H, H-3_{pyr}), 5.30 (s, 4H, CH_2N^+), 3.70 (t, 8H, H-2_{perhydroazep}), 2.64 (t, J = 7.7 Hz, 4H, CH_2 Ph), 1.90 (q, J = 7.7 Hz, 2H, C- CH_2 -C), 1.75 (m, 12H, H-3_{perhydroazep} and H-4_{perhydroazep}). ^{13}C NMR (75.78 MHz): δ 156.84 (C-4_{pyr}), 144.82 (C-1_{Ph}), 143.49 (C-2_{pyr}), 133.61 (C-4_{Ph}), 130.47 (C-3_{Ph}), 129.50 (C-2_{Ph}), 109.26 (C-3_{pyr}), 61.41 (CH_2N^+), 49.08 (C-2_{perhydroazep}), 35.99 (CH_2 Ph), 34.15 (C- CH_2 -C), 26.66 (C-3_{perhydroazep}), 24.93 (C-4_{perhydroazep}). HR LSIMS (thioglycerol + Na^+), calcd m/z for $C_{37}H_{46}N_4Br$ (M-Br)⁺ 625.2906. Found m/z : 625.2907. Anal. for $C_{37}H_{46}N_4Br_2 \cdot 3H_2O$: calcd C, 58.42; H, 6.89; N, 7.37. Found: C, 58.40; H, 6.62; N, 7.26.

Computational methods

Statistical analysis was performed by partial least-squares algorithm using the QSAR module of SYBYL software.³⁵ The clog *P* values were calculated by using the Ghose-Crippen modified atomic contribution system²⁶ (ATOMIC5 option) contained in the PALLAS 2.0 package.²⁷ PALLAS is a package of powerful tools for the prediction of certain physicochemical parameters of organic compounds based solely on structural information. PROLOG is an optional component of the PALLAS system, which predicts the logarithm of the partition coefficient (log *P*) of organic compounds in an *n*-octanol/water system based on chemical structure.

Pharmacology

The ChoK inhibition and anti-proliferative assays against HT-29 cells were followed in accordance with the protocols previously reported.^{6,7} In the few cases for which the two means differed by more than 50%, a third experiment was performed to ascertain the value.

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