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Preliminary communication

Towards a model for the inhibition of choline kinase by a new type of inhibitor

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Abstract

Bispyridinium cyclophanes are novel templates for human choline kinase inhibitors. Molecular modelling of these compounds suggests three anchorage places at the binding site of the enzyme: (i) two anionic centres of the enzyme active site separated from each other at a distance of ≈6.2 Å that bind the two positively charged nitrogen atoms; (ii) a wide hydrophobic pocket that is fulfilled by the upper linker, the benzene ring that links the two amino groups; and (iii) a smaller hydrophobic pocket that can accommodate the lower benzene ring that links both benzylic carbons. This study may be useful for the development of more potent inhibitors of the enzyme.

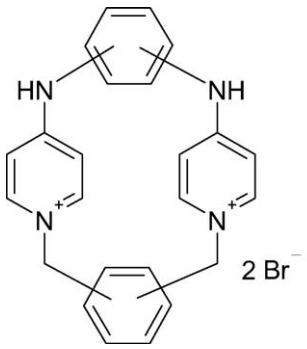
Keywords: Choline kinase; Bispyridinium cyclophanes; Molecular modelling; Binding sites description; Computer-assisted methods

1. Introduction

The discovery of oncogenes is an outstanding fact in cancer therapy [1]. *ras* encogen is one of the most amply studied ones due to its connection in the production of human tumours [2,3]. Upon activation, proteins encoded by *ras* oncogene give rise to the triggering of a series of biochemical pathways that entail an increase in the cellular proliferation [4–6]. Phosphorylcholine (*P*Cho) is one of the second messengers that is involved in the transmission of the mitogenic signal to the nucleus [7]. Very elevated levels of *P*Cho and of the enzyme responsible for its synthesis, choline kinase (ChoK), have been detected when compared with the existing amount in the equivalent non-tumoural cells [8]. Although the mechanism by which *P*Cho carries the mitogenic signal towards the nucleus is still unknown, these results suggest an important role of *P*Cho and ChoK in the production of human tumours, and consequently, that the inhibition of ChoK has therapeutic properties in cells transformed by the oncogene *ras* [9].

Our research group has tackled a rigorous process of molecular variation and design taking as a model the first known ChoK inhibitor: hemicholinium-3 (HC-3) [10,11]. In this programme a high number of symmetrical bispyridinium compounds have been designed, synthesised, characterised and biologically assessed [12–16].

We have recently published the synthesis and biological activity [17] of a set of compounds with the structure of bispyridinium cyclophanes (Fig. 1), which are novel tem-



Compound	Isomer
1	*p,p*
2	*m,m*
3	*p,m*
4	*m,p*

Fig. 1. Structures of cyclophanes **1**–**4**. The first prefix takes into account the upper linker that connects the amino groups, whereas the second one is related to the substitution pattern of the lower benzene ring that links the N^+ atoms.

Abbreviations: ChoK, choline kinase; *P*cho, phosphorylcholine.

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plates for human ChoK inhibitors. One of these cyclophanes, compound **4**, has an $(IC_{50})_{ChoK}$ of 0.3 μM and is the most potent human ChoK inhibitor described to date. The blocking potency of the bispyridium cyclophanes seems to be critically dependent upon the disubstitution model of the upper and lower benzene rings. In this paper, we describe the conformational behaviour of these cyclophane derivatives and initiate the construction of a model for the interaction of these molecules with ChoK.

2. Results and discussion

2.1. Conformational behaviour of cyclophanes

Compound **1** has shown the simplest and very interesting conformational behaviour of all of them, and has been previously described [18]. To summarise, four different conformations that are isoenergetic two by two were found for this molecule forming a conformational equilibrium. Transition states for the interconversion between them have been characterised and related to its spectroscopical properties. Fig. 2 shows the two different conformations for such a compound.

In both conformations the benzene rings are located in a practically parallel and fixed disposition, the main difference between them being the relative orientation of the two pyridine rings. In the most stable conformer (I) the pyridines are located in planes that form an angle of approximately 120°, while in the other conformer (II) both rings are parallel to each other. Both Amber and ab initio calculations agree in the values of the relative energies of both conformers and identify the first one as the most stable conformation for this molecule, unless the energy difference between both is small.

Compound **2** shows the existence of a more complex conformational equilibrium with 10 different conformations, from which six have different energies. Fig. 3 represents the four most stable conformers of these molecules that also show the highest degree of symmetry.

In all these conformations the pyridinium rings are located parallel to each other, whereas the benzene rings occupy more variable positions in the molecule, especially the upper ring that links the two amino groups. In the three first conformers (I–III), both amine NH bonds are parallel to each other whereas in the fourth one (IV), NH bonds are orientated

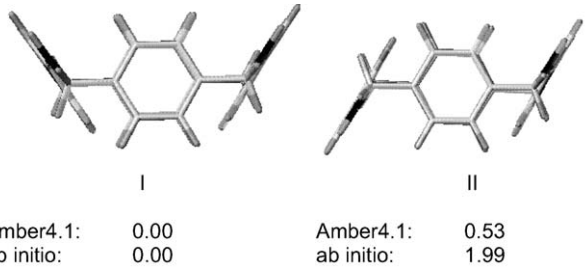

Fig. 2. Representation of the two energetically different conformations of compound **1** showing the disposition of the two pyridinium rings. Relative energies are given in kcal mol^{-1}.

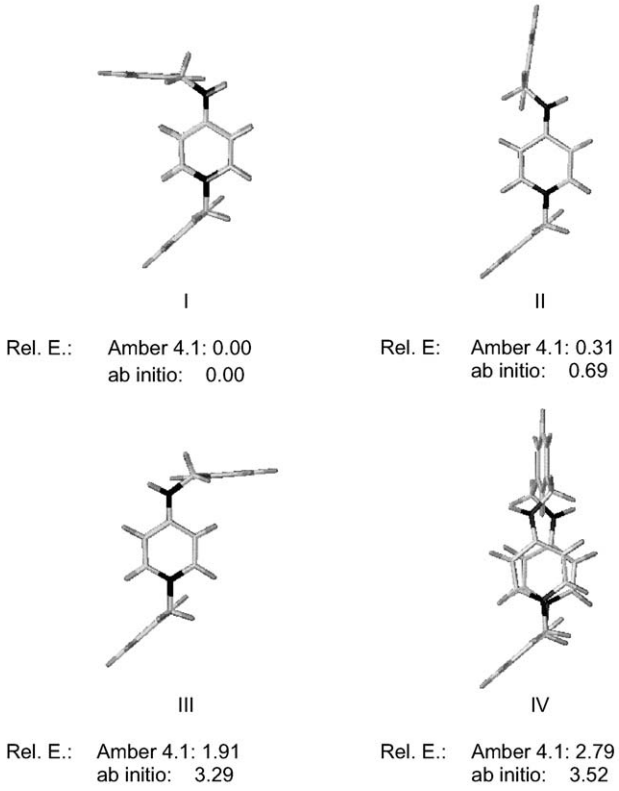

Fig. 3. Representation of the four different conformations for compound **2**, showing the parallel disposition of both pyridinium rings in all conformer. The high mobility of the upper benzene ring. Relative energies are given in kcal mol^{-1}.

in opposite directions. The other conformations found for compound **2** have smaller symmetry degrees, showing non-parallel pyridinium rings. Such conformations are intermediate conformers in the pseudorotation between the more stable conformers, and since their relative energies are elevated (more than 4 kcal mol^{-1}), their conformational populations are small. In fact, calculation of the conformational population using a Boltzman distribution indicates that the two most stable conformers account for more than 85% of the conformational population of this molecule. The main difference between all conformers of compound **2** is the position of the upper benzene ring that shows a high mobility and can adopt several different dispositions.

Four conformers are identified for **3**, although only three of them are of different energy (Fig. 4). The energy order of the two most stable conformations (I and II) depends on the method used in the calculation. Nevertheless, since the new parameters we have developed for Amber are not extensively checked and the ab initio calculation is more accurate, we accept Gaussian results as being more reliable than those achieved by Amber. In any case, the energy differences between conformers are not very high and both of them are very suitable as a possible active conformation. The fourth conformation found for this molecule is symmetrical and isoenergetic to conformation II, and both conformations can be considered as intermediate conformers in the pseudorotation from I to III. Considering the ab initio values for the

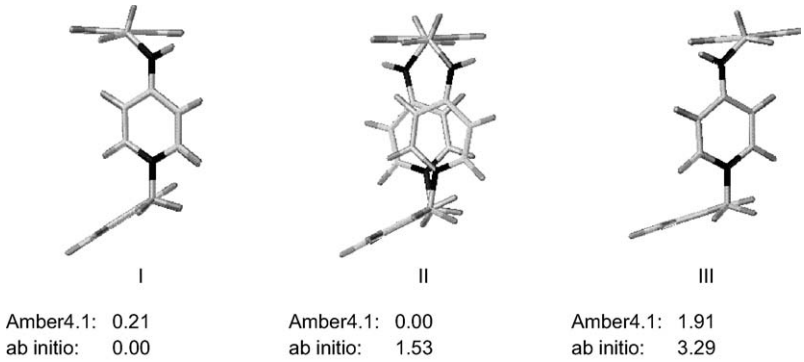

Fig. 4. Representation of the energetically different conformations of compound **3**. Pyridinium rings adopt a non-parallel disposition in this compound while the benzene rings can adopt spatial orientations that are equivalent to those compound **1** (upper ring) and **2** (lower ring). Relative energies are given in kcal mol^{-1}.

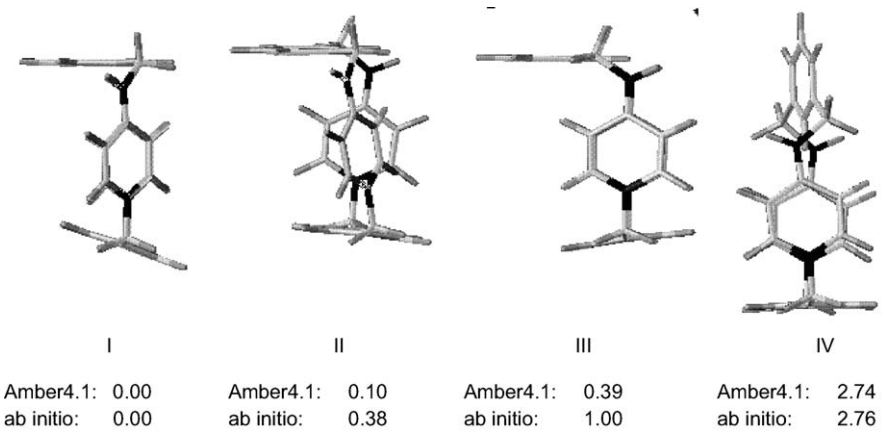

Fig. 5. The four energetically different conformation of compound **4**. Upper benzene rings adopt spatial dispositions equivalent to those of compound **1** while the lower one is equivalent to those of compound **2**. Relative energies are given in kcal mol^{-1}.

relative energy, the Boltzman distribution of the conformation of this molecule indicates that conformation I is the most populated one, representing 93% of the total conformational population. In this molecule, the upper benzene ring also shows higher mobility than the lower one, but it is smaller than that corresponding to compound **2**.

Compound **4** presents the more complex conformational behaviour of all the above-mentioned molecules. The total number of conformations found has been 14, from which only four are energetically different (Fig. 5). The three most stable conformations (I–III) of this compound have very similar energy. Conformations I and III show the NH bond parallel to each other while in conformations II and IV they are orientated in the opposite direction. Conformation II is an intermediate conformer in the pseudorotation from I to III while conformation IV is an intermediate in the transformation between III and its symmetrical conformation (not shown). Pyridinium rings are parallel to each other in conformation I, III and IV, while the benzene rings show a higher mobility, specially the upper benzene ring that occupies several spatial arrangements equivalent to those adopted by the same ring in compound **2**.

2.2. Structure–activity relationships

Table 1 shows the biological activities of compounds **1**–**4** as Chok inhibitors. It can be seen that the value of IC_{50} is structure-dependent, all compounds being good inhibitors of this enzyme. In fact, compound **4** is the most potent inhibitor described to date.

Table 1
ChoK inhibitory activity and distance (Å) between the N^+ atoms of the most stable conformation of cyclophanes

Compound	$(IC_{50})_{ChoK}$ (μM) [a]	Distance N^+–N^+ (Å)
4	0.3	6.21
1	2.1	6.41
2	13.2	5.14
3	24.8	5.25

[a] Taken from Ref. [17].

Our research group has described several ChoK inhibitors [12–16] that are characterised by the presence of two pyridinium moieties joined by a linker of variable length. Our experience indicates that the presence of both positively charged nitrogen atoms is crucial for the inhibition of ChoK, and for this reason we considered that the distance between these atoms must condition the interaction enzyme–inhibitor and consequently the biological activity.

Among all synthesised compounds, cyclophanes **1**–**4** are the most rigid derivatives and compound **4** is also the most potent reported to date. Hence, these molecules could serve as templates for the definition of a model of the interaction with ChoK since all of them show distances between N^+ atoms that are practically constant. The distance between

both atoms in the most stable conformation of each compound has been measured in order to compare it with the biological activity.

Table 1 also shows the distance (Å) between the N^+ atoms for the most stable conformation of cyclophanes **1**–**4**. It can be observed that in the most active compound, the distance between the N^+ atoms is 6.21 Å and that any deviation from this value involves a decrease in the ChoK inhibition potency. If the enzyme inhibition depended only on this structural factor, we could state that with more deviation, less inhibition is reached. The order of activity should have been the following: **4** (6.21 Å) > **1** (6.41 Å) > **3** (5.25 Å) > **2** (5.14 Å). Nevertheless, the order of ChoK inhibition of compounds **2** and **3** seems to be interchanged contrary to what could be expected on the basis of the above reasoning. Moreover, the differences in the distances between the quaternary nitrogen atoms are not so large as to explain such differences in their inhibitory potencies. Consequently, other factors must control the biological activity of these compounds.

Since cyclophanes are very rigid, their conformational flexibilities are very low and the differences observed in the activity must be due to the global shape of the molecule more than to any other factor. Hence, a comparison between the four compounds can explain the experimental inhibition activity.

(a) The quaternary nitrogen atoms of compounds **4** and **1** can be superimposed very tightly and nearly coincide in space. Both compounds show a similar substitution pattern in the lower benzene ring (para) and hence this moiety is completely equivalent in both molecules. The pattern substitutions for the upper ring are different and this feature defines the main difference between both molecules: compound **1** (para substitution) is more rigid than compound **4** (meta substitution) and the upper benzene ring in compound **4** can occupy more variable dispositions. Nevertheless, since **4** is the most potent compound, its higher flexibility is not detrimental to the interaction with ChoK. It can also be affirmed that the higher rigidity of compound **1** does not increase the binding to the enzyme.

(b) Compounds **4** and **2** coincide in the type of substitution (meta) in the upper benzene ring and both compounds show a similar shape in this part of the molecule. The upper benzene rings of compounds **4** and **1** can be superimposed almost perfectly in all conformations of these molecules. On the other hand, the substitution in the lower benzene is different and the quaternary nitrogen atoms occupy more differentiated positions than in the previous couple of compounds. In compound **2**, meta substitution in the lower benzene draws both nitrogen atoms near to each other (5.14 Å) and forces this benzene to be located further outwards than in compound **4**.

(c) Comparing compounds **4** and **3**, it can be seen that the pattern substitution in the upper and in the lower benzene rings is different. Hence, neither the quaternary nitrogen atoms nor the benzene rings coincide.

All these features allow us to rationalise the behaviour of these molecules in the interaction with Chok, and propose a model for the interaction with three anchorage zones (Fig. 6, green zone). One of these would be a wide hydrophobic pocket to allocate the upper benzene ring and allow compounds **4** and **2** to be completely fulfilled since only these compounds possess the appropriate geometry and flexibility in this zone of the molecule.

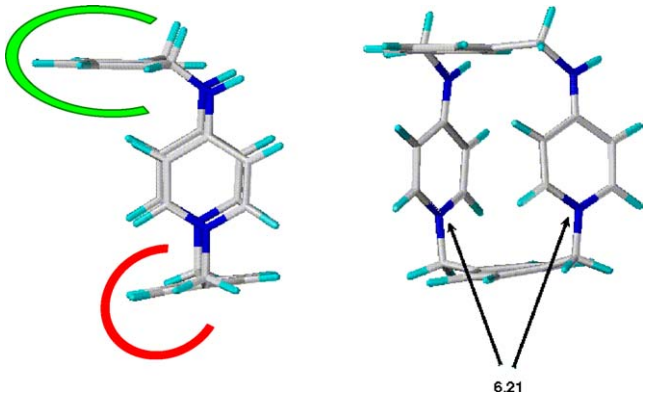

Fig. 6. Compound **4** showing a putative binding model for its interaction with ChoK. The optimal distance between the two quaternary nitrogen atoms is about 6.21 Å. The green zone indicates a wide hydrophobic region that must be filled by the upper benzene ring, whereas the red zone indicates a region of very low steric tolerance, in which an increase in the size of the substituents would provoke a decrease in the enzyme affinity.

The second anchorage point (probably two negatively charged acidic amino acids in the active site of the enzyme) would serve to interact strongly with the two quaternary nitrogen atoms and would be located at a distance of about 6.2 Å (Fig. 6, red zone). Compounds **4** and **1** are those that interact more strongly with this point since their distance between N^+ atom is very appropriate.

Finally, a small steric tolerance must exist in the interaction site in which the lower benzene ring (that links the two benzilic carbon) is located, and this gives rise to a lesser affinity for the enzyme in compounds **2** and **3**.

In short, compound **4** shows the best characteristics for the interaction, that is, N^+ atom distance, geometry and flexibility in the upper benzene and appropriate geometry in the lower one. Compound **1** shows an appropriate N^+ distance and also the substitution in the lower benzene but not an appropriate geometry in the upper benzene, the second being a more potent compound. Compound **2** shows a poor N^+ distance but the meta substitution in the upper benzene gives place to a better interaction with the enzyme than compound **3** and is consequently the third compound in activity. Finally, compound **3** does not possess any of these conditions and will therefore give the worst interactions and the least activity.

3. Conclusions

A model for the interaction of cyclophane with ChoK has been developed, indicating the importance of the three anchorage points: (i) Two negatively charged anionic aminoacids that bind both the cationic centres of cyclophanes, situ-

ated at about 6.2 Å. This interaction seems to be the most important one and governs the whole behaviour of these molecules. (ii) A wide hydrophobic pocket that is fulfilled by the benzene ring that links both amine groups only when the pattern substitution of this ring is meta. This interaction is the second in importance and conditions the strength of the binding. (iii) A second hydrophobic pocket allocates the benzene ring that links both benzylic carbons shows a smaller steric tolerance, since only the para substitution in this benzene gives rise to a strong binding to the enzyme.

4. Experimental

4.1. Computational methodology

Conformational analysis of cyclophanes has been performed using the Sybyl 6.9 [19] modelling package, running on an O2 Silicon Graphics workstation. Initial geometries were constructed from standard fragment libraries of Sybyl and we need to define a new atom type for the N^+ aromatic atom (N.ar4). Amber 4.1 forcefield [20] as implemented in the Sybyl programme has been used for the initial optimisation of the molecules, using N* as the atom type for the pyridine nitrogen, corresponding to a nitrogen with sp^2 hybridisation. A set of new parameters needed for the energy calculation was developed by ab initio calculations using model compounds and has been previously described [18].

Electrostatic interactions were calculated using partial atomic charges that were calculated by means of the AM1 [21] Hamiltonian implemented in the MOPAC 6.0 [22] programme and a distance-dependent dielectric constant with a value of $\epsilon = 1$. The Stewart [23] method was used for the initial minimisation of the starting structures until the energy gradient dropped below a value of 0.01 kcal mol^{-1} $Å^{-2}$.

Conformational search was performed by means of molecular dynamics, using 500 cycles of a simulated annealing simulation by heating the molecule up to 1000 K for 1000 ps, later cooling it down exponentially to 200 K, maintaining it for another 1000 ps, and keeping the final geometry of each cycle. These 500 conformations were optimised again under the same conditions described above and were compared with each other to identify those conformations that were energetically and geometrically different.

Finally, all unique conformations identified for each compound were optimised using ab initio (3–21 G) calculations performed by means of the Gaussian98 [24] programme and used in the subsequent study.

4.2. Synthesis and pharmacology

Synthesis and ChoK inhibition activities of all described cyclophanes have been described previously [17].

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