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Original article

Design, synthesis, theoretical calculations and biological evaluation of new non-symmetrical choline kinase inhibitors

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ABSTRACT

Inhibition of Choline Kinase (ChoK) has been reported as a therapeutical target in the treatment of some kinds of tumor. In this paper, the design and synthesis of new non-symmetrical monocationic ChoK inhibitors is described, bearing a cationic head and an adenine moiety connected by linkers of different lengths. Docking studies indicate that the cationic head of these compounds could be inserted into the choline binding site of the enzyme, while the adenine moiety could be stabilized into the ATP binding site. Docking studies also support the difference of activity of the synthesized compounds, which depends on both the substituent at position 4 of the cationic head and the linker length, being dimethylamine and 1,4-diphenylbutane respectively, the most appropriate ones. Compounds **14** ($IC_{50} = 10.70 \pm 0.40$ μM) and **17** ($IC_{50} = 6.21 \pm 0.97$ μM) are the most potent ChoK inhibitors and suitable for further modification with a view to obtain more potent antitumor compounds.

1. Introduction

Choline (Cho) is phosphorylated by choline kinase (ChoK) to generate phosphocholine (PCho), which represents the first step in the biosynthesis of a membrane phospholipid, phosphatidylcholine [1]. During the tumor formation the phospholipid metabolism is altered, which consequently leads to an increase in the PCho levels [2,3] associated with the overexpression of ChoK. Platelet-derived, epidermal, insulin dependent and vascular endothelial growth factors enhance the ChoK activity during the tumor production [4]. In addition, several oncogenes such as *ras* or *rhoa* increase ChoKα activity resulting in higher intracellular levels of PCho [5,6]. Recently it has been observed that hypoxia can induce ChoK expression in cancer cells [7]. Thus, an activation of ChoK and the resulting increase in PCho levels have been proposed as necessary events for the proliferation of certain cell types [8].

In mammalian cells, the three known isoforms of ChoK (ChoKα1, ChoKα2, and ChoKβ) are encoded by two genes: *chokα* and *chokβ*. None of the isoforms are active as monomers and the active enzyme consists of homo- or heterodimers [1]. Recent studies on the biological function of ChoK isozymes revealed that ChoKα may play a more prominent role in cancer development as compared to ChoKβ, as only ChoKα was upregulated in breast cancer cell lines [9] and specific depletion of the ChoKα isoform by shRNA selectively induced apoptosis in several tumor-derived cell lines without affecting the viability of normal primary cells [10]. The ChoKα isoform has also been proposed as a new prognostic marker for predicting the clinical outcome in patients with non-small-cell lung cancer [11]. These observations have resulted in the development of an antitumoral strategy focused on ChoK inhibition.

The synthesis of several derivatives was based on structural modifications of Cho uptake inhibitor hemicholinium-3 (**HC-3**, Fig. 1). We have previously reported a series of compounds namely bis-pyridinium (**1**–**3**) [12], bis-quinolinium (**4**) [13], tris-pyridinium (**5**) [14], cyclophanes (**6**) [15,16] and bicyclophanes (**7**) [17] as ChoK inhibitors. All these compounds show a symmetrical structure, bearing two or three cationic heads connected through a linker.

Our research group prepared a homology model of the human choline kinase isoform ChoKα [18] based on the 3D structure of *Caenorhabditis elegans* choline kinase (CKA2 PDB id: 1NW1) [19]. In

Abbreviations: Cho, choline; ChoK, choline kinase; PCho, phosphocholine; HC-3, hemicholinium-3; PHC-3, phosphohemicholinium-3; CKA2, Caenorhabditis elegans choline kinase; SAR, structure–activity relationship; HepG2, human hepatocellular carcinoma; FBS, fetal bovine serum; MEM, minimal essential medium; MD, molecular dynamic; SFXC, surflex-dock geomx; LDH, lactate dehydrogenase.

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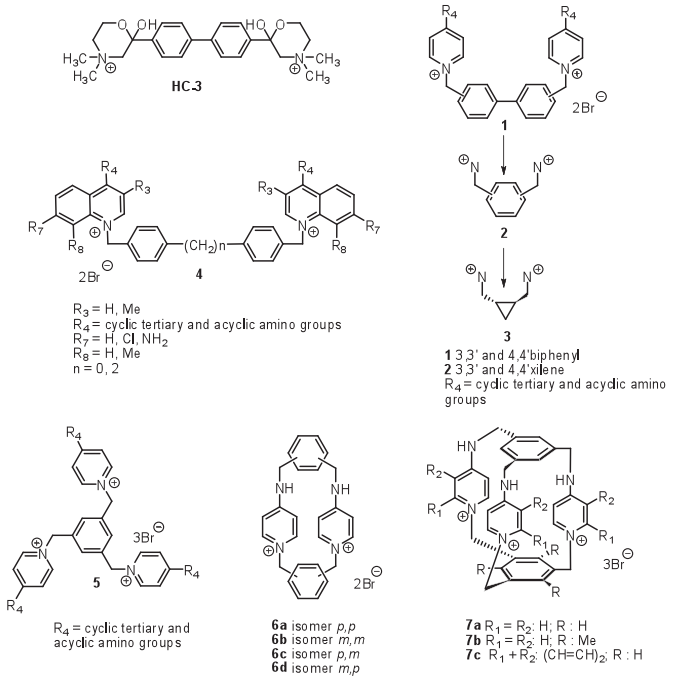


Fig. 1. Chemical structures of bis- and tris-quaternary ammonium salts **1**–**7**.

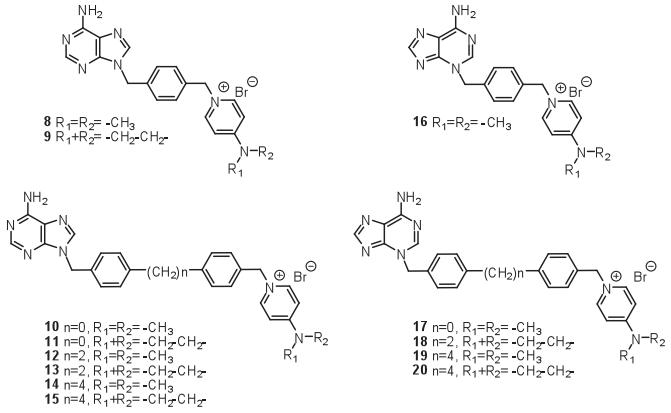


Fig. 2. Chemical structures of mono-quaternary pyridinium salts **8**–**20**.

that paper, preliminary docking studies of some of our previously synthesized ChoK inhibitors were also included. It was observed that the size of symmetrical biscationic inhibitors is appropriated to bind simultaneously in both the ATP and choline putative binding sites of the protein model (Fig. S1, Supplementary data).

Simultaneously to the publication of our ChoKα homology model, the crystal structure of human ChoKα2 isoform was published [20]. Three crystal structures corresponding to the *apo* form (PDB id: 2CKO) and two complexes with the products of the enzymatic reaction, ADP (PDB id: 2CKP) and PCho (PDB id: 2CKQ) were described in this paper. These structures allow the correct identification of both the ATP and Cho binding pockets (Fig. S2).

The preliminary docking studies performed on the homology model, and the fact that the nature of the residues that constitute the ATP binding site is quite different to those residues that form the Cho binding site, suggested the idea of preparing non-symmetric monocationic inhibitors that could bind in both binding sites. Such inhibitors should conserve one cationic head that could be inserted into the Cho binding site, but the second cationic head will be substituted by another fragment that could mimic the ATP adenine moiety.

In this paper, the first family of these new inhibitors is presented (Fig. 2). In these compounds, adenine itself was used to mimic the ATP adenine moiety, and 4-dimethylamino- or 4-pyrrolidino-pyridinium salts were used as cationic head. Both fragments are separated by several linkers that have been chosen to study the influence of the linker size in the inhibition of ChoK.

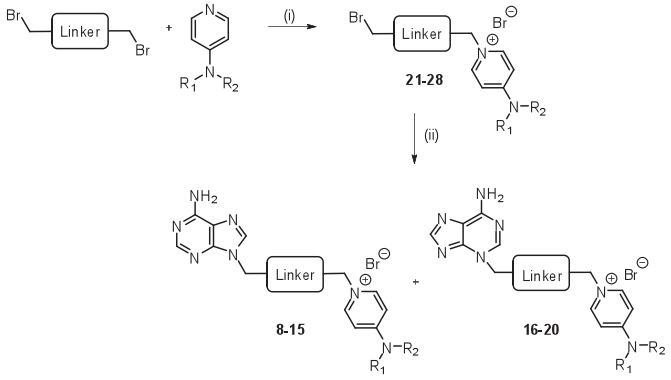
2. Results

2.1. Chemistry

Four different types of linkers have been used, benzene, biphenyl, 1,2-diphenylethane and 1,4-diphenylbutane, that are bisbromomethylated in the adequate position in order to insert both the adenine and the cationic head. 1,4-Bis(bromomethyl) benzene is commercially available. Reaction between biphenyl or 1,2-diphenylethane with formaldehyde and hydrogen bromide in the presence of H_3PO_4 yields 4,4′-bis(bromomethyl)biphenyl [21] and 4,4′-bis(bromomethyl)bibenzyl [22], respectively. Finally, 1,4-Bis[4-(bromomethyl)phenyl]butane was prepared by reduction of *trans,trans*-1,4-diphenyl-1,3-butadiene with Pd/C in glacial acetic acid, followed by the bisbromomethylation in the previous mentioned conditions [22].

The synthesis of the target molecules **8**–**20** has been carried out as described in Scheme 1. Reaction between the appropriate bisbromomethylated linker and the adequate pyridine derivative in butanone, at room temperature during 3 h, yields the intermediates **21**–**28**. ^{1}H NMR of the crude reaction showed the presence of the bisquaternisation structures as side products which have to be separated from the monocationic intermediates by filtration and thorough washing with butanone, ethyl acetate and diethyl ether or flash column chromatography on silica gel using CH_2Cl_2/MeOH (10/0.5) as eluent (see experimental protocols for details).

The reaction between the intermediates **21**–**28** and the purine moiety was carried out in DMF using K_2CO_3 as a base. The reaction mixture was microwave-irradiated at a temperature of 130 °C for 30 min. The preferential N-9 substitution on adenine was accompanied by formation of the corresponding N-3 isomers. In general the formation ratio of the N-9:N-3 isomers is 1.5:1, except for **15** in which the ratio has shown to be reversed (1:1.5). When the total yield of the N-3 isomer is smaller than 10% it was no possible to isolate these structures (N-3 isomers of **9**, **11** and **12**).



Scheme 1. Synthesis of the N-9 and N-3 mono-quaternary pyridinium salts **8**–**20**. Reagents and conditions: i) Butanone, rt, 3 h; ii) Adenine, K_2CO_3, DMF, 130 °C, MW, 30 min.