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Amino and carboxy functionalized modified nucleosides: A potential class of inhibitors for angiogenin

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ABSTRACT

The 3′-amino and carboxy functionalize thymidines execute their ribonucleolytic inhibition activity for angiogenin. These modified nucleosidic molecules inhibit the ribonucleolytic activity of angiogenin in a competitive manner like the other conventional nucleotidic inhibitors, which have been confirmed from kinetic experiments. The improved inhibition constant (K_i) values 427 ± 7, 775 ± 6 μM clearly indicate modified nucleosides are an obvious option for the designing of inhibitors of angiogenesis process. The chorioallantoic membrane (CAM) assay qualitatively suggests that amino functionalized nucleosides have an effective potency to inhibited angiogenin-induced angiogenesis. Docking studies further demonstrate the interaction of their polar amino group with the P_1 site residues of angiogenin, i.e., His-13 and His-114 residues.

1. Introduction

Angiogenin is a member of ribonuclease superfamily protein [1] and is a potent inducer of angiogenesis [2] (growth of new blood vessel). The angiogenesis process is found to operate at an alarming rate during the proliferation of tumor cells for the supply of nutrients necessary for their growth to solid tumor [3]. Angiogenin and ribonuclease A (RNase A) being the members of same superfamily they share a structural homology regarding their ribonucleolytic site but the ribonucleolytic activity of angiogenin is about 10^5-10^6 times weaker than that of RNase A [4]. However, ribonucleolytic activity of angiogenin is crucial for its angiogenic activity [5]. Therefore the enzymatic site (ribonucleolytic site) of angiogenin is an important target from therapeutic point of view. Abolition of the ribonucleolytic activity by low molecular weight inhibitors would therefore tantamount to inhibit the angiogenic activity of angiogenin leading to the inhibition of the undesired neovascularization. The ribonucleolytic site of RNase A and angiogenin is comprised of multiple subsites which bind the phosphate, base, and sugar components of RNA [6–8]. The P_1 subsite where cleavage of the phosphodiester bond occurs is comprised by His-12, Lys-41 and His-119 for RNase A and His-13, Lys-40 and His-114 for angiogenin and it serves as the main catalytic site (Fig. 1). There are two other important B_1 and B_2 subsites which recognize the nucleobases of normal RNA. Among these subsites, B_1 site shows pyrimidine specificity whereas the B_2 site has an affinity for purine nucleobase [9].

Most of the nucleotide-based inhibitors of angiogenin are functionalized with phosphate or pyrophosphate group. These inhibitors inhibit the ribonucleolytic activity of angiogenin by virtue of their structural similarities with its normal substrate [7,10]. However, these conventional nucleotidic inhibitors have a limited success towards angiogenin compared to RNase A [11], as well as, the high negative charge on the phosphate groups is a great disadvantage regarding their cell permeability [12]. Because of the limited success of the nucleotidic inhibitors different non-nucleosidic molecules are tried on angiogenin [13] and also dinucleosides with amide linkage [14].

In literature it has been reported that for RNase A the pK_a values of His-12 and His-119 changes from ~5.22/6.78 to ~6.30/8.10 during its binding with the substrate [15]. Keeping this point in mind we employed a series of 3′-modified nucleosides functionalized with amino and carboxy groups for angiogenin inhibition study. At the physiological pH these polar groups are likely to remain in their conjugate form. Now if these molecules are recognized at the ribonucleolytic site of angiogenin because of the presence of nucleodase and ribose moieties then amino and carboxylic groups have a possibility to interact with the P_1 site residues. However, replacement of the phosphate or pyrophosphate group by one amino or carboxylic group lowers the net negative charge of these molecules compared to their nucleotide analogs.

In this particular report we have selected 2′-deoxynucleoside to protect the amide bond towards the hydrolysis so that they can deliver the free amino and carboxylic groups at the catalytic site.

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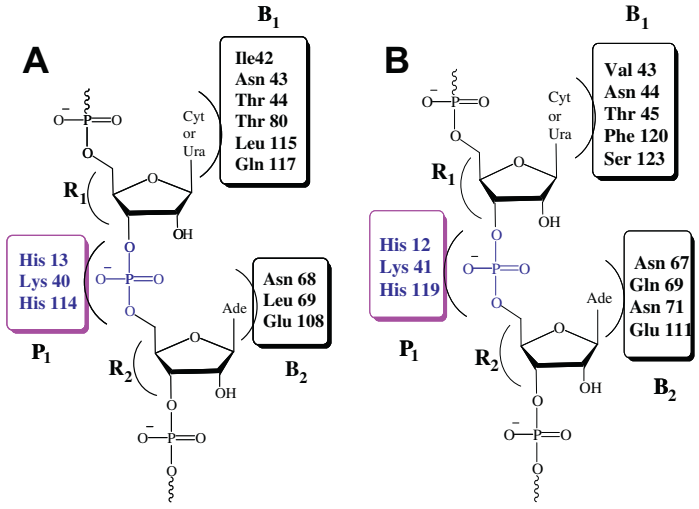

Fig. 1. Key residues of ribonucleolytic site of (A) Human angiogenin and (B) RNase A.

Otherwise in presence of the 2′-OH, the inhibitor molecules can undergo easy hydrolysis of its amide bond as found in case of angiogenin's normal substrate (RNA).

2. Results and discussion

Amino and carboxy functionalize modified nucleosides* showed their inhibitory activity on RNase A [16,17] and it encourages us to extend the inhibition study for angiogenin, a homologous protein of RNase A (structures of the inhibitor molecules of these series is given in Fig. 2). Therefore, the most potential compounds of those series were selected for this study. However, comparison of the inhibitory potential of these two series can also produce valuable information for the further modification of low molecular weight inhibitor of angiogenin.

The ribonucleolytic inhibitory activity of these compounds on angiogenin was quantitatively estimated by precipitation assay [18]. We observed the similar trend of inhibition in case of angiogenin as for RNase A for both class of compounds. Compound **2** and **5** from amino and carboxy functionalized nucleosides respectively showed the best inhibitory activity on angiogenin (22.00% and 23.42% respectively) (Fig. 3). It suggested both amino and carboxy functionalizations have an equal importance in angiogenin inhibition. The bar diagram obtained from this assay (Fig. 3) indicated that for amino functionalize nucleosides inhibitory activity depends upon the nature of the side chain part for respective amino acids. However, for the other set of compounds the chain length of

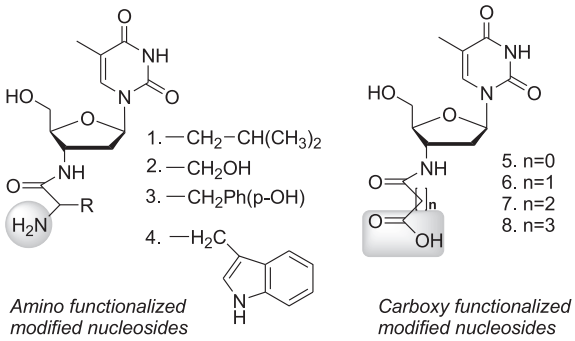

Fig. 2. Structures of amino and carboxy functionalize modified nucleosides. *Note:* Synthesis of these inhibitor molecules has been reported in Refs. [16,17].

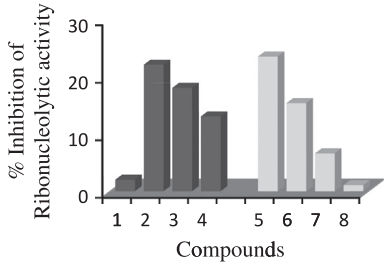

Fig. 3. Percentage inhibition of ribonucleolytic activity of angiogenin by compounds **1**, **2**, **3**, **4** (black) and **5**, **6**, **7**, **8** (gray).

the dibasic acid part played the determining role in inhibition process.

The mode of ribonucleolytic inhibition of angiogenin was further confirmed with the help of kinetic experiment where 6-FAM-dArUdAdA-6-TAMRA was used as the substrate of angiogenin protein [19]. This study was based on the fluorescence quenching of the fluorescein moiety attached to rhodamine by a phosphodiester bond. The enhanced fluorescence of fluorescein moiety was measured during the cleavage of facile phosphate bond by angiogenin. The inhibition constant value (K_i) for compounds **2**, **3**, **5** and **6** was found 427 ± 7, 1251 ± 4, 775 ± 6 and 1,114 ± 3 μM respectively and the nature of the Lineweaver-Burk plots confirmed their competitive mode of inhibition (Fig. 4). The K_i values of these four compounds are much better than 3′-CMP (12,703 ± 0.03 μM), a nucleotidic inhibitor of angiogenin (determined using the same substrate). However, the K_i values obtained from this experiment clearly show the preference of amino group over the carboxy one. The comparative K_i values of these inhibitors for RNase A and angiogenin are given in Table 1.

In view of the results obtained from inhibition of the ribonucleolytic activity of angiogenin based on the fluorimetric assay, it appeared pertinent to observe the effect of the amino functionalized modified nucleosides on the angiogenin induced angiogenesis. The chorioallantoic membrane (CAM) assay [1] was therefore conducted to verify the inhibition of angiogenin-induced angiogenesis by compounds **2** and **3**. As expected, vascularization was enhanced in presence of angiogenin (Fig. 5A). Vascular growth was comparable to the blank (Fig. 5B) for the cases when compounds mixed with angiogenin. It suggested that both **2** (Fig. 5C) and **3** (Fig. 5E) suppress the growth of blood vessels induced by angiogenin. This