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Synthesis and Preliminary Biological Evaluation of [^{3}H]-MRE 3008-F20: the First High Affinity Radioligand Antagonist for the Human A_3 Adenosine Receptors

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Abstract—The synthesis and the preliminary biological evaluation of the first high affinity radioligand antagonist for the human A_3 adenosine receptor, named [^{3}H]-MRE 3008-F20 are reported. [^{3}H]-MRE 3008-20 bound human A_3 receptors expressed in CHO cells with K_D and B_{max} value of 0.82 ± 0.08 nM and 297 ± 28 fmol/mg of protein, respectively. [^{3}H]-MRE 3008-F20 represents a useful tool for a further characterization of A_3 adenosine receptor subtype.

Introduction

Adenosine modulates several physiological functions by interacting with four different receptor subtypes named A_1, A_{2A}, A_{2B} and A_3.[1] While the adenosine A_1 and A_{2A} receptor subtypes have been pharmacologically characterized through the use of selective ligands,[2,3] the A_3 receptor subtype, cloned from different species, is still under investigation due to the recent discovery of highly selective ligands and the lack of labelled form of antagonists.

Activation of adenosine A_3 receptors has been shown to stimulate phospholipase C,[4] and D[5] and to inhibit adenylate cyclase.[2] Stimulation of A_3 adenosine receptors also causes the release of inflammatory mediators such as histamine from mast cells,[6,7] which could be responsible for inflammation[8] and hypotension.[6] In addition, it has been also suggested that the A_3 receptor could play an important role in brain ischemia,[9,10] immunosuppression[11] and bronchospasm in several animal models.[12] On these bases A_3 antagonists could be proposed as anti-inflammatory and anti-asthmatic agents.

In the last few years, several classes of A_3 antagonists have been reported.[13–18] Very recently, our group has reported a series of 5-[[(substituted-phenyl)amino]carbonyl]amino-8-alkyl-2-(2-furyl)-pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*] pyrimidine as highly potent and selective human A_3 adenosine receptor antagonists. In particular two compounds of this series, the 5-[[(4-methoxyphenyl)amino]carbonyl]amino-8-ethyl-2-(2-furyl)-pyrazolo[4,3-*e*] 1,2,4-triazolo[1,5-*c*] pyrimidine (**1**) and the 5-[[(4-methoxyphenyl)amino]carbonyl]amino-8-propyl-2-(2-furyl)-pyrazolo[4,3-*e*] 1,2,4-triazolo[1,5-*c*]pyrimidine (**2**) showed to be the most potent and selective human A_3 adenosine receptor antagonists at the present (Fig. 1).[19]

Starting from these experimental observations, we focused our attention on the synthesis of the first antagonists for the human A_3 receptors in labelled form in order to avoid the complication associated with the high and low agonist affinity states G-protein coupled receptors. Our hypothesis was based on the introduction of an unsaturated chain on N8 pyrazole nitrogen that after reduction with tritium gas could afford the desired radioligand. In this case, we choose the allyl moiety (**3**), which after reduction could afford the labelled form (**4**) of compound **2** (Scheme 1)

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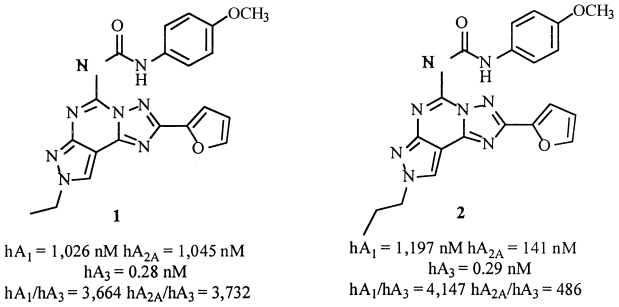
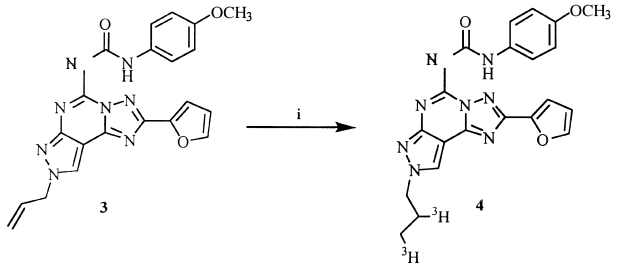


Figure 1. Structures and binding affinities of human A_3 adenosine receptor antagonists.



Scheme 1. Reagents: i: Tritium gas, 10% Pd-C, EtOAc:EtOH 2:1.

Chemistry

Desired compound **3** was prepared according to a well known procedure for the synthesis of the pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as described before.[19,20]

The final compound **3** was obtained in good yield by reduction with tritium gas in presence of Pd-C 10% and purification by HPLC (Column Ultrasphere ODS 250×9.4 mm; eluent A: water:triethylamine 500:0.5; eluent B methanol:triethylamine 500:0.5; gradient 20–100% B over 30 min; 113 mCi, by purification of 357 mCi of crude material) (Scheme 1).[21] The obtained radioligand **4** was analysed by HPLC and mass spectrometry, showing a purity of 99.4% with specific activity of 67 Ci/mmol.

Results and Discussion

Figure 2 shows a saturation curve of [^{3}H]-MRE 3008-F20 (**4**) to the human A_3 adenosine receptors expressed in CHO cells, and the linearity of the Scatchard plot in the inset is indicative, in our experimental conditions, of the presence of a single class of binding sites with K_D value of 0.82 ± 0.08 nM and B_{max} value of 297 ± 28 fmol/mg protein ($n = 4$).

In order to verify the selectivity of this derivative versus the other adenosine receptor subtypes, the unlabelled form (**2**) has been used in inhibition experiments. The receptor binding affinity of compound **2**, has been determined at human A_1, and A_{2A} receptors expressed in CHO cells, using [^{3}H]-1,3-dipropyl-8-cyclopentyl xanthine ([^{3}H]DPCPX),[22] [^{3}H]-5-amino-7-(2-phenyl-ethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4triazolo[1,5-*c*]-pyrimidine, ([^{3}H]-SCH 58261)[23] as radioligands, respectively. Compound **2** has low affinities at human A_1 (K_i 1197 ± 100 nM) and A_{2A} (K_i 141 ± 15 nM) adenosine receptors, respectively, and consequently high selectivity at human A_3 adenosine receptor. Based upon these findings, [^{3}H]-MRE 3008-F20 (**4**) could be considered the first high affinity radioligand antagonist for the human A_3 adenosine receptors.

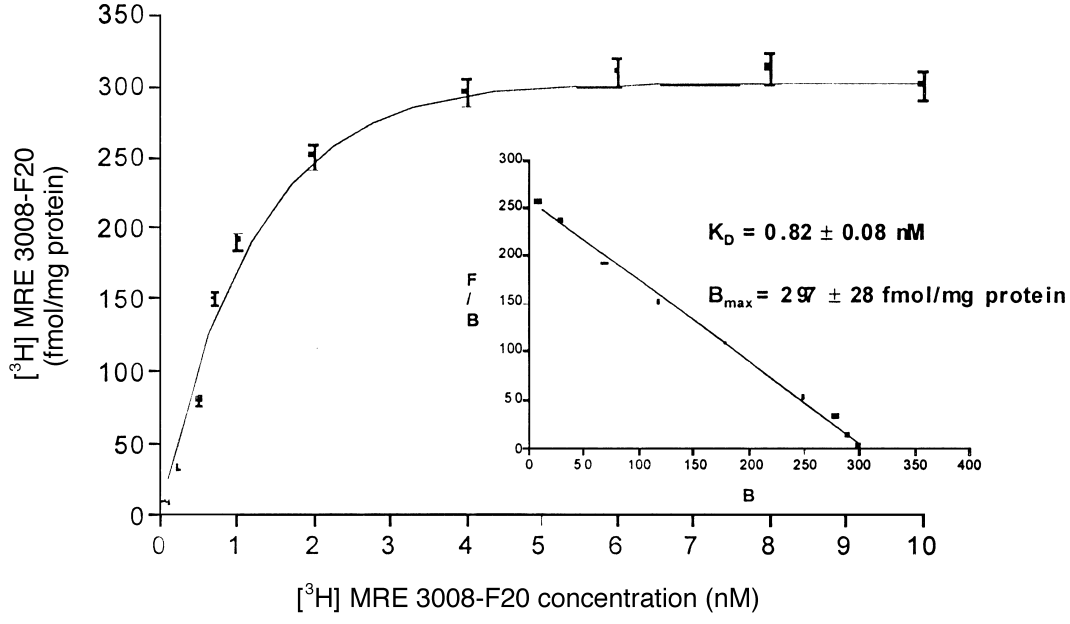


Figure 2. Saturation of [^{3}H]-MRE 3008-F20 (**4**) binding to human A_3 adenosine receptors. Values are the means and vertical lines s.e. mean of four separate experiments performed in triplicate. In the inset the Scatchard plot of the same data is shown. K_D value was 0.82 ± 0.08 nM and B_{max} value was 297 ± 28 fmol/mg protein.

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21. Characterization of compound **3**. Pale yellow solid, mp (EtOAc–light petroleum) 180 °C; IR (KBr) cm^{-1}, 3240–2955, 1660, 1645, 1625, 1605, 1520; ^1H NMR ($CDCl_3$) δ: 3.83 (s, 3H); 5.02 (d, 2H, $J=6$ Hz); 5.44 (dd, 2H, $J=6$ Hz, $J=9$ Hz); 6.08–6.16 (m, 1H); 6.63 (dd, 1H, $J=4$ Hz, $J=1$ Hz); 8.87 (d, 2H, $J=9$ Hz); 7.24 (d, 1H, $J=4$); 7.56 (d, 2H, $J=9$ Hz); 7.67 (d, 1H, $J=1$ Hz); 8.28 (s, 1H); 8.62 (bs, 1H); 10.94 (bs, 1H).
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