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Synthesis and pharmacological characterization of [^{125}I]MRS5127, a high affinity, selective agonist radioligand for the A_3 adenosine receptor

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ABSTRACT

A recently reported selective agonist of the human A_3 adenosine receptor (hA_3AR), MRS5127 (1′R,2′R,3′S,4′R,5′S)-4′-[2-chloro-6-(3-iodobenzylamino)-purine]-2′,3′-O-dihydroxy-bicyclo-[3.1.0]hexane, was radioiodinated and characterized pharmacologically. It contains a rigid bicyclic ring system in place of a 5′-truncated ribose moiety, and was selected for radiolabeling due to its nanomolar binding affinity at both human and rat A_3ARs. The radioiodination of the N^6-3-iodobenzyl substituent by iododestannylation of a 3-(trimethylstannyl)benzyl precursor was achieved in 73% yield, measured after purification by HPLC. [^{125}I]MRS5127 bound to the human A_3AR expressed in membranes of stably transfected HEK 293 cells. Specific binding was saturable, competitive, and followed a one-site binding model, with a K_d value of 5.74 ± 0.97 nM. At a concentration equivalent to its K_d, non-specific binding comprised 27 ± 2% of total binding. In kinetic studies, [^{125}I]MRS5127 rapidly associated with the hA_3AR ($t_{1/2}$ = 0.514 ± 0.014 min), and the affinity calculated from association and dissociation rate constants was 3.50 ± 1.46 nM. The pharmacological profile of ligands in competition experiments with [^{125}I]MRS5127 was consistent with the known structure-activity-relationship profile of the hA_3AR. [^{125}I]MRS5127 bound with similar high affinity (K_d, nM) to recombinant A_3ARs from mouse (4.90 ± 0.77), rabbit (2.53 ± 0.11), and dog (3.35 ± 0.54). For all of the species tested, MRS5127 exhibited A_3AR agonist activity based on negative coupling to cAMP production. Thus, [^{125}I]MRS5127 represents a new species-independent agonist radioligand for the A_3AR. The major advantage of [^{125}I]MRS5127 compared with previously used A_3AR radioligands is its high affinity, low degree of non-specific binding, and improved A_3AR selectivity.

1. Introduction

Modulation of the A_3 adenosine receptor (A_3AR) is being explored in preclinical and clinical studies for the treatment of a variety of diseases [1,2]. Selective agonists **1** and **2** (Fig. 1) are undergoing clinical trials for hepatocarcinoma, rheumatoid arthritis (phase IIB completed), psoriasis, and dry eye disease [3,4]. Other target diseases for selective A_3AR agonists and antagonists that might be the subject of future clinical trials are neurodegeneration [5,6], inflammatory bowel disease [7], other autoimmune inflammatory diseases [8], and cancer [9]. The level of expression of the A_3AR was found to be elevated in tumors,

Abbreviations: AR, adenosine receptor; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle's medium; IB-MECA, N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine; I-AB-MECA, N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine; MRE 3008F20, 5-*N*-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo [4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; MRS1191, 1,4-dihydro-2-methyl-6-phenyl-4-(phenylethynyl)-3,5-pyridinedicarboxylic acid, 3-ethyl-5-(phenylmethyl) ester; MRS1220, *N*-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-yl]benzeneacetamide; MRS1523, 5-propyl-2-ethyl-4-propyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate; MRS5127, (1′R,2′R,3′S,4′R,5′S)-4′-[2-chloro-6-(3-iodobenzylamino)-purine]-2′,3′-O-dihydroxybicyclo-[3.1.0]hexane; MRS1754, 8-[4-[[(4-cyano)phenylcarbamoylmethyl]oxy]phenyl]-1,3-di-(*n*-propyl)xanthine; NECA, 5′-N-ethylcarboxamidoadenosine; PSB-11, 8-ethyl-4-methyl-2-phenyl-(8R)-4,5,7,8-tetrahydro-1H-imidazo[2,1-i]-purin-5-one.

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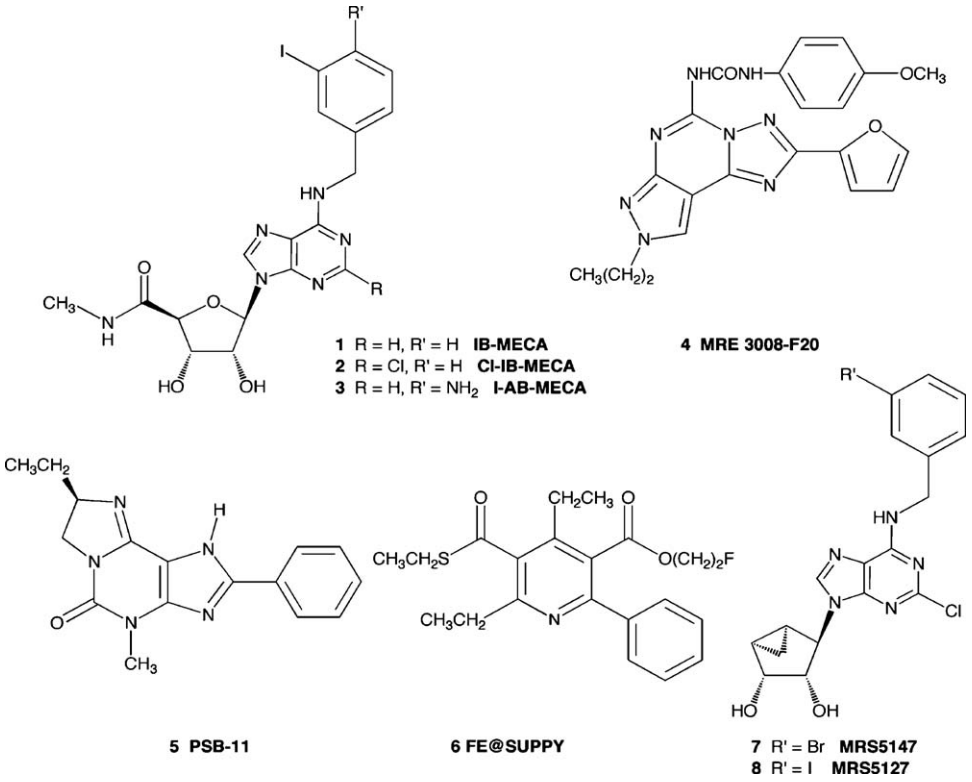

Fig. 1. Structures of nucleoside and non-nucleoside, high affinity ligands for the A_3AR. Compounds **3**–**6** have previously been prepared in radioactive form for use in receptor labeling studies.

neutrophils, and synoviocytes in the disease state [9–12]. The A_3AR expression level correlated to the responsiveness in arthritis patients to therapy with the A_3AR agonist IB-MECA **1** [4].

The most widely used radioligand for the study of the A_3AR is the high affinity agonist [^{125}I]I-AB-MECA **3** ($K_d \sim 1$ nM at human (h), mouse (m), and rat (r) A_3ARs) [13,14]. The disadvantage of this compound is its low selectivity for the A_3AR. Thus, it is useful for characterization of the A_3AR in cell lines overexpressing the receptor and in various cells expressing the A_3AR at high levels, such as eosinophils and neutrophils [15], but not in most native tissues. [^{3}H]HEMADO (2-hexyn-1-yl-N^6-methyladenosine), a tritiated radioligand of high affinity and selectivity was reported to be a useful radioligand for the hA_3AR and demonstrated to have low non-specific binding [16]. However, the greatly decreased affinity of adenosine agonists at the rat A_3AR in comparison to the human A_3AR has been noted consistently for adenosine analogues substituted at the 6 position with small alkyl moieties and at the 5′ and 2 positions with a range of structures [17–20]. Several antagonist radioligands have been used previously in *in vitro* studies, such as the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivative [^{3}H]MRE 3008F20 **4** and the 4,5,7,8-tetrahydro-1*H*-imidazo[2,1-i]purin-5-one derivative [^{3}H]PSB-11 **5** [21,22]. The disadvantage of these structurally diverse heterocyclic antagonists is their low affinity for the A_3AR in nonhuman tissue. For example, the affinity of MRE 3008F20 at the rat A_3AR is >10 μM [23]. Recently, a ^{18}F-labeled radioligand, the 6-phenylpyridine derivative **6**, suitable for PET (positron emission tomography) studies in both human and murine species was reported [24].

A new approach to designing ligands for the A_3AR that bind selectively to several species homologues of this receptor is based on 5′-truncated nucleoside derivatives. Recently, we have extended this truncation approach to selective A_3AR ligands containing the rigid (N)-methanocarba (bicyclo[3.1.0]hexane) ring system as a ribose substitute [25,26]. This bicyclic ring system maintains a conformation that is preferred at the A_3AR increasing selectivity, even in the absence of a 5′-*N*-methyluronamide group. Some members of this series were found to have reduced intrinsic activity for the A_3AR or to function as full antagonists [25,26]. One member of this series, the partial agonist MRS5147 **7**, was labeled with ^{76}Br for use as a PET ligand of high affinity [27]. [^{76}Br]MRS5147 bound to human and rat A_3ARs with K_i values of 0.62 and 5.2 nM, respectively. The corresponding 3-iodo derivative MRS5127 **8** also displays high affinity at both the h and r A_3ARs [25,26]. MRS5127 **8** was highly A_3AR-selective; its affinity at three human AR subtypes was determined: $hA_1 = 3040 \pm 610$ nM, $hA_{2A} = 1080 \pm 310$ nM, $hA_3 = 1.44 \pm 0.60$ nM. By Schild analysis of [^{35}S]GTPγS binding to membranes from CHO cells expressing the hA_3AR, MRS5127 appeared to be an antagonist [25]. However, further analysis determined that it is a partial agonist stimulating cAMP production in transfected cells with 45% efficacy compared to the full agonist NECA [26]. In this study, we have synthesized a radioiodinated form of this truncated rigid carbocyclic nucleoside derivative for *in vitro* studies and have characterized its binding properties at the A_3AR in several species.

2. Materials and methods

2.1. Chemical synthesis

2.1.1. Materials and instrumentation

Hexamethyltin and other reagents, including pharmacological agents, were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO), except where noted. MRS5127 **8** was prepared as reported [25]. Sodium [^{125}I]iodide (17.4 Ci/mg) in NaOH (1.0×10^{-5} M) was supplied by PerkinElmer Life and Analytical Science (Boston, MA). ^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer using $CDCl_3$ and CD_3OD as solvents. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane (δ 0.00) for $CDCl_3$ and water (δ 3.30) for CD_3OD. TLC analysis was carried out on aluminum sheets precoated with silica