ORIGINAL ARTICLE

Synthesis and pharmacological characterization of [125I] MRS1898, a high-affinity, selective radioligand for the rat A₃ adenosine receptor

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Abstract A known selective agonist of the A₃ adenosine receptors (AR), MRS1898 [(1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol], was synthesized in radioactive form and characterized pharmacologically. This agonist ligand series, based on nucleoside analogues containing a rigid, bicyclic ring system in place of the ribose moiety, was selected for radiolabeling due to its high A₃AR affinity across species, with nanomolar binding at both rat and human A₃ARs. The radioiodination of MRS1898 on its N^6–3-iodobenzyl substituent was accomplished in 76% radiochemical yield by iododestannylation of a 3-(trimethylstannyl)benzyl precursor. [125I] MRS1898 bound to the rat A₃AR with a K_d value of 0.17 ± 0.04 nM and a B_{max} value of 0.66 ± 0.15 pmol/mg protein. The competition binding profiles for other agonists and antagonists obtained with this radioligand are similar to those previously obtained with other radioligands. The advantages of [125I]MRS1898 compared with previously used radioligands are primarily its high selectivity and affinity for the rat A₃AR and also its facile synthesis and radiochemical stability; however, a relatively high level of nonspecific binding presents a limitation. Thus, we have introduced the first selective radioligand for the rat A₃AR.

Abbreviations

AR	adenosine receptor
CHO	Chinese hamster ovary
IB-MECA	N^6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine
I-AB-MECA	N^6-(4-amino-3-iodobenzyl)-5′-N-methylcarboxamidoadenosine
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
MRS1898	MRS1898, (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol
NECA	5′-N-ethylcarboxamidoadenosine
Cl-IB-MECA	2-chloro-N^6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine
MRE 3008F20	5-[[(4-methoxyphenyl)amino]carbonyl]amino-8-propyl-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine

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PSB-11   (R)-4-methyl-8-ethyl-2-phenyl-imidazo
                [2,1-i]purin-5-one
HEMADO   2-hexyn-1-yl-N^6-methyladenosine

Introduction

Adenosine regulates many physiological functions through specific cell membrane receptors. The A_3 adenosine receptor (A_3AR) plays an important role in brain ischemia, immunosuppression, and bronchospasm in several animal models [1]. A_3AR agonists, such as Cl-IB-MECA (2, Chart 1), are currently in clinical trials for various metastatic and inflammatory conditions [2–5]. The A_3AR is elevated in tumors, and its expression level correlates to tumor responsiveness to therapy, such as the A_3AR agonist N^6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine (IB-MECA 1) [6, 7].

A number of radioligands have been used for the study of the A_3AR [8–12]. Radioligands for the A_3AR may be promising diagnostic markers for cancer and possibly other diseases, whereas characterization and quantification of the A_3AR in vivo in patients may also be a useful tool in both research and therapeutic studies [13]. The nucleoside N^6-(4-amino-3-iodobenzyl)-5′-N-methylcarboxamidoadenosine

($[^{125}I]$I-AB-MECA 3) ($K_d \sim 1$ nM at human (h) and rat (r) A_3AR) was introduced in 1994 as a radioligand for A_3AR and is the most commonly used radioligand for in vitro studies of that subtype [8]. Several other agonist radioligands have been used in previous studies. All of the A_3AR radioligands so far in use show major deficiencies. For example, the antagonist heterocyclic radioligands, $[^3H]$ MRE 3008F20 4 and $[^3H]$PSB-11 5, have been used for the study of the hA_3AR; however, they do not bind effectively to the rA_3AR or mouse A_3AR [9, 10]. A_3AR agonists are often more consistent than nonnucleoside antagonists in their high affinity at that subtype, independent of species. Nevertheless, most of the agonist radioligands for characterizing the A_3AR are not truly A_3AR selective. Although the agonist N^6-(4-amino-3-iodophenylethyl)-adenosine ($[^{125}I]$I-APNEA) showed reasonable affinity for the rA_3AR ($K_d = 15$ nM), it is even more potent for the rA_1AR ($K_d = 1.32$ nM) [8,21]; $[^{125}I]$I-AB-MECA 3 has a similar affinity for rA_1 and rA_3ARs ($A_3 = 1.48$ nM; $A_1 = 3.42$ nM). 5′-N-ethylcarboxamidoadenosine ($[^3H]$NECA), showed a reasonable binding affinity at the hA_3AR ($K_d = 6$ nM) but is nonselective and weak at the rA_3AR [11]. Recently, a new A_3AR agonist radioligand, $[^3H]$HEMADO 6, which contains an N^6-methyl group, showed high affinity ($K_d = 1.1$ nM at the hA_3AR), selectivity, and low nonspecific
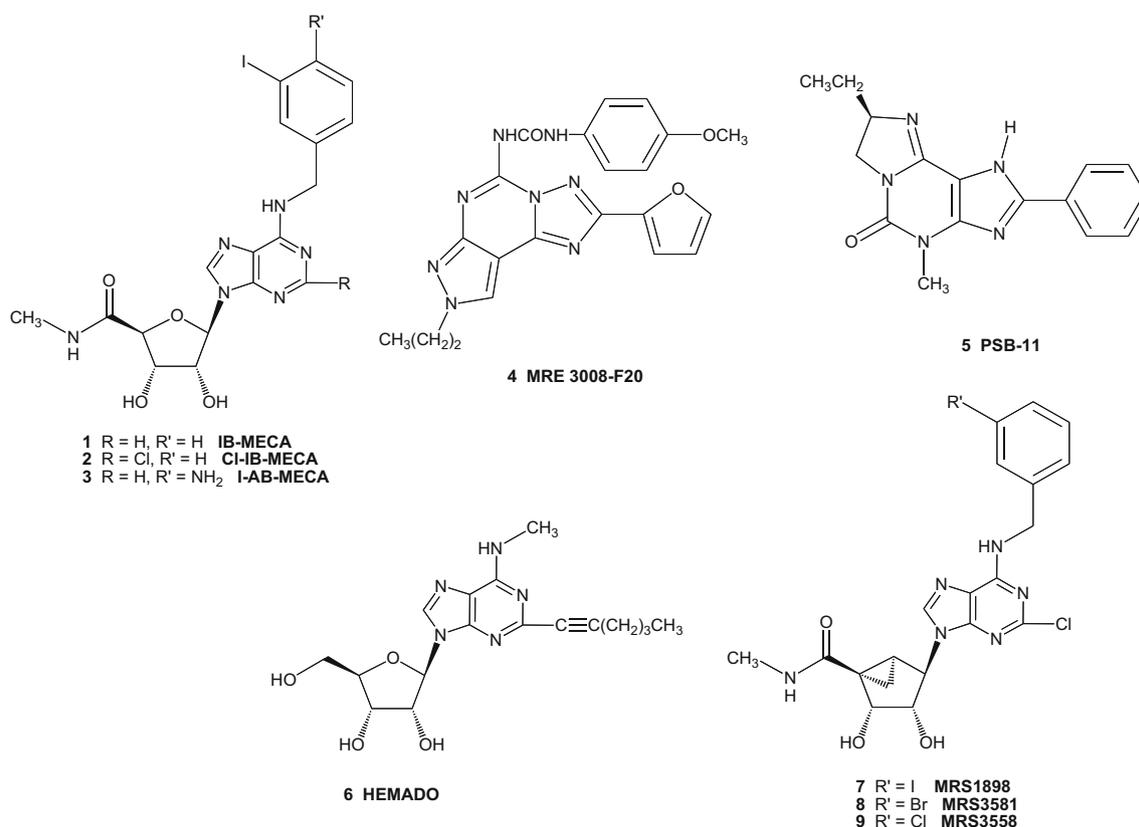
Chart 1 Structures of nucleoside and nonnucleoside, high-affinity ligands for the A_3 adenosine receptor. Compounds 3–6 were previously prepared in radioactive form for use in receptor labeling and characterization

binding. However, it has no effect on the rA$_3$AR [12], which is consistent with previous findings that N^6-methyl-substituted adenosine derivatives are potent at the hA$_3$AR but not rA$_3$AR [14, 15]. For example, the parent nucleoside N^6-methyladenosine has K$_i$ values of 6390 nM at the rA$_3$AR and 9.3 nM at the hA$_3$AR.

Thus, the challenge remains to develop a subtype-selective radioligand for the A$_3$AR in rat and other non-primate species. A new nucleoside derivative, (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol) (MRS1898 7), containing the (N)-methanocarba (bicyclo[3.1.0]hexane) ring system as a ribose substitute, displays high potency and selectivity for the hA$_3$AR compared with other A$_3$AR agonists, such as IB-MECA 1 [16–18]. This bicyclic ring system maintains a conformation that is preferred at the A$_3$AR and thus tends to increase selectivity for the hA$_3$AR. MRS1898 7 has previously been shown to bind with high affinity at the rA$_3$AR. Its affinity profile at three AR subtypes is as follows: rA$_1$=83.9 ± 10.3 nM, hA$_1$=136 ± 22 nM; rA$_{2A}$=1660 ± 260 nM, hA$_{2A}$=784 ± 97 nM; rA$_3$=1.1 ± 0.1 nM, hA$_3$=1.51 ± 0.23 nM. In this study, we synthesized a radioiodinated form of this adenosine derivative for preliminary in vitro studies and characterized its binding properties at the rA$_3$AR.

Materials and methods

General All chemical synthetic reagents and pharmacological agents were purchased from Sigma-Aldrich Chemical Company, except where noted. Sodium [^{125}I]iodide (17.4 Ci/mg) in sodium hydroxide (NaOH) (1.0x10^{-5} M) was supplied by Perkin–Elmer Life and Analytical Science. Iodogen iodination reagent was purchased from Pierce Biotechnology. High-performance liquid chromatography

(HPLC) was performed using an Agilent 1200 Series LC-MS system or a Beckman Gold HPLC system equipped with a Model 126 programmable solvent module, a Model 168 variable wavelength detector, a β–Ram Model 4 radioisotope detector, and Beckman System Gold remote interface module SS420X, using 32 Karat® software. These analyses were performed on Agilent Eclipse XDB-C18 (3.5 μm, 3.0 x 75 mm) and XDB-C18 (5 μm, 4.6 x 250 mm) columns.

Preparation of [^{125}I]MRS1898 [(1′R,2′R,3′Σ,4′R,5′Σ)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol] (10)

MRS1898 (0.018 g, 0.033 mmol), PdCl$_2$(PPh$_3$)$_2$ (5 mg), and hexamethyltin (0.032 g, 0.1 mmol) were mixed together in anhydrous dioxane (3 ml), and the resulting reaction mixture was stirred at 70 ˚C for 2 h. The mixture was concentrated under reduced pressure. The product was purified by preparative thin layer chromatography by using chloroform (CHCl$_3$): MeOH as the eluant to afford the stannyl derivative 1 (0.008 g, 45%) as an oil. High-resolution mass spectrometry (HRMS) (M+ 1)$^+$: calculated 593.1090, found 593.1099. HPLC: R_t=21.95 min. HPLC system: 5 mM TBAP/CH$_3$CN from 80/20 to 60/40 in 25 min, then isocratic for 2 min; flow rate of 1 ml/min.

Regeneration of MRS1898 The trimethylstannyl intermediate 10 (0.1 mg) was reconverted to MRS1898 upon dissolving in MeOH (0.1 ml) followed by treatment with I$_2$ (0.1 M in MeOH, 0.1 ml) for 10 min at room temperature (Scheme 1). The structure was confirmed by HPLC and HRMS. HRMS (M+ 1)$^+$: calculated 555.0408, found 555.0408. HPLC: R_t=15.91 min (same system as above).

Scheme 1 Synthesis of a stannyl precursor 10 and the iodination (reverse) reaction used in nonradioactive generation of [(1′R,2′R,3′Σ,4′R,5′Σ)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol] (MRS1898)
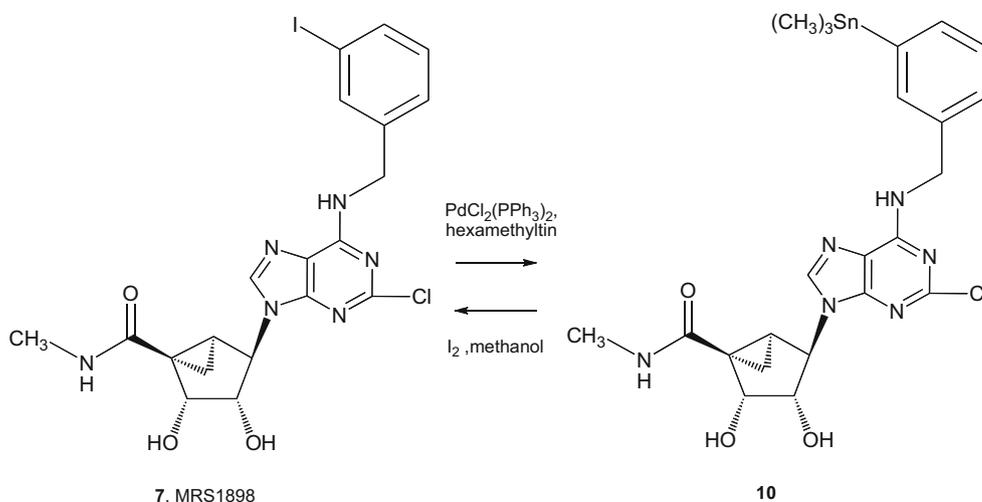
In a separate experiment that was more predictive of the subsequent radioiodination conditions, the trimethylstannyl precursor 10 (0.2 mg, 0.34 μmol) was dissolved in 50 ul of methanol in an Eppendorf vial, and to this solution was added an iodine–methanol solution (100 ul of 8 mg/ml). The vial contents were shaken for 30 min and the crude product analyzed on an Agilent 1200 Series Liquid-Chromatography Mass Spectrometry (LC-MS). MRS1898 eluted at 7.6 min with a molecular weight of 555.1 that showed $[M + H]^+$.

Radiolabeling of MRS1898: method 1 The radioiodination of MRS1898 was performed using *tert*-butyl hydroperoxide (TBHP) as an oxidant (Scheme 2) [19]. Next, 15 ul of TBHP (10% solution in chloroform) was added into a v-vial. To this solution were added an acetic acid solution (4 ul, 3% in chloroform) and a sodium $[^{125}I]$iodide solution (4 ul, 14.8 MBq, 3.7 GBq/ml), followed by the trimethyl-stannyl precursor 10 (50 ul, 0.2 mg in chloroform). The reaction mixture was mixed under vortex for 30 min at room temperature. The final labeled product was separated using a Beckman System Gold HPLC equipped with an Agilent Eclipse XDB-C18 column (5.0 μm, 4.6×250 mm) under the following conditions: solvent A was 0.1% trifluoroacetic acid (TFA) in water; solvent B was 0.1% TFA in acetonitrile. A linear gradient of solvent B from 20% to 50% over 25 min was used at a flow rate of 1 ml/min; $[^{125}I]$MRS1898 eluted at 23 min. The fraction containing $[^{125}I]$MRS1898 was concentrated and loaded onto an Agilent Eclipse XDB-C18 column (3.5 μm, 3.0×75 mm). The mobile phase was the same as above , and a linear gradient of solvent B from 20% to 45% over 12 min was used at a flow rate of 1 ml/min; $[^{125}I]$MRS1898 eluted at 9.0 min. The radiochemical purity was >98%, the specific activity was 3.94 Ci/mg, and the radiochemical yields were 75.6%.

*Radiolabeling of MRS1898: method 2* The second radio-iodination of the trimethylstannyl MRS1898 10 with Na

$[^{125}I]$ iodide was accomplished using iodogen as an oxidant (Scheme 2). Iodogen solution (60 ul, 0.33 mg/ml in chloroform) and sodium $[^{125}I]$iodide solution (3 ul, 11.1 MBq, 3.7 GBq/ml) were transferred into a v-vial. To this solution was added the trimethylstannyl precursor 10 (50 ul, 0.2 mg in chloroform). The reaction mixture was mixed under vortex for 30 min at room temperature, and the $[^{125}I]$MRS1898 was purified using the same conditions as used in method 1. The radiochemical purity was >98%, the specific activity was 3.94 Ci/mg, and the radiochemical yield was 54.5%. The $[^{125}I]$MRS1898 was stored in solution (ethanol: water=9:1, v/v) containing 1% ascorbic acid for inhibition of radiolytic processes.

Pharmacology

The binding experiments were done as previously described [8, 14] on Chinese hamster ovary (CHO) cell membranes expressing the recombinant rA_3AR and on membranes from rat-brain cortex, mainly expressing the A_1AR; and rat striatum, expressing the $A_{2A}AR$ endogenously. In brief, the saturation, displacement and kinetic experiments were performed using membrane preparations from CHO cells expressing rA_3AR in a total assay volume of 100 μl, including 25 μl of radioligand, 50 μl membranes, and 25 μl of test compounds or other ingredients. Nonspecific binding was determined in the presence of 10 μM IB-MECA. The mixtures were incubated at 25°C for 60 min, followed by filtration with a 24-well Brandell MT-24 harvester. Radio-activity was determined in a Beckman 5500B γ-counter. For the dissociation experiment, the mixture was first incubated for 60 min, 10 μM IB-MECA was added, and reaction was terminated at various time points, as indicated. Binding parameters were calculated using Prism 4.0 software (GraphPAD, San Diego, CA, USA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng-Prusoff equation [20]. Data are

Scheme 2 Radiosynthesis of [(1′R,2′R,3′Σ,4′R,5′Σ)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methyla-minocarbonyl)bicyclo[3.1.0]hexane-2,3-diol] ($[^{125}I]$MRS1898)
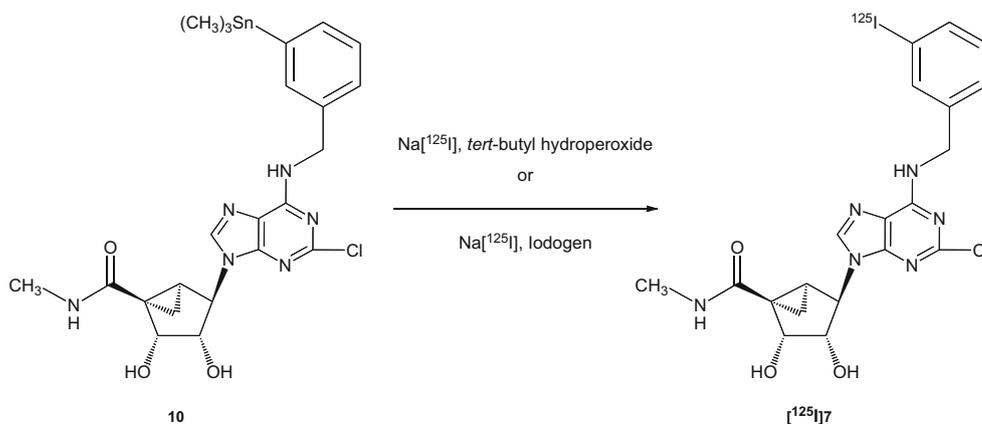
expressed as mean ± standard error. cLogP values were calculated using ChemDraw Ultra (Version 11.0).

Results

Chemistry

It was hoped that a new radioligand would permit better analysis of tissue distribution of the A_3AR. As MRS1898 already contains an iodine atom that is associated with high receptor affinity, that position was selected for convenient radiolabeling. A common method for rapidly introducing radioactive iodine on an aromatic ring is to use a stannyl precursor. The feasibility of this route was demonstrated through a "cold" iodination reaction (Scheme 1). The trimethylstannyl precursor 10 was generated in one step from MRS1898 using a palladium reagent and hexamethyltin. Protection of the hydroxyl groups or the exocyclic amine of this adenosine analogue was not necessary. Compound 10 was stable upon storage at −8°C for several months. This intermediate 10 rapidly reverted to MRS1898 upon treatment with iodine. The radioiodination of MRS1898 was accomplished through a similar iododestannylation of 10 using sources of ^{125}I and by two different radioiodination methods (Scheme 2). The tertiary-butyl hydroperoxide (TBHP) method [19] provided a superior yield in comparison with iodogen. The stability of [^{125}I]MRS1898 solution (ethanol:water=9:1, v/v) containing 1% ascorbic acid over 1 month when stored at −20°C was reliable, as reported in Table 1.
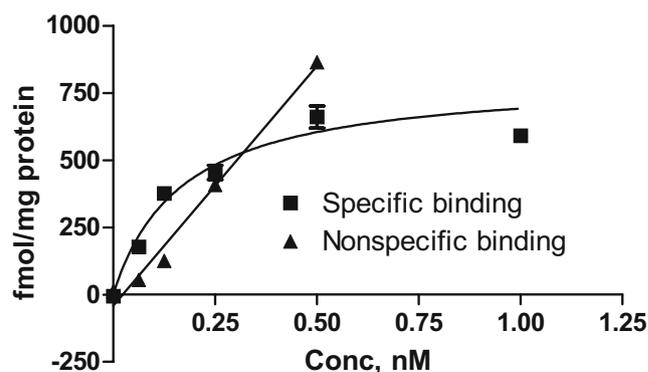
Pharmacology

The new radioligand was examined in standard radioreceptor binding experiments. The rA_3AR was expressed heterologously in CHO cells [21], from which membranes were prepared for binding experiments. Initially, the nonspecific binding of [^{125}I]MRS1898 to CHO cell membranes expressing the rA_3AR was extremely high,

Fig. 1 Saturation of binding of (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol ([^{125}I]MRS1898) at the rat A_3 adenosiner receptor (AR). Experiments were performed using membrane preparations (20 μg protein) from Chinese hamster ovary (CHO) cells expressing rat A_3AR in a total assay volume of 100 μl, including 25 μl of radioligand, 50 μl membranes, and 25 μl buffer (total binding) or 25 μl of 10 μM N^6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine (IB-MECA) (nonspecific binding). The mixtures were incubated at 25°C for 60 min, followed by filtration with a 24-well harvester

almost inseparable from its total binding. After soaking the glass fiber filters with polyethyleneimine (0.1%), the ratio of specific to nonspecific binding (at a concentration of 0.1 nM radioligand) was improved (3–4:1) and acceptable
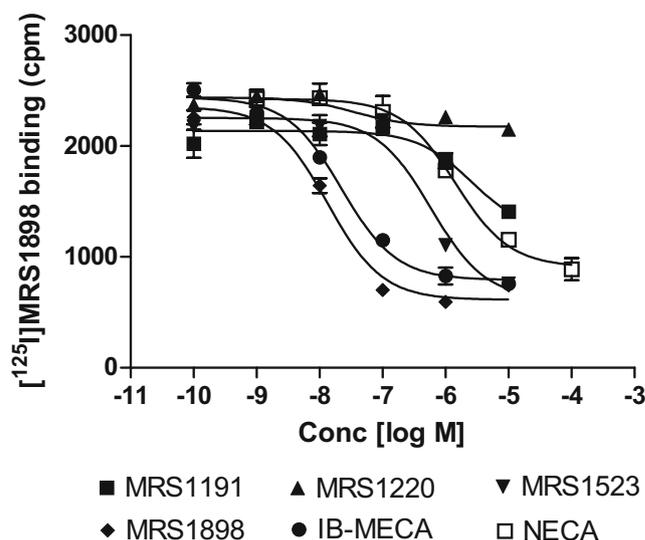
Fig. 2 Competition for binding of (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol ([^{125}I]MRS1898) (0.1 nM) at the rat A_3 adenosine receptor (AR) by A_3AR agonists and antagonists. The y-axis shows radioactivity counts corresponding to total binding. The K_i values (nM ± standard error of the mean) were: 1,4-dihydro-2-methyl-6-phenyl-4-(phenylethynyl)-3,5-pyridinedicarboxylic acid, 3-ethyl- 5-(phenylmethyl) ester (MRS1191) (1,850±386), N-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-yl]benzeneacetamide (MRS1220) (>10,000), 5-propyl-2-ethyl-4-propyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate (MRS1523) (518±236), MRS1898 (7.8±2.5), N^6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine (IB-MECA) (12.9± 3.7) and 5′-N-ethylcarboxamidoadenosine (NECA) (872±251)

Table 1 The radiochemical purities of a (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol ([^{125}I]MRS1898) solution at various time points[a]

Time (days)	2	8	11	20	25	27
Radiochemical purity	98%	98.5%	98.5%	100%	100%	100%

[a] Determined by high-performance liquid chromatography (HPLC) with radioactivity detection. [^{125}I]MRS1898 was present at a concentration of 6.9 nM in solution (ethanol:water=9:1, v/v) containing 1% ascorbic acid and stored at −20°C.

for drug screening, although the ratio was still low when the radioligand concentration was raised.

The specific binding of [^{125}I]MRS1898 to the rA$_3$AR in CHO cell membranes was saturable (Fig. 1), and Scatchard analysis indicated a K$_d$ value of 0.17±0.04 nM and a B$_{max}$ value of 0.66±0.15 pmol/mg protein.

The ability of various known AR agonists and antagonists to compete for [^{125}I]MRS1898 binding to the rA$_3$AR was tested. Figure 2 shows that the rank order of potencies for agonists was MRS1898 ≥ IB-MECA > NECA, and for antagonists 5-propyl-2-ethyl-4-propyl-3-(ethylsulfanyl-carbonyl)-6-phenylpyridine-5-carboxylate (MRS1523) > 1,4-dihydro-2-methyl-6-phenyl-4-(phenylethynyl)-3,5-pyridinedicarboxylic acid, 3-ethyl- 5-(phenylmethyl) ester (MRS1191) > N-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-yl]benzeneacetamide (MRS1220), which is similar to those obtained with other radioligands in previous reports.

In the association kinetic experiment (Fig. 3A), [^{125}I]MRS1898 binding reached a maximum in 30 min, with a t$_{1/2}$ of 7.4±0.9 min. After a 60 min incubation, the dissociation was initiated with the addition of 10 μM IB-MECA at various time points, as indicated in Fig. 3b.
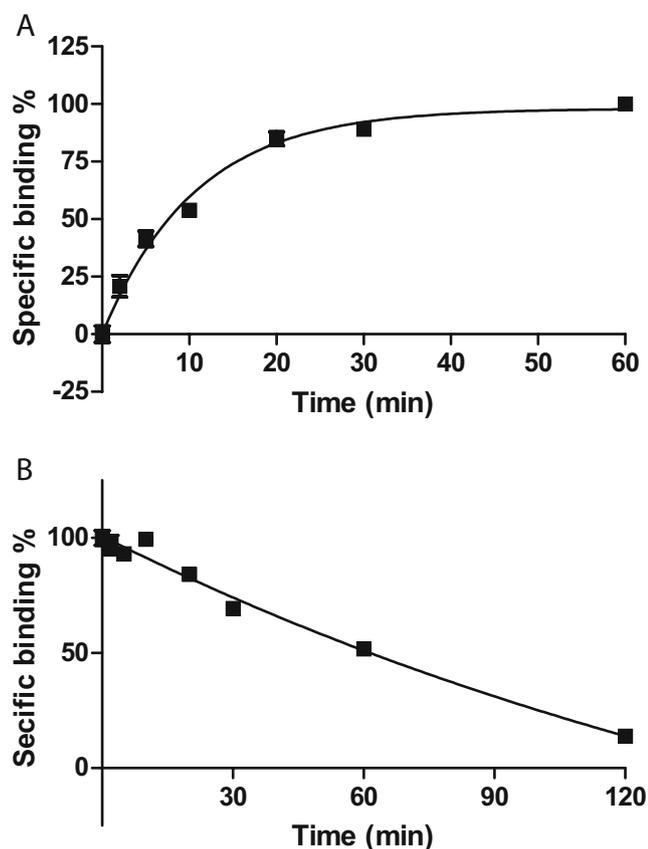
Fig. 3 Kinetics of association (**a**) and dissociation (**b**) of (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol ([^{125}I]MRS1898) (0.1 nM) at the rat A$_3$ adenosine receptor
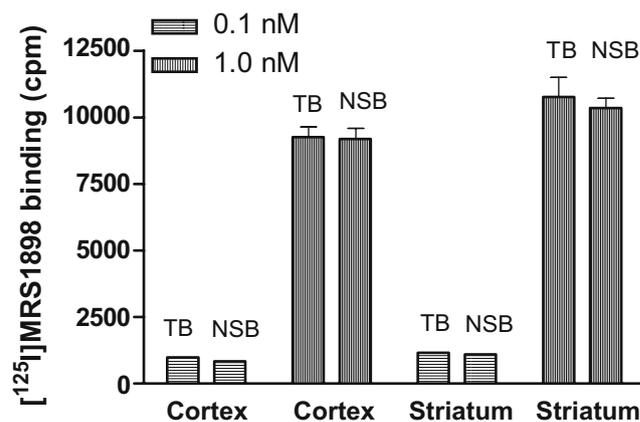
Fig. 4 Binding of (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol ([^{125}I]MRS1898) in membranes from rat-brain cortex [mainly A$_1$ adenosine receptor (AR)] and striatum (expressing both A$_1$AR and A$_{2A}$AR). Two concentrations of [^{125}I]MRS1898 (0.1 and 1.0 nM) were used. The membrane concentration used in the experiment was 200 μg/mg protein). The y-axis shows radioactivity counts. The *first bar (TB, total binding)* in each pair of bars corresponds to total radioligand binding, and the *second bar (NSB, nonspecific binding)* is in the presence of 10 μM N^6-cyclopentyladenosine for A$_1$AR and 10 μM 2-[p-(2-carboxyethyl)phenyl-ethylamino]-5′-N-ethylcarboxamidoadenosine for A$_{2A}$AR. Thus, no specific binding of [^{125}I]MRS1898 to the A$_1$AR and A$_{2A}$AR in rat brain was detected

The binding of [^{125}I]MRS1898 was further tested using membranes prepared from rat-brain cortex (mainly A$_1$AR) and striatum (expressing both A$_1$AR and A$_{2A}$AR). It was found that no specific binding to either the rA$_1$AR or rA$_{2A}$AR could be demonstrated (Fig. 4) at both low (0.1 nM) and high (1.0 nM) concentrations of [^{125}I]MRS1898 using a membrane concentration as high as 200 μg/mg protein.

Discussion

In this study, we synthesized and pharmacologically characterized the new radioligand [^{125}I]MRS1898 for the rA$_3$AR. The agonist ligand series of which MRS1898 is representative, based on nucleoside analogues containing a rigid, bicyclic ring system in place of the ribose moiety, was selected for radiolabeling due to its high A$_3$AR affinity across species. The radioiodination of MRS1898 on its N^6–3-iodobenzyl substituent was accomplished in 76% radiochemical yield by iododestannylation of a 3-(trimethylstannyl)benzyl precursor 10. This precursor was both synthesized and iodinated without protecting groups present at other sensitive positions of the nucleoside.

As a receptor radioligand, [^{125}I]MRS1898 may be useful for compound screening, testing of ligand binding affinity, and for studying association and dissociation kinetics at the rA$_3$AR. Although [^{125}I]MRS1898 could be potentially

useful for the study of the rA_3AR, there are also some potential problems with this radioligand, particularly its high nonspecific binding under the conditions used in this study. The K_d value of the radioligand was somewhat lower than that estimated from the affinity of the nonradiolabeled MRS1898, which may be affected by its hydrophobic nature. The cLogP for MRS1898 was 2.43, which was considerably higher than the cLogP (1.20) for the corresponding 9-riboside, Cl-IB-MECA 2. The frequently used radioligand I-AB-MECA 3 had a cLogP of −0.47. Thus, further efforts are needed toward the design of a similar radioligand with subnanomolar affinity and subtype selectivity, but with lower nonspecific binding, for applications such as autoradiography. Nevertheless, our study demonstrated that [^{125}I]MRS1898 should be useful under certain conditions for the study of the rA_3AR. Its A_3AR selectivity is clearly superior to the widely used [^{125}I]I-AB-MECA.

In summary, a novel selective A_3AR radioligand, [^{125}I]MRS1898, was synthesized and pharmacologically characterized in binding to cell membrane receptors. The advantages of this radioligand compared with other previously used radioligands are its facile synthesis and radiochemical stability and its high selectivity and high affinity for the rA_3AR, which should be applicable for the study of A_3AR in native tissues with mixed AR subtypes. The binding of [^{125}I]MRS1898 to the A_3AR in other species and the feasibility of its use in quantification of the A_3AR from various tissues may now be studied.

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