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Design, Synthesis and Binding Affinity of 3′-Fluoro Analogues of Cl-IB-MECA as Adenosine A_3 Receptor Ligands

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Abstract—Several 3′-fluoro analogues, **1a**, **1b**, and **1c** of selective and potent adenosine A_3 receptor agonist, Cl-IB-MECA were synthesized from D-xylose via highly regioselective opening of *lyxo*-epoxides, **8a** and **8b** with fluoride anion. Compared to the high binding affinity of Cl-IB-MECA to the A_3 adenosine receptor, the corresponding 3′-fluoro derivative showed remarkably decreased binding affinity, indicating that 3′-hydroxyl group acts as hydrogen bonding acceptor, not hydrogen bonding donor like fluorine atom in binding to the A_3 adenosine receptor.

Introduction

Four major subtypes of adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3 have been identified so far,[1,2] among which A_3 adenosine receptors[3] were most recently identified. The A_3 adenosine receptors may play an important role in the regulation of CNS, cardiac, inflammatory, and reproductive functions. Chronic administration of an A_3 agonist showed high cerebroprotection in a model of global cerebral ischemia in gerbrils[4] and significant protection against chemically induced seizures[5] in mice. Since A_3 receptors are expressed on the cardiac ventricular cell, the activation of A_3 receptor is related to the cardioprotective preconditioning response,[6] and thus the A_3 adenosine receptor agonists show a powerful protection against myocardial ischemia. Moreover, adenosine A_3 receptor antagonists may have potential use in treating inflammation since A_3 receptor stimulates the release of histamine on mast cells.[7] The adenosine A_3 receptor antagonists also may be developed as anti-asthma agent since the A_3 receptor is highly expressed on eosinophils in the lung.[8]

A number of compounds have been synthesized and evaluated for binding affinity to adenosine A_3 receptor for the development of therapeutically useful agents.

Among these compounds, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-methylcarboxamide (Cl-IB-MECA) was discovered to be one of the most selective agonists ($K_i = 0.33$ nM)[9] at rat A_3 adenosine receptor from the structure–activity relationship study for N^6- and 5′-substituted adenosine derivatives (Fig. 1).

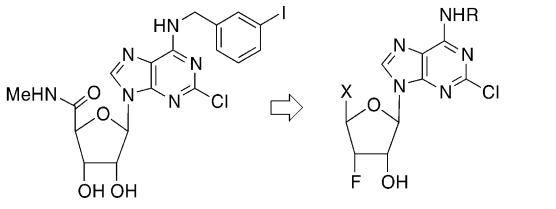

Figure 1. The rationale for the design of the desired nucleosides.

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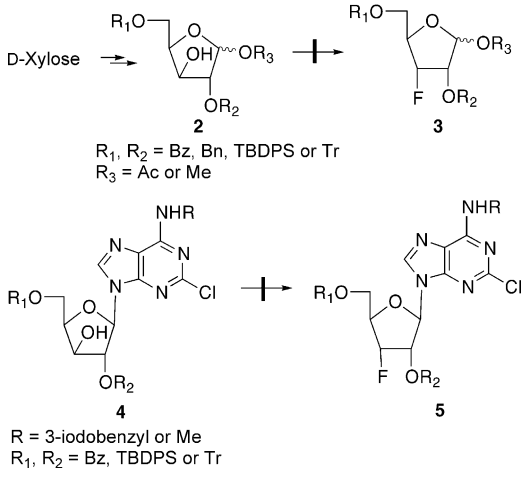
On the basis of its high binding affinity to adenosine A_3 receptor, it was interesting to determine whether 2′- or 3′-hydroxyl group of 2-Cl-IB-MECA is essential for the binding affinity to the receptor or compatible with bioisosteric fluorine for the binding affinity to this receptor. For this purpose, we substituted the 3′-hydroxyl group of Cl-IB-MECA with bioisosteric fluorine. In this communication, we wish to report the synthesis of 3′-fluoro analogue **1c** of Cl-IB-MECA and its related compounds, **1a** and **1b** (Fig. 1) and their binding affinities to adenosine A_3 receptor.

Results and Discussion

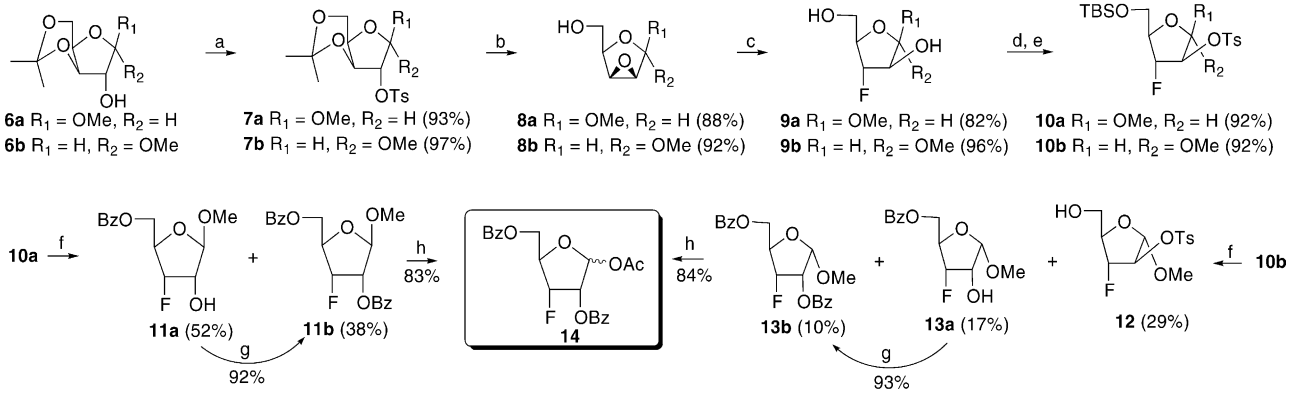
Our original strategy to synthesize the desired 3′-deoxy-3′-fluoroadenosine analogues was directly to fluorinate on carbohydrates **2** or nucleosides **4** with *xylo* configuration, as shown in Scheme 1, but many attempts to fluorinate them under the various reaction conditions failed to give the desired fluorinated compounds, **3** and **5**, respectively.

Thus, synthetic difficulties in fluorinating the compounds with *xylo* configuration made us take alternative synthetic route to utilize the regioselective opening of *lyxo*-epoxides **8a** and **8b** and direct S_N2 displacement of **10a** and **10b** for inversion of stereochemistry at C2 position as key steps (Scheme 2).



Scheme 1.

D-Xylose was converted to anomeric methoxides **6a** and **6b** according to Baker's method.[10] Tosylation of **6a** and **6b** gave **7a** and **7b**, which were converted to *lyxo*-epoxides **8a** and **8b**, respectively, by treating with 80% acetic acid followed by addition of sodium methoxide. Regioselective formation of 3-deoxy-3-fluoro derivatives, **9a** (82%) and **9b** (96%) was cleanly obtained by refluxing of **8a** and **8b** with potassium hydrogen fluoride and sodium fluoride in 1,2-ethylene glycol, respectively. Higher yield of **9b** than **9a** was attributed to the difficulty in attack of the fluoride anion at the C2 position due to the steric effect by the α-methoxy substituent. Interestingly, the same ring-opening reaction on *lyxo*-epoxide with its 5-hydroxyl group protected as benzyl ether[11] or benzoyl group afforded the desired 3-deoxy-3-fluoro analogue in moderate yield. This might be due to many side reactions as well as the separation problem caused by ethylene glycol during silica gel column chromatography. For the conversion of *arabino* configuration to *ribo* configuration, silyl protection of **9a** and **9b** as TBS ethers followed by tosylation afforded **10a** and **10b**, respectively. When β-methoxide **10a** was treated with sodium benzoate in DMSO at 200 °C, the desired **11b** (38%) and its debenzoylated compound **11a** (52%) were obtained as major products. Interestingly, 5-TBS group was transformed to the benzoyl group under the reaction conditions. It is believed that silicon-oxygen bond was first cleaved on thermal conditions to form 5-TBS cation and oxygen anion. TBS cation was then reacted with sodium benzoate to give 5-TBS-benzoate, which was finally attacked by oxygen anion to yield the 5-benzoate derivative. However, under the same reaction conditions, S_N2 displacement of α-methoxide **10b** by sodium benzoate was greatly hindered by the presence of α-methoxy substituent at C1 position, yielding the S_N2 displaced products, **13a** (17%) and **13b** (10%) in low yields with recovered starting material **10b** (16%) and its desilylated compound **12** (29%). Compounds **11b** and **13b** were each converted to the same glycosyl donor **14**.



Scheme 2. Reagents and conditions: (a) TsCl, pyridine, rt, 1–3 days; (b) (i) 80% AcOH 50 °C, 2 h; (ii) NaOMe, MeOH, 0–10 °C, 2–3 days; (c) KHF_2, NaF, 1,2-ethylene glycol, reflux, 0.5–1 h; (c) TBSCl, imidazole, DMF, 0–10 °C, 15 h; (e) TsCl, pyridine, rt, 13 h; (f) NaOBz, 18-crown-6, DMSO, 200 °C, 13 h (g) BzCl, pyridine, rt, 5 h; (h) Ac_2O, AcOH, H_2SO_4, rt, 15 min.

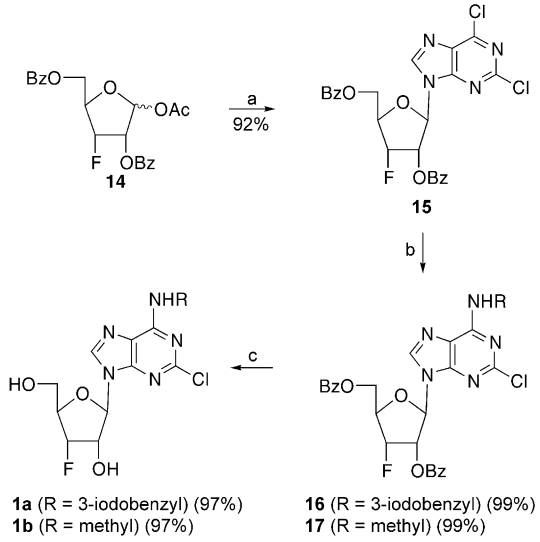
For the synthesis of N^6-substituted adenosine derivatives **1a** and **1b** (Scheme 3), glycosyl donor **14** was condensed with silylated 2,6-dichloropurine to give the protected nucleoside **15**. Treatment of **15** with 3-iodobenzylamine and methylamine in ethanol afforded N^6-(3-iodobenzyl)- and N^6-methyladenosine derivatives **16** and **17**, respectively. The final nucleosides, **1a** and **1b** were obtained after debenzoylation of **16** and **17**, respectively.

For the synthesis of 3′-fluoro analogue of Cl-IB-MECA, another glycosyl donor **22** was synthesized as shown in Scheme 4.

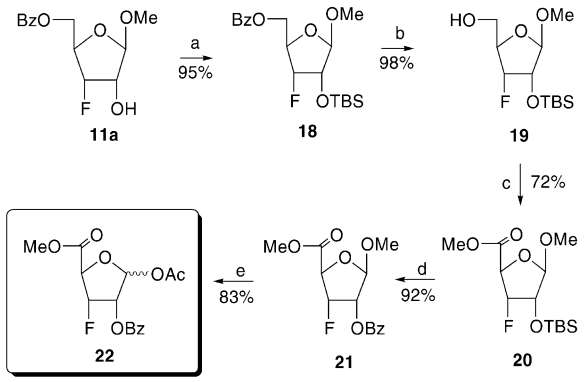
Compound **11a** was protected as TBS ether **18**, in which benzoyl group was removed to give **19**. Oxidation of the primary hydroxyl group of **19** followed by esterification using DCC and methanol afforded methyl ester **20**. For the conversion of anomeric methoxide to anomeric acetate **22** under acidic conditions, TBS group in **20** was changed to the benzoyl group, giving **21**.

The glycosyl donor **22** was condensed with silylated 2-chloro-N^6-(3-iodobenzyl)adenine in the presence of TMSOTf to give the protected nucleoside **23** (Scheme 5). Even on treatment of methyl ester **23** with 2 M methylamine solution in THF for only 4 min, substantial amounts of elimination product **24** (25%) was obtained along with the final nucleoside **1c** (12%), its benzoylated compound **25** (35%) and recovered starting material **23** (20%). Treatment of **25** with 2 M methylamine solution for the removal of benzoyl group afforded the 3′-fluoro analogue of Cl-IB-MECA, **1c** (47%) with concomitant formation of elimination product **24** (38%).

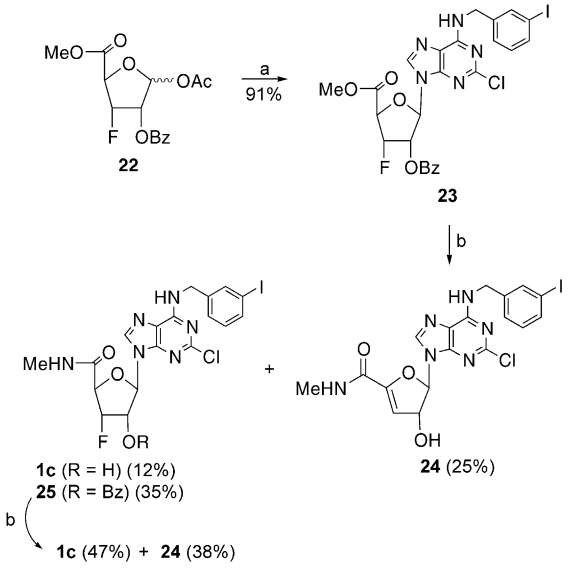
The final nucleosides **1a**–**1c** were tested in radioligand binding assays[12–15] for affinities at rat brain A_1[12] and A_{2A}[13] and human A_3[14,15] adenosine receptors (Table 1). As shown in Table 1, substitution of 3′-hydroxyl group with fluorine resulted in dramatic decrease in binding affinities to adenosine receptors, despite of their bioisosteric relationship. Compared to the high binding affinity ($K_i = 1.0$ nM) of Cl-IB-MECA to the human A_3 adenosine receptor, binding affinities ($K_i = 75$ and 326 nM) of compounds, **1a** and **1b** to A_3 receptor were remarkably diminished. Moreover, conversion of 4′-hydroxymethyl derivative **1a** into its methyl amide **1c** resulted in ca. 5-fold further decrease in binding affinity ($K_i = 406$ nM) to the A_3 receptor, although 4′-uronamide moiety has been generally reported to show better affinity in binding to adenosine receptors than the corresponding 4′-hydroxymethyl moiety. It is attributed that hydrogen bonding capacity of the 4′-uronamide was greatly diminished due to the presence of the strongly electronegative fluorine. However, all tested compounds did not show any binding affinity ($Ki > 10$



Scheme 3. Reagents and conditions: (a) silylated 2,6-dichloropurine, TMSOTf, 0–50 °C, 4 h; (b) 3-iodobenzylamine hydrochloride, Et_3N, EtOH, 50 °C, 5 h for **16**; methylamine hydrochloride, Et_3N, EtOH, rt, 4 h for **17**; (c) NaOMe, MeOH, rt, 1 h.

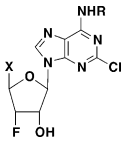


Scheme 4. Reagents and conditions: (a) TBSCl, imidazole, rt, 6 h; (b) NaOMe, MeOH, rt, 2 h; (c) $RuCl_3$, $NaIO_4$, $MeCN/CCl_4/H_2O$ (1/1/1.5), rt, 2 h, then DCC, DMAP, $MeOH/CH_2Cl_2$, rt, 10 h; (d) TBAF/AcOH, THF, rt, 3 days, then BzCl, pyridine, rt, 3 h; (e) Ac_2O, AcOH, H_2SO_4, 0°C to rt, 30 min.



Scheme 5. Reagents and conditions: (a) silylated 2-chloro-N^6-(3-iodobenzyl)adenine, TMSOTf, $ClCH_2CH_2Cl$, 0–55 °C; (b) 2 M $MeNH_2$, THF, rt, 4 min.

Table 1. Binding affinities of the 3′-fluoronucleoside analogues to adenosine receptors



Compd R, X	K_i (nM)		
	rA_1[a]	rA_{2A}[b]	hA_3[c]
Cl-IB-MECA R = 3-iodobenzyl X = CONHMe	820 ± 570	470 ± 365	1.0 ± 0.2
Compound **1a** R = 3-iodobenzyl X = CH_2OH	1,350 ± 350	> 10,000	75 ± 7
Compound **1b** R = CH_3 X = CH_2OH	17,100 ± 5300	> 10,000	326 ± 112
Compound **1c** R = 3-iodobenzyl X = CONHMe	780 ± 280	> 10,000	406 ± 60

[a]Displacement of specific binding of [^{3}H]PIA, unless noted, in rat brain membranes expressed as K_i ± SEM in nM (n = 3–6).
[b]Displacement of specific binding of [^{3}H]CGS 21680, unless noted, in rat brain membranes expressed as K_i ± SEM in nM (n = 3–6).
[c]Displacement of specific binding of [^{125}I]-AB-MECA binding, unless noted, in CHO cells expressing the recombinant receptor as K_i ± SEM in nM (n = 3–5).

μM) to A_{2A} receptor and exhibited similar binding affinities to A_1 receptor except **1b**. This biological results indicate that 3′-hydroxyl group plays an essential role in binding to A_3 and A_{2A} adenosine receptors as a hydrogen bonding acceptor, especially to A_{2A} receptor, but has little effect on binding to A_1 receptor.

In conclusion, we have synthesized novel 3′-fluoro-N^6-substituted adensoine derivatives to substitute 3′-hydroxyl group of Cl-IB-MECA with bioisosteric fluorine via regioselective opening of the *lyxo*-epoxide with fluoride anion and evaluated them for binding affinities to adenosine receptors. From this study, we have found very important and essential role of 3′-hydroxyl group as hydrogen bonding acceptor, not hydrogen bonding donor like fluorine atom in binding to adenosine receptors. This biological finding will provide medicinal chemists with additional important information in designing adenosine receptor ligands.

Acknowledgements

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