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Research Article

Tritium-labelled 8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine ([^{3}H]CPFPX), a potent and selective antagonist for the A_1 adenosine receptor

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Summary

The reduction of 1-allyl-8-cyclopentyl-3-(3-fluoropropyl)xanthine, **7**, with tritium gas catalyzed by 10% Pd-C gave 8-cyclopentyl-3-(3-fluoropropyl)-1-[2,3-^{3}H]propylxanthine ([^{3}H]CPFPX), **8***, a potent and selective antagonist for the A_1 adenosine receptor (A_1AR). The synthesis of **7** proceeded from 6-aminouracil, **1**, which underwent silylation and alkylation with allyl bromide to form 6-amino-3-allyluracil, **2**. Nitrosation led to the 5-nitroso compound, **3**, which underwent reduction to the 4,5-diaminouracil, **4**, and carbodiimide-mediated acylation with cyclopentanecarboxylic acid produced 3-allyl-6-amino-5-cyclopentylcarboxamidouracil, **6**. Alkylation at $N-1$ with 3-fluoro-1-bromopropane and cyclization with alkali completed the synthesis of **7**. [^{3}H]CPFPX had a radiochemical purity of $>$ 98% and a specific activity of $>$ 2.1 TBq/mmol (57 Ci / mmol). [^{3}H]CPFPX bound to the rat, pig and human A_1AR with a K_D of 0.63, 1.37 and 0.71 nM, respectively. The K_D at the rat and human A_{2A}AR was 812 and 940 nM, respectively, thus giving selectivities of $>$ 1200- and $>$ 700-fold. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: A_1 adenosine receptor; xanthines; adenosine antagonist ligand; tritiation

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Introduction

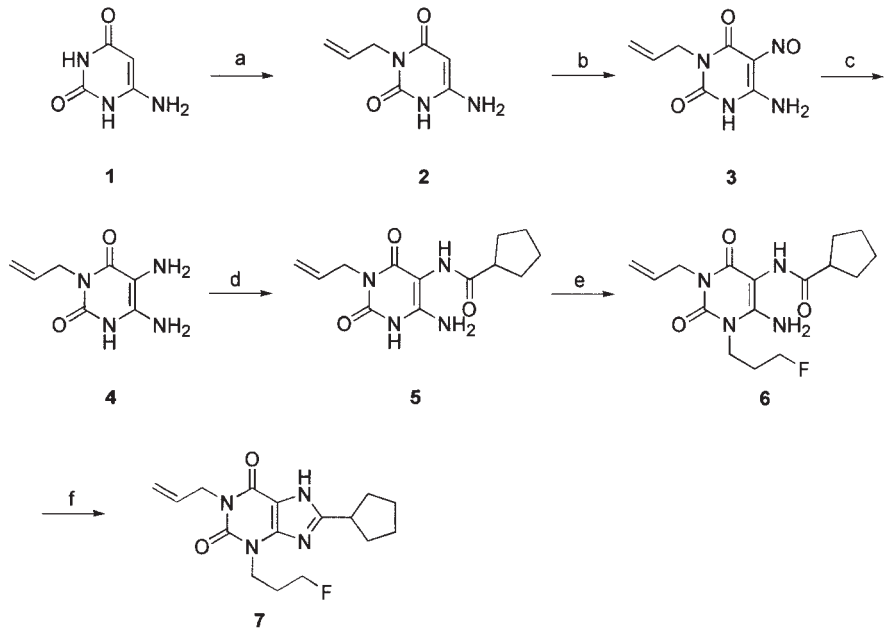
The preclinical evaluation of 8-cyclopentyl-1-propyl-3-(3-[^{18}F]fluoropropyl)xanthine ([^{18}F]CPFPX), a radioligand useful for imaging A_1ARs in the central nervous system (CNS) with positron emission tomography (PET),[1] required the synthesis of the tritiated ligand to support *in vitro* binding studies to that receptor. This report describes the synthesis and tritiation of the precursor 1-allyl-8-cyclopentyl-3-(3-fluoropropyl) xanthine, **7**. The affinity of the target radioligand [^{3}H]CPFPX, **8***, for the A_1AR of 3 species, including man, was similar to that of the 'Gold Standard' antagonist ligand 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

Results and discussion

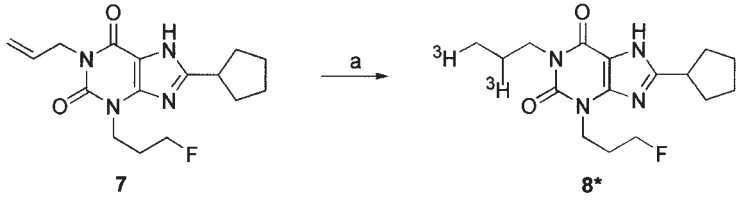
Scheme 1 summarizes the synthesis of **7**, which began with commercially available 6-aminouracil, **1**. Silylation and regioselective alkylation[2,3] of *N*-3 with allyl bromide gave 3-allyl-6-aminouracil, **2**. Nitrosation and reduction with sodium dithionite afforded the unstable 4,5-diaminouracil, **4**, which underwent carbodiimide-mediated coupling with cyclopentanecarboxylic acid to form the 5-carboxamido derivative **5**. Alkylation[4] with 3-fluoro-1-bromopropane introduced a 3-fluoropropyl group at *N*-1 to give **6**. Heating in alkaline solution led to the cyclization of **6** to yield 1-allyl-8-cyclopentyl-3-(3-fluoropropyl)xanthine, **7**.

Reduction of the allyl group over 10% Pd/C in an atmosphere of tritium gas yielded the target compound, **8*** (Scheme 2). HPLC with two systems differing in both stationary and mobile phases showed that the product accounted for all of the radioactivity and $>98\%$ of the UV absorbance. The specific activity was >2.1 TBq/mmol (57 Ci/mmol) as determined by UV-signal comparison with a calibration curve. The radioligand was stable for at least 1 year when stored below -5°C in ethanolic solution.

Radioligand binding assays employed membranes from rat and pig cerebral cortices (rA_1AR and pA_1AR) and corpora striata (rA_{2A}AR and pA_{2A}AR) and also membranes from CHO cells stably expressing the human A_1AR (hA_1AR) or the A_{2A}AR (hA_{2A}AR). The K_D of [^{3}H]CPFPX at the three A_1ARs, as measured by equilibrium binding assays, was 0.63 (rA_1AR), 1.37 (pA_1AR) and 0.71 (hA_1AR) nM, respectively. [^{3}H]CPFPX bound to the rat and human A_{2A}AR with a



Scheme 1. Synthesis of precursor 7



Scheme 2. Radiosynthesis of ligand 8*

K_D of 812 and 940 nM, respectively. Thus, the $A_1AR/A_{2A}AR$ selectivity ratios for [^{3}H]CPFPX in the two species were >1200 and >700. By comparison, the affinity of [^{3}H]DPCPX at the same receptors was 0.9 (rA_1AR), 2 (hA_1AR) and 470 ($rA_{2A}AR$) nM, respectively and the rA_1AR/rA_{2A} AR selectivity ratio was 520.

Experimental

Materials

Melting points were measured on an Electrothermal™ apparatus and are uncorrected. Elemental analyses were performed by the Zentralabteilung für Chemische Analysen at the Forschungszentrum Julich, and are within $\pm$ 0.4% of the calculated composition. Mass spectra (MS), ESI, were obtained on a Finnigan Automass III mass spectrometer (Thermo Quest, Egelsbach). Thin layer chromatography (TLC) employed precoated silica sheets (4×8 cm, Polygram™, Macherey-Nagel, Düren) developed with chloroform/methanol 90/10 (v/v). ^{1}H- and ^{13}C NMR spectra were obtained at 200.13 and 50.32 MHz, respectively, by means of a Bruker DPX-200 spectrometer (Avance 200) in $\approx$ 5% solution at 25°C. Chemical shifts are given in δ ppm using the residual proton signals of the appropriate deuterated solvents as a reference ($\delta_{H(CDCL_3)} = 7.30$, $\delta_{H(DMSO\text{-}d6)} = 2.52$, $\delta_{C(CDCL_3)} = 77.48\delta_{c(DMSO\text{-}d6)} = 40.38$)). The multiplicity symbols s, d, t and m refer to singlet, doublet, triplet and multiplet, respectively. Sigma-Aldrich, Deisenhofen, provided reagents and solvents, which were of the highest purity offered, as well as DPCPX. CPFPX, **8**, was prepared according to a published method.[4] No-carrier-added tritium gas was obtained from NEN Life Sciences, Zaventem, Belgium. Analytical HPLC on a LiChrosphere RP C-18 5μm column eluted with CH_3CN/water, 50/50 (v/v) at a flow rate of 1 ml/min and monitoring UV absorbance of the effluent at 254 nm established the purity of precursor **7**. In this system the capacity factor (k'-value) of **7** was 3.86. Semi-preparative radio-HPLC used a C-18 column (Multospher 120 5μm, 250×8 mm) eluted with acetonitrile/water, 50/50 (v/v) at a flow rate of 3 ml/min. In this system the k'-values of the precursor **7** and the unlabelled ligand **8** were 3.24 and 4.07, respectively. For continuous measurement of radioactivity the outlet of the UV detector was connected to a flow scintillation analyzer (Radiomatic™ 515 TR Series, Packard, Dreieich, Germany) and the recorded data were processed by an integrated software system. Quality control of the product employed a RP 18 Select B column (250×4 mm) eluted with acetonitrile/aqueous KH_2PO_4 (3.3 g/l), 70/30 (v/v) at a flow rate of 1 ml/min. Under these conditions the /(k'-value for **8*** was 2.03. A second system consisted of a Supersphere Si-60 column eluted with ethanol/hexane, 5/95 (vv) at a flow rate of 1 ml/min. Under these conditions the k'-value for **8*** was 2.51. Measurement of the specific activity of **8*** used HPLC, which

measured the mass of product by integrating the UV absorption of the product peak and referenced to a standard curve. The product peak was collected and an aliquot counted in a liquid scintillation counter.

3-*Allyl-6-aminouracil, 2*

A suspension of 6-aminouracil **1** (40 g, 0.314 mol) and $(NH_4)_2SO_4$ (2 g, 15 mmol) in hexamethyldisilazane (HMDS, 400 ml) was stirred at reflux (oil bath temperature ~140°C), until all the uracil had dissolved (~2 h). HMDS was distilled off, initially at normal pressure, later *in vacuo.* After cooling to 60°C allylbromide (30.4 ml, 0.349 mol) and iodine (300 mg) was added, the mixture was heated to reflux (oil bath temperature ~115°C) and stirred at that temperature for 1.5–2h. After cooling to room temperature the viscous syrup was carefully treated with a solution of $Na_2S_2O_4$ (6 g, 25 mmol) in water (20 ml) whereupon the syrup started to form reddish lumps. Under efficient stirring and external cooling with ice the solid was transferred portionwise into a solution of saturated aqueous $NaHCO_3$ (~800 ml). The suspension was stirred overnight at ambient temperature, filtered by suction, washed with water, toluene and ether and dried in a dessicator *in vacuo* over P_4O_{10}. Yield: 42.5 g, 87.4%, mp: 235–236°C (MeOH) (lit.[2]: 236°C). ^{1}H NMR (DMSO-d_6): 4.28 (d, 2 H, CH_2-N), 4.58 (s, 1 H, C^5H), 5.04 (m, 2 H, CH_2=), 5.72 (m. 1 H, C=CH), 6.35 (s_{br}, 2 H, NH_2), 10.53 (s_{br}, 1 H, NH).

3-*Allyl-6-amino-5-nitrosouracil, 3*

Under argon 3-allyl-6-aminouracil **2** (8.4 g, 54 mmol) was suspended in a mixture of dry EtOH (43 ml) and ethanolic HCl (5 N, 33 ml, 165 mmol). When all of the uracil had dissolved the solution was cooled with ice/salt to −2°C and isoamyl nitrite (7.3 ml, 54.3 mmol) was added dropwise in such a way that the temperature remained below 0°C. After completion of the addition the reaction mixture was stirred at 0°C for 1h; during that time product started to precipitate. The solid was filtered by suction and air-dried (3.7 g). Concentrating the mother liquor to near dryness gave a second crop of product that was filtered off, washed with a small volume of ice cold EtOH and air dried (2.9 g). Yield: 6.6 g, 62%, mp:223–224°C (lit.[3]: 225°C).). ^{1}H NMR (DMSO-d6): 4.44 (d, 2 H, CH_2N), 5.14 (m, 2 H, CH_2=*),* 5.87 (m. 1 H, C=CH), 8.37 (s_{br}, 2 H, NH_2), 11.41 (s_{br}, 1 H, *NH).*

3-*Allyl-6-amino-5-cyclopentylcarboxamidouracil, 5*

Under argon the nitroso aminouracil **3** (9.8 g, 50 mmol) was dissolved in 12.5% NH_4OH (250 ml) at 70°C. $Na_2S_2O_4$ (ca 25 g) was added portionwise until the solution became cannary yellow. The reaction mixture was concentrated *in vacuo* until a solid started to precipitate and the mixture was cooled in ice for 6 h. The air sensitive solid was rapidly filtered by suction and dried in a dessicator *in vacuo.* After drying for 2 h the diamine **4** (6.7 g, 37 mmol, 74%) was used immediately for the next step.

A suspension of the diamine **4** (6.7 g, 37 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide (7.5 g, 40.7 mmol, 1.1 eq.) and cyclopentanecarboxylic acid (4.5 g, 40.7 mmol, 1.1 eq.) in MeOH/H_20 (1/1, 240 ml) was stirred at room temperature overnight. The solid was filtered off, washed with a small volume of water and MeOH and air dried. Yield 8.06 g, 78%, mp: 279°C. ^{1}H NMR (DMSO-d_6): 1.72 (m, 8 H, cp-CH_2), 2.75 (m, 1 H, cp-H), 4.29 (d, 2 H, CH_2N), 5.03 (m, 2 H, $CH_2=$), 5.82 (m, 1 H, C=CH), 5.87 (s_{br}, 2 H, NH_2), 8.29 (s, 1 H, amide-NH), 10.46 (s_{br}, 1 H, NH). *m*/*e* 278 (100%). Elemental analysis (FW 278.31): calculated: C 56.10; H 6.52; N 20.13; found: C 56.19; H 6.52; N 20.07.

3-*Allyl-6-amino-1 -(3-fluoropropyl)-5-cyclopentanecarboxamidouracil, 6*

Under argon the amide **5** (5.59 g, 20 mmol) was dissolved in dry DMF (80 ml). Finely ground K_2CO_3 (2.76 g, 20 mmol) was added and the mixture was stirred for 30 min at ambient temperature. A solution of 1-bromo-3-fluoropropane (2.82 g, 1.83 ml, 20 mmol) in dry DMF (20 ml) was added dropwise via a syringe and the mixture was stirred for 72 h at room temperature. The mixture was filtered and the filtrate concentrated *in vacuo* to ~10 ml. The residue was taken up in ethyl acetate (75 ml), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness *in vacuo.* The solid residue was recrystallized from CH_3CN. Yield: 3.52 g, 52%, mp.: 194°C. ^{1}H NMR ($CDCI_3$): 1.72 (m, 10 H, cp–CH_2 + CH_2–CH_2F), 2.79 (m, 1 H, cp-H), 3.67 (m, 2 H, CH_2N^1), 4.04 (t, 2 H, CH_2N^3), 4.44 (m, 2 H, CH_2F), 5.12 (m, 2 H, $CH_2=$), 5.85 (m. 1 H, C=CH), 6.71 (s_{br}, 2 H, NH_2), 8.04 (s, 1 H, amide-NH). *m*/*e* 338 (100%). Elemental analysis (FW 278.31): calculated: C 56.79; H 6.85; N 16.56; found: C 56.81; H 6.87; N 16.51.

1 -*Allyl-8-cyclopentyl-3-(3-fluoropropyl)xanthine, 7*

The fluoropropyl amide **6** (1 g, 3 mmol) was refluxed for 3 h in a mixture of MeOH (100 ml) and 20% $NaOH_{aq}$ (20 ml, 100 mmol). After cooling to room temperature the reaction mixture was acidified to pH 4 with cone HCI and the white precipitate (NaCl) was filtered off. MeOH was evaporated off and the residue was diluted with H_2O (50 ml). The aqueous phase was extracted with dichloromethane (2×75 ml), the pooled organic phases were dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel 60 using DCM/MeOH 88/12 (v/v) as an eluent. The fraction containing the product was collected, solvents were evaporated and the solid residue was recrystallized from CH_3CN. Yield 116 mg, 12%, mp: 201°C. For the tritiation an aliquot of **7** was further purified by HPLC and gave material of > 99% purity. ^{1}H NMR ($CDCl_3$): 1.96 (m, 10 H, cp-CH_2 + CH_2–CH_2F), 3.32 (m, 1 H, cp-H), 4.34 (t, 2 H, CH_2N^3), 4.48 (t, 2 H, CH_2N^1), 4.72 (m, 2 H, CH_2F), 5.23 (m 2 H, $CH_2=$), 5.95 (m, 1 H, C=CH), 12.60 (s_{br}, 1 H, NH). *m/e* 320 (100%). Elemental analysis (FW 320.16): calculated: C 59.99; H 6.61; N 17.49; found: C 59.94; H 6.67; N 17.51.

8-*Cyclopentyl-3-(3-fluoropropyl)-1 -[2,3-^{3}H]propylxanthine 8**

In the 2.5 ml reaction vessel of a tritiation device[5] was placed a solution of 1-allyl-8-cyclopentyl-3-(3-fluoropropyl)xanthine **7** (3 mg, 9.3 μmol) in dry ethyl acetate (1 ml). Palladium on charcoal (10%, 2.2 mg) was added and the vessel was connected to the tritiation apparatus, which was evacuated and flushed with helium (two cycles) before the reaction mixture was carefully degassed (two freeze/thaw cycles). [^{3}H]H_2 (1 Ci, 0.4 ml, 18 μmol) was transferred to the reaction flask by means of a Toeppler pump (three transfer cycles) and the reaction was allowed to proceed with efficient stirring for 6 h at room temperature. The reaction mixture was degassed (two freeze/thaw cycles) and the catalyst was removed by filtration through a syringe filter (Millipore FH, 0.5 μm, 4 mm diameter). The filter was washed with acetone (4×1 ml), the organic filtrates were pooled and labile tritium was removed by adding EtOH (6 ml) and evaporation of the solvents under reduced pressure at 60°C. This cycle was repeated twice. The residue was taken up in CH_3CN (1.6 ml) and water (1ml) and was purified by semi-preparative HPLC. The fractions containing **8*** were collected, pooled and

evaporated to dryness at 60°C under reduced pressure. The product (495 mCi) was taken up in EtOH (6 ml) and aliquots were analyzed by liquid scintillation counting. Quality control revealed a radiochemical purity of 98% and a specific activity of >2.1 TBq/mmol (57 Ci/mmol).

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