SYNTHESIS OF TRITIATED FUNCTIONALIZED CONGENERS OF 1,3-DIPROPYLXANTHINE HAVING HIGH AFFINITY AT ADENOSINE RECEPTORS

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SUMMARY

Two potent ligands for adenosine receptors have been synthesized with non-exchangeable ^3H labeling to high specific activity. Both are adenosine antagonists, one a carboxylic acid congener, 1, and the other an amino congener, 2, and are structurally related to 1,3-dipropyl-8-phenylxanthine. The label was introduced at four positions in the n-propyl groups through catalytic reduction of 1,3-diallyl precursors. Due to favorable aqueous solubility, high potency and selectivity, these ligands are expected to be suitable for competitive binding assays and autoradiography.

Key words: xanthines, functionalized congeners, radioligands, tritiation, adenosine receptor, catalytic reduction.

INTRODUCTION

Recently we have applied a functionalized congener approach (1,2) to the design of ligands for extracellular A_1 and A_2 adenosine receptors. In the antagonist series, the receptor affinity and selectivity of derivatives of 1,3-dipropyl-8-phenylxanthine bearing chains attached at the para position of the phenyl ring illustrated the importance of distal structural changes on these parameters. The effects on biological activity resulting from substituting terminal amino or carboxylic groups on the chain was particularly striking.

The xanthine "functionalized congeners", 1 and 2, synthesized initially as intermediates to be coupled to "carriers" by amide formation, were found

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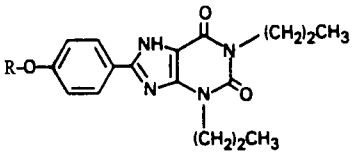
to have high potency (1,3) and greatly improved water solubility (2). We felt these analogues should have potential as improved adenosine receptor radioligands. Thus it was expected that they might overcome the problems of low solubility and non-specific adsorption previously noted in tissue binding assays using radioligands (4,5) related to 8-phenylxanthines. Moreover, the xanthine amine congener (XAC, 2) is 40-fold selective for A_1 adenosine receptors (1).



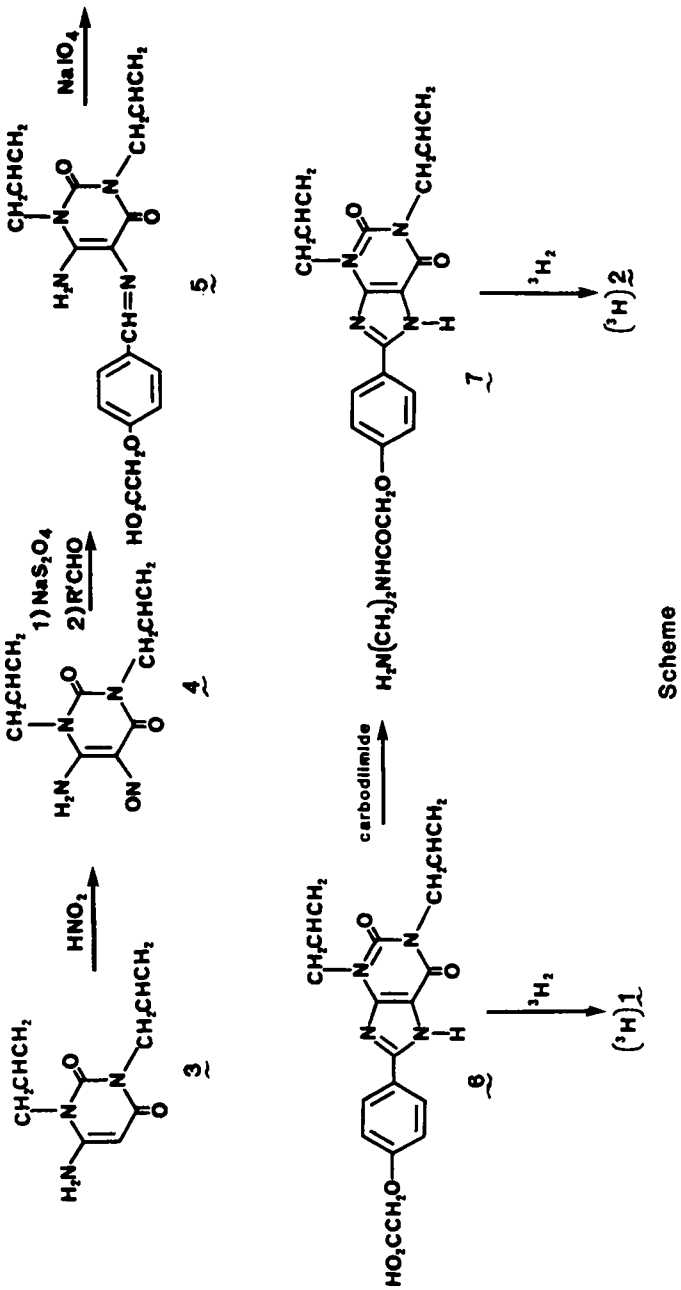
1 R = HO_2C-CH_2- carboxylic congener, XCC

2 = $H_2N-(CH_2)_2-NHCO-CH_2-$ amine congener, XAC

RESULTS AND DISCUSSION

We have incorporated tritium in compounds 1 and 2 through catalytic reduction of the corresponding 1,3-diallyl analogs using tritium gas as shown in the Scheme. The presence of four tritium atoms per molecule allows a marked increase in the maximum specific activity compared to currently commercially available adenosine receptor ligands, eg. [^{3}H]1,3-diethyl-8-phenylxanthine (DPX) which has less than 15 C_i/mmol. This is an important consideration since adenosine receptors are often present at relatively low density (6,7) in peripheral tissue.

The diallyl precursors were prepared by a standard route (Fig. 1) based on condensation of a diaminouracil (8) with a substituted benzaldehyde was chosen. An improvement was made in the conditions required for the oxidative closure of the imidazole ring. In previous reports (1, 5) this closure was mediated by ferric chloride in a non-aqueous, polar solvent. Since iron impurities were difficult to remove completely from the product xanthine, an alternative was sought for the synthesis of the analogous radioligands, in which residual traces of iron might accelerate chemical decay. It was found that substoichiometric quantities of sodium periodate in hot ethanol or dimethylformamide (DMF) led to facile closure of the benzylidene intermediate.



Scheme

The reported route to XAC, 2, consisted of the aminolysis of the ethyl ester derivative of 1 in neat ethylenediamine. When periodate was used as the oxidant, the ring closure reaction stopped at the stage of the carboxylic acid (using ferric chloride also resulted in in-situ esterification). A subsequent esterification step was obviated by use of an alternate amide forming reaction. The 1,3-diallyl carboxylic acid congener, 6, was converted to an active ester in situ using the carbodiimide/N-hydroxysuccinimide method (9) and treated with excess ethylenediamine.

Tritiation of 6 using palladium on charcoal catalyst resulted in a solution of [^{3}H]XCC which required no further purification. After removal of labile tritium, the radiochemical purity, estimated by thin layer chromatography, was 99%. The specific activity was calculated to be 149 C_i/mmol (approx. 75% of the theoretical maximum incorporation).

Tritiation of the 1,3-diallyl amine congener, 7, resulted in an impure mixture, from which the radiochemically pure product could be recovered by reversed phase high pressure liquid chromatography.

Binding of the radioligands was studied in membrane preparations of rat brain. [^{3}H]XAC bound in a saturable and reversible manner to brain membranes. Saturation analysis revealed a dissociation constant (K_D) of 1.23 nM. This K_D-value is nearly identical to the K_i-value of XAC for inhibition of [^{3}H]N^6-cyclohexyladenosine binding to rat brain membranes (1,2). In competition experiments, the unlabeled XAC inhibits radioligand binding of [^{3}H]XAC with a K_i-value of 3 nM, which is in good agreement with the K_D-value from the saturation experiments. Futhermore, the pharmacological profile of the binding sites is consistent with an interaction at A_1-adenosine receptors, eg. N^6-substituted adenosine analogues have high affinities. Binding studies with [^{3}H]XCC gave a nearly identical profile. Therefore, both radioligands satisfy essential criteria for the identification of membrane bound receptors. The diallyl precursors 6 and 7 bound much more weakly to adenosine receptors, with K_i-values of 1070 nM and 47 nM, respectively. Compared to the currently used [^{3}H]1,3-diethyl-8-phenyl-xanthine, [^{3}H]XAC has an approximately 50-fold higher affinity for

A_1-adenosine receptors and an 8- to 10-fold higher specific activity.

EXPERIMENTAL

Diallylaminouracil and sodium periodate were obtained from Aldrich Chemical Co. (Milwaukee, Wisc.). Purity of products was determined by thin layer chromatography on silica plates using a mixture of chloroform: methanol: acetic acid (85:10:5). NMR spectra were measured on a 300 MHz Varian instrument, and results are expressed as parts per million from TMS. Mass spectroscopy (CI-NH_3) was carried out on a Finnigan 1015D instrument. The conditions for high pressure liquid chromatography were a Waters 440 system, Altex ODS 15 X 0.46 cm column, 1.0 ml/min, 60-65% MeOH/0.05M sodium phosphate, pH 5, 254 nm UV detector.

6-Amino-1,3-diallyl-5-nitrosouracil (4). 1,3-Diallyl-6-aminouracil monohydrate (3, 3.77 g, 16.7 mmol) was suspended in 5 ml of a 20% acetic acid solution. Concentrated hydrochloric acid (1.5 ml) and a solution of sodium nitrite (1.15 g, 16.7 mmol, in 5 ml H_2O) were added in alternating small portions such that an acidic pH was maintained. Water was added to the reaction as needed to allow efficient stirring of the purple mixture. After stirring for one hour the solid was filtered, washed thoroughly with water, and dried in vacuo at 60°C to give 4.21 g (100%) of 4, mp 187-188 °C. Analysis ($C_{10}H_{12}N_4O_3$): calc. 50.86% C, 5.12% H, 23.72% N; found 50.68% C, 5.14% H, 23.67% N.

6-Amino-1,3-diallyl-5-[[4-[(carboxymethyl)oxy]benzylidene]amino]uracil (5). Compound 4 (2.12 g, 8.3 mmol) was suspended in 60 ml of ethyl acetate and stirred vigorously with a sodium dithionite solution (11 g in 30 ml of water). After one hour the color lightened, and the phases were separated. The aqueous layer then was extracted 3 times with ethyl acetate, and the combined extracts and organic layer were evaporated to give a clear oil. A mass spectral peak corresponding to 1,3-diallyl-5,6-diaminouracil was observed at 223 z/e.

The oil was then dissolved in 20 ml of methanol and treated with 2 ml of acetic acid. p-Formylphenyloxyacetic acid (ref. 1, 1.5 g, 8.3 mmol) was added with stirring. After several minutes the white precipitate that formed

was filtered to give 5 (2.8 g, 88 % yield), mp 110-112d °C. Mass spec 385. Recrystallized from DMF/ether. Analysis ($C_{19}H_{20}N_4O_5$.DMF.H_2O): calc. 55.57% C, 6.14% N, 14.71% N; found 55.72% C, 5.85% H, 14.71% N.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-diallylxanthine (6). Compound 5 (2.7 g, 7.1 mmol) was dissolved in 50 ml of DMF and treated with 0.33 g of sodium periodate in 2 ml H_2O. The mixture was heated gently on a steam bath for 10 minutes after which time the reaction was judged essentially complete by TLC. Removal of most of the solvent and addition of ether precipitated a first crop of 6, 1.14 g (42%), not melting below 320°C. Mass spec 383. Recrystallized by dissolving in NaOH followed by acidification. Analysis ($C_{19}H_{18}N_4O_5$): calc. 59.68% C, 4.75% H, 14.65% N; found 59.90% C, 5.06% H, 14.57% N.

A sample of 6 was reduced catalytically under conditions used in the tritiation (see below) except with hydrogen gas. Comparison of 6 (retention time = 4 min), the reduction product, and an authentic sample of 1 (9 min) by high pressure liquid chromatography (60% MeOH) demonstrated that the reduction of 6 to 1 had proceeded in 98% yield.

8-[4-[[[[(2-Aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-diallylxanthine (7). Compound 6 (33 mg, 86 μmol) was dissolved in 1 ml DMF and treated with N-hydroxysuccinimide (10 mg, 86 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (34 mg, 0.17 mmol). After stirring for one hour the activated carboxylic acid was added slowly to methanolic ethylenediamine (1 ml of 10% solution) with stirring. After 10 min most of the solvent was evaporated, and a sodium carbonate solution (3 ml of ·3%) and brine (1 ml) were added. The solution was extracted with ethyl acetate, which was discarded, and twice with n-butanol. The butanol extract was evaporated, leaving 24 mg (67%) of crude product. A further purification to chromatographic homogeneity was carried out on Sephadex LH-20, eluting with water. Product melted at 229-232°C. Mass spec: 425, 407, 382. NMR in $(CD_3)_2SO$: 8.27 (t, 1H, CONH); 8.06 (d, J=8Hz, 2H, Ar), 7.08 (d, J=8Hz, 2H, Ar), 5.9 (m, 2H, CH=), 5.1 (m, 4H, CH=), 4.65 (d, J=2.9Hz, 2H, allyl), 4.58 (s, 2H, CH_2O), 4.51 (d, J=3.9Hz, 2H, allyl), 2.78 (t, 2H, $\underline{CH_2}NH$).

Preparation of tritiated 1. A 3 mg sample of 6 dissolved in 2 ml of DMF was reduced by stirring for three hours in an atmosphere of tritium gas in the presence of 10 mg of 5% Pd/C (procedure carried out by NEN Research Products, Boston, Mass.). Labile protons were removed by addition and evaporation of DMF/MeOH, 1:1. The product (radiochemical purity 99%) contained 0.24 C_i tritium, and had an R_f on thin layer chromatography (0.64) identical to that of an authentic sample of 1. The specific activity calculated from UV absorption (e = 34,300 at 319 nm) was 149 C_i/mmol.

Preparation of tritiated 2. A 2.5 mg sample of 7 dissolved in 1 ml of DMF was reduced for one hour in an atmosphere of tritium gas over 10 mg of 10% Pd/C (procedure carried out by Research Products International, Mount Prospect, Ill.). Labile protons were removed by the addition and evaporation of methanol leaving 25 mC_i of tritium. A 0.5 mC_i sample in DMF was purified by high pressure liquid chromatography (65% MeOH) to give 0.12 mC_i of 2 having a calculated specific activity of 103 C_i/ mmol. Thin layer chromatography (Berthold LB 2760 TLC-scanner) revealed a radioactive spot (96% pure) with an R_f (=0.16) identical to that of authentic 2.

Radioligand binding assay: Synaptosomal membranes from rat brain were prepared according to the method of Whittaker (10). Radioligand binding was measured in a total volume of 1 ml containing 50 mM Tris-HCl, pH 7.4, 0.2 unit/ml adenosine deaminase and approximately 100 ug of membrane protein. The final concentrations of the radioligands were 0.5 nM for [^{3}H]XAC and 1 nM for [^{3}H]XCC. Incubation was carried out at 37°C for 90 min and was terminated by rapid filtration through Whatman GF/B glass fiber filters as described previously (1,2). Nonspecific binding, defined in the presence of 1 mM theophylline, was 25% for [^{3}H]XCC. Filter binding was diminished by 80% for [^{3}H]XAC by presoaking the filter in polyethyleneimine by the method of Bruns, et al (11), and by 60% for [^{3}H]XCC by presoaking in 1% 4-fluorobenzoic acid in a 1:1 mixture of dimethylformamide and 50 mM Tris buffer.

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