SYNTHESIS OF THE TRITIUM LABELED SCH 58261, A NEW NON-XANTHINE A_{2A} ADENOSINE RECEPTOR ANTAGONIST

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SUMMARY

The tritium labeled form of 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine (^{3}H-SCH 58261) was obtained by reduction of 5-amino-7-[2-(2',4',5'-tribromo)-phenylethyl]-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine with tritium gas in the presence of 10% Pd-C. Final product was purified by HPLC to give the title ^{3}H-SCH 58261 with radiochemical purity of 99% and specific activity of 68.6 Ci/mmol. ^{3}H-SCH 58261 bound A_{2A} receptors in rat striatal membranes (specific binding > 90%) with K_d and B_{max} value of 0.70 nM and 971 fmol/mg of protein, respectively. ^{3}H-SCH 58261 represents a useful tool for a further characterization of A_{2A} adenosine receptor subtype.

Key words: ^{3}H-SCH 58261, adenosine receptors, A_{2A} adenosine receptors, A_{2A} receptor antagonists, binding assay.

INTRODUCTION

Adenosine modulates a wide range of physiological functions by interacting with different receptor subtypes named A_1, A_{2A}, A_{2B} and A_3 (1).

Binding assay, the most effective and rapid tecnique for investigating drug-receptor interaction, needs radioligands with high affinity and selectivity. In particular, the use of antagonist ligands can avoid the complication associated with the high and low agonist affinity states G-protein coupled receptors. While agonist radioligands are available to label the A_{2A} receptor subtype, the development of antagonist radioligands has long been hampered by the lack of selective compounds.

Only recently, potent and selective antagonists to the adenosine receptor have emerged (2,3). Among these, (E, 18%-Z, 82%)-7-methyl-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine (KF 17837S) has been used in its tritium labeled form to characterize the A_{2A} receptor subtype in rat striatal membranes, but non-specific binding (about 30-40%) still appears high (4).

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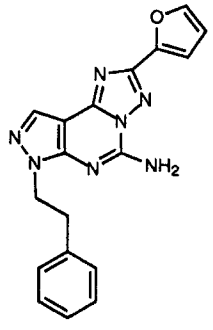
We have recently described the compound 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261) **1** as the first potent (Ki = 2.3 nM) and selective (A_1/A_{2A} ratio of about 50-100 fold) non-xanthine A_{2A} receptor antagonist (5).



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Binding and functional assays indicate that the compound **1** is a competitive A_{2A} antagonist and does not interact with other receptors including the A_{2B} and A_3 receptor subtype (6). These findings make the compound ideally suited for a further characterization of the A_{2A} receptor subtype and thus the tritium labeled form of SCH 58261 has been prepared.

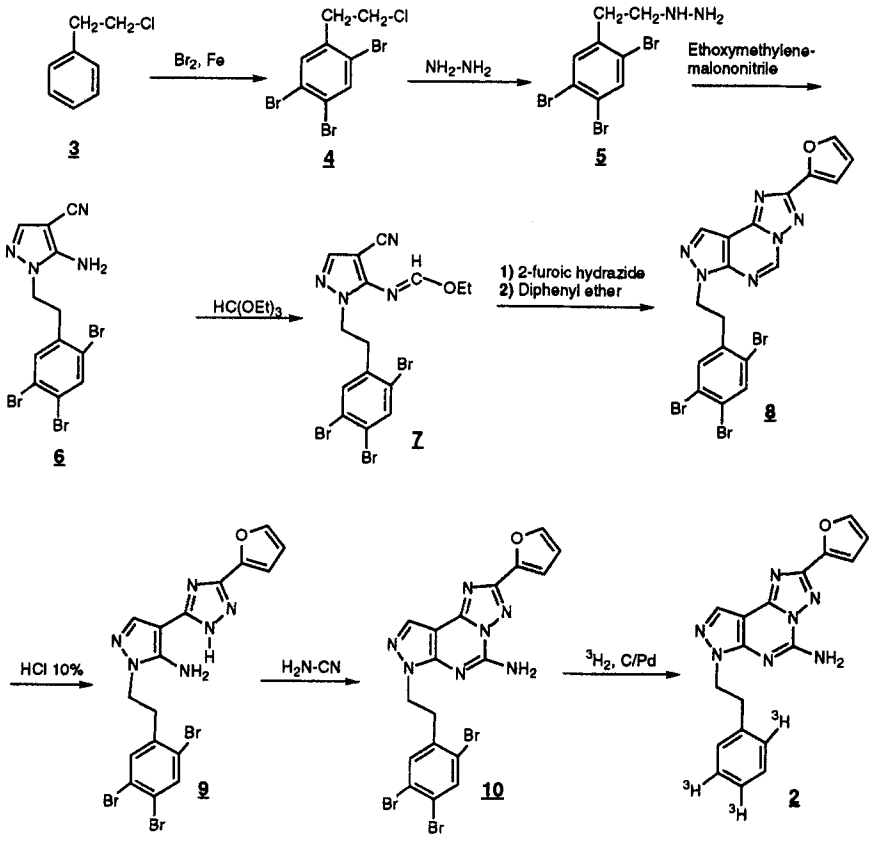
CHEMISTRY

The synthesis of labeled ^{3}H-SCH 58261 **2** was performed following the generale procedure depicted in scheme 1.

The commercially available β-phenethylchloride **3** was brominated, in presence of a catalytic amount of iron, to give the desired tribromo derivative **4**. Treatment of this compound with hydrazine hydrate in refluxing ethanol for three days afforded **5**, which was reacted with ethoxymethylenemalononitrile to obtain the 1-substituted pyrazole **6**.

The designed compound **10** was synthesized according to Gatta et al.(7) for the synthesis of pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidines. Imidate **7**, obtained by refluxing **6** in triethylorthoformate, was reacted with 2-furoic acid hydrazide in refluxing 2-methoxyethanol to provide the pyrazolo[3,4-d]pyrimidine intermediate. The latter compound was converted through a thermally induced cyclization in diphenyl ether to the derivative **8** in good overall yield. Treatment of **8** with dilute hydrochloric acid at reflux temperature induced pyrimidine ring opening to furnish the tribromo derivative **9** in good yield.

Scheme 1



This derivative was converted into the compound **10** by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 160 °C.

Tritiation of **10** was performed with tritium gas in the presence of 10% Pd/C. Final product was purified by HPLC on a Zorbax silica column to give the title compound **2** with radiochemical purity of 99% and specific activity of 68.6 Ci/mmol.

DISCUSSION

As reported above, we have performed an efficent synthetic method for the preparation of a new labeled probe for the A_{2A} adenosine receptor subtype (8 steps, overall yield 14.3 %).

As extensively reported elsewhere (8), radioligand binding assay showed that ^{3}H-SCH 58261 labels a single class of receptors in rat striatal membranes with a specific binding of 92%. K_d and apparent B_{max} values were 0.70 nm and 971 fmol/mg of protein, respectively. a representative saturation isotherm of ^{3}H-SCH 58261 binding to rat striatal membranes (8).

Several adenosine agonists inhibited ^{3}H-SCH 58261 binding to rat striatal membranes with the following order of potency: 5'-N-ethyl-carboxamidoadenosine (NECA) > 2-[4-(2-carboxyethyl)-phenetyl-amino]-5'-N-ethylcarboxamidoadenosine (CGS 21680) > 2-phenylaminoadenosine (CV 1808) > R-N^6-2-phenylisopropyladenosine (R-PIA) > cyclohexyladenosine (CHA) > S-N^6-2-phenylisopropyladenosine (S-PIA). The ability of several xanthine and non xanthine adenosine receptor antagonists in competing ^{3}H-SCH 58261 binding was also examined. Their order of potency was: 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-c]quinazoline (CGS 15943) > SCH 58261 > KF 17837S = xanthine amine congener (XAC) > 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

^{3}H-SCH 58261 binding for both adenosine agonists and antagonists showed an order of potency similar to that observed using the agonist radioligand ^{3}H-CGS 21680 and was consistent with a selective interaction at the A_{2A} receptors.

For these reasons, ^{3}H-SCH 58261, which labels the A_{2A} striatal receptor, appears to be an excellent probe for studying this adenosine receptor subtype in mammalian brain. Clear advantages over other A_{2A} antagonist radioligands proposed for this purpose (4,9,10) include high receptor affinity, good selectivity and low non-specific binding. Moreover, ^{3}H-SCH 58261 possesses the characteristics to become a useful tool for the investigation of A_{2A} receptors distributed in peripheral tissues.

EXPERIMENTAL

General: Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with iodine or aqueous potassium permanganate. Infrared spectra (IR) were measured on a Perkin Elmer 257 instruments. UV absorption spectra were recorded with a Perkin Elmer λ 19 spectrometer. ^{1}H NMR and ^{13}C NMR were determined in DMSO-d_6 solution with a Bruker AC 200 spectrometer, peaks positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and J values are given in Hz. Light petroleum refers to the fractions boiling at 40-60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed with Merck 60-200 mesh silica gel. All solvents were from Carlo Erba Reagenti, Milano. All reagents were from Aldrich Chemical Co. All products reported showed IR and ^{1}H NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara. Tritiation was performed by Dupont-New England Nuclear Products (Boston, MA), HPLC

and TLC purifications were carried out using a Zorbax RX C8 silica column and silica gel GHLF, respectively. Tritium gas was obtained from EGG Mouds.

2-(2',4',5'-tribromo)-phenylethyl chloride 4 The 2-phenylethyl chloride **3** (2.5 ml, 0.02 mol) was dissolved in dry CCl_4 (20 ml) and iron powder (780 mg, 0.014 mol) was added. The resulting suspension was cooled at 0°C and a solution of bromine (4.3 ml, 0.088 mol) in dry CCl_4 (5 ml) was added dropwise and stirred at room temperature for 18 h and refluxed for 4 h. Then the mixture was washed with a solution of sodium thiosulfate to eliminate the bromine excess. The organic phase was dried and evaporated under reduced pressure to afford the compound **4** as a brown oil (5.28 g, 70%). IR (neat) cm^{-1}: 1610, 1520, 1415; ^1H NMR ($CDCl_3$) δ: 3.11 (t, 2H, J=8); 3.71 (t, 2H, J=8); 7.51 (s, 1H); 7.79 (s, 1H). Anal. Calcd. for $C_8H_6Br_3Cl$: %C 25.68; %H 1.62. Found: %C 25.62; %H 1.60.

2-(2',4',5'-tribromo)-phenylethyl hydrazine hydrochloride 5 Tribromo derivative **4** (5g, 13.2 mmol) was dissolved in absolute ethanol (20 ml) and hydrazine hydrate (3.2 ml, 66 mmol) was added. The resulting solution was refluxed for three days, then the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (20 ml) and washed several times with saturated NaCl (7x10 ml). The organic phase was dried and concentrated under reduced pressure. The remaining thick oil was diluted with dry ether (30 ml) and treated at 0°C with anhydrous 2M HCl in ether (6.6 ml), to give the correspondent hydrochloride salt **5** (4.27 g, 85%) as a white solid. mp 190-192°C (CH_2Cl_2-ether). IR (KBr) cm^{-1}: 3550-2950, 1615, 1510, 1415; ^1H NMR (DMSO d_6) δ: 2.91-2.97 (m, 2H); 3.07-3.12 (m, 2H); 3.80 (bs, 1H); 7.60 (s, 1H); 8.01 (s, 1H); 8.80 (bs, 3H). Anal. Calcd. for $C_8H_{10}Br_3ClN_2$: %C 23.66; %H 2.48; %N 6.90. Found: %C 23.60; %H 2.51; %N 6.87.

1-[2-(2',4',5'-tribromo)-phenyl-ethyl]-4-cyano-3-amino pyrazole 6 The hydrazine hydrochloride **5** (0.2 g, 0.53 mmol) was dissolved in EtOH (5 mL), triethylamine (0.15 ml, 1.06 mmol) and ethoxymethylene malono nitrile (65 mg, 0.53 mmol)) was added in little portions. Then the mixture was heated at 70°C for 18 h before evaporating the solvent. The solid residue was purified by chromatography (EtOAc/light petroleum 1:4) to afford the product **6** as a light yellow solid (0.183 g, 77%). mp 202-205°C (CH_2Cl_2-ether). IR (KBr) cm^{-1}: 3300-3150, 2200, 1610, 1520, 1410; ^1H NMR (DMSO d_6) δ: 1.41 (bs, 2H), 3.14-3.21 (m, 2H); 4.12 (t, 2H, J=8); 7.29 (s, 1H); 7.52 (s, 1H); 7.82 (s, 1H). Anal. Calcd. for $C_{12}H_9Br_3N_4$: %C 32.30; %H 2.03; %N 12.56. Found: %C 32.38; %H 2.01; %N 12.57.

1-[2-(2',4',5'-tribromo)-phenyl-ethyl]-4-cyano-3-ethoxymethylenamino pyrazole 7 The amino pyrazole **6** (0.7 g, 1.55 mmol) was dissolved in triethyl orthoformate (20 ml) and the

resulting solution was refluxed under nitrogen for 8 h. Then the solvent was removed under vacuum and the oily residue was dissolved in ether and roughly purified on silica gel (EtOAc-light petroleum 1:1)to afford the corresponding iminoethers 7 as a dark oil (0.685 g, 88%). IR (neat) cm^{-1}: 2220, 1630, 1550, 1410, 1370; ^1H NMR ($CDCl_3$) δ: 1.38 (t, 3H, J=8), 3.15 (t, 2H, J=6.5); 4.22-4.35 (m, 4H); 7.06 (s, 1H); 7.69 (s, 1H); 7.75 (s, 1H); 8.05 (s, 1H). Anal. Calcd. for $C_{15}H_{13}Br_3N_4O$: %C 35.87; %H 2.61; %N 11.16. Found: %C 35.94; %H 2.59; %N 11.17.

7-[2-(2',4',5'-tribromo)-phenyl-ethyl]-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine 8 Iminoether 7 (1g, 1.98 mmol)) was dissolved in 2-methoxyethanol (10 ml) and 2-furoic acid hydrazide (0.274 g, 2.18 mmol) was added. The mixture was refluxed for 6 h, then, after cooling, the solvent was removed under reduced pressure and the dark oily residue was dissolved in diphenyl ether (20 ml) and heated at 260°C using a Dean-Stark for the azeotropic elimination of water produced in the reaction. After 1.5 h, the mixture was poured onto hexane (150 ml) and cooled. The precipitate was filtered off and purified by chromatography (EtOAc-light petroleum 1:1) to afford 8 as a light yellow solid (0.725 g, 65%). mp 216-218°C (CH_2Cl_2-ether). IR (KBr) cm^{-1}: 1620, 1550, 1510, 1415, 1350; ^1H NMR ($CDCl_3$) δ: 3.36 (t, 2H, J=6.5); 4.79 (t, 2H, J=6.5); 6.59-6.62 (m, 1H); 6.98-7.03 (m, 2H); 7.30 (s, 1H); 7.65 (d, 1H, J=0.8); 8.41 (s, 1H); 9.04 (s, 1H). Anal. Calcd. for $C_{18}H_{11}Br_3N_6O$: %C 38.31; %H 1.97; %N 14.90. Found: %C 38.34; %H 1.93; %N 14.87.

1-[2-(2',4',5'-tribromo)-phenylethyl]-4-[3 (2-furyl)-1,2,4-triazol-5-yl]-5-amino pyrazole 9 A solution of 8 (60 mg, 0.1 mmol)) in aqueous 10% HCl (5 ml) and dioxane (10 ml) was refluxed for 3 h. Then the solution was cooled and basified with conc. ammonium hydroxide at 0°C. The compounds were extracted with EtOAc (3x10 ml), the recombined organic layers were dried and evaporated under vacuum. The residue was purified by chromatography (EtOAc-light petroleum 1:1) to afford 9 as a white solid (55 mg, 98.7%). mp 187-188°C (CH_2Cl_2-ether). IR (KBr) cm^{-1}: 3400-3150, 1645, 1560, 1500, 1410, 1320; ^1H NMR ($CDCl_3$) δ: 3.18 (t, 2H, J=6.8); 4.19 (t, 2H, J=6.8); 5.17 (bs, 2H); 6.47-6.49 (m, 1H); 6.97 (d, 1H, J=4); 7.42 (s, 1H); 7.49 (s, 1H); 7.76 (s, 1H); 7.84 (s, 1H); 10.50 (bs, 1H). Anal. Calcd. for $C_{17}H_{13}Br_3N_6O$: %C 36.83; %H 2.37; %N 15.17. Found: %C 36.87; %H 2.41; %N 15.15.

5-Amino-7-[2-(2',4',5'-tribromo)-phenylethyl]-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c] pyrimidine 10 To a solution of 9 (0.92 g, 1.65 mmol) in N-methyl pyrrolidone (10 ml), cyanamide (0.416 g, 9.9 mmol) and p-toluenesulfonic acid (0.47 g, 2.47 mmol) were added and the mixture was heated at 160°C for 4 h. Then cyanamide (0.416 g, 9.9 mmol) was added again

and the solution was heated overnight. Then the solution was diluted with EtOAc (60 ml) and the precipitate (excess of cyanamide) was filtered off; the filtrate was concentrated under reduced pressure and washed with water (3x20 ml). The organic layer was dried and evaporated under vacuum. The residue was purified by chromatography (EtOAc-light petroleum 1:1) to afford the final product **10** as a white solid (0.623 g, 65%). mp 285°C (DMF-water). IR (KBr) cm^{-1}: 3300-3150, 1650, 1540, 1500, 1430, 1310; ^1H NMR (DMSO d_6) δ: 3.24 (t, 2H, J=6); 4.50 (t, 2H, J=6); 6.72-6.74 (m, 1H); 7.22 (d, 1H, J=3); 7.47 (s, 1H); 7.94 (s, 1H); 7.99 (s, 1H); 8.07 (bs, 2H); 8.15 (s, 1H). Anal. Calcd. for $C_{18}H_{12}Br_3N_7O$: %C 37.31; %H 2.09; %N 16.93. Found: %C 37.33; %H 2.11; %N 16.88.

5-Amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c] pyrimidine [Phenyl-^{3}H(N)] 2 A solution of tribrominated precursor **10** (25mg, 0.04 mmol) in DMF (2 ml) and THF (2 ml) containing triethylamine (0.05 ml) was reacted at room temperature with 10% Pd/C (20 mg) and tritium gas (60 Ci of Carrier free gas (5£ Ci/mmol) at 1 atm pressure. After 24 h, the catalyst was filtered off and labile tritium was removed by several evaporations with methanol/THF (1:1). The resulting crude product was diluted in methanol and purified by HPLC on a Zorbax Silica column (9.4 mm x 25 cm I.D.) eluted with hexane/ethyl acetate (60:40). The combined fractions were concentrated at rotary evaporator, and diluted with ethanol to give the title compound **2** (85% yield, 21% radiochemical yield based on tribrominated precursor **10**) with radiochemical purity of 99% determined by liquid scintillation assay (HPLC, Zorbax RX C8 column eluted with 1% Tiethylammonium acetate pH=4/MeCN (1:1), TLC, silica gel GHLF eluted with ethyl acetate/hexane (2:1), and specific activity (determined by UV spectroscopy) of 68.6 Ci/mmol).

REFERENCES

1. Fredholm B.B., Abbracchio M.P., Burnstock G., Daly J.W., Harden T.K., Jacobson K.A., Leff P., Williams M. Pharmacol. Rev. 46: 143 (1994).

2. Nonaka Y., Shimada J., Nonaka H., Nonaka H., Koike N., Aoki N., Kobayashi H., Kase H., Yamaguchi K., Suzuki F. J. Med. Chem. 36: 3731 (1993).

3. Poucher S.M., Keddie J.R., Singh P., Stoggal S.M., Caulkett P.W.R., Jones G., Collis M.G. Br. J. Pharmacol. 115: 1096 (1995).

4. Nonaka H., Ichimura M., Takeda M., Nonaka Y., Shimada J., Suzuki F., Yamaguchi K., Kase H. Eur. J. Pharmacol. 267: 335 (1994).

5. Baraldi P.G., Manfredini S., Simoni D., Zappaterra, L., Zocchi C., Dionisotti, S., Ongini E., Bioorg. & Med. Chem. Lett. 4, 2539 (1994).

6. Zocchi C., Ongini E., Conti A., Monopoli A., Negretti A., Baraldi P.G., Dionisotti S. J. Pharmacol. Exp. Ther. In Press.

7. Gatta F., Del Giudice M.R., Borioni A., Borea P.A., Dionisotti S., Ongini E. Eur. J. Med. Chem. 28: 569 (1993).

8. Zocchi C., Ongini E., Ferrara S., Baraldi P.G., Dionisotti S. Br. J. Pharmacol. In Press.

9. Bruns R.F., Fergus J.H., Badger E.W., Bristol J.A., Santay L.A., Hartman J.D., Hays S.J., Huang C.C. Naunyn-Schmiederberg's Arch. Pharmacol. 335: 59 (1987).

10. Ji X.D., Stiles G.L., Van Galen P.J.M., Jacobson K.A. J. Recept. Res. 12: 149 (1992).