SYNTHESIS OF A RADIOBROMINATED ANALOG OF SCH 23390, A SELECTIVE DOPAMINE D_1/DA_1 ANTAGONIST

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SUMMARY

A radiobrominated analog of SCH 23390, a selective dopamine D_1/DA_1 antagonist was prepared. Radiochemical yields of up to 60% were obtained within 15 minutes reaction at room temperature. The synthesis was validated by performing both physical (NMR and mass spectroscopy) and biological tests on the product. Preliminary studies suggest that ^{75}Br-labelled SCH 23390 analog would be a useful ligand for the studies of CNS dopamine D_1 receptors by positron emission tomography.

Keywords: Radiobromination, SCH 23390, dopamine D_1 receptors, positron emission tomography.

INTRODUCTION

Dopamine (DA) receptors in the CNS have been hypothesized to be involved in the pathophysiology of several neuropsychiatric disorders (1-4). The past few years have seen the development of high affinity and very selective ligands which are useful as probes of DA receptors. Studies using these probes have led to proposals on multiple classes of DA receptors. Kebabian and Calne have subdivided DA receptors into those which have linkage to the enzyme adenylate cyclase (AC), D_1 receptors, and those which do not, the D_2 receptors (5).

Peripheral DA receptors have likewise been classified into two subtypes, DA_1 and DA_2, by Goldberg et al. (6). This classification was based primarily on pronounced differences in the potency series of agonists active on both receptors and the demonstration of selective antagonism. The pharmacology of DA_1 and DA_2 receptors are similar but not exactly identical to that of CNS

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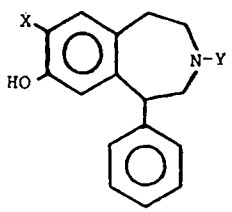
dopamine D_1 and D_2 receptors (7).

It is evident that ligands capable of differentiating classes of DA receptors are needed to clarify the number of subtypes, their function and location, and their role in movement and psychiatric disorders.

Recently, a group of benzazepines (Figure 1) have been shown to be highly selective ligands for DA D_1 receptors, either as agonists or antagonists (7). One of these compounds, SCH 23390, has been shown to be an excellent probe for D_1 receptors (8-11) with weak or negligible interactions with acetylcholine, norepinephrine, GABA, histamine, and serotonin receptors (10). The 7-bromo analog of SCH 23390, SCH 24543 (also known as SKF 83566), has also been shown to be a specific and stereoselective antagonist of striatal D_1 receptors (12, 13) and a selective vascular DA_1 antagonist (14,15).



X	Y	NAME	CLASS
-OH	-H	SKF 38393	Agonist
-Cl	$-CH_3$	SCH 23390	Antagonist
-Br	$-CH_3$	BrSCH (or SKF 83566)	Antagonist

Figure 1. Benzazepine Compounds - Specific Dopamine D_1 Ligands

We have prepared a positron emitting 7-bromo analog of SCH 23390 labelled with either ^{75}Br or ^{76}Br. Our goal in preparing this radioligand is to assess its potential for brain imaging using positron emission tomography (PET). To date, PET studies on CNS dopamine receptors have utilized positron emitting analogs of butyrophenones, such as spiroperidol which are predominantly D_2 antagonists (16-22). Non-invasive studies of both classes of cerebral DA receptors may provide valuable insights into their role of normal and diseased states.

METHODS AND MATERIALS

I. Production of Bromine Radioisotopes.

^{75}Br ($t_{1/2}$ = 100 min) and ^{76}Br ($t_{1/2}$ = 16 hr), both positron emitters, were prepared via the (p,α) or (d, nα) reaction on enriched ^{78}Kr gas target (23). Details of this preparation will be reported elsewhere.

II. Synthesis of 7-bromo-8-hydroxv-2,3,4,5-terahydro-3-methyl-1-phenyl-1H-3-benzazepine (BrSCH).

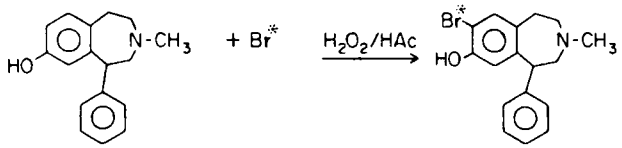
Milligram quantities of BrSCH were prepared by the electrophilic bromination of SCH 23982 using stoichiometric amounts of Br^- and glacial acetic acid:H_2O_2 (2:1 v/v) from a previously reported procedure (24,25). The extent of product formation was followed by analysis of the reaction mixture at time intervals using high performance liquid chromatography (HPLC) in a system previously calibrated with authentic samples. When the reaction reached equilibrium, separation of the desired product was carried out by HPLC using a semi-preparative column. The purified product was then recrystallized from methanol. Both this product and an authentic BrSCH sample, SKF R-83566 (provided by Smith Kline & French Laboratories, Philadelphia, PA), were subjected to mass spectrometric analysis, using Cf-252 plasma desorption mass spectrometer (Argonne National Laboratory) and an EI-MS (The University of Chicago). Proton NMR analysis of both samples was also perfomed.

III. Biological Evaluation of BrSCH using the Method of DA-induced Vasodilation in Anesthetized Dog.

Different doses of the test drugs, SCH 23390, BrSCH, and SKF 83566 were co-injected with 48 nanomoles of DA into the renal artery of a pentobarbital-anesthetized dog which had been pre-treated with phenoxybenzamine, an alpha-adrenergic blocking agent. The percent inhibition of DA induced renal vasodilation was then measured for each dose of the test drugs using an electromagnetic flow-meter and procedures reported by Goldberg et al. (26). The selectivity of BrSCH as a DA_1 antagonist was also tested by measuring its potency as a blocker of dipropyldopamine-induced femoral vasodilation, a DA_2 mediated response, in an anesthetized dog.

IV. Radiosynthesis of ^{75}Br or ^{76}Br labelled BrSCH.

Glacial acetic acid and hydrogen peroxide (2:1 v/v) were premixed at least one hour before use (27). $^{75}Br^-$ or 76-bromide was dissolved in ca. 200 ul 1M H_2SO_4 with 10-30 ug Br^- carrier added. One milligram SCH 23982 was dissolved in the HOAc-H_2O_2 mixture and the two solutions were mixed. Reaction time was usually twenty minutes at room temperature after which the mixture was diluted to 2 ml with water and injected directly into the HPLC system. UV absorption at 280 nm and radioactivity, measured by a flow detector, were simultaneously recorded.



Scheme 1

RESULTS AND DISCUSSION

Electrophilic radiobromination using glacial acetic acid and H_2O_2 mixture, first reported by Katzenellenbogen et al. (24) and used in the preparation of a radiobrominated analog of spiperone (25), was found to be equally effective in the preparation of ^{75}Br or ^{76}Br-labelled 7-bromo analog of SCH 23390 (Scheme 1). Radiochemical yields of up to 60% were obtained within 15 minutes reaction at room

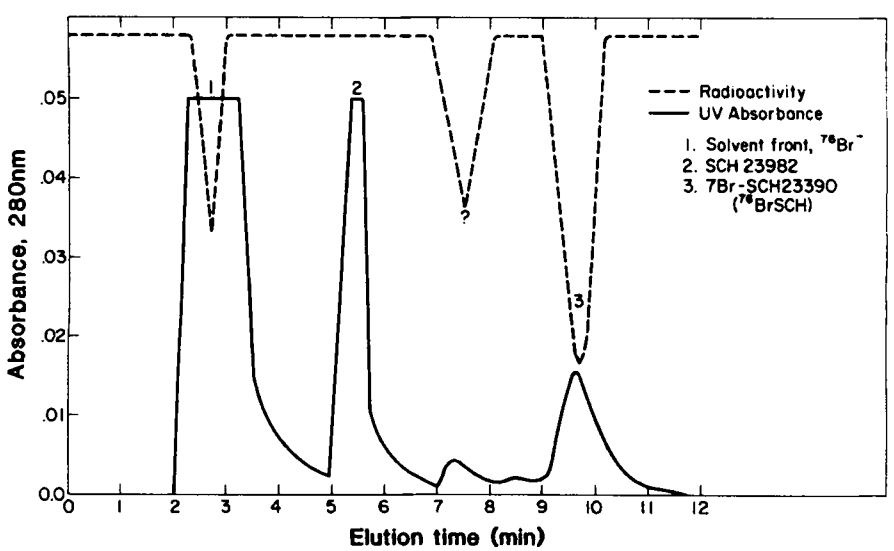


Figure 2. Separation of Radiobrominated SCH 23390 analog by high performance liquid chromatography. Conditions : Alltech C-18 column (25 cm L x 4.6 mm id), ethanol:0.2M NH_4OAc:HOAc (60:39:1) mobile phase, 1.4 ml/min flowrate.

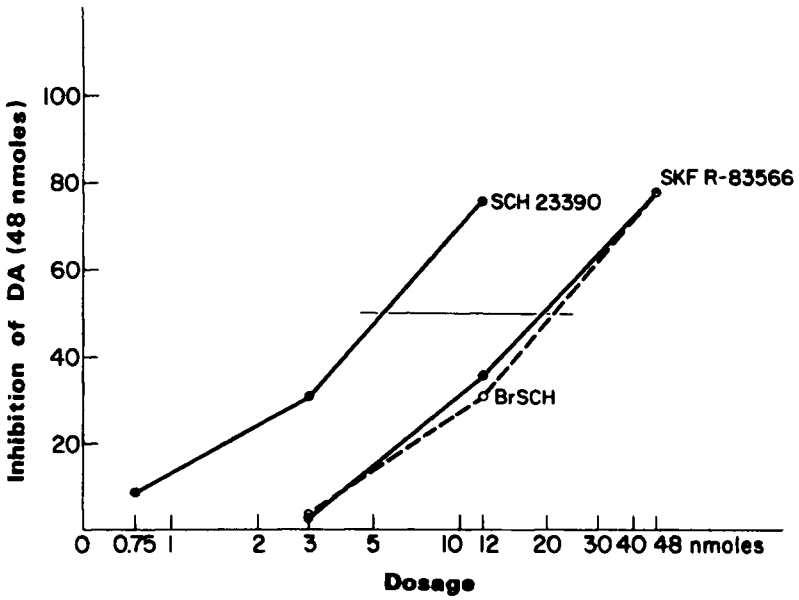


Figure 3. Comparison of the pharmacological activity of SCH 23390, SKF R-83566, and the BrSCH product using a bioassay method. (See text for details).

temperature. No improvement in the yields was observed with longer reaction times. This reaction was carried out under carrier-added conditions and the specific activity range of the product at the end of synthesis was 10-20 Ci/mmole. Under no carrier-added conditions, reduction in radiochemical yields was observed similar to that reported in a previous study (29). Studies are currently under way to reduce or eliminate the need for carrier addition and also the use of higher purity reagents in order to improve the specific activity of the product.

A radioactive by-product of the reaction, amounting to a radiochemical yield of about 30% but with a small UV absorption, was found to elute between the starting material and the product (Figure 2). Using a less polar mobile phase, the starting material eluted at 9 minutes, the unknown by-product at 12.5 minutes and BrSCH at 35 minutes (not shown). Recently, Wyrick et al. (28) reported that the reaction of Br_2 and SCH 23390 in glacial acetic acid resulted in bromination at the 9-position. Thus it is very likely that the minor product in our synthesis is 9-bromo-SCH 23982, since the same peak was observed, in addition to the 7-bromo product, in the bromination of SCH 23982 with Br_2 in glacial acetic acid. However, the baseline separation that is achieved by the HPLC purification gives a pure product and this has been confirmed by the analysis of the product by analytical HPLC.

Milligram quantities of the product were synthesized using scaled-up amounts of the reactants. Preparative HPLC purification of the product gave a compound whose mass spectrum, using two techniques, Cf-252 plasma desorption and electron-ionization, were identical to an authentic BrSCH sample, SKF R-83566. Nuclear magnetic resonance spectroscopic analysis likewise confirmed that our product was identical to SKF R-83566.

In addition to physical methods, our product was also characterized in terms of its pharmacological activity using a bioassay technique developed by Goldberg et al. (26). This method measured the inhibition of DA induced renal vasodilation in an anesthetized dog using different doses of our BrSCH product, SKF R-83566 and SCH 23390. As shown in Figure 3, our product antagonized DA with an ID_{50}, the dose which inhibits 50% of DA activity, of approximately 20 nmolar. This is identical to that observed with SKF R-83566. It is interesting to note that SCH 23390 is about four times more potent (ID_{50} = 5 nmolar) than BrSCH according to this bioassay method. The selectivity of BrSCH as a DA_1 antagonist was shown by the failure of the drug, up to a dose of 3000 nmol, to block dipropyldopamine-induced femoral vasodilation, a DA_2 effect (30).

Preliminary studies on the biodistribution of BrSCH in the mouse brain showed that the uptake of this drug is consistent with mediation by dopamine receptor (in preparation). These mice studies showed that the D_1 sites were not saturated when the animals were given 100 ug/kg Br-75-BrSCH. This could be related to the higher density D_1 in the striatum compared to D_2 sites found by Boyson et al. (31). PET studies on the distribution of [^{76}Br]-BrSCH in the brain of a rhesus monkey provided evidence that D_1 sites like D_2 sites are localized in the basal ganglia (32).

In summary, a positron emitting 7-bromo analog of SCH 23390 has been prepared. With this radioligand, binding studies using either non-invasive techniques such as PET or postmortem sample analysis may help elucidate the role of CNS DA receptors, both D_1 and D_2, in the pathophysiology of motor and psychiatric disorders.

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