Synthesis and labelling of epidepride

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Abstract S-(-)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (proposed generic name, epidepride) is a very potent dopamine D2 antagonist. It was synthesized by five steps from 3-methoxysalicylic acid. [^{131}I]epidepride was obtained in 97.3% radiochemical yields from the corresponding 5-(tributyltin) derivative using hydrogen peroxide as the oxidant. The aryltin precursor was prepared from non-labelled epidepride by palladium-catalyzed stannylation using bis(tri-n-butyltin) in triethylamine. [^{131}I]epidepride was stable under 4°C, and partition coefficient was 72.3 at pH 7.40. The biodistribution study in rats exihibited high localization in the striatum of the brain with the striatum/cerebellum ratio reaching 237/1 at 320 min postinjection. All these results suggest that [^{131}I]epidepride may be used widely as a useful dopamine D2 receptor imaging agent for SPECT.

Keywords [^{131}I]epidepride, Dopamine D2 antagonist, Synthesize, Aryltin precursor, Biodistribution, SPECT

***CLC* numbers** *O628.5*$^{+}$*1, O629.7, O621.3*

1 INTRODUCTION

Nuclear medicine is rapidly establishing itself as an important tool in the *in vivo* study of brain neurochemistry. In particular, several radioligands for the dopamine D2 receptor have been developed and these have offered valuable new insights into the role of dopamine in disorders of the locomotor function, psychiatry and genetic disease.

Epidepride, S-(-)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide, is a potential dopamine D2 antagonist. It has high affinity (Kd=24pmol/L) for the D2 receptor and low lipophilicity (lgKw=2.05), the striatum/cerebellum ratio can reach 234/1 in rats. [^{123}I] and [^{11}C] epidepride have been successively used in dopamine D2 receptor SPECT and PET imaging, as good clinical tools in brain research. Particularly in extrastriatal imaging, diagnosis of pituitary adenomas and prediction of the response of pituitary adenomas to dopamine agonist therapy, [^{123}I]epidepride is better than [^{123}I]IBZM[1~4].

In China, there is no report of epidepride and [^{123}I] is not easy to be available. So we worked to develop [^{131}I] epidepride as dopamine D2 receptor SPECT imaging agent. Recently we have synthesized epidepride. Detailed synthesis and labeling of epidepride are given in this paper.

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2 MATERIALS

2.1 Instruments

A YANADIMOTO melting point instrument (made in Japan), FT-IR spectrometer (made in USA), PE2400 elemental analyzer (made in USA), Varian Model AM-400 Proton Nuclear Magnetic Resonance Spectrometer (made in USA), Bio-Rad HPLC (made in USA), and Pakard Cobra γ-counter(made in USA) were used in the present work.

2.2 Reagents

s-(-)-N-ethyl-2-(aminomethyl)pyrrolidine was prepared in this group[5]. 3-methoxysalicylic acid, Bu_6Sn_2 and $(Ph_3P_4)Pd$ were from Aldrich. Other reagents were from Shanghai Chemical Co. and all were of reagent grade.

3 METHODS AND RESULTS

3.1 The preparation of epidepride and its aryltin precursor[6~9]

The synthesis is followed by the sequences of reaction outlined in Fig.1.

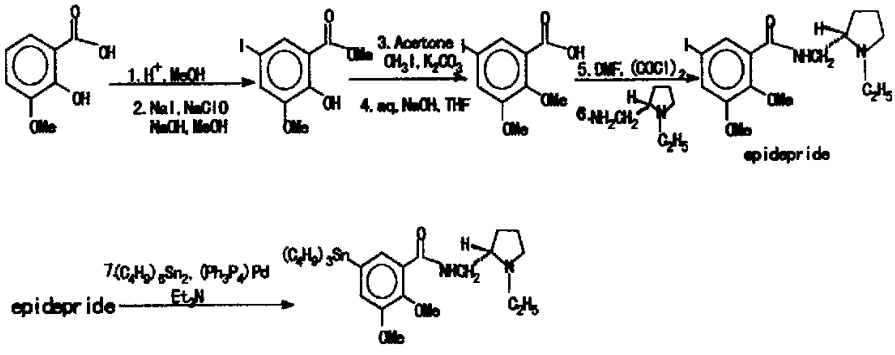

Fig.1 Preparation scheme of epidepride and its aryltin precursor

3.1.1 3-methoxysalicylic acid, methyl ester (1) The 3-methoxy-salicylic acid (15.03g, 89.4 mmol) was dissolved in methanol(250 mL), and a catalytic amount of 18 mol/L H_2SO_4(1 mL) was added. The mixture was warmed to reflux and stirred for 58 h.The reaction was cooled and the solvent was evaporated. The residue was dissolved in CH_2Cl_2(200 mL) and washed with saturated $NaHCO_3$(100 mL), the aqueous layer was back-extracted with CH_2Cl_2(3×25 mL),and the combined organic layers were dried, filtered, and concentrated to a brown solid. Distillation of the crude solid under reduced pressure afforded **1** as a white solid, mp 56~58°C.

3.1.2 Methyl 5-Iodo-3-methoxysalicylate(2) Compound **1** (9.2g, 50.5 mmol) was dissolved in methanol. Sodium iodide (11.36 g, 75.73 mmol) and sodium hydroxide(2.02 g,

50.5 mmol) were added, and the solution was cooled to 0°C. To this solution was added aqueous sodium hypochlorite (142mL) dropwise. The pale yellow slurry mixture was stirred for 1 h at 0~3°C and then treated with 10% aqueous sodium thiosulfite. The mixture was adjusted to pH 7 using HCl. Ether was added, and the layers were separated. The ether layer was washed with brine and dried over anhydrous sodium sulfate. After the ether was evaporated, the crude orange solid was crystallized from ethyl acetate/hexane(2/3),producing **2** as a white crystal, mp:108~110°C.

3.1.3 Methyl 5-Iodo-2,3-dimethoxysalicylate(3) Compound **2** (1.13 g, 3.66 mmol) was dissolved in dried acetone, and potassium carbonate(1.5 g, 10.86 mmol) was added, followed by methyl iodine(2.8 g, 19.68 mmol). The mixture was refluxed with stirring for 48 h. Acetone was evaporated and the residue was dissolved in dichloromethane, washed several times with water, and dried over anhydrous sodium sulfate. Dichloromethane was evaporated under reduced pressure. The product **3** was obtained as a white solid, mp:50~52°C.

3.1.4 5-Iodo-2,3-dimethoxybenzoic acid(4) The ester **3** (0.88 g, 3.43 mmol)was dissolved in THF, and then 0.5 mol/L NaOH (100 mL) was added, and the solution was warmed with stirring for 2 h. The product **4** was isolated by first washing the aqueous reaction mixture with Et_2O, back-extracting the Et_2O layer with 1 mol/L NaOH(100 mL), acidifying the combined aqueous layers (0°C) to pH 2 with H_3PO_4 and then extracting the acidic aqueous phase with chloroform (1×100 mL, 5×25 mL). The organic portion was dried and filtered, and the solvent was evaporated to provide **4** as a white solid, mp:124~127°C.

3.1.5 Epidepride(5) Compound **4** (600 mg, 1.95 mmol) was dissolved in CH_2Cl_2(10 mL), and to this solution was added DMF(2 drops) and oxalyl chloride (0.425 mL, 4.87 mmol). The mixture was stirred at room temperature(1 h), and the solvent was removed in vacuo affording the corresponding acid chloride as a yellow residue (not characterized). The residue was dissolved in CH_2Cl_2(10 mL), and the s-(-)-N-ethyl-2-(aminomethyl) pyrrolidine in CH_2Cl_2(5 mL) was added. The reaction mixture was allowed to stir for 1 h(20°C), and the solvent was removed under reduced pressure. The crude product was redissolved in CH_2Cl_2(20 mL) and washed with 1 mol/L NaOH(20 mL), and the phases were separated. The organic portion was dried and filtered, and the solvent was removed in vacuo to afford a yellow oil. That was purified by column chromatography (silica gel; 1:9 MeOH-CH_2Cl_2) to yield epidepride as a yellow oil. IR(cm^{-1}):3369,1658; ^1H NMR($CDCl_3$): δ(ppm) 8.30(br, s, 1H); 8.00(d, 1H); 7.25(d, 1H,); 3.86(s, 3H); 3.85(s, 3H); 3.75-1.58(m, 11, pyrrolidine-H); 1.11(t, 3, N-Et).MS(m/z):419(M+ 1), 98(100%) Theo Anal: C 45.9%, H 5.54%, N 6.7%; Found: C 45.6%, H 5.52%; N 6.5%.

3.1.6 S-5-(tri-n-butyltin)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3 -dimethoxybenzamide(6) To a solution of epidepride (0.42 g, 1.0 mmol) in dry Et_3N(20 mL) was added solid $(Ph_3P)_4Pd$ (0.06 g, 0.05 mmol) followed by Bu_6Sn_2(0.58 g, 1.0 mmol). The mixture was heated to reflux for 3.5 h. After cooling, the solvent was removed by evaporation and the residual oil was subjected to chromatographic separation on silica gel in

hexane-EtOAc(1:1) to give the product 6. IR(cm^{-1}):3378, 2958-2799, 1658

3.2 The preparation of [^{131}I]epidepride[1]

Aqueous hydrogen peroxide (20 μL, 3%V/V) was added to a mixture of 20 μL of compound 6 (1 mg/mL of EtOH), 20μL of 4 mol/L HCl, and 10 μL of ^{131}I-sodium iodide (37~74MBq, no carrier added) in a sealed vial . The reaction was allowed to proceed at 25°C for 15 min, and then it was terminated by the addition of 20 μL saturated sodium metabisulfite solution. The reaction mixture was neutralized via the addition of 20 μL of 4 mol/L ammonium hydroxide.

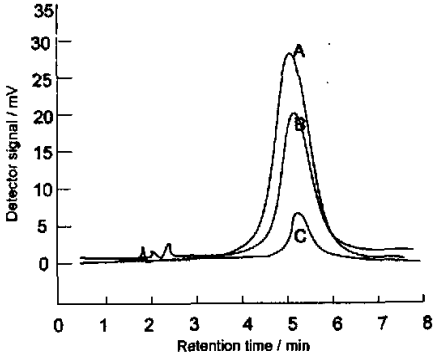

Fig.2 Overlaid chromatograms:
(A) Radioactivity of 370 KBq ^{131}I-epidepride,
(B) 2μL of 2.39 mmol epidepride,
(C)UV absorption at 235 nm of ^{131}I-epidepride

3.3 Determination of radiochemical purity (*RCP*) and radiolabelling yield (*RLY*)

Thin-layer chromatography (TLC): silica gel plate, and silica gel paper, with developing system of $CH_2Cl_2/CH_3OH(9/1)$. The R_f values of [$^{131}I^-$] and [^{131}I] epidepride were 0.0~0.1 and 0.3~0.5, respectively. *RCP* and *RLY* of [^{131}I]epidepride were over 95%. High performance liquid chromatography (HPLC):C-18 reverse-phase column (ϕ4 mm×300 mm) using the eluent (ethanol/0.01 mol/L phosphate buffer pH 6.3=7/3) at a flow rate of 1.0 mL/min. The result was described in Fig.2

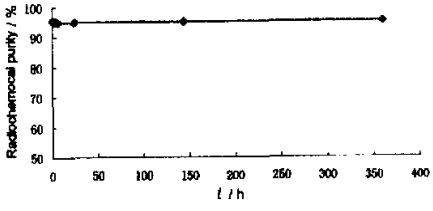

Fig.3 Stability of ^{131}I-epidepride solution

3.4 The stability of [^{131}I]epidepride

[^{131}I] epidepride was diluted by saline (including some stabilizer and ethanol), then stored at 4°C. Radiochemical purity was measured with paper chromatography (silica gel paper, CH_2Cl_2/CH_3OH=9/1) at 0, 2 h, 4 h, 6 h, 24 h, 6 d and 15 d. The result was given in Fig.3.

3.5 Determination of partition coefficient

To a mixture of phosphate buffer (3 g, 0.1 mol/L, pH7.4) and n-octanol (3 g) was added [^{131}I]epidepride (*RCP* >95%, approximately 5×10^5 counts/min), vortexed each for 3× 1 min, centrifuged for 5 min at approximately 1500 rpm. Took out 1 mL of buffer solution and 1 mL of n-octanol solution, weighed and counted. The partition coefficient

was 72.3 and $\lg PC$=1.86.

3.6 Biodistribution in rats

Groups of four 200~250 g male Harlan-Sprague-Dawley rats were injected with 20 μCi of ^{131}I labeled epidepride via tail vein. The animals were killed at 5, 20, 40, 80, 160, 320 min after injection. The organs of interest were rapidly removed, weighed and the radioactivity was counted with a Pakard Cobra γ-counter. The percentage dose/g of the sample was calculated . The target to nontarget ratio in brain (striatum contains high density of D2 dopamine receptors, while cerebellum is essentially devoid of D2 dopamine receptors) was determined by regional dissection. The uptake ratio of each region was obtained by dividing the percentage dose/g of each region by that of the cerebellum.

The results were listed in Table 1. Moderate brain uptake (0.42% dose/gram at 5min) was observed. At later time points, a large portion of the radioactivity was washed out from brain(0.21%dose/gram at 20 min postinjection). The liver uptake remained high for the first 40 min and rapidly washed out 80 min postinjection.

Table 1 Biodistribution of ^{131}I-epidepride in rats after intravenous injection

Organ	% dose/g					
	5	20	40	80	160	320
Blood	0.16±0.025	0.13±0.035	0.1±0.012	0.052±0.0054	0.035±0.0030	0.028±0.0044
Heart	0.48±0.15	0.25±0.11	0.13±0.028	0.063±0.0082	0.024±0.0028	0.011±0.0015
Liver	0.74±0.18	1.2±0.26	1.09±0.50	0.46±0.078	0.2±0.022	0.097±0.0033
Spleen	0.53±0.31	1.24±0.36	0.44±0.17	0.23±0.029	0.061±0.0091	0.051±0.029
Lung	2.11±1.05	1.21±0.45	0.81±0.19	0.28±0.061	0.16±0.034	0.035±0.014
Kidney	1.13±0.43	1.02±0.49	0.71±0.36	0.43±0.090	0.24±0.14	0.087±0.030
Thyroid	0.51±0.099	0.57±0.17	0.66±0.22	1.37±0.43	2.23±0.65	4.89±1.15
Muscle	0.29±0.052	0.21±0.048	0.076±0.023	0.04±0.0043	0.02±0.0061	0.011±0.0015
Brain	0.42±0.18	0.21±0.058	0.18±0.042	0.14±0.013	0.087±0.010	0.045±0.015
			Regional uptake			
Cerebellum	0.34±0.10	0.12±0.058	0.093±0.017	0.041±0.0064	0.011±0.0034	0.0027±0.0014
Striatum	0.73±0.21	0.92±0.18	0.71±0.097	0.71±0.18	0.67±0.089	0.64±0.16
Hippocampus	0.38±0.10	0.17±0.086	0.13±0.028	0.064±0.021	0.024±0.014	0.0075±0.0038
Frontal cortex	0.49±0.12	0.34±0.17	0.14±0.065	0.057±0.010	0.018±0.0045	0.0051±0.0022
ST/CB	2.1	7.6	7.6	17.3	61	237

Peak striatal uptake was seen at 20 min, at that time uptake of epidepride was 0.92%/g of striatum. Striatal uptake of epidepride varied only 9.86% from 40 to 320 min following injection, at that time a peak striatum : cerebellum ratio of 237/1 was seen. The striatum/cerebellum (ST/CB) ratio of ^{131}I-epidepride dramatically increased with time(Table 1), while this type of profound increase in target to nontarget ratio vs time was not observed for the other two regions, hippocampus and cortex.

4 DISCUSSION

Epidepride and its aryltin precursor were synthesized with good yield. The purity of the product obtained was characterized. Compared with Hoberg *et al*[10] and Yue *et al.*[6] methods for epidepride, this method is simpler and the reaction condition is moderate. In the course to prepare compound 2, crystallization was used to replace the chromatography (silica gel), for it could shorten the time and increase the yield.

[^{131}I]epidepride was easily prepared in the presence of hydrogen peroxide at room temperature. The *RCP* and *RLY* were over 95% determined by TLC and HPLC after oxidized 10 min. 15 min was selected as the optimal time. In HPLC, the retention time of [^{131}I]epidepride was involved in pH of the solution. In acidic condition, the retention time was shorten(<5 min); in basic condition, the retention time was lengthen(>5 min). Paper chromatography (silica gel paper, CH_2Cl_2/CH_3OH=9/1) is simple and fast, so it can be used widely to measure the purity of [^{131}I]epidepride in clinic.

At room temperature, after diluting by saline , [^{131}I]epidepride could be stable in 4 h. At 24 h, the *RCP* was only 82% and some yellow precipitate could be seen. [^{131}I]epidepride was stable under 4°C for 15 d postpreparation (Fig.3) after some stabilizer and ethanol added . The biodistribution studies in rats suggested that in regions with nonspecific association, i.e. regions low in dopamine receptors , the agent was washed out rapidly, whereas the striatum (rich in dopamine receptors) showed prolonged retention. The study on *ex vivo* autoradiography of the compound (data not shown) also confirmed the selective regional distribution with high striatal localization reflecting D2 dopamine receptor distribution.

5 CONCLUSION

The synthesis and labeling of epidepride are simple, fast and may provide high purity product. This work will be valuable for widely domestic clinic use of [^{131}I]epidepride as a dopamine D2 receptor for SPECT imaging agent in the future.

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