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Synthesis of S-[3-(4(5)-Imidazolyl)propyl],N-[2-(4-[^{125}I]-Iodophenyl)ethyl]Isothiourea Sulfate (^{125}I-Iodophenpropit), a New Probe for Histamine H_3 Receptor Binding Sites.

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Summary:

The synthesis of the first high specific activity [^{125}I] labelled selective H_3 antagonist, Iodophenpropit- i.e., S-[3-(4(5)-imidazolyl)propyl],N-[2-(4-[^{125}I]-iodophenyl)ethyl]isothiourea sulfate is reported.
The radiolabelled compound was prepared by Cu(I)assisted nucleophilic non-isotopic exchange from the corresponding bromo derivative (VUF 4598). The ^{125}I-Iodophenpropit was purified using reverse phase HPLC and obtained in a chemical and radiochemical purity >98% and a specific activity of 1900 Ci/mmol.

Keywords: Histamine, Na^{125}I, H_3 receptors, Copper(I)catalyzed halogen exchange, ^{125}I-Iodophenpropit.

Introduction:

The chemical messenger histamine is involved in various physiological and pathological processes, through the stimulation of (at least) three classes of receptors, called H_1, H_2 and H_3 respectively[1], the latter of which has been identified in 1983 by Arrang et al[2] as a presynaptic autoreceptor in the central nervous system (CNS). Very potent and selective agonists, for example, (R) α-methylhistamine[3] and N^{α}-methylhistamine[4], and antagonists, for example, thioperamide[5] and N-arylalkyl,S-imidazolylalkylisothiourea [6] have been reported. So far, in binding assays, only the tritiated H_3 agonists (R)-α-methylhistamine and N^{α}-methylhistamine have been used to visualize and assay

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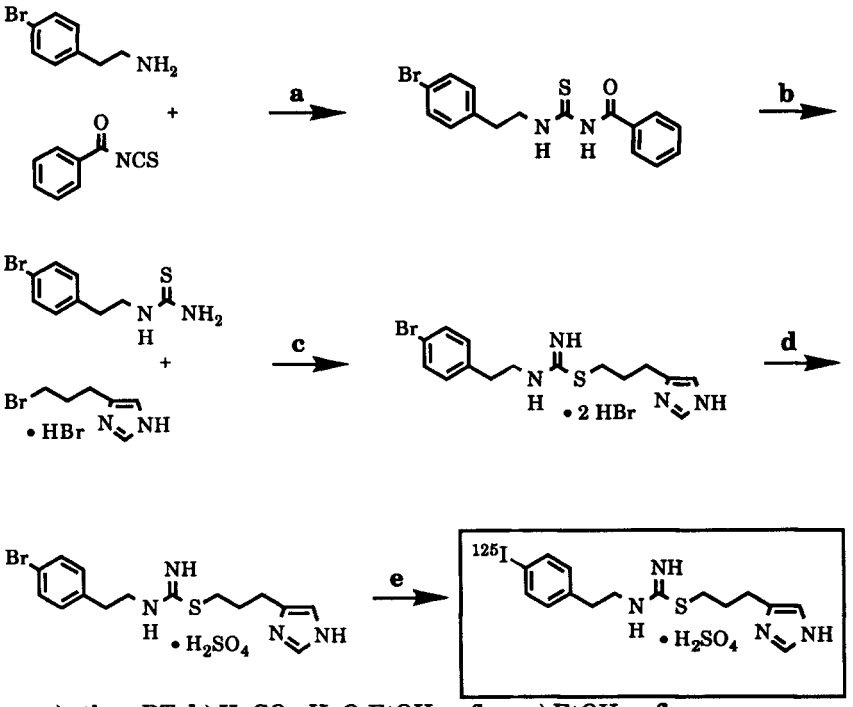
Scheme 1: Synthesis of ^{125}I-Iodophenpropit.



a) ether, RT; **b**) K_2CO_3-H_2O-EtOH, reflux; **c**) EtOH, reflux;
d) Ag_2SO_4-H_2O; **e**) Na^{125}I-Cu(I)-H_2O-120°C

the H_3 receptor[4,5]. However, a word of caution is in place against the use of a radiolabelled agonist for the purposes of assaying the receptor. As it is not known whether the H_3 receptor is present in one or two affinity states[7], a potent radiolabelled antagonist would constitute a "safer" probe. Therefore a {^{125}I} labelled antagonist was developed on the basis of the S-imidazolylpropyl,N-phenylalkylisothiourea series of antagonists [6]: ^{125}I-Iodophenpropit (see scheme 1). The "cold" Iodophenpropit has a pA_2 of 9.3 on the histamine H_3 receptor[12]. The activities on histamine H_1 and H_2 receptors[13,14] were 5.4 and 5.6 respectively, and this clearly demonstrates the great selectivity of the compound for the H_3 receptor. Full pharmacological data on this new label will be published elsewhere.

Synthesis:

The new {^{125}I}-labelled H_3 antagonist ^{125}I-Iodophenpropit was prepared starting from the commercially available 2-(4-bromophenyl)-ethyl amine as visualized in scheme 1. The amine was converted into

the corresponding thiourea via reaction with N-benzoyl isothiocyanate, followed by hydrolysis of the intermediate N'-benzoyl isothiourea. Reaction of this thiourea with 4(5)-(3-bromopropyl)imidazole hydrobromide afforded the dihydrobromide salt of N-[2-(4-bromophenyl)-ethyl],S-[3-(4(5)-imidazolyl)propyl] isothiourea (VUF4598). The introduction of (^{125}I) was achieved by Cu(I)assisted nucleophilic non-isotopic exchange[8a,b,c] of the bromide in VUF4598 for (^{125}I). Since the introduction of (^{125}I) was not feasible in the presence of free bromide ions it was converted into the sulfate salt prior to the exchange reaction. The crude ^{125}I-Iodophenpropit was purified by semi preparative reverse phase HPLC from the excess VUF4598. "Cold" Iodophenpropit was synthesized analogously starting from 2-(4-iodophenyl)ethyl amine.

Experimental:

2-(4-bromophenyl)ethyl amine was obtained from Janssen Chimica. 4(5)-(3-bromopropyl)imidazole hydrobromide[9a,b], N-benzoyl isothiocyanate[10] and 2-(4-iodophenyl)ethyl amine[11] were prepared according to reported procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 200 MHz spectrometer and the chemical shifts are expressed in ppm relative to tetramethylsilane as internal standard. Melting points were determined on a Mettler FP5 apparatus with microscope. HPLC Equipment: The equipment consisted of a Rheodyne injector (50 µl loop for the analitical and 500 µl loop for the semi preparative column), a LKB 2150 pump, a LKB LCC-2252 controller, a LKB VWM-2141 variable wavelenght UV-monitor and a NaI(Tl) detector and electronics (ORTEC). The chromatographic data were captured and analyzed by computer (Drew Scientific DS 4000 Chromatographic System version 5.0).

N-Benzoyl, N'-[2-(4-Iodophenyl)ethyl] Thiourea:
4.45 g (18.0 mmol) 2-(4-iodophenyl)ethyl amine in 25 ml ether was added dropwise to 3.10 g (19 mmol) N-benzoyl isothiocyanate in 50 ml ether and stirred overnight at room temperature. The cream colored product was filtered, washed with ether and dried in vacuo and recrystallized from ethanol. Yield 5.76 g (78%) N-benzoyl, N'-[2-(4-iodophenyl)ethyl] thiourea. ^{1}H NMR ($CDCl_3$): δ 2.96 (t,2H,CH_2Ph), 3.91 (t,2H,CH_2N), 6.95-7.85 (m,9H,Phenyl-H), 8.97 (br s,1H,NH), 10.72 (br s,1H,NH)

N-[2-(4-Iodophenyl)ethyl] Thiourea:
The crude N-benzoyl,N'-[2-(4-iodophenyl)ethyl] thiourea (5.70 g, 14 mmol) was suspended in 100 ml ethanol and 40 ml 1 M K_2CO_3 and refluxed for 4 hrs. The reaction mixture was concentrated in vacuo diluted with 100 ml water and extracted with 2x100 ml ethyl acetate. The

organic layers were washed with 25 ml water, 25 ml brine and dried with Na_2SO_4. Filtration and concentration afforded 3.00 g (70%) of white solid N-[2-(4-iodophenyl)ethyl] thiourea. Mp = 184-185.5°C. ^{1}H NMR (DMSO-d_6): δ 2.74 (t,2H,CH_2Ph), 3.56 (m,2H,CH_2N), 6.94 (br s,2H,NH_2), 6.95-7.10 and 7.55-7.70 (m,4H,Phenyl-H), 7.50 (br s,1H,NH).

Iodophenpropit.2HBr
1.53 g (5.00 mmol) N-[2-(4-iodophenyl)ethyl] thiourea and 1.35 g (5.00 mmol) 4(5)-(3-bromopropyl)imidazole hydrobromide were refluxed in 25 ml ethanol. After 4 days the solvents were removed in vacuo and the crude product was purified by column chromatography (SiO_2; ethyl acetate-methanol 2:1) to give 2.02 g (70%) of Iodophenpropit.2HBr as a colorless oil. This material was crystallized from 2-propanol and subsequently recrystallized from ethanol. mp = 171.8-174.0°C. ^{1}H NMR (DMSO-d_6): δ 1.89 (m,2H,CCH_2C), 2.74 (t,2H,CH_2Ph), 2.83 (t,2H,CH_2Im), 3,24 (t,2H,CH_2N), 3.57 (t,2H,CH_2S), 7.05-7.20 (m,2H,Phenyl-H), 7.50 (s,1H,Im-H_5), 7.60-7.75 (m,2H,Phenyl-H), 9.03 (s,1H,Im-H_2), 9.25 (br s,2H,NH), 9.66 (br s,1H,NH), 14.05 (br s,2H,Im-NH). ^{13}H NMR (D_2O): δ 23.90 ($C\underline{C}H_2C$), 28.63 (CH_2Im), 32.01 (CH_2S), 34.08 (CH_2Ph), 45.87 (CH_2N), 93.28 , 117.11 (Im-C_5), 130.34, 132.52 (2xC), 133.80 (Im-C_4), 134.63 (Im-C_2),139.01, 139.22 (2xC), 168.25(Isothiourea-C).

Iodophenpropit.H_2SO_4:
501 mg (0.87 mmol) of Iodophenpropit.2HBr was dissolved in 25 ml water and titrated with a solution of 272 mg (0.87 mmol) of silver sulfate in 70 ml water. The precipitated silver bromide was filtered and washed with 15 ml water. The filtrate was concentrated in vacuo. Recrystallization of the crude product from ethanol afforded 300 mg (67%) of Iodophenpropit.H_2SO_4. mp = 125.0-127.0°C. ^{1}H NMR (D_2O): δ 1.84 (m,2H,CCH_2C), 2.76 (t,2H,CH_2Ph), 3.03 (m,4H,CH_2Im+CH_2N), 3.73 (t,2H,CH_2S), 7.05-7.20 (m,2H,Phenyl-H), 7.24 (s,1H,Im-H_5), 7.55-7.75 (m,2H,Phenyl-H), 8.66 (s,1H,Im-H_2).

N-Benzoyl, N'-[2-(4-Bromophenyl)ethyl] Thiourea:
This compound was prepared in 90% yield in a procedure similar to N-benzoyl, N'-[2-(4-iodophenyl)ethyl] thiourea. ^{1}H NMR ($CDCl_3$): δ 2.98 (t,2H,CH_2Ph), 3.94 (t,2H,CH_2N), 6.95-7.87 (m,9H,Phenyl-H), 8.97 (br s,1H,NH), 10.98 (br s,1H,NH).

N-[2-(4-Bromophenyl)ethyl] Thiourea:
N-[2-(4-bromophenyl)ethyl] thiourea was obtained by hydrolysis of the N-benzoyl, N'-[2-(4-bromophenyl)ethyl] thiourea in 97% of . mp = 165-166°C. ^{1}H NMR (DMSO-d_6): δ 2.77 (t,2H,CH_2Ph), 3.58 (t,2H,CH_2N), 7.03 (br s,2H,NH_2), 7.15-7.60 (m,4H,Phenyl-H), 7.63 (br s,1H,NH).

N-[2-(4-Bromophenyl)ethyl],S-[3-(4(5)-Imidazolyl)propyl] Isothiourea Dihydrobromide:

2.30 g (8.90 mmol) N-[2-(4-bromophenyl)ethyl] thiourea and 2.40 g (8.90 mmol) 4(5)-(3-bromopropyl)imidazole hydrobromide were dissolved in 50 ml ethanol and heated under reflux. After 4 days the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, ethyl acetate-methanol 2:1). The oily product was crystallized from 2-propanol and subsequently recrystallized from ethanol. mp =202.2-203.5°C. ^{1}H NMR (D_2O): δ 1.76 (m,2H,CCH_2C), 2.73 (t,2H,CH_2Ph), 2.96 (t,2H,CH_2Im), 3.02 (t,2H,CH_2N), 3.73 (t,2H,CH_2S), 7.15-7.55 (m,4H,Phenyl-H), 7.22 (s,1H,Im-H_5), 8.60 (s,1H,Im-H_2). ^{13}C NMR (D_2O): δ 23.93 ($C\underline{C}H_2C$), 28.62 (CH_2Im), 31.99 (CH_2S), 33.98 (CH_2Ph), 46.01 (CH_2N), 117.10 (Im-C_5), 121.73, 132.34 (2xC), 133.09 (2xC), 133.69 (Im-C_4), 134.55 (Im-C_2),138.35, 168.27(Isothiourea-C).

N-[2-(4-Bromophenyl)ethyl],S-3-[(4(5)-Imidazolyl)propyl] Isothiourea Sulfate (VUF 4598):

530 mg (1.00 mmol) of the hydrobromide salt was dissolved in 30 ml water and titrated with a solution of 310 mg (1.00 mmol) silver sulfate in 75 ml water. The precipitated silver salts were filtered and washed with 25 ml water. The filtrate was concentrated in vacuo and the crude product was recrystallized from ethanol. 270 mg (58%) of a white solid was obtained. mp = 118.8-120.0 °C. ^{1}H NMR (D_2O): δ 1.86 (m,2H,CCH_2C), 2.73 (t,2H,CH_2Ph), 2.96 (t,2H,CH_2Im), 3.00 (t,2H,CH_2N), 3.72 (t,2H,CH_2S), 7.10-7.55 (m,4H,Phenyl-H), 7.22 (s,1H,Im-H_5), 8.64 (s,1H,Im-H_2).

^{125}I-Iodophenpropit:

0.8 mg (1.7 μmol) VUF4598, 3 mg (20 μmol) 2,5-dihydroxybenzoic acid, 5 mg (23 μmol) citric acid, 0.3 mg (1.4 μmol) Tin(II)sulfate were dissolved in water (± 250 μl) in a 0.5 ml reaction vial. 15 μl of a 15 mM Copper(II) sulfate solution and 1.5 mCi $Na^{125}I$ (± 20 μl, specific activity 1900 Ci/mmol) were added and this solution was flushed with nitrogen for 20 minutes at RT. Subsequently the reaction mixture was heated in an oil bath at 120°C for 45 minutes. The reaction mixture was analyzed by HPLC (Merck RP-Select B column, 125 mm x 4 mm, flow 1 ml/min, the eluent was methanol/water/trimethylamine/acetic acid (35/65/0.12/0.2, vol/vol) with 0.5 g Na_2SO_4/l; pH = 4.8). Iodophenpropit (retention time, 10 min) was well separated from the starting material (VUF4598, retention time 6 min). Usually, about 80-85% of the initial radioactivity was recovered in this peak. The reaction mixture was filtered using a Millipore 0.22 micron filter and submitted to HPLC purification on a semi preparative Merck RP Select B column (250 mm x 10 mm). The

eluent was methanol/water/trimethylamine/acetic acid (29/71/0.12/0.2) with 0.5 g Na_2SO_4/l, pH = 4.8, and the flow rate was 4 ml/min. ^{125}I-Iodophenpropit (retention time, 22 min) was well separated from the starting material (VUF4598, retention time 13 min). The product fraction was collected and diluted with water to 50 ml and submitted to a homemade RP-18 (Lichrosorb, Merck) mini column washed with water and eluted with ethanol/0.5 M H_2SO_4 (50:4, vol/vol). 0.3 ml solution with 700 μCi ^{125}I-Iodophenpropit with a specific activity of 1900 Ci/mmol was obtained. The product was carrier free and had a radiochemical purity of > 99% as verified by quality control on analytical HPLC.

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