SYNTHESIS OF ^{35}S- AND ^{14}C-LABELED FAMOTIDINE, A NEW POTENT HISTAMINE H_2 RECEPTOR ANTAGONIST

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SUMMARY

Famotidine, 3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N^2-sulfamoylpropionamidine, a new potent histamine H_2 receptor antagonist, was labeled with ^{35}S and ^{14}C for metabolic studies at specific activities of 45.7 μCi/mg (VIb) and 47.6 μCi/mg (VIc) respectively.

Key words: Carbon-14, Sulfur-35, Famotidine,
Histamine-H_2-receptor-antagonist,
Sulfamoylpropionamidine, Thiazole derivatives

INTRODUCTION

Famotidine, 3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N^2-sulfamoylpropionamidine (VIa), is a new potent histamine H_2 receptor antagonist synthesized in our laboratories by Yanagisawa et al.[1] The inhibitory effect of famotidine was about 40 times more potent than that of cimetidine on gastric response to histamine and food in the conscious dog with Heidenhain pouch.[2] As a matter of course, studies on the metabolic fate of this promising agent required preparation of the radioactive compound. For preliminary studies we synthesized [^{35}S]famotidine (VIb) labeled at the side-chain sulfide S atom from the readily available [^{35}S]thiourea (Ib). For

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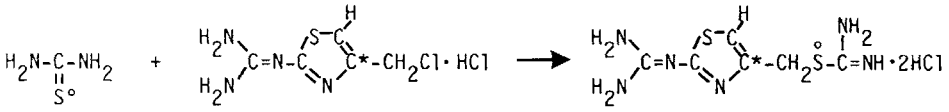
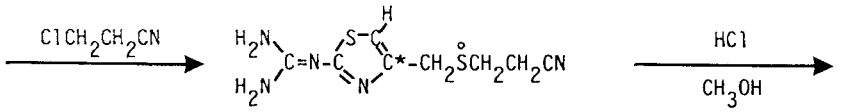
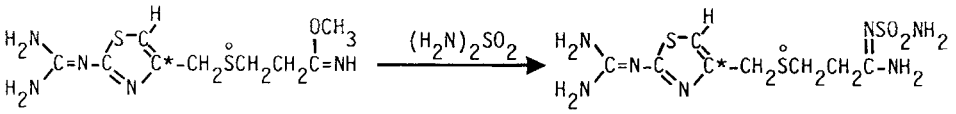
detailed metabolic studies [thiazole-4-^{14}C]famotidine (VIc) was prepared from 4-chloromethyl-2-[(diaminomethylene)amino][4-^{14}C]thiazole hydrochloride. The synthetic route was shown in the following Scheme.



Ia,b IIa,c IIIa,b,c



IVa,b,c



Va,b,c VIa,b,c

a: non-labeled

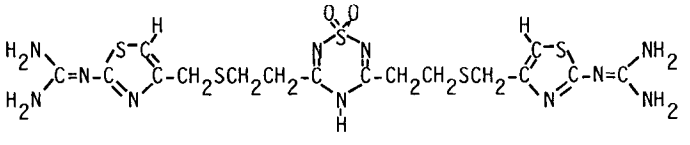
b: ^{35}S-labeled at °

c: ^{14}C-labeled at *

Scheme

RESULTS AND DISCUSSION

The optimum yield of S-[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]-[^{35}S]isothiourea dihydrochloride (IIIb) was achieved by the reaction of equimolar quantities of [^{35}S]thiourea (Ib) and 4-chloromethyl-2-[(diaminomethylene)amino]thiazole hydrochloride (IIa)[3] in isopropanol at 80°. Similarly the ^{14}C-labeled compound IIIc was obtained by the reaction of IIc with thiourea (Ia). Variations of the molar ratio I/II reduced the yield of III. The reaction products (IIIb and IIIc) without isolation were allowed to react with 3-chloropropionitrile under basic conditions with cooling to afford IVb and IVc respectively. Addition reaction of methanol to the cyano group of IVb and IVc in the presence of a large excess of hydrogen chloride at -30° to 0° gave Vb and Vc respectively, which were isolated as crystals by prompt treatment with aqueous potassium carbonate solution at low temperature. From the mother liquor of Vb after standing at room temperature, a second crop of crystals were obtained, which were identified as an unexpected ester i.e. methyl 3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl][^{35}S]thio]-propionate probably produced by hydrolysis of imino group of Vb. Reactions of Vb and Vc with sulfamide in methanol at room temperature overnight yielded the desired [^{35}S]famotidine (VIb) and [thiazole-4-^{14}C]famotidine (VIc) respectively in moderate yields. When the reaction was carried out at an elevated temperature (60°) in methanol, V was consumed within 30 min to give VI in a low yield with many by-products. The use of N,N-dimethylformamide as a solvent provided a homogeneous slow reaction but no improvement in the yield of VI. The reactivity of the amino group of sulfamide to the imidate carbon of V seemed to be similar to that of the sulfamoyl amino group in VI to the same carbon. Therefore, the formation of bis-thiazole compound VII as a



VII

by-product was due to a competitive reaction between sulfamide and the sulfamoyl group of VI. High molar ratio of sulfamide : V was necessary to decrease the formation of VII, but too much sulfamide caused difficulties in the isolation of the desired VI. HPLC analysis on LiChrosorb® Si 60 showed formation of VIa in 70% yield in the optimum molar ratio (5 equivalent mole of sulfamide).

Overall radiochemical yield, chemical yield and analytical data of VIb and VIc are summarized in the Table.

Table Overall radiochemical yield, chemical yield and batch analysis data of [^{35}S]- and [^{14}C]famotidine (VIb and VIC)

	VIb	VIc
Overall radiochemical yield	23.6%	59.9% [a]
Overall chemical yield	34.2%	60.6% [a]
Specific activity [b]	45.7μCi/mg	47.6μCi/mg
Radiochemical purity	98% [c]	98% [c,d]
Chemical purity	98% [e]	99% [e,f]

a) The yield of VIc was better than that of VIb due to the modification of the isolation method of the intermediate IVc and by the recovery of an additional amount of VIc from the mother liquor using Lobar® column chromatography (see Experimental section).

b) determined by liquid scintillation counting with a Tri-Carb Liquid Scintillation Spectrometer 3255 (Packard).

c) determined by radioactivity scanning of TLC plates with a Radio-TLC Scanner LB 2733 (Berthold).

d) determined by liquid scintillation counting of HPLC fractions collected every 1 min.

e) determined by TLC analysis on silica gel (60 F_{254}, 0.25 mm, 5 x 20 cm^2,

Merck) using solvent systems : (i) ethyl acetate/ethanol/water/acetic acid (10:4:1:1, v/v), (ii) ethyl acetate/benzene/methanol/conc. ammonia solution (16:8:6:3, v/v).

f) determined by HPLC analysis on (i) normal phase column (LiChrosorb® Si 60, 5µm, 4mm x 25 cm, Cica-Merck) using n-hexane/ethanol/iso-propylamine/water (65:35:1:1), (ii) reverse phase column (Nucleosil $5C_{18}$, 5µm, 4mm x 15 cm, Marcherey-Nagel) using acetonitrile/methanol/0.01M sodium n-hexanesulfonate adjusted to pH 3 with acetic acid (6:1:25, v/v).

EXPERIMENTAL

[^{35}S]Thiourea (Ib) and 4-chloromethyl-2-[(diaminomethylene)amino][4-^{14}C]-thiazole hydrochloride (IIc, custom preparation)[4] were purchased from Amersham International plc, Amersham, England. The labeled products were characterized by co-chromatography (TLC and/or HPLC) with non-radioactive standards. HPLC analysis was performed on a Waters Associates, Inc., HPLC equipment (Model 6000A pump, Model 440 Absorbance Detector, 254 nm). All evaporations were carried out under reduced pressure.

3-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl][^{35}S]thio]propionitrile (IVb)

A mixture of IIa (227 mg)[3], thiourea (Ia, 38.9 mg) and [^{35}S]thiourea (Ib, 20 mCi = 37.2 mg) in isopropanol (1.5 ml) was stirred at 78° to 80°. After 10 min the reaction mixture became a clear solution and within a further 10 min crystals of IIIb began to precipitate. To the mixture, after 3 hr, were added water (2 ml) and 3-chloropropionitrile (107 mg = 98 ul) at room temperature, followed by 1M NaOH (3.2 ml) dropwise with stirring over 40 min at 0° to 5° under an atmosphere of nitrogen. Towards the end of the addition, IVb was deposited as an oil which gradually crystallized. After 2 hr water (5.8 ml) was added to the mixture and after stirring overnight at 4°, the crystals of IVb were collected by filtration, washed with water and dried over P_2O_5 in vacuo. Yield: 184.7 mg (76.6 %).

3-[[[2-[(Diaminomethylene)amino]-4-[4-^{14}C]thiazolyl]methyl]thio]propionitrile (IVc)

Thiazole derivative IIc (20 mCi = 279.7 mg, 97% radiochemically pure), Ia (102 mg) and 3-chloropropionitrile (144 mg) were treated as described above. The reaction mixture was extracted with ethyl acetate (10 ml x 3). The combined organic layers were evaporated to dryness and the residue applied to a Lobar® column (Si 60, B size, Merck) using chloroform/methanol (3:1, 3x1 ml) through a silica gel pre-column (Wakogel C-200, 3 g). The column was eluted with the same solvent at a flow rate of 10 ml/min with UV monitoring at 280 nm. The fraction (12 to 17 min) was collected and evaporated to give colorless crystals of IVc. Yield: 275.3 mg (92.7%).

Methyl 3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl][^{35}S]thio]propionimidate (Vb)

A solution of IVb (184.7 mg) in a mixture of methanol (1.1 ml) and chloroform (2.2 ml) was saturated with dry hydrogen chloride at -20° to -30° protected from moisture. The resulting mixture tightly sealed with a rubber stopper was allowed to stand at 0° for 3 days. The mixture was evaporated carefully below -30° and the residue was treated rapidly with aqueous potassium carbonate solution (2.4 g/6 ml) under ice-water cooling. The oil which was deposited was crystallized by scratching. After standing for 1 hr at 0°, the crystals of Vb were filtered, washed with water and dried over phosphorus pentoxide in vacuo. Yield: 182.6 mg (87.3%). The small crop of crystals which precipitated in the mother liquor after standing overnight were characterized as methyl 3-[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]-[^{35}S]thio]propionate by co-chromatography with the non-radioactive authentic sample (Rf=0.72, chloroform/ methanol = 2:1, v/v).

Methyl 3-[[[2-[(diaminomethylene)amino-4-[4-^{14}C]thiazolyl]methyl]thio]propionimidate (Vc)

Nitrile IVc (275.3 mg) was converted to imidate Vc by the same method

described above. Yield: 283.8 mg (91.0%).

3-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl][^{35}S]thio]-N^2-sulfamoyl-propionamidine, [^{35}S]Famotidine (VIb)

A suspension of Vb (182.6 mg) and sulfamide (320 mg) in methanol (1.3 ml) was warmed at 35° on a water bath to give a clear solution and allowed to stir at room temperature. After 3 hr VIb began to precipitate as fine crystals. The reaction mixture was stirred overnight and diluted with ethanol (2.6 ml). The crystals were collected by filtration and washed with ethanol (0.5 ml x 4). The filter cake was dissolved in a mixture of ethanol (0.62 ml), water (1.12 ml) and acetic acid (25.2 µl). The resulting mixture was filterd and the filtrate was neutralized with 1M NaOH (0.44 ml). The crystals of VIb soon precipitated were collected by filtration and dried over phosphorus pentoxide under reduced pressure. Yield: 115.2 mg. (51.1%).

3-[[[2-[(Diaminomethylene)amino]-4-[4-^{14}C]thiazolyl]methyl]thio]-N^2-sulfamoyl-propionamidine, [Thiazole-4-^{14}C]Famotidine (VIc)

Imidate Vc (283.8 mg) was allowed to react with sulfamide (500 mg) to afford VIc by the procedure described above. Yield: 200.3 mg (57.1%).

Further crystals of VIc were recovered from the mother liquor as follows. The mother liquor of VIc was evaporated to dryness, and the residue in dimethylformamide (1 ml) was applied to a Lobar® column (LiChroprep® Si 60, size B, Merck) through a silica gel pre-column (Wakogel C-200, 3 g). The column was eluted with n-hexane/ethanol/iso-propylamine/water (65:35:1:1, v/v) at a flow rate of 10 ml/min with UV monitoring at 280 nm. The fraction (86 to 113 min) was collected and evaporated. The residue was transferred to a small flask with methanol (2 ml x 4) and evaporated to dryness. The residual syrup was crystallized by the addition of ethanol. The crystals were filtered and desiccated over phosphorus pentoxide to give an additional amount of VIc (51.3 mg). The radiochemical purity and specific activity of VIc thus obtained were

identical to those of the first crop. Total yield of VIc : 251.6 mg (71.8%).

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2) Takagi T., Takeda M. and Maeno H.-Arch. Int. Pharmacodyn. Ther. 256: 49-58 (1982).

3) British patent 2001624 (Imperial Chemical Industries Ltd., C.A. 90 87452d (1979))

4) This compound was synthesized by the reaction of amidinothiourea and 1,3-dichloro[2-^{14}C]acetone essentially according to the method in the reference 3.