SYNTHESIS OF CARBON-14 LABELED DISUPRAZOLE

W.T. Stolle, J.C. Sih and R.S.P. Hsi*
Research Laboratories of The Upjohn Company
Kalamazoo, MI 49001, U.S.A.

SUMMARY

This report describes the synthesis of carbon-14 labeled title compound from [^{14}C]CS_2. The substituted benzimidazole was labeled at the C-2 position of the benzimidazole moiety.

Key Words: Synthesis, carbon-14, substituted benzimidazole

INTRODUCTION

Disuprazole, 2-[(4-ethylthio-3-methyl-2-pyridinyl)methylsulfinyl]-1H-benzimidazole (*1*), is a long acting antisecretory agent with clinical potential for treating duodenal ulcers. Its mode of action is believed to resemble that of other substituted benzimidazoles, such as omeprazole and timoprazole. These compounds inhibit gastric acid secretion by inhibiting the enzyme (H^+-K^+)-ATPase in the gastric mucosa, which catalyzes the transport of H^+ from inside the parietal cells of the stomach walls into the gastric lumen in exchange for K^+(1). We synthesized carbon-14 labeled *1* for conducting biotransformation and drug disposition studies with this agent in test animals and man.

*To whom correspondence should be addressed.

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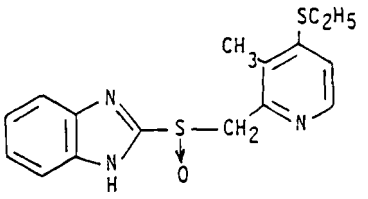

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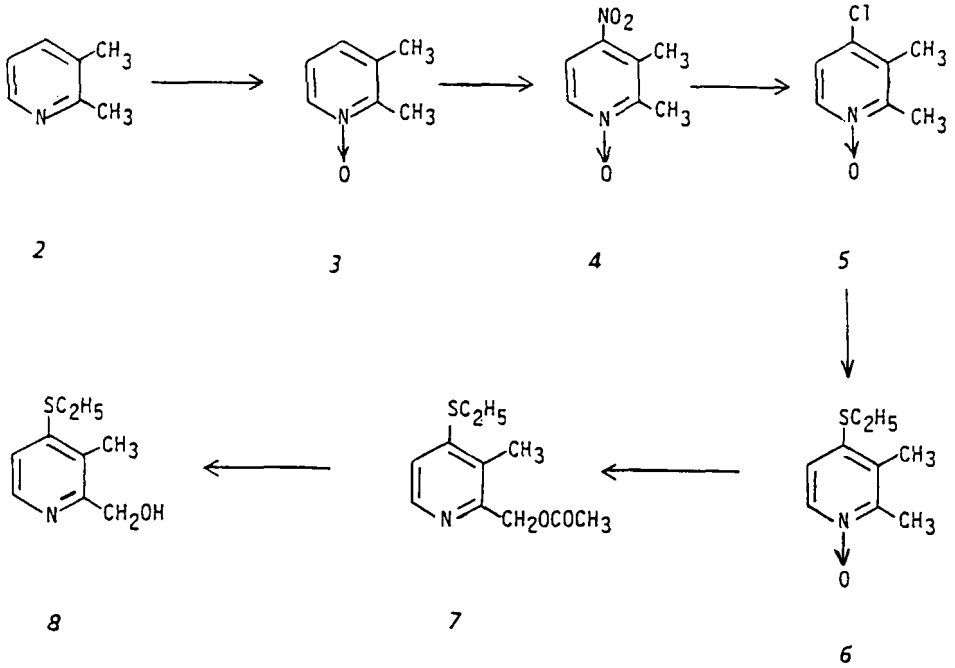
DISCUSSION AND RESULTS

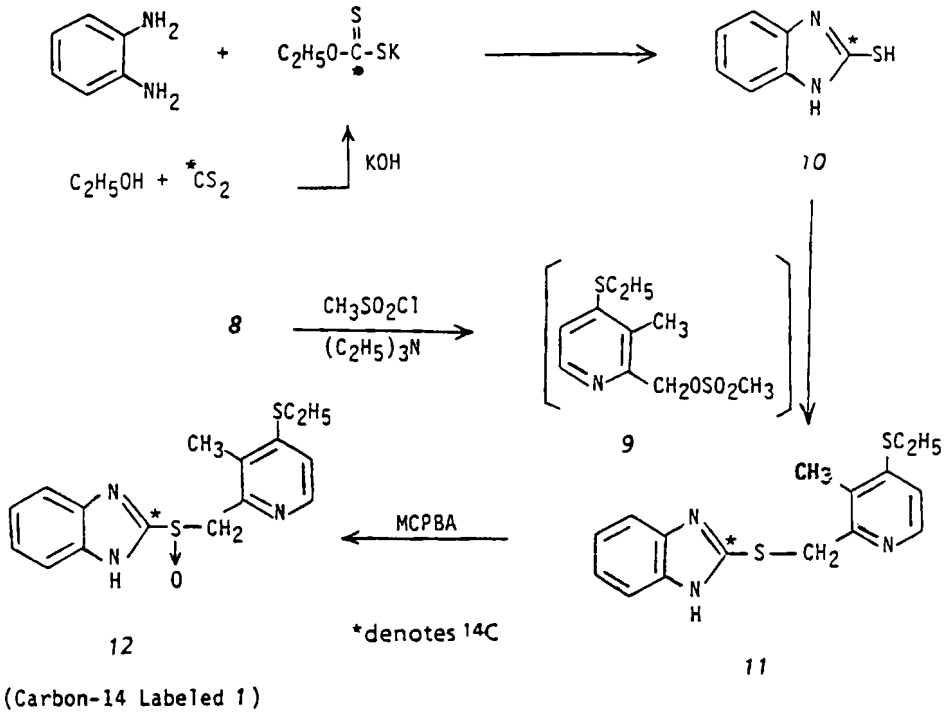
Compound *1* consists of two principal building blocks, benzimidazole and a substituted pyridine. The pyridine portion of the molecule was constructed as shown in Scheme 1. The N-oxide of 2,3-lutidine (*3*) was nitrated with fuming nitric acid in concentrated sulfuric acid to give 2,3-dimethyl-4-nitropyridine N-oxide (*4*). Treatment of *4* with hydrogen chloride gas afforded 2,3-dimethyl-4-chloropyridine N-oxide (*5*). Displacement of chlorine with ethyl mercaptan gave 2,3-dimethyl-4-ethylthiopyridine N-oxide (*6*). Heating *6* with acetic anhydride effected rearrangement of the N-oxide to the acetate *7* (*2*) which was hydrolyzed to the alcohol *8*.

Scheme 1



2 *3* *4* *5* *6* *7* *8*

Scheme 2



(Carbon-14 Labeled 1)

Carbon-14 was incorporated into an appropriately functionalized benzimidazole by first converting carbon-14 labeled carbon disulfide into potassium ethyl xanthate *in situ* and reacting the latter with *o*-phenylenediamine to give 2-mercapto[2-^{14}C]benzimidazole (*10*) as shown in Scheme 2, according to the procedure of VanAllan and Deacon (3). The thiol *10* was then alkylated with the mesylate *9* generated *in situ* from the alcohol *8* to produce the radioactive sulfide *11*. Finally, selective oxidation of *11* with *m*-chloroperbenzoic acid afforded carbon-14 labeled sulfoxide *12*. The product, with specific activity of 156.7 μCi/mg, was obtained in overall 51.7% radiochemical yield. High performance liquid chromatographic (HPLC) analysis of this material showed radiochemical purity in excess of 99%.

EXPERIMENTAL SECTION

The radioactivity determinations were made with an LKB Instruments Model 1217

Rackbeta liquid scintillation spectrometer, using the external standard method. Diotol (Burdick and Jackson Laboratories, Muskegon, Michigan, U.S.A.) was the scintillation cocktail. Thin layer chromatographic (TLC) analyses were carried out with 2.5 x 10 cm glass plates precoated with 250 μm layer of silica gel GF (Analtech, Newark, Delaware, U.S.A.). The developed zones were visualized with 254 nm UV light. Radioactive zones were detected with a Packard Model 7220 Radiochromatogram Scanner equipped with a Model 7222 thin layer plate scanner. High performance liquid chromatographic (HPLC) analyses were carried out with a Spectra Physics Model 8700 Solvent Delivery System and a Supelcosil LC-18 (5μ) analytical column (4.6 mm I.D. x 25 cm). The eluate was analyzed with a Spectra Physics Model 8440 Variable Wavelength UV Detector set at 254 nm and a Berthold LB-503 HPLC Radioactivity Monitor with FLO-SCINT II (Radiomatic Instruments and Chemical Co., Inc. Tampa, FL, U.S.A.). Quantification of the chromatograms was done with a Spectra Physics Model 4270 Computing Integrator. The mobile phase, pumped isocratically at 1.5 ml/min, consisted of 64.7:34.8:0.5 v/v methanol:water:triethylamine. The apparent pH of the mixed solvents was adjusted with phosphoric acid to 7.0.

2,3-Dimethylpyridine-1-oxide (*3*)

To a stirred solution of 40 g of 2,3-lutidine (*2*, 0.374 mol, Fairchild Chemical Co.) in 500 ml of chloroform at 0-5°C was added 84.4 g of *m*-chloroperbenzoic acid (0.383 mol, Aldrich 80-85%) in portions over 15 minutes. The reaction mixture was stirred at 0-5°C for 25 minutes and poured with stirring into a mixture of 100 ml of 10% sodium sulfate and 200 ml of saturated sodium bicarbonate solutions. The layers were separated and the aqueous phase was exhaustively extracted with 9:1 v/v chloroform-methanol. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure. The crude product was chromatographed on a column of 750 g of silica gel eluted first with a 95:95:10 v/v mixture of chloroform:acetone:methanol to remove *m*-chlorobenzoic acid, then with a 9:9:2 v/v mixture of the same solvents, to give 37.9 g of white solids, 82% yield; TLC R_f 0.19 with 95:95:10 v/v chloroform:acetone:methanol; 1H-NMR δ

($CDCl_3$,TMS): 2.33(s,3H,$C\underline{H}_3$ at C-3), 2.50 (s, 3H, $C\underline{H}_3$ at C-2), 7.13 (m, 2H, aryl H at C-4 and C-5), 8.23(m, 1H, aryl H at C-6).

2,3-Dimethyl-4-nitropyridine-1-oxide (*4*)

Compound *3* (37.9g, 0.308 mol) was cautiously added to 96 ml of concentrated sulfuric acid. When the temperature of the exothermic mixture declined to 45°C, 51 ml of fuming nitric acid was added dropwise with stirring over 10 minutes. The mixture was stirred at 95°C for 1.5 hour, cooled in an ice bath, poured into 700 ml of crushed ice, basified with 45% KOH (~340 ml) to pH 12, and extracted three times with chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to give 48.3 g (93% yield) of *4* as yellow solids; TLC R_f 0.22 with 6:1 v/v methylene chloride:acetone; ^{1}H-NMR δ ($CDCl_3$, TMS): 2.60 (s,6H,$C\underline{H}_3$ at C-2 and C-3), 7.80 (d, j = 6Hz, 1H, aryl H at C-5), 8.32 (d, j = 6Hz, 1H, aryl H at C-6).

4-Chloro-2,3-dimethylpyridine-1-oxide (*5*)

Hydrogen chloride gas was bubbled into 700 ml of absolute ethanol until ~150 g was absorbed. To the warm solution was added 48.3 g (0.287 mol) of *4* with 100 ml of hot absolute ethanol as a rinse. The mixture was refluxed for 3.25 hour and purged vigorously with nitrogen gas to remove excess hydrogen chloride. The mixture was neutralized with 400 ml of saturated sodium bicarbonate solution followed by solid potassium carbonate until evolution of carbon dioxide ceased, and extracted three times with chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure. The resulting crude orange solids were chromatographed on a column of 600 g of silica gel packed in and eluted with 1l of 3:1 v/v methylene chloride:acetone, followed by 1l each of 2:1, 3:2, and 1:1 v/v methylene chloride:acetone, the last with 7% methanol added, to give 39.9 g (88% yield) of *5*, yellow solids, mp 103-5°C; TLC R_f 0.25 with 95:95:10 v/v methylene chloride:acetone:methanol; ^{1}H-NMR δ ($CDCl_3$,TMS): 2.40 (s, 3H, $C\underline{H}_3$ at

C-3), 2.57 (s, 3H, $C\underline{H}_3$ at C-2), 7.23 (d, j = 6 Hz, 1H, aryl H at C-5), 8.20 (d, j = 6 Hz, 1H, aryl H at C-6).

2,3-Dimethyl-4-ethylthiopyridine-1-oxide (*6*)

To a stirred ice cold suspension of 12.2 g of sodium hydride (60% emulsion in mineral oil, 0.305 mol, 1.2 equiv.) in 420 ml of dry dimethylformamide was added dropwise 22.1 g of ethyl mercaptan (0.356 mol, 1.4 equiv.) in seven minutes. The mixture was stirred at room temperature for 1 hour and treated with 39.9 g of *5* (0.25 mol) which was added with 65 ml of dimethylformamide. After stirring at room temperature for 30 minutes, the mixture was chilled and poured into 350 ml of 9:1 v/v chloroform:methanol. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to afford a quantitative yield of *6* as yellow solids which were of sufficient purity for the next step;TLC R_f 0.15 with 95:95:10 v/v chloroform:acetone:methanol; ^{1}H-NMR δ ($CDCl_3$,TMS): 1.37 (t, j = 7.5 Hz, 3H, $C\underline{H}_3$ of $-CH_2\underline{C}H_3$), 2.33 (s, 3H, $C\underline{H}_3$ at C-3), 2.55 (s,3H, $C\underline{H}_3$ at C-2), 2.98 (q, j = 7.5 Hz, 2H, $C\underline{H}_2$ of $-C\underline{H}_2CH_3$), 7.00 (d, j = 6 Hz, 1H, aryl H at C-5), 8.17 (d, j = 6 Hz, 1H, aryl H at C-6).

2-Acetoxymethyl-4-ethylthio-3-methylpyridine (*7*)

The compound 6 from above (46.5 g, 0.254 mol) was heated at 100-110°C with 250 ml of acetic anhydride for 15 minutes and the hot mixture was poured into 300 ml of methanol. After being stirred at room temperature for 1.6 hour, the mixture was basified with 500 ml of saturated sodium bicarbonate solution followed by solid sodium bicarbonate until evolution of carbon dioxide ceased, and extracted with chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure. The resulting residue was chromatographed on a column of 850 g of silica gel packed in and initially eluted with 4 l of 10:1 v/v methylene chloride:acetone, then with 6:1 mixture of the same solvents, to give 41.4 g of *7* as brown oil, 72% yield, which was used in the next step

without further purification; TLC R_f 0.33 with 6:1 v/v methylene chloride:acetone; ^{1}H-NMR δ ($CDCl_3$, TMS): 1.42 (t, j = 7.5 Hz, 3H, $C\underline{H}_3$ of -CH_2CH_3), 2.13 (s, 3H, $C\underline{H}_3$ at C-3), 2.28 (s, 3H, $C\underline{H}_3$-C = O), 3.01 (q, j = 7.5 Hz, 2H, $C\underline{H}_2$ of -CH_2CH_3), 5.30 (s, 2H, $OC\underline{H}_2$), 7.13 (d, j = 6 Hz, 1H, aryl H at C-5), 8.08 (d, j = 6 Hz, 1H aryl H at C-6).

4-Ethylthio-2-hydroxymethyl-3-methylpyridine (*8*)

To an ice cold solution of 41.4 g of acetate *7* (0.184 mol) in 500 ml of methanol was added with stirring 44.2 ml of methanolic sodium methoxide (Aldrich, 25% in methanol, 0.193 mol, 1.05 equiv.). After 15 minutes the mixture was diluted with 1 l of chloroform and washed with 220 ml of ice water. The aqueous layer was extracted with 300 ml of chloroform. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to give 31.8 g of crude *8*. A portion of crude *8* (4g) was purifed by chromatography on a column of 150 g of silica gel packed in and eluted with 4:1 v/v methylene chloride:acetone at 3 ml per minute. The eluate was collected in 17.5 ml fractions. Fractions 34-59 were pooled and concentrated at reduced pressure. The resulting residue was crystallized from a methylene chloride-hexane mixture to give 2.87 g of pure *8*, mp 76.5-77^{0}C; TLC R_f 0.30 with 6:1 v/v methylene chloride:acetone; ^{1}H-NMR δ ($CDCl_3$ TMS): 1.40 (t, j = 7.5 Hz, 3H, $C\underline{H}_3$ of -CH_2CH_3), 2.15 (s, 3H, $C\underline{H}_3$ at C-3), 3.00 (q, j = 7.5 Hz, 2H, $C\underline{H}_2$ of -CH_2CH_3), 4.71 (s, 2H, -$OC\underline{H}_2$), 4.93 (broad, 1H, OH), 7.07 (d, j = 6 Hz, 1H, aryl H at C-5), 8.03 (d, j = 6 Hz, 1H, aryl H at C-6).

2-Mercapto [2-^{14}C]benzimidazole (*10*)

O-Phenylenediamine (409 mg, 3.78 mmol, Aldrich) was dissolved in 2.0 ml of 95% ethanol in a 25 ml screw top round bottom flask (Wheaton Glass Co.). Carbon-14 labeled carbon disulfide (nominally 200 mCi from Pathfinder Laboratories, Inc.) in a break-seal tube was frozen in a liquid nitrogen bath before opening the tube, and dissolved in 3.9 ml of 0.98 M potassium hydroxide in ethanol to form a yellow xanthate solution. This solution was transferred to the 25 ml screw top flask by pipet with the aid of an additional 2 ml of ethanol. The flask was capped with a

Teflon valve (Wheaton Glass Co.) and stirred in an oil bath at 78°C. A thick-walled (1/2 in) Pyrex chromatography jar was inverted over the reaction flask to contain any possible rupture of the closed system. After 20 hours, the reaction mixture was cooled and transferred to a 50 ml flask with 5 ml of water, and 12 ml of 1:2 v/v acetic acid:water was added to give a mixture of pH4. The addition caused some foaming which was followed by crystal formation. The ethanol was removed at 30°C and 30 torr and the remaining aqueous mixture was cooled for 1 hour at 0°C. The crystals were collected, washed with 7 ml of water, and dried under vacuum overnight at room temperature to give 497 mg of *10* (94.8% chemical yield), specific activity 340 μCi/mg. The total activity of 169 mCi represented a 84.5% radiochemical yield. A TLC radiochromatogram (ethyl acetate, R_f 0.75) showed the product was radiochemically homogeneous, and identical to an authentic sample of benzimidazole.

2-[(4-Ethylthio-3-methyl-2-pyridinyl)methylthio]-1H-[2-^{14}C]benzimidazole (*11*)

A solution of 590 mg of the alcohol *8* (3.22 mmol) in 9 ml of methylene chloride under a nitrogen atmosphere was cooled at 0°C in an ice bath. Methanesulfonyl chloride (Aldrich Chemical Co.), 0.262 ml (3.38 mmol), was added to the cold solution, followed by 0.7 ml (5.0 mmol) of triethylamine. This mixture was stirred for 40 minutes at 0°C, whereupon TLC analysis showed conversion of *8* to the methanesulfonate *9* was complete (4:1 v/v methylene chloride:acetone, R_f 0.44 for 9, 0.37 for 8; 1:1 v/v ethyl acetate:hexane, R_f 0.14 for 9, 0.27 for *8*). To this mixture was added with stirring a solution of 490 mg of *10* (3.22 mmol, 167 mCi) dissolved in 13 ml of absolute ethanol and 0.5 ml of triethylamine (3.5 mmol). After being stirred at room temperature for 16 hours, the solution was concentrated at reduced pressure and the residual semi-solid was chromatographed on a column of 80 g of silica gel packed in and eluted with 4:1 v/v methylene chloride:acetone at 3 ml per minute. The eluate was collected in 12 ml fractions. Pooling of fractions 28-52 and removal of solvents afforded crude *11* which was crystallized from acetone-hexane to give 929 mg of pure *11* as fluffy white crystals, sp. act. 163 uCi/mg, 90.7% radiochemical yield. From pooled fractions 18-24 there was obtained 28 mg (7.8

mCi) of unreacted *10*. HPLC analysis of *11* showed the material was in excess of 99% radiochemically pure; TLC R*f* 0.36 with 4:1 v/v methylene chloride:acetone, identical with authentic sample of unlabeled *11*; mass spec. M/Z 316 (M + 1); ^{1}H-NMR δ ($CDCl_3$, TMS) 1.42 (t, j = 7.5 Hz, 3H, $C\underline{H}_3$ of $-CH_2C\underline{H}_3$), 2.37 (s, 3H, $C\underline{H}_3$ at C-3 of pyridine ring), 3.00 (q, j = 7.5 Hz, 2H, $C\underline{H}_2$ of $-C\underline{H}_2CH_3$), 4.47 (s, 2H, aryl CH_2), 7.10 (d, j = 6 Hz, 1H, H at C-5 of pyridine ring), 8.03 (d, j = 6 Hz, 1H, H at C-6 of pyridine ring), 7.27 (m, 4H, H of phenyl ring), 7.60 (broad s, 1H, N-H).

2-[(4-Ethylthio-3-methyl-2-pyridinyl)methylsulfinyl]-1H-[2-^{14}C]benzimidazole (*12*)

A mixture of 619 mg of *11* (1.95 mmol, 100.9 mCi), 252 mg of anhydrous sodium bicarbonate, and 15 ml of chloroform was cooled to -13°C (acetone-ice bath). A solution of 421 mg (1.95 mmol) of *m*-chloroperbenzoic acid (80%, Aldrich Chemical Co.) in 6 ml of chloroform was added dropwise with stirring over 20 minutes. The mixture was stirred 20 minutes longer and the reaction was quenched with 10 ml of cold 10% sodium sulfite and 5 ml cold saturated sodium bicarbonate solutions. The aqueous layer was extracted with 10 ml of chloroform. The organic layers were combined, washed with brine, and dried over sodium sulfate. Triethylamine (0.5 ml) was added to the mixture to stabilize the crude product. The dried extract was concentrated at 35°C and 30 torr to an oil, which was chromatographed on a column of 80 g silica gel packed in and eluted with 5:10:85:0.5 v/v isopropanol:acetone:methylene chloride:triethylamine. The eluate was collected in 12 ml fractions at 4 ml per minute. Fractions 18-23 contained 59 mg of unreacted *11*, (9.5%, 9.6 mCi). Fractions 32-52 containing the desired product *12* were pooled and concentrated at 35°C and 30 torr. The residue was dissolved in methylene chloride and decolorized with activated charcoal to remove a distinct violet color. The mixture was filtered and the yellow filtrate was concentrated. The residue was dissolved in a hot mixture of 20 ml of methylene chloride, 15 ml of acetone, and 4 ml of ethyl acetate, and the solution was boiled. As methylene chloride was distilled off, it was replaced with additional acetone, which caused crystals to form. The resulting slurry was cooled in an ice bath and 4 ml of ether was added. The crystals were filtered and washed with ether and hexane to give 393 mg of *12*, mp

147-149°C; sp. act. 156.7 μCi/mg, 67.4% radiochemical yield after correction for recovered starting material. HPLC analysis showed *12* was in excess of 99% radiochemically pure; TLC *f* 0.32 with 85:10:5 v/v methylene chloride:acetone:isopropanol, 99% radiochemically pure and identical to an authentic sample of unlabeled *12*; mass spec. M/Z 332 (M + 1); 1H-NMR δ ($CDCl_3$, TMS): 1.38 (t, j = 7.5 Hz, 3H, CH_3 of $-CH_2CH_3$), 2.22 (s, 3H, $C\underline{H}_3$ at C-3 of pyridine ring), 2.95 (q, j = 7.5 Hz, 2H, $C\underline{H}_2$ of $-CH_2CH_3$), 4.87 (s, 2H, aryl CH_2), 3.02 (d, J = 6 Hz, 1H, aryl H at C-5 of pyridine ring), 8.3 (d, j = 6 Hz, 1H, aryl H at C-6 of pyridine ring), 7.3 (m, 4H, H of phenyl ring), 7.63 (s, broad, 1H, N-H).

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