SYNTHESIS AND STABILITY OF S-(2-CARBOXYETHYL)-N-(2-t-BUTYL-5-METHOXY-BENZTHIAZOL-6-YL)-[^{14}C]DITHIOCARBAMATE [CGP 20376 (CGI 16483)]

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SUMMARY

For pharmacokinetic and metabolism studies in laboratory animals, CGP 20376 labelled with carbon-14 on the dithiocarbamoyl carbon atom in the side chain at 6-position of the benzthiazolyl ring system and having a specific activity of 6.13 μCi/mg (2.35 mCi/mmol) was synthesised, in four steps, starting with potassium[^{14}C]thiocyanate. At physiological pH (*in vitro*) [^{14}C] CGP 20376 dissociated rapidly into its biologically active isothiocyanate precursor [^{14}C] CGP 20308, indicating that it might behave as a pro-drug.

Key words : CGP 20376, carbon-14, side chain, synthesis, antifilarial, pro-drug

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* Contribution No.805 from Hindustan CIBA-GEIGY Research Centre.

0362-4803/87/080957-13$06.50

Received September 10, 1986
Revised December 19, 1986

INTRODUCTION

CGP 20376 (CGI 16483), 5-(2-carboxyethyl)-N-(2-t-butyl-5-methoxy-benzthiazol-6-yl) dithiocarbamate is an experimental antifilarial compound exhibiting potent micro and macrofilaricidal activity[1] in rodents and cattle and currently being evaluated in man[2].

CGP 20376 is a pale yellow crystalline substance having a molecular formula $C_{16}H_{20}N_2O_3S_3$, a molecular weight 384 and a melting point 150-152°C from aqueous acetic acid. In methanol solution it exhibits UV absorption at 293 nm (ε 17,752), 315 nm (ε 12,590) and 326 nm (ε 11,646). It is sparingly soluble in water (~ 24 µg/ml at room temperature) and insoluble in hexane (ligroine). However, it dissolves in several organic solvents on warming.

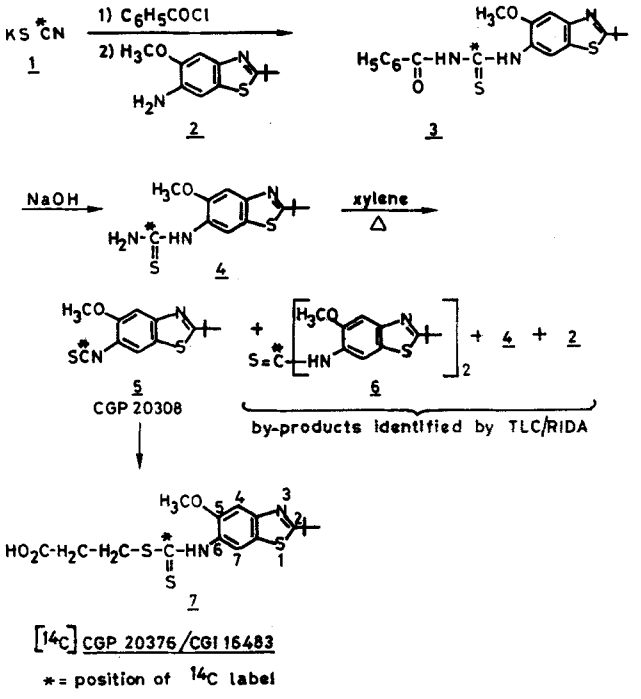
For pharmacokinetic and metabolism studies in laboratory animals CGP 20376 was labelled with carbon-14 on the dithiocarbamoyl carbon atom in the side chain at 6-position of the benzthiazolyl ring system.

The labelling was carried out on a 2.06 mmol scale starting with 5 mCi of potassium[^{14}C]thiocyanate according to the scheme outlined.

DISCUSSION

Potassium[^{14}C]thiocyanate (1) was reacted with benzoyl chloride in acetone at 65-70°C and the benzoyl[^{14}C]-isothiocyanate formed *in situ* on further reaction with 6-amino-2-t-butyl-5-methoxybenzthiazole (2) gave N-benzoyl-N'-benzthiazolyl[^{14}C]thiourea (3) which on mild alkaline hydrolysis with NaOH aq afforded the benzthiazolyl[^{14}C]-thiourea (4). Pyrolysis of (4) at the reflux temperature

Synthetic Scheme:



[14C] CGP 20376/CGI 16483

* = position of 14C label

of xylene in the presence of ammonium sulphate led to the benzthiazolyl[^{14}C]isothiocyanate, CGP 20308 (5), which on condensation with 3-mercaptopropionic acid yielded the adduct, [^{14}C] CGP 20376 (7) having a specific activity of 6.13 μCi/mg in an overall radiochemical yield of 45% based on potassium[^{14}C]isothiocyanate started with.

The results from stability studies carried out with ^{14}C-labelled CGP 20376 (7) are presented in Tables 1-3.

From the data shown in Table 1 (7) is observed to be stable in solutions in water, acetic acid , chloroform, ethyl acetate and PEG 200†. However, the stability is affected when (7) is dissolved in other common organic solvents viz. acetone, acetonitrile, dimethylformamide and

† a vehicle used for drug administration.

methanol as revealed by the considerably diminished amounts of (7). This observation precludes thus use of such solvents for extraction/analysis of (7) from biological fluids etc.

Table 1.

Stability of ^{14}C-labelled CGP 20376 in freshly prepared solutions of some common organic solvents and in water.

S. No.	Solvent	Stability (% CGP 20376*)
1	Acetic acid (aq)	97.7
2	Acetone	24.4
3	Acetonitrile	46.5
4	Chloroform	97.6
5	Dimethylformamide (DMF)	59.8
6	Ethyl acetate	99.7†
7	Methanol	38.5
8	PEG-200	100
9	Water††	98.9

* Values determined by RIDA following dissolution at room temperature (concn. 100 µg/10 ml)

† Stability diminished on storage for long periods, even in a refrigerator (% CGP 20376 : after one month - 90%; after 2 months - 14%).

†† Saturation solubility of [^{14}C]CGP 20376 in water at room temperature (25°C) : 24 µg/ml.

Radiometric TLC of ^{14}C-labelled (7) on silica using the solvent mixture containing ethyl acetate, hexane and acetic acid (92 : 8 : 0.2, v/v/v) for development showed the formation of the isothiocyanate (5) besides other unidentified polar products (Fig. 1). This suggested that silica (pH 4.5) contributed to the dissociation of the

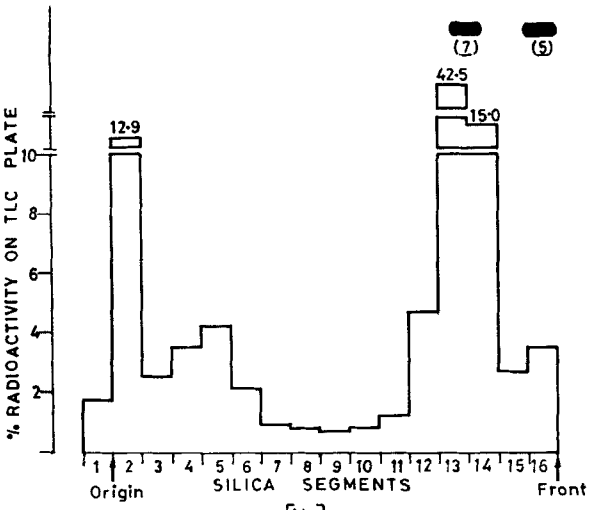


Fig.1 Radiochromatogram of [^{14}C]CGP 20376
TLC : Silica HF_{254}/Ethyl acetate-hexane- acetic acid (92:8:0·2 v/v)
Reference compounds:(7) - CGP 20376; (5) - CGP 20308

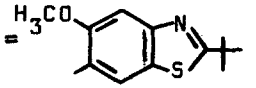
dithiocarbamate (7) since the solvent mixture selected for development was not expected to have caused any decomposition.

Data from pH dependent stability studies carried out at room temperature (25°C) with ^{14}C-labelled (7) are shown in Table 2. The instability of (7) over the body pH range was observed and its corresponding isothiocyanate (5) which possesses anthelminthic and microbicidal[3] and antifilarial activities[4] , was produced to the extent of 5%, 36% and 25% at artificial gastric, intestinal and plasma pH (1.5, 7.6 and 7.4 respectively).

Further, following incubation at 37°C at physiological pH 7.4, the dissociation of (7) to its isothiocyanate (5) was faster (Table 3). Thus, following incubation for one hour the formation of (5) was observed to be as much as 82% in the absence of plasma (i.e. in buffer only) and 52% in the presence of plasma. These results confirming the facile formation of (5) nonenzymatically from (7) indicated that the latter compound might behave as a pro-drug[4].

Table 2. pH dependent stability of ^{14}C-labelled CGP 20376 at room temperature. [Concentrations of [^{14}C] CGP 20376 = 10 µg/ml]

Body pH range (artificial)	pH	Amounts* detected: % Intact CGP 20376 (dithiocarbamate) HOOC·H_2C·H_2C-S-C(=S)-HN-R	Amounts* detected: % CGP 20308 (corresponding isothiocyanate) S=C-N-R
Gastric	1.5	91.38	4.96
Intestinal	7.6	59.18	35.70
Plasma (Phosphate buffered dsaline, PBS)	7.4	70.58	25.33
	10.0†	43.62	47.80

*Determined by RIDA; R = 

Required amounts of [^{14}C] CGP 20376 (from evaporation of suitable aliquots of freshly prepared ethylacetate solution) were vortexed for 20 min. at room temperature at elected pH, prior to analysis.

†An aqueous solution of [^{14}C] CGP 20376 was brought to ∽pH 10, prior to analysis.

Table 3. Effect of incubation at 37°C on ^{14}C-labelled CGP 20376 in the presence/absence of plasma (PBS buffer pH 7.4).

Incubation period	Amounts expressed as % radioactivity incubated			
	Plasma (*)		PBS Buffer pH 7.4 (†)	
	Intact CGP 20376	CGP 20308	Intact CGP 20376	CGP 20308
1/2 h	66.67	23.82	30.59	61.03
1 h	28.15	52.19	10.81	82.30

Concentrations of [^{14}C]CGP 20376 in : * = 2 µg/ml; † = 12 µg/ml

EXPERIMENTAL

Melting and boiling points are uncorrected.

Potassium[^{14}C]thiocyanate (19.7 mg; specific activity 24.5 mCi/mmol; 5 mCi) was obtained from Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

Authentic nonradioactive compounds (2) (CGA 93069; m.p. 118-120°C)[3], (4) (CGP 20312; m.p. 214-216°C)[3], (5) (CGP 20308; m.p. 74-76°C)[3] and (7) (CGP 20376; m.p. 150-152°C)[1] were synthesized at CIBA-GEIGY AG, Basel, Switzerland.

(6) was synthesized by reacting (2) and (5) in xylene under reflux with stirring for 5 hrs. (m.p. 188-189°C; chloroform-methanol). Analysis : Found : N, 11.08; $C_{25}H_{30}N_4O_2S_3$ requires N, 10.89%).

3-Mercaptopropionic acid (puriss) was purchased from Fluka AG, Buchs, Switzerland.

Benzoylchloride (b.p. 198°C) was distilled and stored in sealed ampoules.

All other solvents obtained locally were distilled before use.

Radioactivity measurements were made on a Packard TRICARB (Model 460 CD) Liquid Scintillation Counter operating at a carbon-14 efficiency of 94%, by the channels ratio method and by internal standardisation.

The scintillator (cocktail) contained 4 g PPO and 0.05 g POPOP per litre of toluene.

Artificial gastric and intestinal fluids (buffers) prepared as described[5] were of the following composition :

(Gastric, pH 1.5) : Sodium chloride, 2 g; concentrated hydrochloric acid, 7 ml; distilled water, 1000 ml.

(Intestinal, pH 7.6) : Anhydrous sodium monohydrogen phosphate, 8.05 g; sodium dihydrogen phosphate, $2H_2O$, 1.65 g; distilled water, 1000 ml.

PBS (Phosphate buffered saline, pH 7.4) : Sodium monohydrogen phosphate 12 H_2O, 6.81 g; sodium dihydrogen phosphate $2H_2O$, 12.64 g; sodium chloride 9.0 g; distilled water 1000 ml.

Radiometric TLC of [^{14}C]CGP 20376 (2µg; 27000 dpm) was done on glass-plates (20 x 20 cm) precoated with silica-gel HF_{254} (tlc grade; E. Merck) of 250-300 µ in thickness, using a solvent mixture of ethyl acetate-hexane-acetic acid (92:8: 0.2 v/v) and development distance of 15 cm. Unlabelled (7) and (5) served as reference compounds. Silica segments (2.5 x 1 cm) of the radiochromatogram were subjected to radiometric assay after mixing each with methanol (5 ml) and PPO-POPOP cocktail (10 ml). (7) and (5) were visualised under UV light (254 nm).

Reversed isotope dilution analysis (RIDA) ; compounds (4), (5), (6), (7) : Solutions of the test^{14}C-samples were mixed with solutions of the respective analytically pure unlabelled compounds (50-100 mg) and the carrier samples were isolated either by precipitation with hexane or water or by evaporation

to dryness in vacuo and purified further to constant specific activity as follows.

(4) : crystallisation from acetone-water (1:1) three times

(5) : extraction with hexane, chromatography on silica column and crystallisation of residue from hexane eluates from isopropanol-water (1:1, v/v), three times

(6) : crystallisation from acetone, three times

(7) : crystallisation from acetic acid-water (1:1, v/v), three times.

5 mg of the RIDA samples were dissolved in methanol (5 ml), mixed with cocktail (15 ml) and counted for radioactivity.

2-(t-Butyl-5-methoxy-benzthiazol-6-yl)-[^{14}C]thiourea (4)

To a mixture of potassium[^{14}C]thiocyanate (1) (19.7 mg; 24.5 mCi/mmol; 5 mCi) and nonradioactive potassium thiocyanate (180 mg), in dry acetone (10 ml) was added benzoyl chloride (0.25 ml) and the suspension stirred under gentle reflux for 30 min. A solution of 6-amino-2-(t-butyl)-5-methoxybenzthiazole (2) (0.47 g) in dry acetone (10 ml) was added dropwise while maintaining the gentle reflux. The mixture was stirred under reflux for a further 1 h and the solvent evaporated off under a stream of nitrogen. The dry residue was stirred with ice-water (50 ml) for 2 h. The solid was filtered, washed with water and dried to give (3), 0.75 g, yield 91%.

(3) was stirred with aqueous sodium hydroxide (8%; 4 ml) at 110-120°C (oil-bath) for 15 min and cooled. The mixture was diluted with water (50 ml) and stirred for 30 min. The solid was filtered, washed with water and dried to give the [^{14}C]thiourea (4), 0.5 g, yield 90%.

2-t-Butyl-6-[^{14}C]isothiocyanato-5-methoxybenzthiazole (CGP 20308) : (5)

A suspension of (4) (0.5 g), powdered ammonium sulphate (100 mg) and xylene (30 ml) was stirred under reflux for 6 h with a gentle stream of nitrogen being passed through the stirred reaction mixture. As the reaction proceeded, a clear solution resulted gradually. After cooling the xylene solution was adsorbed on a column of silica gel (100-200 mesh; 30 g) equilibrated with petroleum ether (b.p. 60-80°C). The column was eluted, as follows, fractions being collected :

Fraction No.	Eluant	Eluate volume
1	Pet. ether bp (60-80°C)	100 ml
2	- do -	100 ml
3	Pet. ether-acetone (90:10, v/v)	100 ml
4	- do -	100 ml
5	Pet. ether-acetone (60:40, v/v)	100 ml
6	- do -	100 ml

All these fractions were examined by TLC*.

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*TLC : Silica F_{254}/Solvent systems

A. Pet. ether (bp 60-80°C) : acetone (90:10, v/v)

B. Pet. ether (bp 60-80°C) : acetone (70:30, v/v)

C. Chloroform : methanol (98:2, v/v)

Compound	Rf/Solvent system		
	A	B	C
(2)	0.23	0.70	-
(4)	0.05	0.35	0.25
(5)	0.80	-	-
(6)	-	-	-

- : not determined

Fraction 1, contained only the solvent, xylene, used in the reaction. Besides (5) the by-products (2), (4) and (6)

formed due to a disproportionation reaction, could be detected in Frs. 5 and 6.

By reversed isotope dilution analysis with authentic synthetic compounds from an aliquot of xylene solution of the total reaction product from the pyrolysis reaction, the amounts of (5), (6) and (4) formed were observed to be 80.2%, 1.1% and <1.0% respectively. (2) could not be quantitated as it was devoid of ^{14}C label. However, TLC indicated its presence to be in traces only.

The fractions (Nos. 3 and 4) containing the pure isothiocyanate were combined and evaporated to dryness to give (5) initially, as a viscous oil which however changed into a white crystalline solid (0.4 g) on drying in vacuo, yield 80%.

S-(2-Carboxyethyl-N-(2-t-butyl-5-methoxybenzthiazol-6-yl)-[^{14}C]-dithiocarbamate, CGP 20376/CGI 16483 (7)

A solution of the purified isothiocyanate (5) (0.4 g) in dimethylformamide (5 ml) was treated at room temperature (RT) (25°C) in an oxygen-free nitrogen atmosphere with 3-mercaptopropionic acid (0.4 g) under vigorous stirring. After stirring for 6 h, the reaction mixture was allowed to stand at RT overnight and diluted with water (50 ml). The gummy material which separated was triturated with a glass rod occasionally. After 4-5 h, the resulting pale yellow solid† was filtered on a Buchner funnel washed with water and dried in vacuo.
The material sticking to the sides of the reaction flask was extracted with ethyl acetate (30 ml), the extract dried over anhydrous sodium sulphate, filtered and evaporated to dryness. To this residue the earlier solid (†) was added and redissolved in ethyl acetate. The solution was filtered and concentrated to 5 ml and diluted with petroleum ether (b.p. 60-80°C; 20 ml). Crystallisation was induced by

scratching with a nickel spatula, and cooled in ice. The crystals were filtered after 1 h, washed with ethyl-acetate-petroleum ether (1:4, v/v; 10 ml) and dried *in vacuo* to give (7), 340 mg, yield 61%, specific activity 6.13 μCi/mg.

From the filtrates, dilution with nonradioactive CGP 20376 and crystallisation afforded a second sample of (7), 68 mg, having a lower specific activity (2.37 μCi/mg). The radiochemical purity* as assessed by RIDA procedure only was observed to be >98%. The overall radiochemical yield starting from potassium[^{14}C]thiocyanate was 45%.

ACKNOWLEDGEMENT

Grateful acknowledgement is extended to CIBA-GEIGY AG, Basel, Switzerland for the generous supply of the authentic nonradioactive compounds CGA 93069 (2), CGP 20312 (4), CGP 20308 (5) and CGP 20376 (7) required for standardization of the synthetic reaction sequence, TLC comparisons and RIDA.

The author is also grateful to Dr. V. Ramachandra Rao of the Research Centre for helpful discussions and Mr. S. M. Shah and Mr. G. Thiruvarasu of the department for technical assistance.

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* Assessment by radiometric TLC was not feasibile for reasons explained under stability studies.

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