Eur. J. Med. Chem. **24** (1989) 493–496

Original paper

Ro 11–2933, a potential drug in the treatment of cancer and malaria: synthesis and physicochemical properties; potential metabolites

Henri RAMUZ*, Walter SCHMUTZ and Rolf HUBER

F. Hoffmann-La Roche & Co., Ltd., Pharmaceutical Research Department, 4002 Basel, Switzerland

(Received 24 December 1988, accepted 6 March 1989)

Summary — Ro 11–2933, **4b**, a compound with minor cardiovascular activity has been shown to reverse multidrug resistance in cancer cells as well as chloroquine resistance in *Plasmodium falciparum*. A useful synthesis of the drug is described. Alkylation of dithiane **(2)** with chloroalkylamine **(3)** affords **4b** as a crystalline compound in a very good yield. The synthesis of its potential metabolites **7** and **11** has also been carried out.

Résumé — **Le Ro 11–2933, médicament potentiel du traitement du cancer et de la malaria. Synthèse et propriétés physico-chimiques; métabolites potentiels.** *Ro 11–2933, **4b**, un composé dont l'activité cardiovasculaire est peu importante, possède la propriété d'inverser la résistance des cellules cancéreuses à l'action des agents anticancéreux et la résistance du* Plasmodium falciparum *à l'action de la chloroquine. Nous décrivons ici une synthèse de la substance. L'alkylation du dithiane **(2)** avec la chloralkylamine **(3)** fournit le composé cristallin **4b** avec un très bon rendement. Nous avons également effectué la synthèse des composés **7** et **II**, deux métabolites potentiels de Ro 11–2933.*

synthesis of Ro 11–2933 / potential metabolites of Ro 11–2933 / reversal of multidrug resistance / reversal of chloroquine resistance

Introduction

Since 1981, several Ca^{2+} entry blockers have been investigated for their role in reversing multidrug resistance in cancer cells [1, 2]. The mechanism of action of verapamil [3, 4], its enantiomers [5], nifedipine and other cardiovascular drugs is very likely to be correlated with an inhibition of the active efflux of the drug from resistant cells. Martin and collaborators [6] have shown that verapamil efficiently reverses chloroquine resistance in *Plasmodium falciparum*. Although transport systems in parasites are different from those in neoplastic cells, a similar biochemical basis (inhibition of enhanced chloroquine eflux from parasite acidic vacuoles) has been proposed [7]. Nevertheless, it seems obvious that these cardiovascular drugs are not suitable to reverse drug resistance since the *in vitro* concentration at which they are effective can probably not be achieved *in vivo* without inducing considerable side effects.

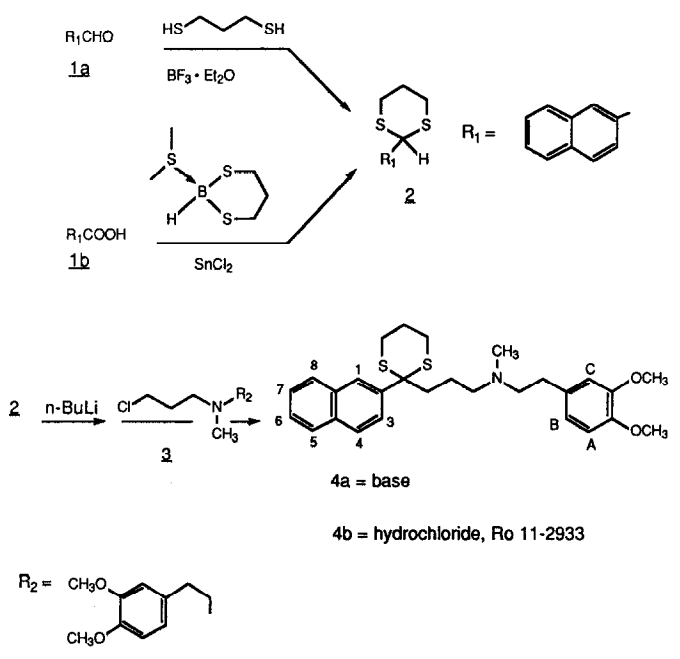
In the course of our research work in the field of calcium entry blockers, we have identified, in addition to several compounds with a high profile of cardiovascular activity, some other compounds having important binding activity to calcium channels but displaying only minor or short lasting cardiovascular activity following intravenous and oral administration in animals. Although detailed biological results related to Ro 11–2933 will not be presented here, the following short statements can be made. On the basis of IC_{50} values, Ro 11–2933, **4b** has the same potency as (±)-verapamil in the classical [^{3}H]-nifedipine binding test ($4.0 \cdot 10^{-7}$M, displacement: 81% *vs* $4.5 \cdot 10^{-7}$M, displacement: 65%). While verapamil (30 mg·kg^{-1}, *p.o.*) elicits a strong and sustained decrease in blood pressure in spontaneously hypertensive rats (SHR), Ro 11–2933 is inactive at that dose. In anaesthetized open chest dogs, both drugs are active following intravenous administration. But verapamil exerts hypotensive, bradycardic and marked coronary vasodilating effects at doses 30 times smaller than those of Ro 11–2933 (0.03–0.1 mg·kg^{-1}). Ro 11–2933 has a very high lipophilic character and is therefore expected to penetrate the cell membrane. For this reason, we selected this compound as a potential membrane modifying agent and proposed it to several academic institutes and research centers for biochemical and biological investigation. The compound has been shown to be 2–10 times more effective than verapamil *in vitro* in reversing daunorubicin resistance in multiple drug resistant P–388 / ADR murine leukemia cells by inhibiting drug efflux [8–10]. Ro 11–2933 has also been investigated by Martin and collaborators and characterized as a potent reversing agent for chloroquine resistance in *Plasmodium falciparum*. In preliminary experiments, oral coadministration of chloroquine and Ro 11–2933 has resulted in parasite clearance in the Aotus monkey model [11]. A reverse phase HPLC method to measure simultaneously chloroquine, its metabolites, Ro 11–2933 and verapamil has been developed

[12]. It seems therefore appropriate to describe a useful synthesis of Ro 11−2933 and its potential metabolites and to give some indications on their physicochemical properties.

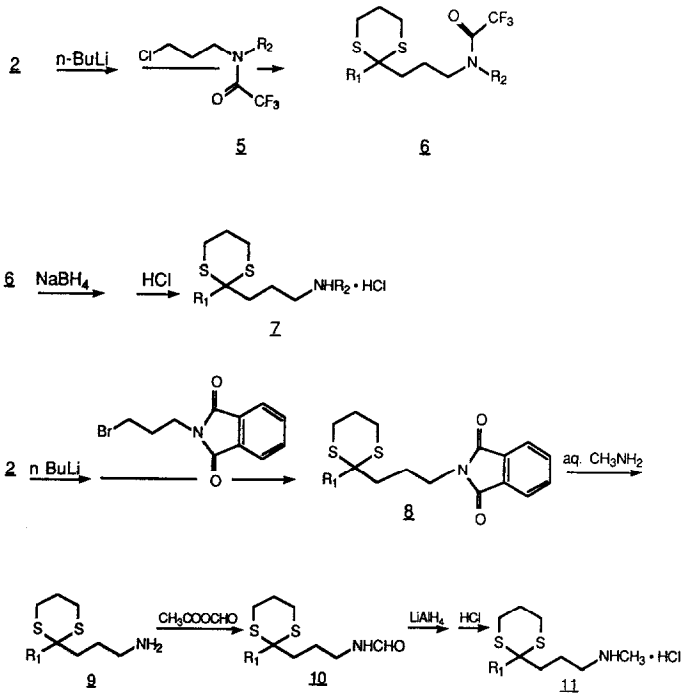
Syntheses of Ro 11−2933, its metabolites and physicochemical properties

The synthesis of Ro 11−2933, **4b**, is similar to that leading to tiapamil [13] (Scheme 1). The dithiane **2** was easily prepared from 2-naphthaldehyde **1a** and 1,3-propanedithiol in the presence of boron trifluoride etherate. A direct conversion of 2-naphthoic acid **1b** (obtained from 2-naphthylmagnesium bromide and CO_2) into **2** using 1,3,2-dithiaborinane-dimethylsulfide as a remarkable reagent [14] has been developed to provide an easy access to the ^{14}C-labelled drug corresponding to **4b**. The reaction of the chloroalkylamine **3** [13] with the lithium salt of **2** provides **4a** as an oil. The corresponding hydrochloride, Ro 11−2933, **4b**, can be obtained as a crystalline compound with an overall yield of 87%, starting from the commercially available aldehyde **1a**. Since the potential metabolites of **4b** cannot be conveniently synthesized by usual methods [13], other ways had to be investigated (Scheme 2). By reacting the *N*-3-chloropropyl derivative **5** (from *N*-trifluoroacetylhomoveratrylamide [15]) with the lithium salt of **2**, the trifluoracetamide **6** was obtained as an oil.

After scission of the protecting group of **6** with sodium borohydride in EtOH [16] the compound **7**, a potential metabolite of **4**, was characterized as a crystalline hydrochloride. The primary amine **9** was obtained in 2 steps from **2** *via* **8** whose phthalimido group was cleaved with aqueous CH_3NH_2. Formylation of **9** gave **10**, which was reduced with $LiAlH_4$ to **11**, a potential metabolite of **4**,



Scheme 1.



Scheme 2.

which can be crystallized as hydrochloride salt. Ro 11−2933, **4b**, *N*-(3,4-dimethoxyphenethyl)-*N*-methyl-2-(2-naphthyl)-*m*-dithiane-2-propylamine hydrochloride is a white crystalline substance with a melting point (mp) of 197−199°C. It has no generic name as yet. To its formula $C_{28}H_{35}NO_2S_2$. HCl has a molecular weight of 518.174. The compound is very slightly soluble in water at 25°C, slightly soluble in EtOH and soluble in MeOH and DMSO. A 3% solution in 1,2-propanediol (propylene glycol), a solvent generally accepted for i.v. or s.c. administration in animals, can be prepared by a short warming of **4b** in the solvent at 80°C.

Experimental protocols

All the experiments were conducted under an argon atmosphere. The usual method of isolation means extraction with an organic solvent, washing with water to pH 5−6, drying on anhydrous sodium sulfate and distilling the solvent under reduced pressure.

Column chromatography was carried out by using silica gel 60, (0.04−0.063 mm; Merck). Melting points were determined in the Tottoli apparatus and not corrected. ^1H NMR spectra: AS−250 MHz), WM−400 (400 MHz), ∂ values in ppm relative to TMS; coupling constants J in Hz. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. All the NMR and MS data are in accordance with the proposed structures.

Chemistry

2-(2-Naphthyl)-m-*dithiane* **2**

a) A solution of 30 g (0.192 mol) of 2-naphthaldehyde and 21.4 ml

(0.213 mol) of 1,3-propanedithiol in 400 ml dry $CHCl_3$ was cooled to 0°C, treated with 10 ml of boron trifluoride etherate and left overnight at 0°C. A cooled aqueous solution of KOH was then added and the organic solution treated as usual. Recrystallisation of the solid residue from cyclohexane gave 44.7 g (94% yield) of **2**, mp 112–114°C.

b) A solution of 1.72 g (10 mmol) of 2-naphthoic acid and 3.15 g (16.6 mmol) of anhydrous $SnCl_2$ in 25 ml dry THF was stirred and treated with 30 ml of the standard solution of the 1,3,2-dithiaborinane-dimethylsulfide complex [14]. That operation was repeated after 24 and 48 h. A small residue was filtered and the solvent evaporated under reduced pressure. The residual oil was dissolved in Et_2O, the solution treated with a 3 N NaOH solution and worked up as usual. The dithiane **2** (2.40 g, 97% yield) had an mp of 112–114°C after recrystallisation from cyclohexane. $C_{14}H_{14}S_2$; mw = 246.386; (C, H, S).

N-*(3,4-Dimethoxyphenethyl))*-N-*methyl-2-(2-naphthyl)*-m-*dithiane-2-propylamine hydrochloride* ***4b*** *Ro 11–2933*

85 ml (0.136 mol) of a solution of *n*-BuLi in hexane was added to a solution of 33.6 g (0.136 mol) of **2** in 450 ml of THF which has been cooled to −70°C. The temperature was maintained at −20°C for 2 h. The reaction mixture was then cooled to −70°C, treated dropwise with a solution of 30.9 g (0.114 mol) of the chloride **3** [13], stirred for 2 h at room temperature and finally evaporated under reduced pressure. The oily residue was taken up in EtOAc, the organic solution treated 3 times with a 5% aqueous solution of CH_3SO_3H and with H_2O. A 3 N NaOH solution was added to the aqueous extracts to adjust the pH to 12. After the usual procedure, an oily residue was obtained, which after treatment with 300 ml absolute EtOH and 40 ml dioxane containing HCl was transformed into a crystalline compound (54.3 g, 92% yield based on **3**) with an mp of 197–199°C. $C_{28}H_{35}NO_2S_2$. HCl; mw 518.174; (C, H, N, Cl, S).

^{1}H NMR 400 MHz (DMSO–d_6): 1.67 ppm, m(1), $-NCH_2CH_2CH_2-$, 1.89 ppm, ~ quintett, J ~ 5.5 Hz, $-SCH_2CH_2-$; 2.31 ppm, m(1), $-NCH_2CH_2CH_2-$; 2.65 ppm, s, $-NCH_3$; ~ 2.67 ppm, m and 2.98 ppm ddd, J_{gem} = 15 Hz, J_{vic} = 5.5 Hz, $-SCH_2-$; 2.89 ppm, ~ t, J = 8 Hz and 2.9–3.2 ppm, m, $-NCH_2-$, benzylic–CH_2-; 3.712 and 3.716 ppm, 2 × s, $-OCH_3$; 6.64 ppm, dd, J_{ortho} = 8.2 Hz, J_{meta} = 1.6 Hz, H_B; 6.81 ppm, d, H_A; 6.84 ppm, d, H_C; 7.52–7.57 ppm, m, H_6, H_7; 7.92 ppm, dd, J_{ortho} ~ 8 Hz, J_{meta} ~ 2 Hz, H_3; ~ 7.92 ppm, m, H_5; 7.97 ppm, d, H_4; ~ 8.00 ppm, m, H_8; 8.31 ppm, d, H_1; 10.8 ppm, 1, ,HCl.

N-*(3-Chloropropyl)*-N-*(3,4-dimethoxyphenethyl)-2,2,2-trifluoroacetamide* ***5*** *[15, 17]*

1.68 g (0.03 mol) pulverized KOH were added to a solution of 4.0 g (0.0144 mol) *N*-trifluoroacetylhomoveratrylamide in 50 ml of acetone. The suspension was warmed to boiling point and vigorously stirred whilst adding 11.33 g (0.072 mol) of 1,3-bromochloropropane dropwise. After 2 h, the reaction mixture was evaporated under reduced pressure and the residue taken up in Et_2O. After the usual work up and chromatography of the residue on silicagel using cyclohexane-Et_2O (3:2) as eluant, 2.87 g (56% yield) of **5**, as a colourless oil, were obtained. $C_{15}H_{19}ClF_3NO_3$; mw = 353.768; (C, H, N).

^{1}H NMR 250 MHz ($CDCl_3$): mixture of rotamers 1.9–2.2 ppm, m, $ClCH_2CH_2-$; 2.88 ppm, m, benzylic–CH_2-; 3.3–3.6 ppm, m, $ClCH_2-$, $-NCH_2-$; 3.87 and 3.89 ppm, 2 × s (but not 1:1), $-OCH_3$; 6.69–6.85 ppm, m, aromat. H.

N-*(3,4-Dimethoxyphenethyl)-2,2,2-trifluoro*-N-*[3-[2-(2-naphthyl)*-m-*dithian-2-yl]propyl-]acetamide* ***6***

As with the preparation of **4a**, 1.95 g (7.9 mmol) of **2**, 5.0 ml (8 mmol) of a solution of *n*-BuLi in hexane and 2.80 g (7.9 mmol) of **5** were reacted in 40 ml of dry THF. After chromatography of the residue on silicagel using cyclohexane-Et_2O (3:1) as eluant, 3.50 g (79% yield) of **6**, as a colourless oil, were obtained. $C_{29}H_{32}F_3NO_3S_2$; mw = 563.693; (C, H, N).

^{1}H NMR 250 MHz ($CDCl_3$): (mixture of rotamers ~ 3:2) 1,5–2,2 ppm, m, $-NCH_2CH_2CH_2-$, $-SCH_2-CH_2-$; 2.66–2.85 ppm, m, $-SCH_2-$, benzylic–CH_2; 3.05–3.45 ppm, m, $-NCH_2-$; 3.805 and 3.847 ppm, s, $-OCH_3$ of rotamer I; 3.838 and 3.857 ppm, s, $-OCH_3$ of rotamer II; 6.5–6.8 ppm, m, H_A, H_B, H_C; ~ 7.5 ppm, m, H_6, H_7; ~ 7.8–8.0 ppm, m, H_3, H_4, H_5, H_8; 8.35 ppm, ~ d, J ~ 2 Hz, H_1.

N-*(3,4-Dimethoxyphenethyl)-2-(2-naphthyl)*-m-*dithiane-2-propanamine hydrochloride* ***7***

A solution of 3.20 g (5.7 mmol) of **6**, 0.10 g (2.7 mmol) of sodium borohydride in 45 ml EtOH was stirred for 64 h at room temperature. After addition of 30 ml acetone, the solvent was distilled under reduced pressure, the residue worked up as usual and purified by chromatography using CH_2Cl_2MeOH (98:2) as eluant. A pure fraction corresponding to 1.20 g (45% yield of a heavy oil) was transformed into the corresponding hydrochloride salt **7** by treatment with dioxane containing HCl. Mp = 159–162°C. $C_{27}H_{33}NO_2S_2$·HCl 504.147 (C, H, N, Cl, S).

^{1}H NMR 250 MHz ($CDCl_3$): (as a base) 1.58 ppm, m, $-NHCH_2CH_2CH_2-$; 1.91 ppm, m, SCH_2CH_2; 2.10 ppm, m, $-NHCH_2CH_2CH_2-$; 2.57 ppm, t, J ~ 7 Hz, $-NHCH_2CH_2CH_2-$; 2.64–2.8 ppm, m, $-SCH_2-$, benzylic–CH_2CH_2; ~ 3.6 ppm, l, $-NH-$; 3.82 and 3.83 ppm, $-OCH_3$; 6.66 ppm, dd, J_{ortho} ~ 8 Hz, J_{meta} ~ 2 Hz, H_B; 6.67 ppm, d, H_C; 6.74 ppm, d, H_A; ~ 7.5 ppm, m, H_6, H_7; ~ 7.8–7.9 ppm, m, H_4, H_5, H_8; 7.95 ppm, dd, J_{ortho} = 8 Hz, J_{meta} = 2 Hz, H_3; 8.35 ppm, d, H_1.

N-*[3-[2-(2-Naphthyl)*-m-*dithian-2-yl]propyl]-phthalimide* ***8***

As with the preparation of **4a**, 2.46 g (10 mmol) of **2**, 8.0 ml (12.8 mmol) of a solution of *n*-BuLi in hexane and 2.68 g (10 mmol) of the commercially available 3-bromopropylphthalimide were reacted in 55 ml of dry THF. After usual work up and chromatography of the residue on silicagel using cyclohexane-Et_2O (3:2) as eluant, 3.0 g (69% yield) of an oil which was crystallized from diisopropylether were obtained: mp = 181–182°C. $C_{22}H_{23}NO_2S_2$; mw = 433.584 (C, H, N).

^{1}H NMR 250 MHz ($CDCl_3$): 1.69 ppm, m, $-N-CH_2CH_2-$; 1.92 ppm, m, $-SCH_2-CH_2-$; 2.14 ppm, m, $-N-CH_2CH_2CH_2-$; 2.6–2.8 ppm, m, $-S-CH_2-$; 3.56 ppm, m, $-N-CH_2$; 7.48 ppm, m, H_6, H_7; 7.65–7.98 ppm, m, H_3, H_4, H_5, H_8 and Phthalimid–=CH–; 8.34 ppm, d, J_{meta} = 2 Hz, H_1.

2-(2-Naphthyl)-m-*dithiane-2-propanamine hydrochloride* ***9***

A solution of 1.90 g (4.38 mmol) of **8**, 10 ml of MeOH and 20 ml of aqueous CH_3NH_2 (40%) was stirred for 72 h at room temperature. After evaporation of the solvent under reduced pressure, the residue was taken up in Et_2O–CH_2Cl_2 (9:1), the organic solution extracted with a 1 N NaOH solution and treated as usual. The remaining oil was dissolved in Et_2O and the solution treated with dioxane containing dry HCl. 1.25 g (84% yield) of a solid with an mp of 130°C (dec.) were obtained. $C_{17}H_{21}NS_2$·HCl; mw = 339.943; (C, H, N, Cl, S).

^{1}H NMR 250 MHz (DMSO–d_6): as hydrochloride 1.48 ppm, m, $-N-CH_2CH_2-$; 1.90 ppm, m, $-S-CH_2CH_2-$; 2.24 ppm, m, $-N-CH_2CH_2CH_2-$; 2.65 and 2.90 ppm, m, J_{gem} = 15 Hz, $-SCH_2-$; ~ 2.65 ppm, m, $-N-CH_2-$; 7.54 ppm, m, H_6 H_7; ~ 7.85 ppm, 1, $-NH^{+}_{3}$; ~ 7.8–8.05 ppm, m, H_3, H_4, H_5, H_8; 8.29 ppm, d, J_{meta} ~ 2 Hz, H_1.

N-*[3-[2-(2-Naphthyl)*-m-*dithian-2-yl]propyl]formamide,* ***10***

A solution of 0.70 g (2.3 mmol) of the base corresponding to **9** in 4.8 ml HCOOH (98%) was cooled to 5°C, treated with 1.69 ml acetic anhydride and left overnight at room temperature. After distillation of the solvent under reduced pressure and chromatography of the residue on silicagel using cyclohexane-EtOAc (1:1) as eluant, 0.45 g (59% yield) of **10** as a foam were obtained. $C_{18}H_{21}NOS_2$; mw = 331.492 (C, H, N).

^{1}H NMR 250 MHz ($CDCl_3$): (mixture of rotamers ~ 5:1). 1.50 ppm, m, $-N-CH_2CH_2-$; 1.95 ppm, m, $-S-CH_2CH_2-$; 2.11 ppm, m, $-N-CH_2CH_2CH_2-$; 2.71 ppm, m, $-SCH_2-$; 3.07 ppm, dt, J = 7.5 Hz, J = 7.5 Hz, $-NCH_2-$ of rotamer II; 3.18 ppm, dt, J = 7.5 Hz, J = 7.5 Hz, $-N-CH_2-$ of rotamer I; ~ 5.4 ppm, 1, $-NH-$; 7.50 ppm, m, H_6, H_7; 7.81–7.90 ppm, m, H_4, H_5, H_8 and $-CHO$ of rotamer II; 7.97 ppm, dd, J_{ortho} = 8 Hz, J_{meta} = 2 Hz, H_3; 8.05 ppm, d, J ~ 1.5 Hz, $-CHO$ of rotamer I; 8.36 ppm, d, H_1.

N-*Methyl-2-(2-naphthyl)*-m-*dithiane-2-propanamine hydrochloride,* ***11***

A solution of 431 mg (1.3 mmol) of **10** in 2 ml of dry THF was added, with vigorous stirring, to a boiling suspension of 197 mg (5.2 mmol) of $LiAlH_4$ in 10 ml of dry THF. The mixture was refluxed for 6 h. After addition of a saturated solution of Na_2SO_4, the reaction mixture was extracted with Et_2O. The organic extracts were shaken several times with a 1 M aqueous tartaric acid solution. The aqueous extracts were treated with a concentrated solution of NaOH and extracted several times with Et_2O. After usual work up and chromatography of the residue on silicagel using a mixture of CH_2Cl_2–MeOH–25% aqueous NH_3 (94:5:1) as eluant, a very pure fraction corresponding to 0.2 g (48%

yield) of an oil was isolated, which was transformed as usual into the corresponding hydrochloride salt of mp = 158–163°C. $C_{18}H_{23}NS_2 \cdot HCl$; mw 353.97 (C, H, N, Cl).

^1H NMR 250 MHz (DMSO–d_6): 1.50 ppm, m, $-N-CH_2-CH_2-$; 1.87 ppm, m, $-SCH_2CH_2-$; 2.25 ppm, m, $-N-CH_2-CH_2-CH_2-$; 2.41 ppm, s, 1, $-NCH_3$; 2.66 ppm and 2.92 ppm, m, J_{gem} = 15 Hz, $-SCH_2-$; 2.80 ppm, m, $-N-CH_2-$; 7.56 ppm, m, H_6, H_7; ~ 7.9–8.05 ppm, m, H_3, H_4, H_5, H_8; 8.31 ppm, d, J_{meta} ~ 2 Hz, H_1; ~ 8.62 ppm, 1, $-NH_2^+-$.

Acknowledgments

The authors thank Drs W. Arnold, A. Dirscherl†, W. Meister and W. Vetter for the spectroscopic determinations and microanalyses.

†*Deceased.*

References

1 Hall T.C. (ed.) (1985) Cancer drug resistance. *in: Progress in Clinical and Biological Research,* Vol. 223
2 Gottesman M.M. & Pastan I. (1988) *Trends Pharmacol. Sci.* 91, 54–58
3 Kessel D. & Wilberding C. (1984) *Biochem. Pharmacol.* 33, 1157–1160
4 Kessel D. & Wilberding C. (1985) *Cancer Res.* 45, 1687–1691
5 Gruber A., Peterson C. & Reizenstein P. (1988) *Int. J. Cancer* 41, 224–226
6 Martin S.K., Oduola A.M.J. & Milhous W.K. (1987) *Science* 235, 899–901
7 Krogstad D.J., Gluzman I.Y., Kyle D.E., Oduola A.M.J., Milhous W.K. & Schlesinger P.M. (1987) *Science* 238, 1283–1285
8 Kessel D. & Wilberding C. (1985) *Cancer Treat. Rep.* 69, 673–676
9 Broxterman H.J., Pinedo H.M., Kuiper C.M., Kaptein L.C.M., Schuurhuis G.J. & Lankelma J. (1988) *Fed. Am. Soc. Expr. Biol. J.* 2, 2278–2282
10 Radel S., Bankusli I., Mayhew E. & Rustum Y.M. (1988) *Cancer Chemother. Pharmacol.* 21, 25–30
11 Milhous W.K., Kyle D.E., Oduola A.M.J., Martin S.K., Boudreau E., Schuster B.G., Davidson D.E. & Rossan R.N. (1987) 3rd International Conference on Malaria and Babesiosis, Annecy, France, September 7–11
12 Williams H.L., Johnson D.J., Collins H.H., Martin S.K. & Ramuz H. (1989) *Anal. Biochem.* (in press)
13 Ramuz H. (1978) *Arzneim.-Forsch. / Drug Res.* 28, 2048–2051
14 Kim S., Kim S.S., Lim S.T. & Shim S.C. (1986) *J. Org. Chem.* 52, 2114–2116
15 Ramuz H. (1975) *Helv. Chim. Acta* 58, 2050–2060
16 Weygand F. & Frauendorfer E. (1970) *Chem. Ber.* 103, 2437–2449
17 Johnstone R.A.W., Payling D.W. & Thomas C. (1969), *J. Chem. Soc. C.* 2223–2224