Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 2, pp. 73-77, 1994

0968-0896/94 $6.00 + .00

0968-0896(94)E0002-J

Indolocarbazole Protein Kinase C Inhibitors from Rebeccamycin

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Abstract—Structural modifications were carried out on rebeccamycin, an antitumour antibiotic, to obtain analogues. The inhibitory potencies of these analogues against protein kinase C are compared. The method described represents an alternative route to the staurosporin aglycone, a potent protein kinase C inhibitor.

Introduction

Rebeccamycin **1** is an antitumour antibiotic isolated from cultures of *Saccharotrix aerocolonigenes* (ATCC 39243).[1,2] This bacterial metabolite has no inhibitory activity on protein kinase C (PKC) unlike other structurally related metabolites such as staurosporin,[3] TAN 999 and 1030A,[4] RK286C,[5] UCN 01 and 02,[6] K252a and b,[7] where the two indole nitrogens are linked to a heterocycle, and which are potent PKC inhibitors.

Bromo- or fluoro- analogues have also been isolated from cultures of this bacterial strain supplemented respectively with potassium bromide[8] or fluorotryptophan.[9]

Besides total syntheses of rebeccamycin,[10,11] structural modifications have been carried out to obtain water soluble analogues by introducing an aminoalkyl group on the imide or on the free indole nitrogens,[12] and also to obtain the rebeccamycin aglycone as a precursor for the synthesis of PKC inhibitors.[13]

We report here structural modifications of rebeccamycin yielding analogues. The inhibitory activities of these were tested against PKC.

Results and Discussion

Refluxing rebeccamycin with zinc amalgam[14] in ethanol/HCl according to the method described by Toullec *et al.*[15] yielded the isomeric mixture of **1** and **1'** which could not be separated by chromatography (Figure 1). The ratio of the isomers determined from ^{1}H NMR spectrum was 2:1. The N-glycoside bond was cleaved using 70% perchloric acid affording **2** in a high yield. The amide **2**, described by Kleinschroth *et al.*[16] was prepared by these authors according to the method of Brenner *et al.*[17] from dibromomaleimide and chloroindole; however, no characteristic data were given.

Dechlorination of **2** by refluxing in formic acid/dimethylformamide in the presence of catalytic amounts of palladium on charcoal gave the staurosporin aglycone **3**. The spectroscopic data for **3** were identical in all respects with literature data. [13]

Hydrogenolysis of rebeccamycin using Raney nickel in aqueous sodium hydroxide according to the method described by Buu-Hoï[18] yielded **4** quantitatively. Dechlorination of **1–1'** using Raney nickel proved to be difficult leading to mixtures of dechlorinated products and unreacted **1–1'** whereas using Pd/C in the conditions described for the obtention of **3** yielded the isomeric mixture of **5–5'**, the ratio of which, determined from ^{1}H NMR spectrum, was 1:1.

Besides affording intermediates **1–1'**, **2**, **4** and **5–5'** useful for structure–activity studies, this short reaction sequence provides an alternative route to the staurosporin aglycone **3**, a potent PKC inhibitor.

The inhibitory potencies of **1–5**, **5'** towards PKC and PKA were determined using histones III_S and II_A respectively as substrates, according to the method described by Ricouart *et al.*[19] IC_{50} values are reported in the table; the isoquinoline sulfonamide **H-7** was tested as reference.[20]

Unlike rebeccamycin and **4** which are inactive against PKC, the mixture of amide analogues (**1–1'**) and (**5–5'**) was found to be active. As the two isomers may not have identical activities, one of them might be a more effective PKC inhibitor.

Removal of the sugar moiety led to loss of inhibitory potency for compound **2**. The chlorinated staurosporin aglycone **2**, like the rebeccamycin aglycone **6**,[13] has no activity against PKC, and dechlorination of **2** and **6** (Figure 2) led to **3** and **7** which are active compounds (**3**: IC_{50} = 2.45 μM; **7**: IC_{50} = 44.7 μM[13]).

A marked insolubility of **2** even in DMSO was observed. The inactivity observed with chlorinated compounds may be due to their insolubility: even if the small quantities necessary for the IC_{50} determinations were soluble in DMSO, they could precipitate when diluted with the buffer used for the tests. The sugar moieties in **1–1'** and in **5–5'** may enhance solubility in water and account for the biological activity observed

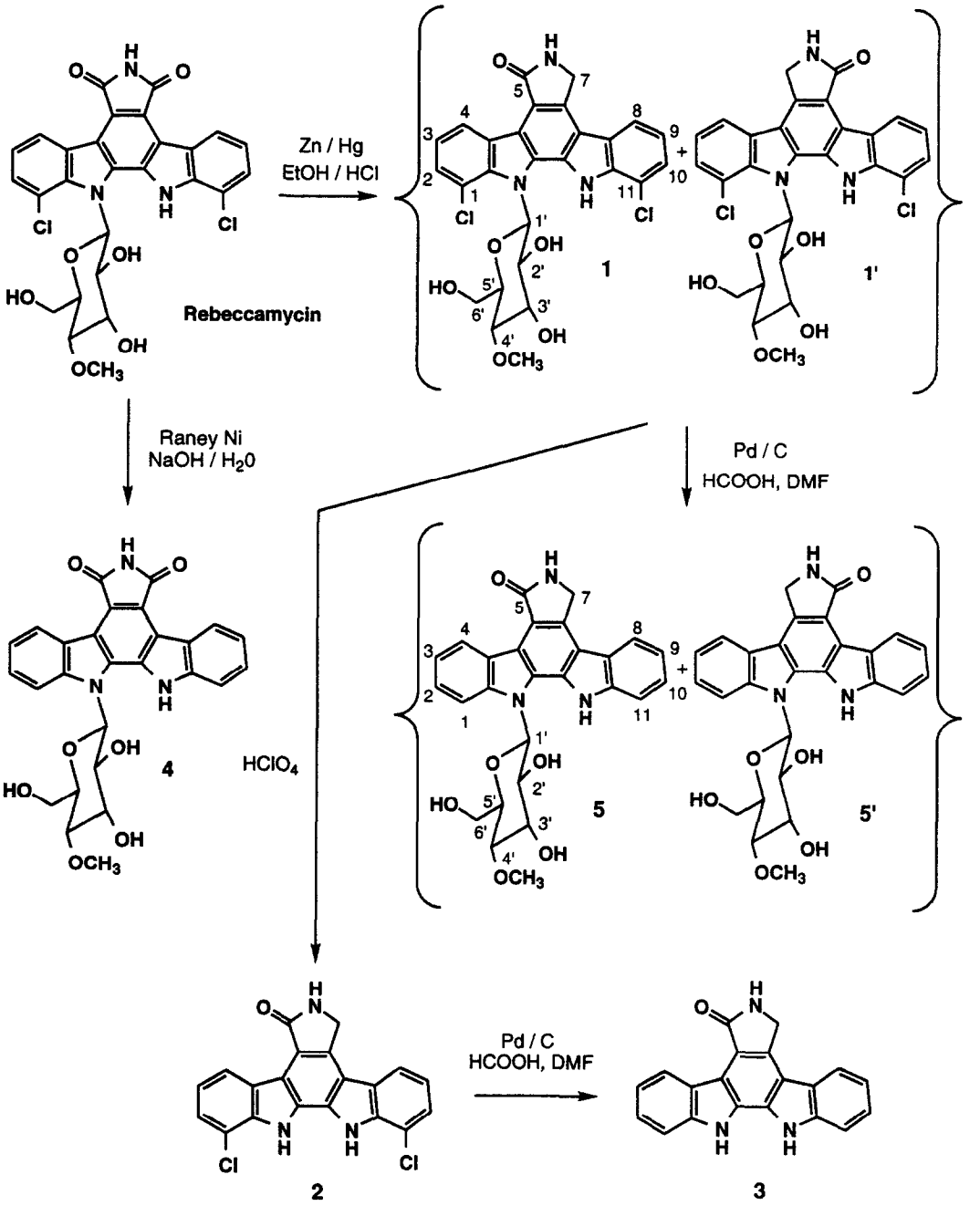

Figure 1.

Table. Inhibitory potencies for compounds 1–5, 5' (IC_{50} μM)

Compound	PKC	PKA
H-7	9.1	3.3
Rebeccamycin	> 100	n.d.
1-1'	28.9	> 100
2	> 100	> 100
3	2.45	25.7
4	> 100	> 100
5-5'	3.7*	n.d.

*Determined from another batch of PKC.

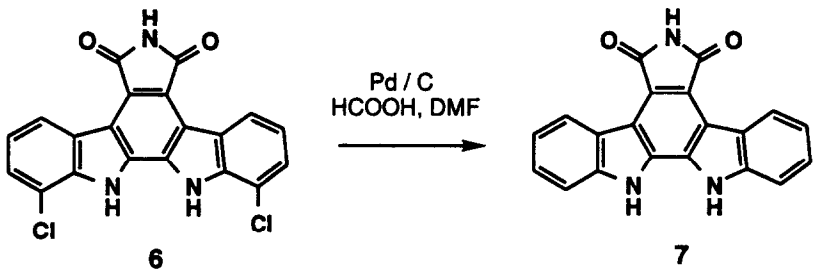

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Figure 2.

By comparison of the activity of **5–5'**, **1–1'** and **3** respectively to the activity of **4**, rebeccamycin and **7**,[21] it can be concluded that the reduction of the carbonyl function in 7-position induces a large increase of activity towards PKC in indolocarbazole series.

Such results are not obtained in the bis-indole series.[21] These data parallel that seen for staurosporin and 7-oxostaurosporin, the activity of the latter towards PKC being 10 times weaker than that of staurosporin.[22]

Experimental Section

All reactions were carried out under argon atmosphere using dry solvents. The solvents were dried according to known procedures. IR spectra were recorded on a Perkin–Elmer 881 spectrometer (ν in cm^{-1}), NMR spectra on a Bruker MSL 300 (^1H: 300 MHz, ^{13}C: 75.45 MHz) (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), tertiary carbons (C tert.), quaternary carbons (C quat.)). Mass spectra (EI, CI and FAB^+) were determined at the Service Central d'Analyses, CNRS (Vernaison) on a VG.ZAB SEQ and at CESAMO (Talence) on a high resolution Fisons Autospec-Q spectrometer. Chromatographic purifications were performed with flash Geduran SI 60 (Merck) 0.040–0.063 mm and silicagel plates (Kieselgel 60 F_{254} Merck). Rebeccamycin was from our laboratory stock sample.

Histone III_S and II_A, phosphatidylserine, diacylglycerol and PKA were purchased from Sigma. [γ ^{32}P] ATP was from Amersham. PKC was from Calbiochem. PKC phosphorylation assays were performed in a reaction mixture (80 µL) containing histone III_S (2.4 mg/mL), $MgCl_2$ (10 mM), $CaCl_2$ (0.1 mM), phosphatidylserine (10 mg/mL), diacylglycerol (10 mg/mL), Tris/HCl buffer (pH 7.5), ATP (10 µM, 1000–2000 cpm/pmol), PKC (0.5 µg/mL). Stock solutions of inhibitors were prepared in DMSO. In each assay, data points were determined in triplicate.

1,11-Dichloro-12(4-O-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c] carbazole, 1 and 1'

Zinc-amalgam (2.5 g) was added to rebeccamycin (300 mg, 0.527 mmol) in a solution of ethanol (35 mL) and HCl (6 mL). The mixture was refluxed for 3 h, then cooled, poured into water and extracted with AcOEt. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was removed and the residue purified by flash chromatography (AcOEt/cyclohexane 90:10) to yield the isomeric mixture of **1** and **1'** as a white powder (160 mg; 0.288 mmol; 55% yield). IR $\nu_{C=O}$ 1700, ν_{N-H} and ν_{OH} 3200, 3600; m.p. > 300 °C. Mass (FAB^+) $(M + H)^+$: 556 (100%). Exact mass calculated for $C_{27}H_{24}Cl_2N_3O_6$ $(M + H)^+$ 556.1042 found 556.1035. ^1H NMR (DMSO-d_6): 3.58 (3H, s, OCH_3); 3.40–4.10 (6H, m, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H, $C_{6'}$-H_2); 4.99–5.03 (3H, C_7-H_2, OH); 5.31–5.44 (2H, 2OH); 6.93 and 6.98 (1H, 2d, J = 10 Hz, $C_{1'}$-H); 7.31 and 7.35 (2H, 2t, J = 7 Hz); 7.51–7.65 (2H, m); 8.00 and 8.05 (1H, 2d, J = 7 Hz); 8.76 and 8.80 (1H, 2s, N_{amide}-H); 9.32 and 9.69 (1H, 2d, J = 7 Hz); 10.40 and 10.52 (1H, 2s, N_{indole}-H). ^{13}C NMR (DMSO-d_6): 45.3 and 45.6 (C_7); 59.9 ($C_{6'}$); 60.3 (OCH_3); 72.4 ($C_{2'}$); 77.7 ($C_{3'}$); 79.3 ($C_{5'}$); 80.1 ($C_{4'}$); 84.2 ($C_{1'}$); 115.9; 116.4; 117.6; 118.2; 119.9; 120.2; 124.4; 124.7; 126.1; 126.3; 126.7; 128.3; 128.4; 133.6; 135.7; 136.3; 136.6; 136.8 (Cquat.); 120.5; 120.9; 121.6; 121.9; 122.4; 124.6; 125.6; 125.7; 125.9; 128.5 (Ctert.); 171.5 and 171.7 (C = O).

1,11-Dichloro-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole, 2

An isomeric mixture of **1** and **1'** (300 mg; 0.540 mmol) in $HClO_4/H_2O$ 70:30 (50 mL) was heated at 100 °C for 2 h. After pouring into water and extraction with AcOEt, the organic phase was washed with saturated aqueous $NaHCO_3$ and brine. Purification by flash chromatography (AcOEt) afforded **2** as a white solid (178 mg; 0.470 mmol; 87% yield). IR $\nu_{C=O}$ 1700, ν_{N-H} 3200, 3320; m.p. > 300 °C. Exact mass (EI) calculated for $C_{20}H_{11}Cl_2N_3O$: 379.0279, found 379.0289. ^1H NMR (DMSO-d_6): 4.90 (2H, s); 7.21 (1H, t, J = 7 Hz); 7.23 (1H, t, J = 7 Hz); 7.48 (2H, t, J = 7 Hz); 7.90 (1H, d, J = 7 Hz); 8.54 (1H, s, N_{amide}-H); 9.11 (1H, s, J = 7 Hz); 11.45 (1H, s, N_{indole}-H); 11.63 (1H, s, N_{indole}-H). ^{13}C NMR (DMSO-d_6): 45.2 (C_7); 114.7; 115.2; 115.9; 116.0; 119.8; 124.1; 125.2; 125.3; 127.6; 133.7; 135.8; 135.9; (C quat.); 115.6; 120.0; 120.1; 121.0; 124.4; 124.5 (C tert.); 171.9 (C = O).

6,7,12,13-Tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole, 3

Compound **2** (60 mg; 0.158 mmol) was dissolved in DMF (10 mL). 85% HCOOH (1 mL) was added followed by catalytic amounts of Pd/C. The mixture was refluxed for 3

h. After filtration, the solid residue was washed with hot water, the filtrate was extracted with AcOEt washed with 10% aqueous $NaHCO_3$ and brine. After drying over $MgSO_4$ and removal of the solvent purification of the residue by flash chromatography gave **3** as a white powder (15 mg; 0.048 mmol; 30% yield).

12-(4-O-Methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro-indolo[2,3-a]pyrrolo[3,4-c] carbazole-5,7-dione, 4

Rebeccamycin (121 mg; 0.212 mmol) in water (20 mL) and NaOH (137 mg) were refluxed. Raney nickel (137 mg) was added portionwise and then reflux was carried out for 3 h. After filtration and washing the solid residue with hot water, the filtrate was acidified with HCl 0.5 N and the resulting yellow solid precipitate was extracted with AcOEt. Purification using TLC silicagel plates (eluent AcOEt) gave **4** as a yellow solid (105 mg; 0.210 mmol; 99% yield). IR $\nu_{C=O}$ 1750, ν_{OH} and ν_{N-H} 3250–3600; m.p. > 260 °C. Mass (CI) $(M + H)^+$ 503, $((M + H)^+-H_2O)$ 485. ^1H NMR (DMSO-d_6): 3.68 (3H, s, OCH_3); 3.40–4.10 (6H, m, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H, $C_{6'}$-H_2); 5.08 (1H, d, J = 2 Hz, OH); 5.37 (1H, s, OH); 6.28 (1H, s, OH); 6.39 (1H, d, J = 10 Hz); 7.46 (2H, t, J = 7 Hz); 7.66 (2H, t, J = 7 Hz); 7.80 (1H, d, J = 7Hz); 8.50 (1H, d, J = 7 Hz); 8.92 (1H, d, J = 7 Hz); 8.99 (1H, d, J = 7 Hz); 11.89 (1H, s, N-H). ^{13}C NMR (DMSO-d_6): 59.3 ($C_{6'}$); 60.8 (OCH_3); 73.3 ($C_{2'}$); 76.9 ($C_{3'}$); 78.0 ($C_{4'}$, $C_{5'}$); 84.9 ($C_{1'}$); 117.9; 118.1; 119.4; 119.6; 121.2; 121.7; 129.9; 131.2; 141.5; 142.9 (C quat.); 121.8; 121.9; 122.1; 128.2; 128.4 (C tert.); 165.4 (C=O).

12(4-O-Methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c] carbazole, 5 and 5'

The isomeric mixture of **1–1'** (100 mg; 0.179 mmol) was treated as described for the preparation of **3**. The mixture was refluxed for 3 days. After identical work up as for **3**, purification by flash chromatography (AcOEt/cyclohexane 90:10) yielded the isomeric mixture of **5–5'** as an off-white powder (35 mg; 0.072 mmol; 40% yield). IR $\nu_{C=O}$ 1670, ν_{N-H} and ν_{OH} 3300–3550; m.p. 235–240 °C. Mass (FAB$^+$) $(M + H)^+$: 488. Exact mass calculated for $C_{27}H_{26}N_3O_6$ $(M + H)^+$ 488.1821, found 488.1824. ^1H NMR (DMSO-d_6): 3.72 and 3.74 (3H, 2s, OCH_3); 3.59–4.12 (6H, m, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H, $C_{6'}$-H_2); 4.92–5.08 (3H, C_7-H_2, OH); 5.35 (1H, OH); 6.25–6.34 (2H, $C_{1'}$-H, OH); 7.29 and 7.37 (1H, 2t, J = 8 Hz); 7.49 and 7.53 (1H, 2t, J = 8 Hz); 7.70 and 7.75 (1H, 2d, J = 8 Hz); 7.91 and 7.97 (1H, 2d, J = 8Hz); 8.06 and 8.12 (1H, 2d, J = 8 Hz); 8.61 and 8.67 (1H, 2s, N_{amide}-H); 9.37 and 9.48 (1H, 2d, J = 8 Hz); 11.35 and 11.48 (1H, 2s, N_{indole}-H). ^{13}C NMR (DMSO-d_6): 45.2 and 45.3 (C_7); 58.6 ($C_{6'}$); 60.0 and 60.1 (OCH_3); 73.2 and 73.3 ($C_{2'}$); 76.5; 77.0; 77.1; 77.4 ($C_{3'}$, $C_{4'}$, $C_{5'}$); 84.2 ($C_{1'}$); 111.1; 111.6; 111.7; 112.1 (C_1, C_{11}); 119.1; 119.4; 120.0; 120.4; 121.0; 121.1; 124.9 (2C); 125.0; 125.1; 125.3; 125.5 (C tert.); 115.3; 116.4; 117.0; 118.6; 118.8; 120.1; 122.1; 122.5 122.8; 124.8; 126.0; 127.0; 128.1; 128.4; 132.5; 134.2; 139.2; 139.7; 141.0; 141.1 (C quat.); 172.1 and 172.2 (C=O).

Acknowledgements

We are grateful to RIOM-Laboratoires CERM for financial support and to N. Grangemare for her technical assistance.

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(*Received* 24 *May* 1993; *accepted* 6 *January* 1994)