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Short communication

A three-step synthesis from rebeccamycin of an efficient checkpoint kinase 1 inhibitor

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Abstract

Rebeccamycin derivative **1** bearing a sugar moiety linked to both indole nitrogens and an amino substituent on the carbohydrate unit was synthesized in three steps from the bacterial metabolite. This compound was found to be a highly potent checkpoint kinase 1 inhibitor with an IC_{50} value of 2.8 nM.

Keywords: Rebeccamycin; Staurosporine; Antitumor agents; Indolocarbazoles; Chk1 inhibitors

1. Introduction

Rebeccamycin is a microbial metabolite isolated from cultures of *Saccharothrix aerocolonigenes* [1]. Its antiproliferative activity was shown to be linked to its capacity to inhibit topoisomerase I [2,3]. Topoisomerase I is a ubiquitous enzyme necessary for DNA replication and transcription. This nuclear enzyme catalyses DNA unwinding by cleaving one strand of the DNA, allowing the other strand to pass through the break and resealing the cleaved strand. Rebeccamycin, like camptothecins, is a topoisomerase I poison which stabilizes the ''cleavable complex'' and prevents the religation of the cleaved strand [4,5]. Rebeccamycin possesses a maleimide indolocarbazole framework onto which is attached a 4-*O*-methyl glucopyranose via a β-*N*-glycosidic bond. Structure–activity relationship studies have been performed and several families of rebeccamycin analogues were prepared either by semi-synthesis or by total synthesis [2,6–12]. Among these families, a series of staurosporine analogues were obtained from rebeccamycin by semi-synthesis [13,14]. Staurosporine is a microbial metabolite, structurally related to rebeccamycin (Fig. 1).

Both compounds possess an indolocarbazole chromophore but the main differences are the function in the upper heterocycle, lactam in staurosporine and imide in rebeccamycin, and the sugar moiety linked to both indole nitrogens in staurosporine and to only one indole nitrogen in rebeccamycin. Moreover the sugar part of staurosporine bears an amino substituent [15]. In contrast with rebeccamycin, staurosporine is not a topoisomerase I inhibitor but exhibits a non-selective inhibitory activity toward kinases including checkpoint kinase 1 (Chk1), a kinase involved in the regulation of the G2 cell cycle checkpoint [15–17]. Among the staurosporine analogues obtained from rebeccamycin, compound **1** was especially interesting because of its amino substituent on the carbohydrate moiety that could mimic the one of staurosporine. Therefore, compound **1** was prepared by semi-synthesis from rebeccamycin but the first synthetic pathway required more than six steps [14]. In this paper, we report a three-step semi-synthesis of compound **1** from rebeccamycin. The inhibitory activity of compound **1** toward Chk1 and tyrosine kinase Src was determined.

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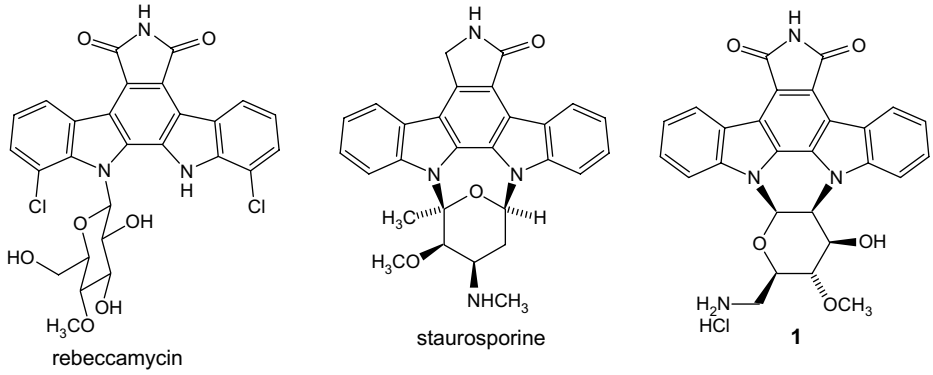


Fig. 1. Bacterial metabolites rebeccamycin and staurosporin, and rebeccamycin derivative **1**.

2. Chemistry

In a previous work [14], we investigated the synthesis of compound **1** in six steps as shown in Scheme 1, the last step would be the reduction of azide **8** to the corresponding amine. Monotosylation was performed at the 2′ position of the carbohydrate, then reaction with sodium azide led to cyclized compound **3** as the major product. Dechlorination using ammonium formate in the presence of Pd/C gave compound **5**. Chlorination at the 6′ position was carried out using CCl_4 and PPh_3 in pyridine but the required compound **6** was obtained as the minor product of the reaction in only 23% yield. Treatment of compound **6** with sodium azide led to azide **8** in 38% yield, but as in the case of the preparation of compound **6**, the major product of the reaction was anhydro **7**. The overall yield in the synthesis of azide **8** from rebeccamycin was about 2%.

To avoid the formation of by-products in steps 4 and 5 which lowered the overall yield, selective protection–deprotection sequence on the sugar moiety was investigated (Scheme 2) [14]. The hydroxy groups of the carbohydrate were protected as acetates, then a selective deprotection at the 6′ position was carried out using BF_3–etherate in wet acetonitrile. Chlorination and nucleophilic substitution with sodium azide were performed as in the first synthesis. Reduction of the azide was then carried out by catalytic hydrogenation. Deprotection of the hydroxy group at the 3′ position was performed by aminolysis and the amine transformed into hydrochloride **1**. In this second way, the overall yield for the synthesis of **1** was less than 2%, and the synthesis needed nine steps.

In this paper, a three-step synthesis of compound **1** from rebeccamycin is reported. Ditosylated compound **13** was obtained in 35% yield by reaction of rebeccamycin with 5 equiv. of tosyl chloride and K_2CO_3. By the treatment of **13** with sodium azide (20 equiv.) both nucleophilic substitution at the 6′ position and at the 2′ position occurred to give compound **14** in 53% yield. Reduction of the azide and dechlorination occurred simultaneously on treatment with ammonium formate and Pd/C. Hydrochloride **1** was obtained from **14** in 36% yield. The overall yield from rebeccamycin was improved (about 7%) and the synthesis needed only three steps (Scheme 3).

3. Kinase inhibitory activities

The Chk1 inhibitory activity of compound **1** was evaluated. The percentage of Chk1 inhibition at a drug concentration of 0.1 μM was 90%. The IC_{50} value toward Chk1 was found to be 2.8 nM. To get an insight into the kinase selectivity, the inhibitory activity of compound **1** was evaluated toward the Src tyrosine kinase. The percentage of Src inhibition at a drug concentration of 1 μM was 35%, much less than that toward Chk1.

4. Conclusion

In summary, a new synthesis of a staurosporine analogue, with the sugar unit linked to both indole nitrogens and bearing an amino function at the 6′ position on the carbohydrate moiety, was performed in three steps from rebeccamycin. This rebeccamycin derivative is especially interesting as a highly potent Chk1 inhibitor, with an IC_{50} value in the nanomolar range.

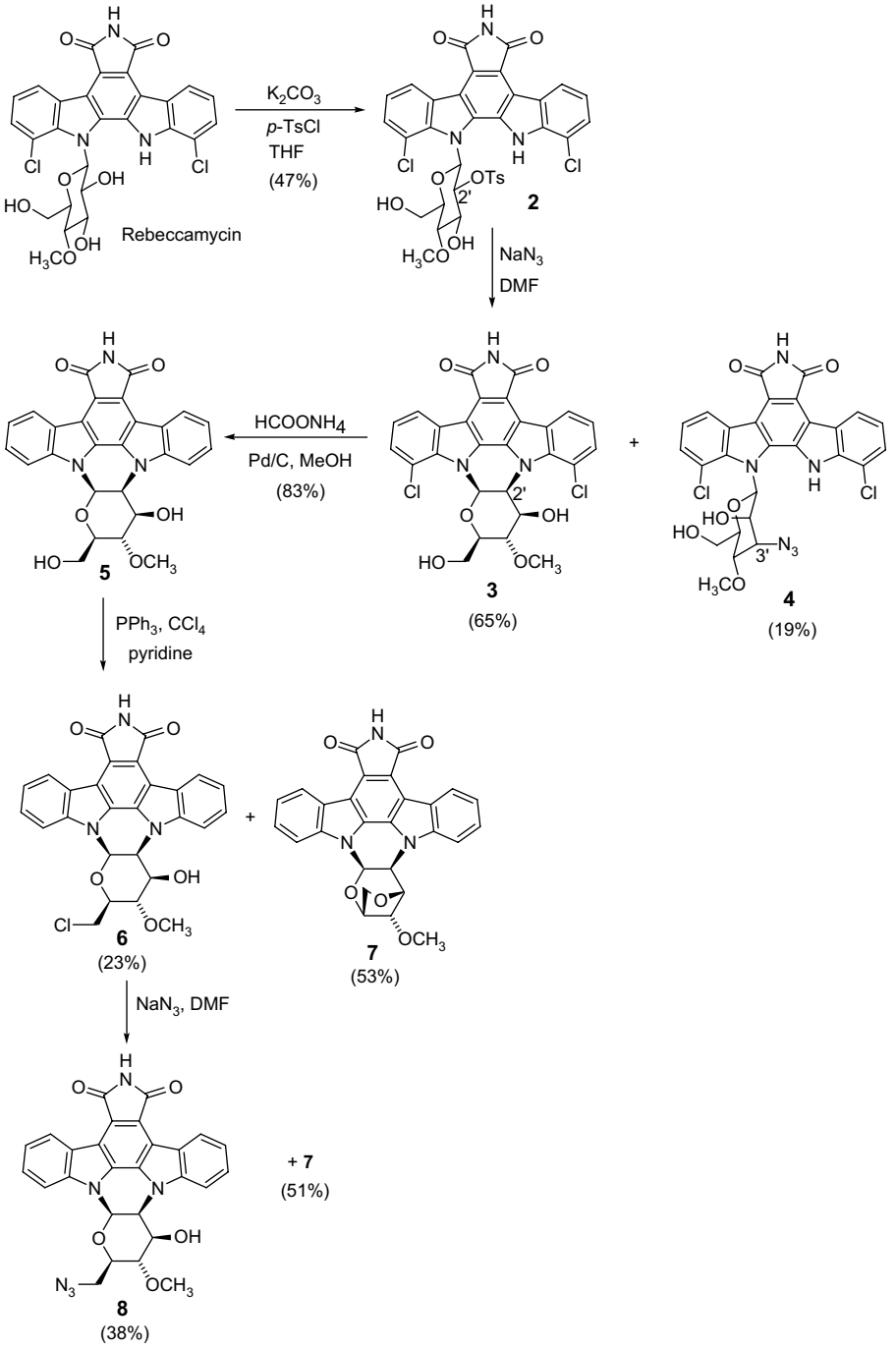
5. Experimental

5.1. Chemistry

IR spectra were recorded on a Perkin–Elmer 881 spectrometer ($\overline{\nu}$ in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 and AVANCE 500 (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), broad signal (br s), doublet (d), doubled doublet (dd), doublet of doublet of doublet (ddd), triplet (t), multiplet (m), quadruplet (q), tertiary carbons (C tert), quaternary carbons (C quat)). HRMS (FAB+) were determined on a high resolution Fisons Autospec-Q spectrometer at CESAMO (Talence, France). Chromatographic purifications were performed by flash silica gel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography.

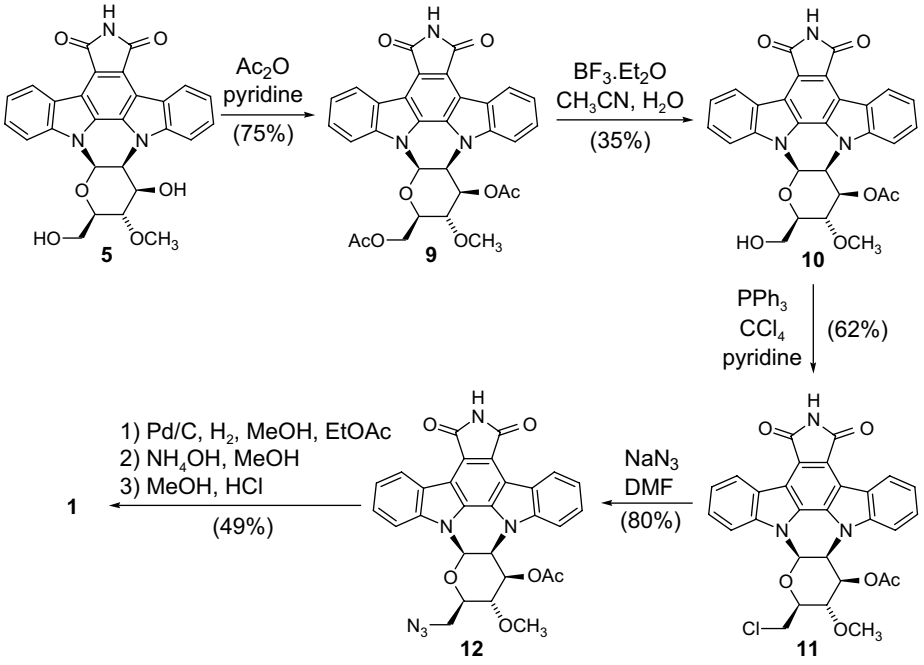
5.1.1. 7,10-Dichloro-8-(4-O-methyl-1-deoxy-2,6-di-O-tosyl-β-D-glucopyranos-1-yl)-8,9-dihydro-1H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-1,3(2H)-dione (13)

To a solution of rebeccamycin (100 mg, 0.175 mmol) in THF (34 mL) were added K_2CO_3 (121 mg, 0.877 mmol,



Scheme 1. First attempt for the synthesis of compound **1** from rebeccamycin.

5 equiv.) and *p*-tosyl chloride (167 mg, 0.877 mmol, 5 equiv.). The mixture was refluxed for 4 days. After removal of the solvent, the residue was purified by two successive flash chromatography on silica gel (eluents, cyclohexane/EtOAc, 8:2 and dichloromethane/EtOAc, 97.5:2.5) to give **13** (54.1 mg, 0.062 mmol, 35% yield) as a yellow solid. Mp 140–143 °C. IR (KBr) $\overline{\nu}_{C=O}$ 1715, 1760 cm^{-1}, $\overline{\nu}_{NH,OH}$ 3300–3600 cm^{-1}. HRMS (FAB+) $(M)^+$ calcd for $C_{41}H_{33}N_3O_{11}S_2Cl_2$, 877.0934; found 877.1060. ^1H NMR (400 MHz, DMSO-d_6): 1.68 (3H, s, CH_3), 2.23 (3H, s, CH_3), 3.57 (3H, s, OCH_3), 3.71 (1H, t, $J = 8.5$ Hz), 4.10 (1H, q, $J = 7.5$ Hz), 4.49 (1H, ddd, $J_1 = 8.5$ Hz, $J_2 = 6.5$ Hz, $J_3 = 2.0$ Hz), 4.58 (1H, dd, $J_1 = 11.5$ Hz, $J_2 = 6.5$ Hz), 4.72 (1H, dd, $J_1 = 11.5$ Hz, $J_2 = 2.0$ Hz), 4.91 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 8.0$ Hz), 6.28 (1H, d, $J = 7.5$ Hz), 6.56 (2H, d, $J = 8.0$ Hz), 6.66 (2H, d, $J = 8.5$ Hz), 6.75 (2H, d, $J = 8.0$ Hz), 7.37–7.42 (4H, m), 7.52 (1H, t, $J = 8.0$ Hz), 7.61 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz), 7.77 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz), 9.10 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz), 9.12 (1H, d, $J = 7.5$ Hz), 9.81 (1H, s, NH), 11.49 (1H, s, NH). ^{13}C NMR (100 MHz,

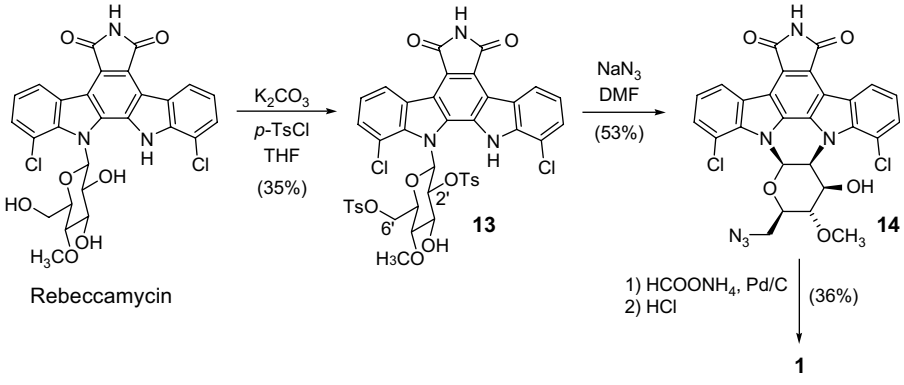


Scheme 2. Synthesis of compound **1** in nine steps from rebeccamycin.

DMSO-d_6): 20.4, 21.0 (CH_3), 60.0 (OCH_3), 69.1 (CH_2), 73.5, 76.5, 78.8, 80.0, 80.7 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 115.2, 116.4, 118.0, 119.1, 120.6, 122.9, 123.3, 124.9, 128.0, 128.8, 131.9, 132.3, 135.3, 137.2, 144.1, 144.3 (C quat arom), 122.4, 122.6, 123.7, 124.3, 125.6 (2C), 127.0 (3C), 129.1 (4C), 130.1 (C tert arom), 170.3, 170.4 (C=O).

*5.1.2. 7,10-Dichloro-8,9-(4-O-methyl-6-azido-1,2,6-trideoxy-β-D-mannopyranos-1,2-diyl)-8,9-dihydro-1H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-1,3(2H)-dione (**14**)*

A mixture of **13** (100 mg, 0.114 mmol) and NaN_3 (148 mg, 20 equiv.) in DMF (3 mL) was heated at 70 °C for 1 week. Water was added. After extraction with EtOAc, the organic phase was washed with water and dried over $MgSO_4$, the solvent was removed and the residue purified by flash chromatography (eluent, THF/dichloromethane, 2.5:97.5 then 5:95). After evaporation of the solvents, the solid was washed with diethylether to give **14** (34.9 mg, 0.060 mmol, 53% yield) as a yellow solid. Mp > 240 °C (decomposition). IR (KBr) $\overline{\nu}_{C=O}$ 1710, 1750 cm^{-1}, $\overline{\nu}_{N_3}$ 2105 cm^{-1}, $\overline{\nu}_{NH,OH}$ 3300–3600 cm^{-1}. HRMS (FAB+) $(M+H)^+$ calcd for $C_{27}H_{18}N_6O_5Cl_2$, 576.0716; found 576.0721. ^1H NMR (400 MHz, DMSO-d_6): 3.38 (1H, m), 3.48 (3H, s, OCH_3), 3.47 (1H, dd, $J_1 = 13.5$ Hz, $J_2 = 7.5$ Hz), 3.64 (1H, dd, $J_1 = 13.5$ Hz, $J_2 = 3.5$ Hz), 4.04 (1H, m), 4.64 (1H, m), 4.96 (1H, d, $J = 6.5$ Hz), 5.96 (1H, dd, $J_1 = 6.5$ Hz, $J_2 = 3.0$ Hz), 6.91 (1H, d, $J = 7.0$ Hz), 7.44 (1H, t, $J = 8.0$ Hz), 7.47 (1H, t, $J = 8.0$ Hz), 7.66 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz), 7.70 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz), 8.67 (1H, d, $J = 8.0$ Hz), 8.72 (1H, d, $J = 8.0$ Hz), 11.15 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 52.8 (CH_2), 56.4, 57.2, 68.6, 75.2, 79.5, 80.7 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.1 (2C), 116.6,



Scheme 3. Three-step synthesis of compound **1** from rebeccamycin.

117.6, 120.3, 121.0, 126.0, 126.6, 128.1, 130.0, 136.1, 136.8 (C quat arom), 122.3, 122.7, 122.9, 123.5, 127.7, 127.9 (C tert arom), 170.3 (2 C=O).

*5.1.3. 8,9-(4-O-Methyl-6-amino-1,2,6-trideoxy-β-D-mannopyranos-1,2-diyl)-8,9-dihydro-1H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-1,3(2H)-dione hydrochloride (**1**)*

A light-protected mixture of **14** (216 mg, 0.374 mmol), 10% Pd/C (648 mg) and $HCOONH_4$ (648 mg) in methanol (53 mL) was stirred under argon atmosphere at room temperature for 4 days. After filtration over Celite, the solid residue was washed with EtOAc and methanol. The filtrate was evaporated and the residue dissolved in EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, and then was acidified with 1 N HCl. The aqueous phase was washed with EtOAc and treated with saturated aqueous $NaHCO_3$. After extraction with EtOAc, the organic phase was dried over $MgSO_4$. The solvent was removed, the residue dissolved into methanol and 1 N HCl (500 μL) was added before evaporation. Dichloromethane was added to the residue. Filtration gave compound **1** (69 mg, 0.133 mmol, 36% yield). For spectroscopic data of compound **1** see Ref. [14].

5.2. Chk1 inhibitory assays

Human Chk1 full-length enzyme with an N-terminal GST sequence was either purchased from Upstate Biochemicals (no. 14-346) or purified from extracts of Sf9 cells infected with a baculovirus encoding GST-Chk1. Assays for compound testing were based upon the method described by Davies et al. [18].

5.3. Src inhibition assays

The drug was diluted with a Tecan Evo150 robot. The kinase assay was performed with 4 μL of inhibitor (10% dimethylsulfoxide, DMSO), 10 μL of kinase assay buffer 4× concentrated (80 mM $MgCl_2$, 200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.4 mM ethylenediamine-tetraacetic acid (EDTA), 2 mM DL-dithiothreitol (DTT)), 10 μL of substrate peptide (KVEKIGEGYYGVVYK, 370 nM) and 6 μL of Src kinase (stock GTP purified diluted with 1× kinase assay buffer to 200 nM). 10 μL of co-substrate (40 μM ATP with 0.2 μCi P^{33}-γ-ATP) was added with a Precision 2000 (Biotek Robotic). The assay was incubated for 20 min at 30 °C, stopped by adding 200 μL of 0.85% orthophosphoric acid, and then transferred to a phosphocellulose filter microplate (Whatman – P81). The plate was washed three times with 200 μL of 0.85% orthophosphoric acid and dried with 200 μL of acetone. The remaining activity was measured on a Topcount with 25 μL of scintillation solution (Packard UltimaGold).

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