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Synthesis of indolo[2,3-*a*]carbazole glycoside analogs of rebeccamycin: inhibitors of cyclin D1-CDK4

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Abstract—The synthesis and structure–activity relationships of a new series of indolo[2,3-*a*]carbazole glycosides, analogs of rebeccamycin, derived from the natural sugars (glucose, fucose, mannose, xylose, rhamnose, and galactose) is described.

1. Introduction

Indolo[2,3-*a*]carbazole glycosides are divided into two classes based upon their structure and mechanism of action (Fig. 1). The staurosporine (**1**) and K252a (**2**) class, containing two glycosidic bonds linked to the indolocarbazole heterocycle are inhibitors of protein kinases.[1] In contrast, indolo[2,3-*a*]carbazole glycosides with only one glycosidic linkage to the indolocarbazole (e.g., rebeccamycin (**3**),[2,3]) bind to DNA and possess anti-tumor properties. This latter class are currently under evaluation in the clinic for treatment of a variety of malignancies including advanced renal carcinoma, metastatic or locally recurrent colorectal cancer and stage IIIB or IV breast cancer.

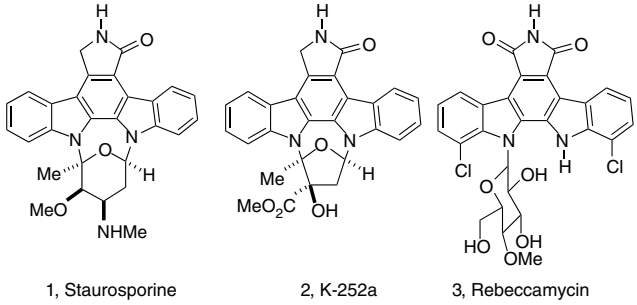

Figure 1. Chemical structures for (+)-staurosporine, K252a, and rebeccamycin derivatives.

As part of our continued effort to identify novel and selective inhibitors of cyclin D1-CDK4 for the treatment of cancer,[4–9] we wanted to evaluate the synthesis and biological activity of rebeccamycin analogs that lack the aryl chlorine groups and in which the 4-*O*-methylglucose moiety has been replaced by a naturally occurring sugar, for example glucose, fucose, mannose, xylose, rhamnose, and galactose. Despite multiple reports on the synthesis[10–12] and structure–activity relationships of rebeccamycin,[13–17] no work has been reported on the simpler analogs of this class. Since the sugar is often the critical determinant of the key biological activity, an efficient strategy to prepare this class of compounds would be valuable.

2. Results and discussion

A number of methods for glycosylation of indoles and indolocarbazoles have been reported.[8,10–12,17–23] Of these procedures the Mannich dimerization of indoles[24] is an important strategy because selective glycosidation can

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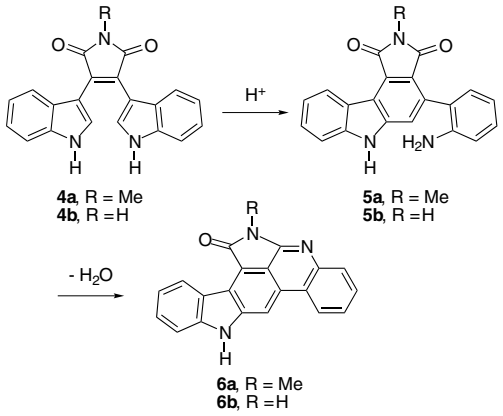


Scheme 1. Synthesis of indolocarbazole glycosides.

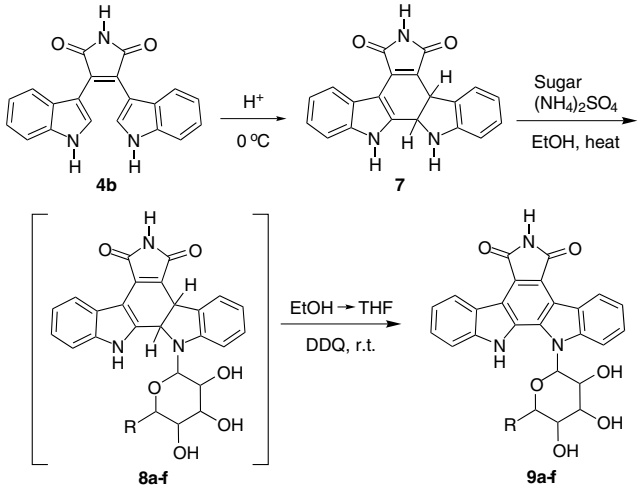
be achieved using unprotected, unactivated carbohydrates.[25–27] Mannich dimerization of *N*-methyl arcyriaubin A (**4a**),[28,29] or its maleimide equivalent (**4b**), has been reported to afford predominantly carbazole **5** unless the indoline intermediate is oxidized in situ (Scheme 1).[28,30] Once formed, **5** undergoes intramolecular cyclization to **6** under the reaction conditions. For this reason, to successfully perform glycosidations on an indole-indoline derived from **4**, it has been reported that the maleimide double bond must be reduced to the corresponding succinimide.[22]

To develop an efficient strategy to prepare the glycosidated rebeccamycin analogs, we identified **7** as a late stage synthetic intermediate that could be glycosidated in one step. Since the maleimide NH is known to be required for biological activity, we wanted to directly access this compound and avoid any deprotection steps that would be required if the corresponding NMe derivative was employed. Upon evaluation of the Mannich dimerization of **4b** previously reported, we confirmed that at room temperature **6b** was the major product. However when the reaction was performed at 0 °C indole-indoline **7** was obtained in 80% yield. In fact, **7** proved to be a stable compound, that was not prone to oxidation by air or light, either in solution or as a crystalline solid. This extra stability of **7** allowed us to evaluate the glycosidation of this substrate. Using conditions similar to those developed by Chisholm and Van Vranken,[22] we identified an efficient synthesis of **8a**–**f** by heating an EtOH solution of **7** with excess sugar (5 equiv) and ammonium sulfate (5 equiv) until the reaction was complete (3–72 h). However, isolation of **8a**–**f** proved extremely difficult due to the polarity of these compounds and their ability to form solvates and hydrates. Thus to eliminate this problem a one-pot procedure, in which the glycosidated indole-indolines (**8a**–**f**) were used directly in the DDQ oxidation step without isolation was developed (Table 1). Once the glycosidation reaction to **7** was complete, EtOH was removed in vacuo, and DDQ (1 equiv) in THF was added. After oxidation, indolo[2,3-*a*]carbazole glycosides **9a**–**f** were isolated in 49–97% yield from **7** (Scheme 2).[31]

Table 1. One-pot synthesis of indolo[2,3-*a*]carbazole glycosides

Entry	Compound	Sugar[a,b]	Yield (%) from **4**
1	**9a**	D-Glucose	88
2	**9b**	D-Galactose	96
3	**9c**	L-Fucose	97
4	**9d**	L-Rhamnose	60
5	**9e**	D-Xylose	49
6	**9f**	D-Maltose	91

[a] Glycosidation was performed with 5 equiv of saccharide and $(NH_4)_4SO_4$ in EtOH.
[b] Oxidations were performed in THF with 1 equiv DDQ.



Scheme 2. Synthesis of indolo[2,3-*a*]carbazole glycosides.

Each saccharide exhibited differing reactivity and stability during the reaction. For D-glucose formation of **8a** was 75% complete within 5 h, but could not be advanced past 85%. Alternatively, D-galactose and L-fucose provided highly stable glycosylated intermediates and oxidized products (**8** and **9b**,**c**, respectively). L-Rhamnose was sluggish during the glycosylation step to prepare **8d** and demonstrated instability during the oxidation reaction. D-Xylose afforded a quantitative yield of **8e** within 5 h but was also found to be unstable to the oxidation conditions, as demonstrated by the lower yield for **9e** (49%). In analogy with literature precedent,[27,32] compounds **9a**–**f** are believed to have the sugar in the axial configuration.

Indolo[2,3-*a*]carbazole glycosides **9a**–**f** were evaluated for their D1-CDK4 inhibitory activity in an enzymatic assay by measuring phosphorylation of Rb^{ING} substrate according to standard protocols.[33] In addition, other assays such as B-CDK1, E-CDK2, PKA, and CAMII were also conducted to determine the selectivity profile. The IC_{50} values determined were compared to staurosporine (**1**) and K252a (**2**) used as a control (Table 2).

As reported in the literature, staurosporine (**1**) is a potent and nonselective kinase inhibitor.[34–36] Although staurosporine potently inhibits D1-CDK4 ($IC_{50} =$ 59 nM), it also potently inhibits other kinases tested (E-CDK2, B-CDK1, CAMKII, PKA, and PKC).

Table 2. IC_{50} values for indolo[2,3-*a*]-carbazole glycosides **9a**–**f**

Compound	D1-CDK4 (Rb^{ING})	B-CDK1 (Histone)	E-CDK2 (Rb^{ING})	CAMKII (Histone)	PKA (Histone)	PKCβ II
9a	0.785	9.75	6.880	0.565	>20	0.594
9b	1.762	5.24	2.063	1.098	>20	5.268
9c	1.600	>20	1.830	2.066	>20	2.04
9d	0.076	3.16	0.296	0.621	>20	1.145
9e	0.130	1.66	0.404	0.135	>20	0.318
9f	0.803	>20	16.18	0.665	>20	0.748
1	0.059	0.012	0.021	0.002	0.038	0.007
2	0.28	0.141	0.299	NT	0.38	0.104

However, in analogy to staurosporine **1**, the simple indolo[2,3-*a*]carbazole glycosides **9a**–**f**, have good inhibitory activity for D1-CDK4, however they also show improved selectivity toward several other kinases (B-CDK1, E-CDK2, PKA, and PKC) (Table 2). Of the compounds prepared **9d**, derived from L-rhamnose, was found to be the most selective and potent analog for D1-CDK4 ($IC_{50} = 76$ nM).

3. Conclusion

An efficient two-step process to prepare indolo[2,3-*a*]carbazole glycoside analogs of rebeccamycin **9a**–**f** in 40–78% yield is reported. These compound are selective inhibitors of cyclin D1-CDK4.

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31. (**7**): Arcyriarubin A (**4b**) (72.5 g, 0.221 mol, 1.0 equiv) was added to cold (0 °C) TFA (1.7 L) with stirring. The resulting dark red solution was stirred under N_2 at 0 °C for ~6 h until the reaction was complete. Cold (0 °C) hexanes (1.3 L) was added slowly, and the TFA salt of **7** precipitated. The slurry was allowed to warm to room temperature, filtered, and rinsed with hexanes. After brief air drying, the salt was partitioned between EtOAc and satd $NaHCO_3$ solution. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 58.0 g (80%) **7** as a red-orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 10.68 (s, 1H), 8.28 (dd, 1H, $J = 6.59$ Hz, 3.29 Hz), 7.46 (dd, 1H, $J = 5.90$ Hz, 2.2 Hz), 7.22–7.11 (m, 3H), 6.97 (app t, 1H, $J = 7.5$ Hz), 6.63–6.57 (m, 2H), 6.27 (br s, 1H), 5.52 (d, 1H $J = 11.7$ Hz), 4.70 (d, 1H, $J = 11.7$ Hz); HRMS (ES) exact mass calcd for $C_{20}H_{13}N_3O_2$ M+327.1018, found 327.1017. General procedure for the synthesis of indolo[2,3-*a*]carbazole glycosides (**9**): Indole-indoline **7** (1.0 equiv), saccharide (5 equiv), and $(NH_4)_2SO_4$ (3 equiv) were combined with EtOH and heated to reflux. After reaction completion the solvent was removed in vacuo. The crude mixture was dissolved in THF and DDQ (1.1 equiv) added. The reaction was stirred at room temperature for 24 h, then quenched with satd $NaHCO_3$ solution, diluted with EtOAc, and the layers separated. The aqueous layer was extracted with EtOAc and the organic layer dried ($MgSO_4$), filtered, and concentrated onto silica gel. Purification by silica gel chromatography (40% acetone–hexanes) yielded **9**.
(**9a**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.68 (s, 1H), 11.11 (s, 1H), 9.19 (d, 1H, $J = 8.3$ Hz), 9.11 (d, 1H, $J = 8.3$ Hz), 7.99 (d, 1H, $J = 8.8$ Hz), 7.72 (d, 1H, $J = 8.3$ Hz), 7.62–7.55 (m, 2H), 7.42–7.35 (m, 2H), 6.30 (d, 1H, $J = 9.2$ Hz), 6.01 (br s, 1H), 5.40 (d, 1H, $J = 8.3$ Hz), 5.14 (br s, 1H), 4.93 (d, 1H, $J = 5.0$ Hz), 4.14–3.97 (m, 2H), 3.88–3.81 (m, 1H), 3.66–3.54 (m, 2H), 3.37 (br s, 2H); HRMS calcd for $C_{26}H_{22}N_3O_7$ 488.1458, found 488.1449.
(**9b**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 1H), 11.10 (s, 1H), 9.22 (d, 1H, $J = 9.9$ Hz), 9.13 (d, 1H, $J = 9.9$ Hz), 7.98 (d, 1H, $J = 9.9$ Hz), 7.73 (d, 1H, $J = 9.9$ Hz), 7.61–7.65 (m, 2H), 7.43–7.33 (m, 2H), 6.87 (d, 1H, $J = 3.3$ Hz), 6.22 (d, 1H, $J = 9.9$ Hz), 5.36 (br s, 1H), 5.23 (d, 1H, $J = 6.6$ Hz), 4.88 (br s, 1H), 4.31–4.23 (m, 1H), 4.18–4.12 (m, 2H), 3.88–3.84 (m, 1H), 3.78–3.66 (m, 2H), 3.36 (br s, 1H); HRMS calcd for $C_{26}H_{22}N_3O_7$ 488.1458, found 488.1469.
(**9c**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.22 (s, 1H), 11.12 (s, 1H), 9.25 (d, 1H, $J = 9.8$ Hz), 9.13 (d, 1H, $J = 9.8$ Hz), 7.96 (d, 1H, $J = 9.8$ Hz), 7.77 (d, 1H, $J = 9.8$ Hz), 7.65–7.57 (m, 2H), 7.44–7.35 (m, 2H), 6.87 (d, 1H, $J = 3.3$ Hz), 6.18 (d, 1H, $J = 9.8$ Hz), 5.32 (d, 1H, $J = 3.3$ Hz), 5.19 (d, 1H, $J = 6.4$ Hz), 4.33–4.24 (m, 2H), 3.92 (br s, 1H), 3.88 (br s, 1H), 3.37 (br s, 1H); HRMS calcd for $C_{26}H_{21}N_3O_6$ 472.1509, found 472.1516.
(**9d**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.87 (s, 1H), 9.42 (d, 1H, $J = 8.2$ Hz), 9.23 (d, 1H, $J = 8.2$ Hz), 7.68 (d, 1H, $J = 8.2$ Hz), 7.61–7.44 (m, 3H), 7.36 (t, 1H, $J = 8.2$ Hz), 7.27 (t, 1H, $J = 8.2$ Hz), 6.37 (s, 1H), 5.23 (d, 1H, $J = 4.3$ Hz), 4.68 (s, 1H), 4.45 (d, 1H, $J = 4.2$ Hz), 4.23 (s, 1H), 3.85 (br s, 2H), 3.59 (br s, 1H), 1.59 (d, 3H, $J = 6.0$ Hz); HRMS calcd for $C_{26}H_{21}N_3O_6$ (M+H) 472.1508, found 475.1502.
(**9e**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.80 (s, 0.6H), 11.14 (s, 0.4H), 11.09 (s, 0.6H), 10.61 (s, 0.4H), 9.22 (d, $J = 8.0$ Hz, 0.6H), 9.19–9.10 (m, 1.4H), 8.00–7.91 (m, 1.4H), 7.79 (d, $J = 8.0$ Hz, 0.4H), 7.59–7.53 (m, 2H), 7.39–7.35 (m, 2H), 6.27 (d, $J = 9.0$ Hz, 0.6H), 6.09 (d, $J = 9.0$ Hz, 0.4H), 5.6 (br s, 1H), 5.25 (d, $J = 5.0$ Hz, 0.6H), 5.26–5.17 (m, 1H), 4.92 (d, $J = 5.0$ Hz, 0.4H), 4.54–4.50 (m, 0.6H), 4.09–3.92 (m, 1.4H), 3.89–3.67 (m, 1.6H), 3.73–3.62 (m, 1.4H); HRMS calcd for $C_{25}H_{20}N_3O_6$ 458.1352, found 458.1366.
(**9f**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.61 (s, 1H), 11.12 (s, 1H), 9.15 (d, 1H, $J = 8.9$ Hz), 9.06 (d, 1H, $J = 8.9$ Hz), 7.95 (d, 1H, $J = 8.9$ Hz), 7.74 (d, 1H, $J = 8.9$ Hz), 7.59–7.52 (m, 2H), 7.39–7.31 (m, 2H), 6.35 (d, 1H, $J = 8.9$ Hz), 6.03 (br s, 1H), 5.71 (d, 1H, $J = 3.3$ Hz), 5.54 (d, 1H, $J = 5.9$ Hz), 5.28 (d, 1H, $J = 3.3$ Hz), 5.08–4.96 (m, 3H), 4.74–4.66 (m, 1H), 4.26–4.13 (m, 3H), 3.91–3.72 (m, 3H), 3.62–3.41 (m, 4H), 3.33 (s, 1H), 3.19–3.12 (m, 2H); HRMS calcd for $C_{32}H_{32}N_3O_{12}$ 650.1986, found 650.1979.
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