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The affinity of RSK for cylitol analogues of SL0101 is critically dependent on the B-ring *C*-4′-hydroxy†

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Abstract

Five cyclitol analogues of SL0101 with variable substitution at the *C*-4′ position (*i.e.*, OH, Cl, F, H, OMe) were synthesized. The series of analogues were evaluated for their ability to inhibit p90 ribosomal S6 kinase (RSK) activity. The study demonstrated the importance of the B-ring *C*-4′ hydroxy group for RSK1/2 inhibition.

The Ser/Thr protein kinase family, RSK, are downstream effectors of ERK1/2.[1,2] RSK activity has been correlated with a number of disease etiologies, including cancer, but no RSK inhibitor has yet transitioned to the clinic.[3] SL0101 (**1**) is a flavonoid glycoside natural product that has been identified as a selective inhibitor of RSK1/2 (K_i ~ 1 μm).[4] To date, SL0101 (1) is the only RSK1/2 inhibitor available, which is advantageous as RSK3/4 inhibitors may act to suppress tumor formation.[3] The high affinity binding of SL0101 (1) for the N-terminal kinase domain of RSK is dependent on a conformation change in the protein. This conformational change generates a unique binding pocket for the inhibitor and may explain the specificity of SL0101 for RSK1/2.[5] In this work we investigated modifications to the B-ring in an effort to further identify the regions of SL0101 that are critical for RSK1/2 interaction and to improve bioavailability (Scheme 1).[5,6]

This combined medicinal chemistry/structural biology effort[7] led to the discovery of a *C*-6″-substituted pyranose analogue (**2**)[8] that possessed improved *in vitro* kinase inhibitory and anti-cancer activity.[9] In an effort to find improved inhibitors with improved bioavailability,

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Conflicts of interest

There are no conflicts to declare.

we identified a *C*-6″-substituted carbasugar (**3a**) analogue[10] that retained the RSK-kinase inhibitory activity and demonstrated *in vivo* efficacy.[11,12]

The cyclitol variants of SL0101 (**3**) were designed to be isosteres of improved SL0101 analogue **2** with a pseudo-anomeric bond that is resistant to acid or enzyme catalysed S_N1/S_N2 hydrolysis.[10] In a continued effort to find analogues with improved bioavailability, we became interested in a possible oxidative hydrolysis mechanism that could lead to net hydrolysis of the cyclitol anomeric bond (Scheme 2). Specifically, if the B-ring *C*-4′ phenol was oxidized *in vivo* it could lead to net hydrolysis *via* addition of water to the *C*-3 position of the A-ring to yield an oxidized aglycon **5** and the free carbasugar **6**. The vinylogous quinone functionality of **5** could be biologically reduced to give the aglycon.

Therefore, we became interested in finding structural congeners (**3b—e**) with *C*-4′ B-ring substitution that would impart resistance to metabolic B-ring oxidation. Previously we found that removal of the *C*-4′ OH group in SL0101 (**1**) led to analogues with reduced RSK inhibitory activity.[13] Our modeling based upon crystallographic structure of SL0101 bound to the RSK2 NTKD suggested the *C*-6″ substitution in **2** and **3** would lead to rotation of the B-ring out of plane with the A-ring, which in turn could affect the hydrogen bonding of the *C*-4′ OH-group. Thus, in addition to the deoxy-variant **3b**, we also targeted electronically similar analogues (**3c—e**) with hydrogen bond accepting methoxy group (**3c**) and variably sized halogens (*i.e.*, the smaller fluorine **3d** and larger chlorine **3e**).

Retrosynthetically, we envisioned the synthesis of a small library of five B-ring analogues **3b—e** to follow our previously reported synthesis of **3a**.[7a] Thus, analogues **3a—e** could be prepared from **7a—e** which could result from a Pd-catalyzed cyclitolization reaction between **8** and **9b—f**.[14] Previously, we described the synthesis of **8** from quinic acid **12** and the aglycon with a free *C*-3 alcohol could be prepared in 5-steps from benzoic acids **10b—f** and acetophenone **11**, which can be prepared in two additional steps from phloroglucinol. Herein we disclose the synthesis of cyclitol analogues **3a—e** as well as their relative RSK2 *in vitro* and cell-based inhibitory activity (Schemes 3 and 4).

The synthesis of the analogues begins with a Pd-**π**-allyl coupling between **8** and the various *C*-4″ substituted aglycons **9b—f** to give **7b—f** in generally good yield (60–80% yield). The alkenes in **7b—f** was then diastereoselectively dehydroxylated to give diols **13b—f** with *rhamno*-stereochemistry in 56 to 72% yields. We then looked into the regioselective introduction of the *C*-3″-acetates to form the *C*-3″/4″ diacetate **14b—f** from diols **13b—f**. This step was most easily accomplished by using Taylor's borinate catalyst (10% $Ph_2BOCH_2NH_2$, AcCl/Hünig's base).[15] This reaction selectively gave **14b—f**, with a *C*-3″ equatorial acetate over **15b—f** with a *C*-2″ axial acetate in a 3 : 1 to 5 : 1 ratio. These compounds were then regioselectively acylated at the *C*-3″ position *via* a borinate catalysis with AcCl. With the exception of chloride **14d** (40% yield), the remaining diols **14b—c** and **14e—f** were isolated in good yield after flash chromatography (58 to 64%).

Finally, the benzyl protecting groups were selectively removed from **14b—f** under hydrogenolysis condition to give the desired analogues **3a—e** (Scheme 5). For the tris-benzyl substrate **14b** this occurred, 1 atm H_2 using 5% Pd/C to cleanly give **3a** in a 70%

yield. Similarly, the bis-benzyl substrates **14b—d** reacted under identical conditions to give analogues **3b—d** in good yields (71–72%). Unfortunately, under the same conditions (1 atm H_2 using 5% Pd/C), the chlorine substituted analogue **14e** occurred with a significant amount of concomitant reduction of the *C*-4′ chloride to afford **3b**. Careful monitoring of the reaction conditions by lowering the amount of catalyst and reaction times increased the amount of desired analogue **3e** being produced. Thus, under these optimized conditions, the desired Cl-substituted analogue **3e** could be isolated in a 40% yield along with 30% of **3b**.

With synthetic access to the series of *C*-4′-substituted SL0101 cyclitol analogues **3a—e**, we evaluated them as RSK2 inhibitors in an *in vitro* kinase assay. The results are outlined in Table 1. Interestingly, loss or replacement of the hydroxyl group in the B-ring with any other substitutient dramatically decreased the affinity for RSK2. To investigate whether the *C*-4′ series had potential anti-cancer targets independent of RSK their ability to inhibit the proliferation of the breast cancer cell line, MCF-7, was evaluated. In this analysis **3b** and **3e** had an IC_{50} ~ 25 μM and **3d** an IC_{50} ~ 15 μM compared to **3a** with an IC_{50} ~ 10 μM. The compound **3c** had minimal inhibitory activity at the highest soluble concentration (50 μM), which may be due to poor cell permeability. These data indicate that *C*-4′ substitutions inhibit MCF-7 proliferation through a pathway independent of RSK1/2 activity.

In conclusion the asymmetric synthesis of a series of *C*-4′ substituted cyclitol analogues **3a—e** was described. The five syntheses were accomplished in 13 longest linear steps (from D-quinic acid) and 20 total steps (from two commercially available starting materials; quinic acid and phloroglucinol). The convergent nature of the synthesis and the late stage point of divergence significantly reduced the impact of the number of synthetic steps. Thus, final products **3a—e** were prepared for **8** and **9a—e** in five unique 4-step syntheses. This synthetic effort provided access to four novel SL0101 analogues, which allowed the effects of the *C*-4″ B-ring substitution to be evaluated. Specifically, the importance of the B-ring phenol OH group was revealed to be essential for the high affinity interaction with RSK.

Acknowledgments

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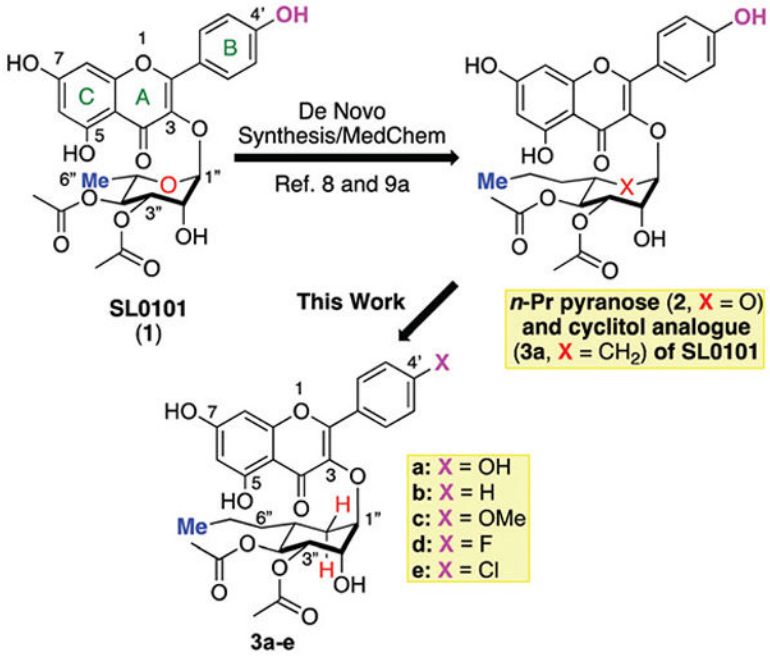
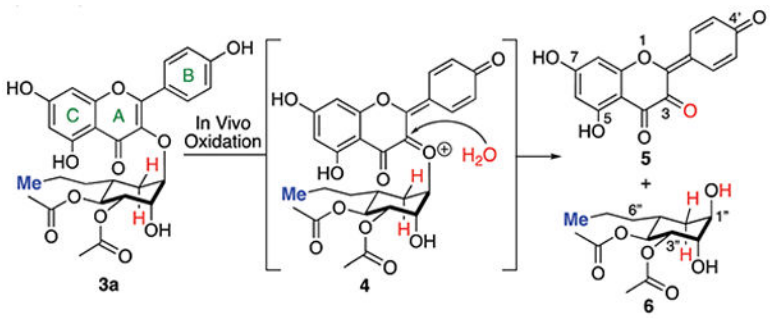
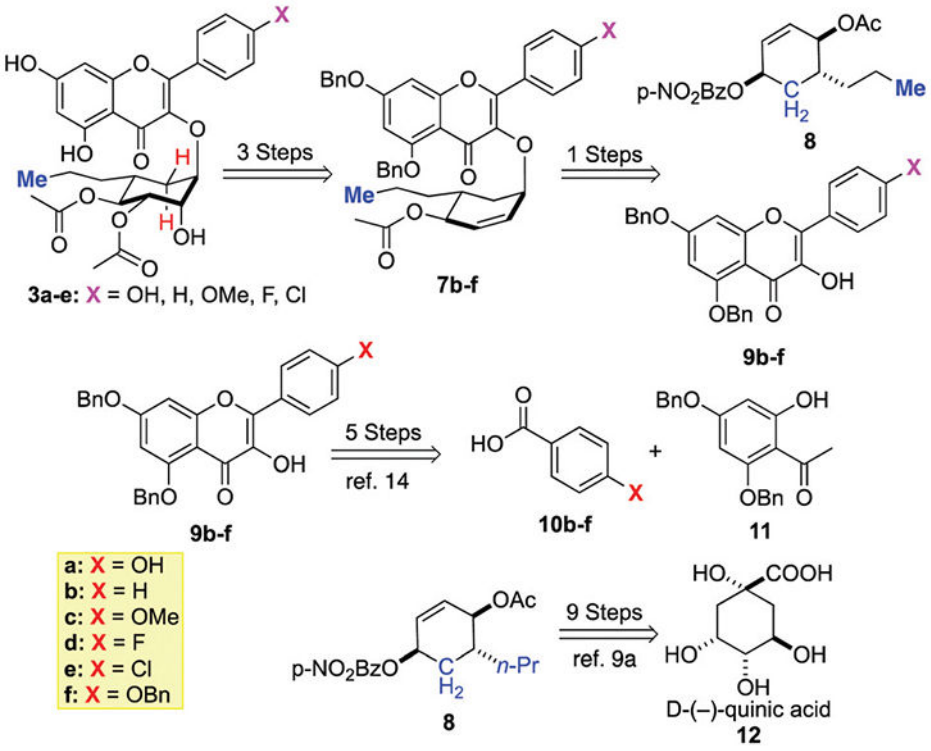


Scheme 1.
SL0101 structure activity relationship studies.

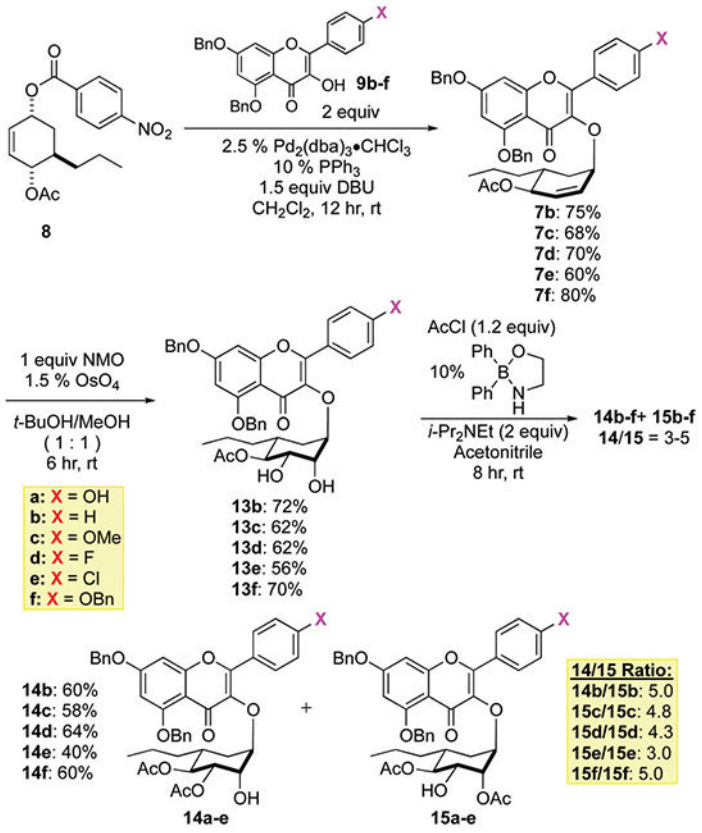


Scheme 2.
Proposed oxidative mechanism of aglycon hydrolysis.

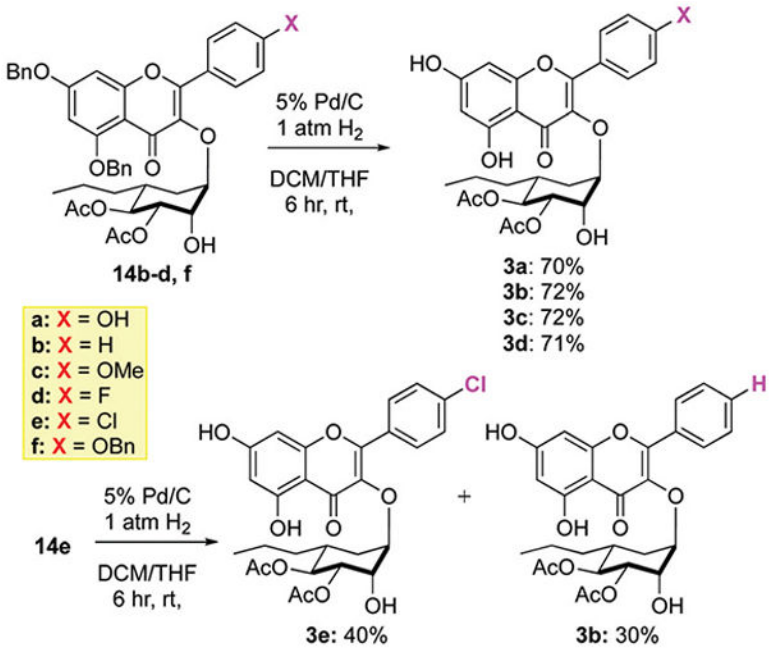


Scheme 3.

De novo approach to SL0101 cyclitol B-ring analogues.



Scheme 4.
Synthesis of protected analogues.



Scheme 5.
Global deprotection of analogues.

Table 1

In vitro RSK inhibitory activity of B-ring analogues

Analogue	X =	RSK2 inhibition IC_{50}, μM[a]	
3a	OH	0.58	±0.2
3b	H	8.23	±2.7
3c	OMe	ND	
3d	F	15.6	±3.5
3e	Cl	ND	

[a]RSK2 IC_{50}: concentration needed for 50% RSK2 inhibition ($n > 3$; quadruplicate: mean, 95% confidence interval. ND = IC_{50} could not be determined because at the maximum soluble concentration in the kinase buffer (30 μM) only 30% inhibition was achieved. The IC_{50} is a relative value and to facilitate comparisons **3a** was included in each assay as a positive control. The value for **3a** is significantly higher than in other reports[11a,12] and this variation is due to batch-to-batch differences.