ChemComm

The Affinity of RSK for Cylitol analogues of SL0101 is Critically Dependent on the B-Ring C-4'-Hydroxy

Journal:	*ChemComm*
Manuscript ID	CC-COM-01-2020-000128.R1
Article Type:	Communication

Journal Name

ARTICLE

The Affinity of RSK for Cylitol analogues of SL0101 is Critically Dependent on the B-Ring *C*-4'-Hydroxy.

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

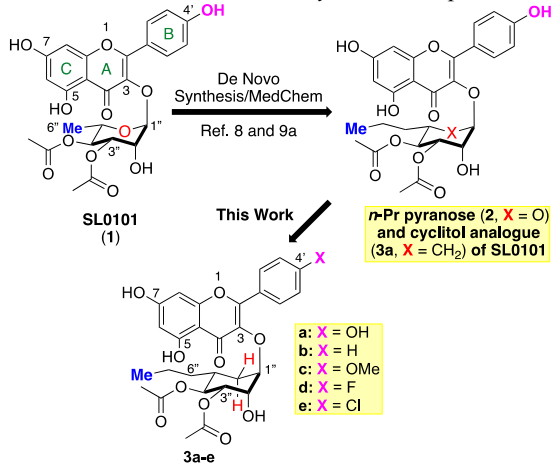
www.rsc.org/

Yu Li,[†,a] Pedro Seber,[†,b] Eric B. Wright, [†c] Sharia Yasmin,[†d] Deborah A. Lannigan*[,b,c,d] and George A. O'Doherty*[,a]

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.[12] 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 (Ki ~ 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.[4] 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]

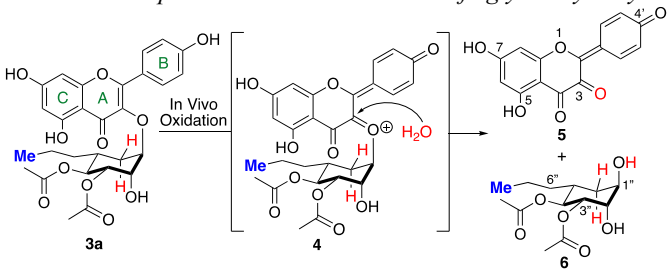
Scheme 1: *SL0101 Structure activity relationship studies*



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 anticancer activity.[9] In an effort to find improved inhibitors with improved bioavailability, 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 resistance 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.

Scheme 2: *Proposed oxidative mechanism of aglycon hydrolysis*

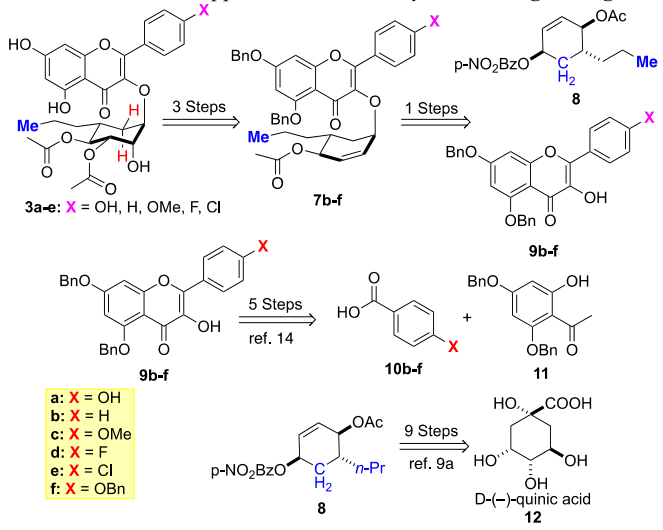


Therefore, we became interested in finding structural congeners (**3b-e**) with *C*-4' B-ring substitution that would impart resistance to metabolic B-ring oxidation. Previously we found that removal of the *C*-4' OH group in SL0101 (**1**) led to analogues with reduced RSK inhibitory activity.[13] Our 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**).

[a.] *Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115.*
[b.] *Departments of Pathology, Microbiology & Immunology, Vanderbilt University School of Medicine [c] Biomedical Engineering and [d] Cell & Developmental Biology , Nashville, TN 37232.*
† Co-first authors, the order is alphabetical.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

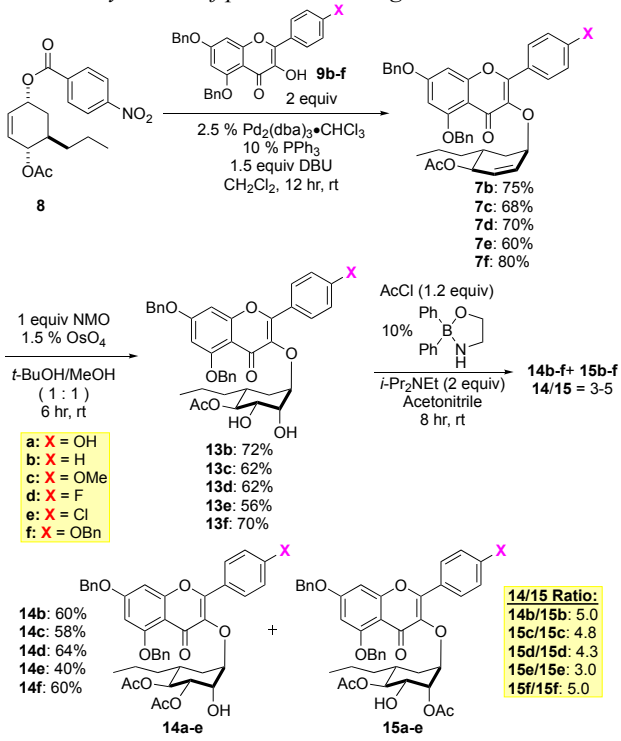
Retrosynthetically, we envisioned the synthesis of a small library of five B-ring analogues **3b-e** to follow our previously reported synthesis of **3a**.[9a] Thus, analogues **3a-e** could be prepared from **7a-e** which could result from a Pd-catalyzed cyclitolization reaction between **8** and **9b-f**.[14] 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.

Scheme 3: *De Novo approach to SL0101 cyclitol B-ring analogues*



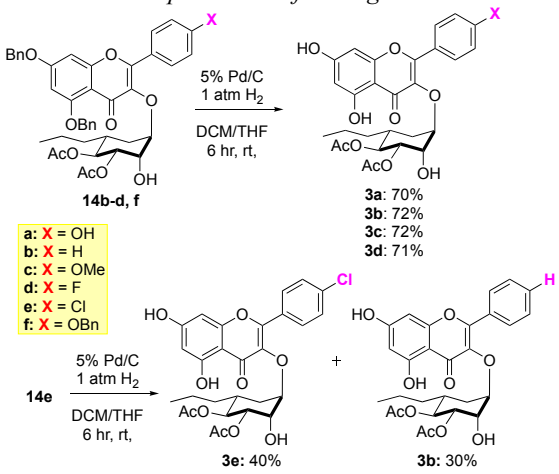
The synthesis of the analogues begins with a Pd-π-allyl coupling between **8** and the various *C*-4" substituted aglycons **9b-f** to give **7b-f** in generally good yield (60-80% yield). The alkenes in **7b-f** was then diastereoselectively dehydroxylated to give diols **13b-f** with *rhamno*-stereochemistry 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%).

Scheme 4: *Synthesis of protected analogues*



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**.

Scheme 5: *Global deprotection of analogues*



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.

Table 1. *In vitro* RSK inhibitory activity of B-ring analogues

Analogue	X =	RSK2 Inhibition IC_{50} μM	
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.

Conclusions

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.

Acknowledgements

The authors gratefully acknowledge the National Science Foundation (CHE-1565788 (G.A.O.)), and the National Institutes of Health (AI146485 (G.A.O.), AI144196 (G.A.O.), AI142040 (G.A.O.) and CA213201 (D.A.L.)) for their support of this work.

Notes and references

Eisinger-Mathason, T. S.; Andrade, J.; Lannigan, D. A. *Steroids* **2010**, *75*, 191–202.

2 (a) Doehn, U.; Hauge, C.; Frank, S. R.; Jensen, C. J.; Duda, K.; Nielsen, J. V.; Cohen, M. S.; Johansen, J. V.; Winther, B. R.; Lund, L. R.; Winther, O.; Taunton, J.; Hansen, S. H.; Frödin, M. *Mol. Cell* **2009**, *35*, 511–522. (b) Larrea, M. D.; Hong, F.; Wander, S. A.; da Silva, T. G.; Helfman, D.; Lannigan, D.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 9268–9273. (c) Vial, D.: McKeown-Longo, P. J.; *J. Biol. Chem*. **2012**, *287*, 40371–40380. (d) Gawecka, J. E.; Young-Robbins, S. S.; Sulzmaier, F. J.; Caliva, M.J.; Heikkila, M. M.; Matter, M.L.; Ramos, J. W. *J. Biol. Chem*. **2012**, *287*, 43424–43437.

3 Ludwik, K.A., Lannigan, D.A. Ribosomal S6 kinase (RSK) modulators: a patent review. *Expert Opin. Ther. Pat.* **2016**, *26*, 1061-1078.

4 Smith, J. A.; Poteet-Smith, C. E.; Xu, Y.; Errington, T. M.; Hecht, S. M.; Lannigan, D. A. *Cancer Res.* **2005**, *65*, 1027-1034.

5 Utepbergenov, D.; Derewenda, U.; Olekhnovich, N.; Szukalska, G.; Banerjee, B.; Hilinski, M. K.; Lannigan, D. A.; Stukenberg, P. T.; Derewenda, Z. S. *Biochemistry* **2012**, *51*, 6499-6510.

6 Mrozowski, R. M.; Vemula, R.; Wu, B.; Zhang, Q.; Schroederd, B. R.; Hilinski, M. K.; Clarke, D. E.; Hecht, S. M.; O'Doherty G. A.; Lannigan, D. A. *ACS Med. Chem. Lett*. **2012**, *4*, 175-179.

7 (a) Maloney, D. J.; Hecht, S. M. *Org. Lett*. **2005**, *7*, 1097-1099. (b) Shan, M.; O'Doherty, G. A. *Org. Lett.* **2006**, *8*, 5149-5152.

8 (a) Wang, H. Y.; Wu, B.; Zhang, Q.; Kang, S.-W.; Rojanasakul, Y.; O'Doherty, G. A. *ACS Med. Chem. Lett*. **2011**, *2*, 259-263. (b) Aljahdali, A. Z.; Shi, P.; Zhong, Y.; O'Doherty, G. A. *Adv. Carbohydr. Chem. Biochem.* **2013**, 69, 55-123.

9 (a) Li, M.; Li, Y.; Mrozowski, R. M.; Sandusky, Z. M.; Shan, M.; Song, X.; Wu, B.; Zhang, Q.; Lannigan, D. A. and O'Doherty, G. A. *ACS Med. Chem. Lett*. **2015**, *16*, 95–99. (b) Mrozowski, R. M. M. Sandusky, Z. M. Vemula, R. Wu, B. Zhang, Q. Deborah A. Lannigan, D. A. and O'Doherty, G. A. *Org. Lett*. **2014**, *16*, 5996–5999. (c) Mrozowski, R. M.; Vemula, R.; Wu, B.; Zhang, Q.; Schroederd, B. R.; Hilinski, M. K.; Clarke, D. E.; Hecht, S. M.; O'Doherty G. A.; Lannigan, D. A. *ACS Med. Chem. Lett*. **2013**, *4*, 175-179.

10 (a) Shan, M.; O'Doherty, G. A. *Org. Lett*. **2010**, *12*, 2986-2989. (b) Shan, M.; O'Doherty, G. A. *Synthesis*, **2008**, 19, 3171-3179. (c) Shan, M.; O'Doherty, G. A. *Org. Lett*. **2008**, *10*, 3381-3384. (d) Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9492-9495.

11 (a) Li, M.; Li, Y.; Ludwik, K. A.; Sandusky, Z. M.; Lannigan, D. A.; O'Doherty. G. A. *Org. Lett.* **2017**, *19*, 2410-2413. (b) Mrozowski, R. M.; Vemula, R.; Wu, B.; Zhang, Q.; Schroederd, B. R.; Hilinski, M. K.; Clarke, D. E.; Hecht, S. M.; O'Doherty G. A.; Lannigan, D. A. *ACS Med. Chem. Lett*. **2013**, *4*, 175-179.

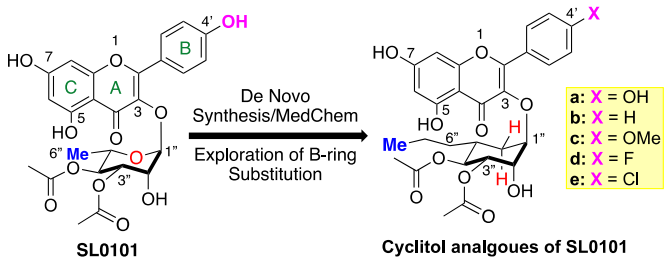
12 For detailed *in vivo* studies of **3a** see: Ludwik, K. A.; Campbell, J. P.; Li, M.; Li, Y.; Sandusky Z. M; Pasic, L.; Sowder, M. E.; Brenin D. R; Pietenpol J. A.; O'Doherty G. A.; Lannigan, D. A. *Mol. Cancer Ther*. **2016**, *15*, 2598-2608.

12 Smith, J. A.; Maloney, D. J.; Sidney M. Hecht, S. M.; Lannigan, D. A. Bioorg. Med. Chem. **2007**, *15*, 5018–5034.

14 The synthesis of compounds **9a-e** is described in a manuscript under review, the synthesis follows a protocal described in, see: (a) Yang, W.; Sun, J.; Lu, W.; Li, Y.; Shan, L.; Han, W.; Zhang, W.-D.; Yu, B. *J. Org. Chem*. **2010**, *75*, 6879–6888. (b) Li, Y.; Yang, W.; Ma, Y.; Sun, J.; Shan, L.; Zhang, W.D.; Yu, B. *SYNLETT* **2011**, *7*, 0915-0918.

15 (a) Chan, L.; Taylor, M. S. *Org. Lett*. **2011**, *13*, 3090–3093. (b) Lee, D.; Williamson, C. L.; Chan, L.; Taylor, M. S. *J. Am. Chem. Soc*. **2012**, *134*, 8260−8267. (c) Taylor, M. S. *Acc. Chem. Res*. **2015**, *48*, 295−305. (d) Bajaj, S. O.; Sharif, E. U.; Akhmedov, N. G.; O'Doherty, G. A. *Chem. Sci.* **2014**, *5*, 2230-2234.

TOC Graphic:



The De Novo asymmetric synthesis of carbohydrates for the SAR-study of the anticancer natural product, SL0101.

1 Eisinger-Mathason, T. S.; Andrade, J.; Lannigan, D. A. *Steroids* **2010**, *75*, 191–202.

2 (a) Doehn, U.; Hauge, C.; Frank, S. R.; Jensen, C. J.; Duda, K.; Nielsen, J. V.; Cohen, M. S.; Johansen, J. V.; Winther, B. R.; Lund, L. R.; Winther, O.; Taunton, J.; Hansen, S. H.; Frödin, M. *Mol. Cell* **2009**, *35*, 511–522. (b) Larrea, M. D.; Hong, F.; Wander, S. A.; da Silva, T. G.; Helfman, D.; Lannigan, D.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 9268–9273. (c) Vial, D.: McKeown-Longo, P. J.; *J. Biol. Chem*. **2012**, *287*, 40371–40380. (d) Gawecka, J. E.; Young-Robbins, S. S.; Sulzmaier, F. J.; Caliva, M.J.; Heikkila, M. M.; Matter, M.L.; Ramos, J. W. *J. Biol. Chem*. **2012**, *287*, 43424–43437.

3 Ludwik, K.A., Lannigan, D.A. Ribosomal S6 kinase (RSK) modulators: a patent review. *Expert Opin. Ther. Pat.* **2016**, *26*, 1061-1078.

4 Smith, J. A.; Poteet-Smith, C. E.; Xu, Y.; Errington, T. M.; Hecht, S. M.; Lannigan, D. A. *Cancer Res.* **2005**, *65*, 1027-1034.

5 Utepbergenov, D.; Derewenda, U.; Olekhnovich, N.; Szukalska, G.; Banerjee, B.; Hilinski, M. K.; Lannigan, D. A.; Stukenberg, P. T.; Derewenda, Z. S. *Biochemistry* **2012**, *51*, 6499-6510.

6 Mrozowski, R. M.; Vemula, R.; Wu, B.; Zhang, Q.; Schroederd, B. R.; Hilinski, M. K.; Clarke, D. E.; Hecht, S. M.; O'Doherty G. A.; Lannigan, D. A. *ACS Med. Chem. Lett*. **2012**, *4*, 175-179.

7 (a) Maloney, D. J.; Hecht, S. M. *Org. Lett*. **2005**, *7*, 1097-1099. (b) Shan, M.; O'Doherty, G. A. *Org. Lett.* **2006**, *8*, 5149-5152.

8 (a) Wang, H. Y.; Wu, B.; Zhang, Q.; Kang, S.-W.; Rojanasakul, Y.; O'Doherty, G. A. *ACS Med. Chem. Lett*. **2011**, *2*, 259-263. (b) Aljahdali, A. Z.; Shi, P.; Zhong, Y.; O'Doherty, G. A. *Adv. Carbohydr. Chem. Biochem.* **2013**, 69, 55-123.

9 (a) Li, M.; Li, Y.; Mrozowski, R. M.; Sandusky, Z. M.; Shan, M.; Song, X.; Wu, B.; Zhang, Q.; Lannigan, D. A. and O'Doherty, G. A. *ACS Med. Chem. Lett*. **2015**, *16*, 95–99. (b) Mrozowski, R. M. M. Sandusky, Z. M. Vemula, R. Wu, B. Zhang, Q. Deborah A. Lannigan, D. A. and O'Doherty, G. A. *Org. Lett*. **2014**, *16*, 5996–5999. (c) Mrozowski, R. M.; Vemula, R.; Wu, B.; Zhang, Q.; Schroederd, B. R.; Hilinski, M. K.; Clarke, D. E.; Hecht, S. M.; O'Doherty G. A.; Lannigan, D. A. *ACS Med. Chem. Lett*. **2013**, *4*, 175-179.

10 (a) Shan, M.; O'Doherty, G. A. *Org. Lett*. **2010**, *12*, 2986-2989. (b) Shan, M.; O'Doherty, G. A. *Synthesis*, **2008**, 19, 3171-3179. (c) Shan, M.; O'Doherty, G. A. *Org. Lett*. **2008**, *10*, 3381-3384. (d) Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9492-9495.

11 (a) Li, M.; Li, Y.; Ludwik, K. A.; Sandusky, Z. M.; Lannigan, D. A.; O'Doherty. G. A. *Org. Lett.* **2017**, *19*, 2410-2413. (b) Mrozowski, R. M.; Vemula, R.; Wu, B.; Zhang, Q.; Schroederd, B. R.; Hilinski, M. K.; Clarke, D. E.; Hecht, S. M.; O'Doherty G. A.; Lannigan, D. A. *ACS Med. Chem. Lett*. **2013**, *4*, 175-179.

12 For detailed *in vivo* studies of **3a** see: Ludwik, K. A.; Campbell, J. P.; Li, M.; Li, Y.; Sandusky Z. M; Pasic, L.; Sowder, M. E.; Brenin D. R; Pietenpol J. A.; O'Doherty G. A.; Lannigan, D. A. *Mol. Cancer Ther*. **2016**, *15*, 2598-2608.

13 Smith, J. A.; Maloney, D. J.; Sidney M. Hecht, S. M.; Lannigan, D. A. Bioorg. Med. Chem. **2007**, *15*, 5018–5034.

14 The synthesis of compounds **9a-e** is described in a manuscript under review, the synthesis follows a protocal described in, see: (a) Yang, W.; Sun, J.; Lu, W.; Li, Y.; Shan, L.; Han, W.; Zhang, W.-D.; Yu, B. *J. Org. Chem*. **2010**, *75*, 6879–6888. (b) Li, Y.; Yang, W.; Ma, Y.; Sun, J.; Shan, L.; Zhang, W.D.; Yu, B. *SYNLETT*. **2011**, *7*, 0915-0918.

15 (a) Chan, L.; Taylor, M. S. *Org. Lett*. **2011**, *13*, 3090–3093. (b) Lee, D.; Williamson, C. L.; Chan, L.; Taylor, M. S. *J. Am. Chem. Soc*. **2012**, *134*, 8260−8267. (c) Taylor, M. S. *Acc. Chem. Res*. **2015**, *48*, 295−305. (d) Bajaj, S. O.; Sharif, E. U.; Akhmedov, N. G.; O'Doherty, G. A. *Chem. Sci.* **2014**, *5*, 2230-2234.