

Organic & Biomolecular Chemistry

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



Journal Name

ARTICLE

Application of Mannich Bases to the Synthesis of Hydroxymethylated Isoflavonoids As Potential Antineoplastic Agents

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

DOI: 10.1039/x0xx00000x

www.rsc.org/

Mykhaylo S. Frasinuk,^{a,b,c} Galyna P. Mrug,^a Svitlana P. Bondarenko,^d Vitaliy M. Sviripa,^{b,c} Wen Zhang,^{b,e} Xianfeng Cai,^{b,e} Michael V. Fiandalo,^f James L. Mohler,^f Chunming Liu,^{b,c} and David S. Watt^{b,c,e*}

The regiospecific Mannich aminomethylation of 7-hydroxyisoflavonoids using bis(*N,N*-dimethylamino)methane afforded C-8 substituted *N,N*-dimethylaminomethyl adducts, and the regioselective aminomethylation of 5-hydroxy-7-methoxyisoflavonoids afforded predominantly the C-6 substituted *N,N*-dimethylaminomethyl adducts. Acetylation of these C-6 or C-8 Mannich bases with potassium acetate in acetic anhydride provided access to the corresponding acetoxyethyl derivatives that were subsequently converted to hydroxymethyl- and methoxymethyl-substituted 5-hydroxy- or 7-hydroxyisoflavonoids related to naturally occurring flavonoids. The C-8 acetoxyethyl, hydroxymethyl or methoxymethyl-substituted isoflavonoids possessed promising inhibitory potency in the low micromolar range in a prostate cancer PC-3 cell proliferation assay.

Introduction

Considerable lore surrounds the health benefits associated with the consumption of foods rich in natural products in the isoflavone family. In particular, soy products containing 7-hydroxyisoflavones, such as daidzein (**1**) and genistein (**2**) (Figure 1), captured attention for alleged benefits with respect to cancer prevention and treatment of prostate cancer.¹⁻⁴ Unfortunately, naturally occurring isoflavones and their metabolites possess numerous biological activities in addition to their effects on either androgen receptor expression or enzymes associated with androgen metabolism.⁵ Three pathways operate to produce testicular androgens, testosterone and 5 α -dihydrotestosterone (DHT) from other sterol precursors (Figure 2). First, the “frontdoor” pathway involves the reduction of pregnenolone to dehydroepiandrosterone (DHEA) and the conversion of DHEA,

in succession, to 5-androsten-3 α ,17 β -diol, testosterone (T) and DHT. Second, the primary “backdoor” pathway utilizes the conversion of pregnenolone, in several steps, to androsterone (AND) and 5 α -androstan-3 α ,17 β -diol (DIOL), and the ultimate conversion of DIOL to DHT. Finally, the secondary “backdoor” pathway involves the reduction of DHEA to 5-androstene-3,17-dione (ASD) and affords, in succession, 5 α -androstane-3 α ,17 β -dione (5 α -DIONE) and finally DHT.⁶⁻⁸

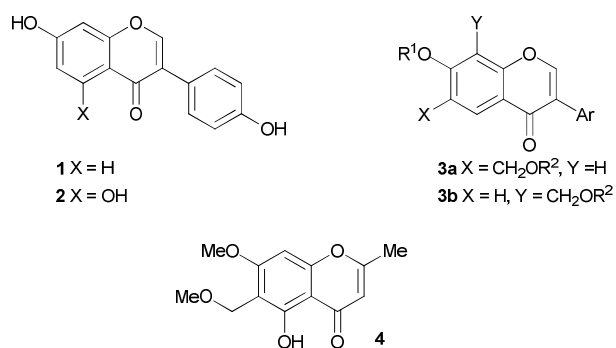


Figure 1. Naturally occurring 7-hydroxyisoflavones, daidzein (**1**) and genistein (**2**), synthetic C-6 or C-8 substituted isoflavonoids **3**, and 6-(methoxymethyl)eugenin (**4**).

The critical, characteristic feature of the two backdoor pathways is the production of DHT in a route that does not directly involve testosterone as an intermediate. The backdoor

^a Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Science of Ukraine, Kyiv 02094, Ukraine

^b Department of Molecular and Cellular Biochemistry, College of Medicine, University of Kentucky, Lexington, KY 40536-0509, USA

^c Center for Pharmaceutical Research and Innovation, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0509, USA

^d Taras Shevchenko Kyiv National University, Kyiv 01601, Ukraine

^e Lucille Parker Markey Cancer Center, University of Kentucky, Lexington, KY 40536-0509, USA

^f Department of Urology, Elm & Carlton Streets, Roswell Park Cancer Institute, Buffalo, New York 14263, USA

*Email, dwatt@uky.edu

Electronic Supplementary Information (ESI) available: Copies of NMR data for all synthesized compounds are available online. See DOI: 10.1039/x0xx00000x

pathways play a sinister role in producing sufficient DHT to activate androgen receptors in advanced prostate cancer during androgen-depletion therapy (*i.e.*, medical castration). Efforts to interdict these backdoor pathways remain a worthy

goal for prolonging time-to-relapse for patients who either fail post-radical prostatectomy or radiation therapy or patients who present with advanced prostate cancer.

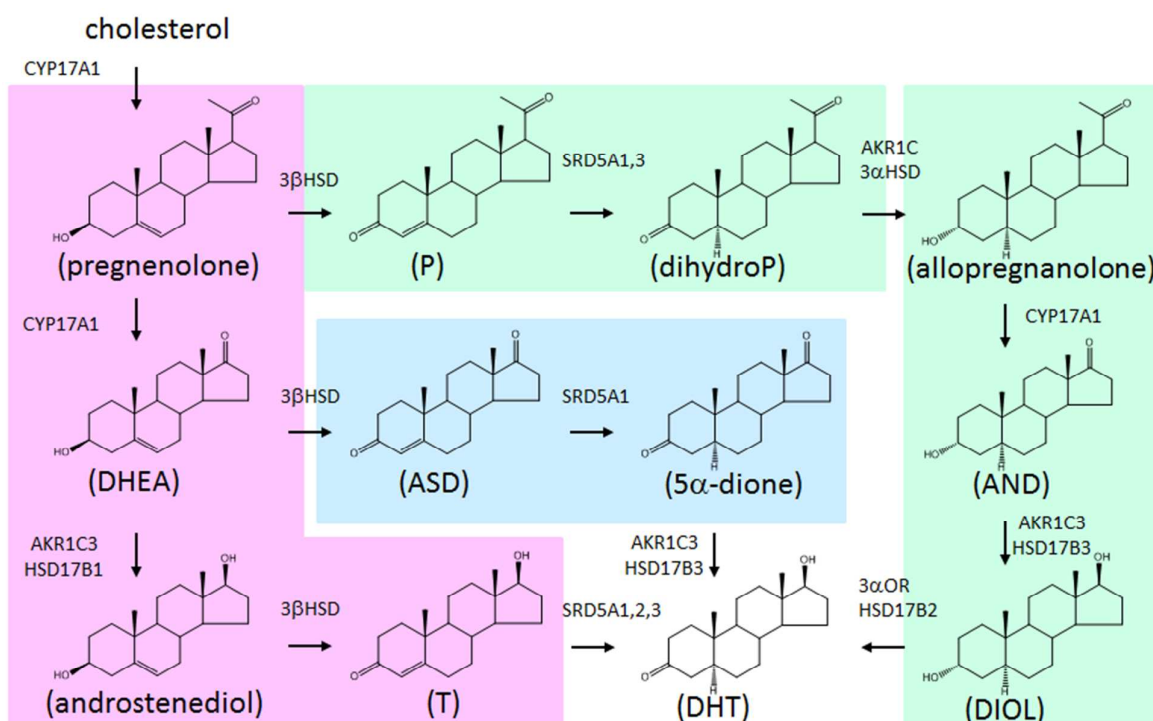


Figure 2. Frontdoor (pink), primary backdoor (green) and secondary backdoor (blue) pathways to 5 α -dihydrotestosterone (DHT). Enzymes: Cytochrome P450 17A1 or steroid 17 α -monooxygenase (CYP17A1); aldo-keto reductase-3 (AKR1C3), 3 β -hydroxysteroid dehydrogenase/ Δ^5 -4 isomerase (3 β HSD); 17 β -hydroxysteroid dehydrogenase (HSD17B2; HSD17B3); steroid 5 α -reductase or 3-oxo-5 α -steroid Δ^4 -dehydrogenase alpha (SRD5A1, 2, 3); 3 α -hydroxysteroid dehydrogenase (3 α OR). Enzymatic reactions are reversible and single arrows were used only for clarity.

As part of a program to develop antineoplastic agents with enzyme targets in the backdoor pathways B and C, we evaluated isoflavonoids **3** (Figure 1) with modifications at either position C-6 or C-8 for their activity in a cell-proliferation assay using castration-resistant⁹ PC-3 cells. An effect on cell proliferation is, of course, no guarantee that we are inhibiting the enzymes (Figure 2) in these backdoor pathways. Alternatively, we could have screened libraries against a suitable fluorescent-based, specific enzyme assay or screened libraries using a computational model of the active site of a dehydrogenase. Absent these assays, which are under development, or an X-ray structure involving co-crystallization of a dehydrogenase with an isoflavone, we turned to a cell-proliferation assay. Our past experience along these same lines in developing inhibitors that affect Wnt signaling encouraged these efforts.^{10,11}

Apart from general literature reports¹⁻⁴ on the toxicity of isoflavones, we noted that genistein (**2**) displayed IC₅₀ values in the 15-100 μ M range against different cancer cell lines and 8-(methoxymethyl)eugenin (**4**) (Figure 1), which is a related natural product in the chromone family,¹²⁻¹⁶ showed cytotoxicity in P388 leukemia cells.¹⁶ The molecular targets of

these natural products were unknown, and the potency was inadequate, in our experience, to launch studies to identify these targets. An evaluation of genistein (**2**) showed 74% inhibition at 15 mM concentration in a PC-3 proliferation assay. Although these results suggested that **2** was active against a prostate cancer cell line, we required a more potent isoflavonoid **3** for studies of the molecular-level target.

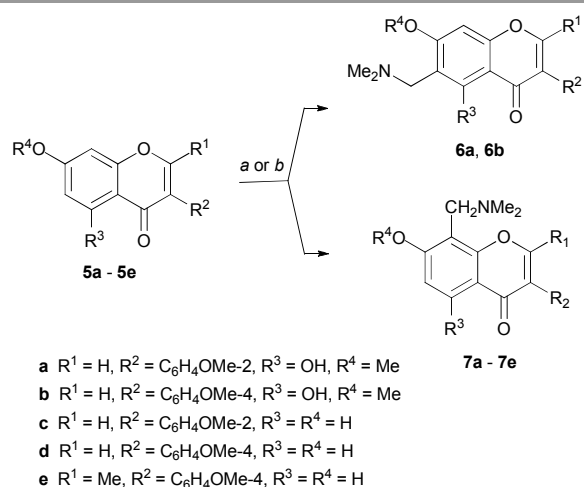
Reported routes to C-8 substituted isoflavonoids **3b** focused on the hydrolysis of 8-bromomethyl analogs,¹⁷ but these starting materials were not readily available in an efficient process. As a consequence, we required a synthetic route to either C-6 or C-8 substituted 7-hydroxyisoflavonoids **3** (Figure 1), and we employed a proliferation assay using a prostate cancer PC-3 cell line for probing structure-activity (SAR) relationships. We now report on these SAR studies that served as a necessary first step in identifying potent isoflavonoids and in setting the stage for the identification of the molecular target(s) of these isoflavonoids.

Results and discussion

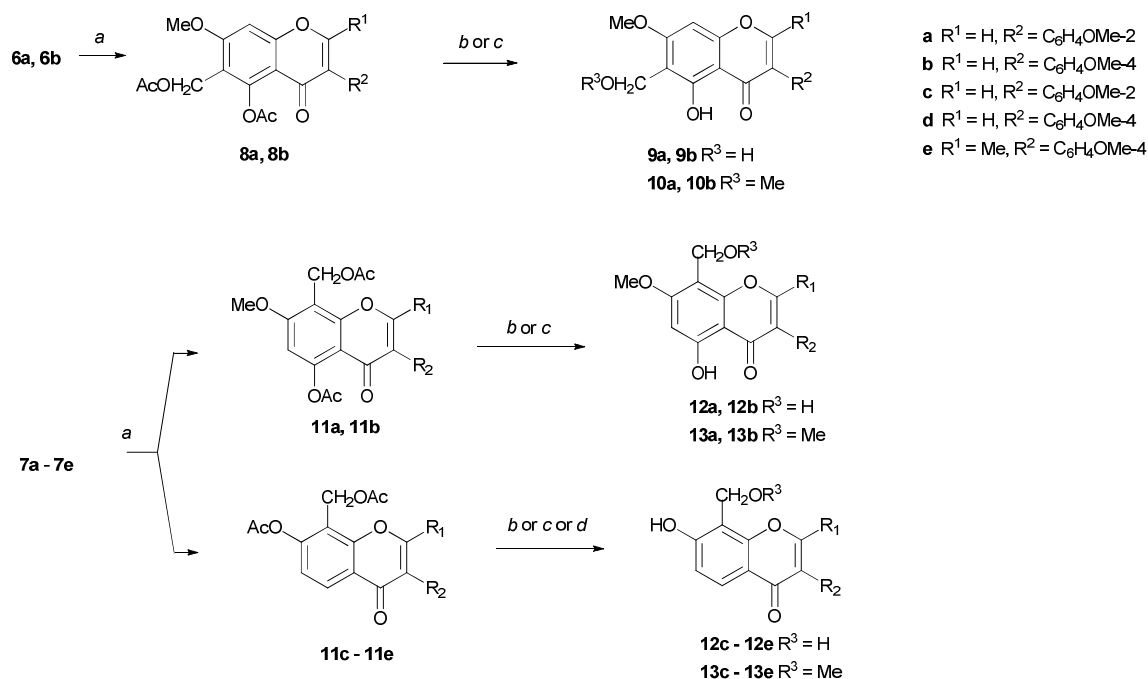
In accord with reported applications of the aminomethylation reaction to phenols¹⁸⁻²⁵ and β -naphthols,²⁶⁻³⁴ heating hydroxylated isoflavonoids with bis(*N,N*-dimethylamino)methane in either 1,4-dioxane or isopropanol furnished the desired *N,N*-dialkylaminomethyl-substituted derivatives. Synthesis of appropriate starting materials involved the regioselective methylation of the C-7 hydroxy group in 5,7-dihydroxy-2'-methoxy or 4'-methoxyisoflavonoids to afford 5-hydroxy-2',7-dimethoxyisoflavone (**5a**) or 4',7-di-*O*-methylgenistein³⁵ (**5b**) (Scheme 1) using dimethyl sulfate in the presence of potassium carbonate.³⁶ The 5,7-dihydroxyisoflavonoids underwent bis-aminomethylation reactions,³⁷⁻³⁹ and the C-5 hydroxylated isoflavonoids **5a** and **5b** underwent the Mannich reaction to give a mixture of the C-6 and C-8 mono-aminomethylation products **6a-6b** and **7a-7b**, respectively, in which the C-6 isomer predominated (Scheme 1). The structures of these isomers were established by HMBC NMR spectroscopy. The 6-(*N,N*-dimethylamino)methyl derivatives **6a** and **6b** have cross-peaks for H-2 with C-8a and for H-8 with C-8a. Similar cross-peaks were observed for H-2 with C-8a and for the methylene protons at C-8 with C-8a in compounds **7a** and **7b**. Heating individual isomers **6b** or **7b** with bis(*N,N*-dimethylamino)methane in 1,4-dioxane failed to cause their interconversion, unlike the interconversions reported in a related chromone system.⁴⁰

The C-7 hydroxylated isoflavonoids **5c-5e** underwent Mannich reactions to give exclusively the C-8 substituted *N,N*-dimethylamino derivatives **7c-7e**. In summary, both 5-

hydroxylated and 7-hydroxylated isoflavonoids underwent the desired aminomethylations using bis(*N,N*-dimethylamino)methane and exhibited regioselectivity in the C-5 hydroxylated cases in favor of the C-6 (dimethylamino)methyl derivatives **6** and regiospecificity in the C-7 hydroxylated cases in favor of the C-8 (dimethylamino)methyl derivatives **7**.



Scheme 1. Aminomethylation reaction of C-5 or C-7 monohydroxylated isoflavonoids **5**. Legend: *a*, $CH_2(NMe_2)_2$, *i*-PrOH, reflux, *b*, $CH_2(NMe_2)_2$, 1,4-dioxane, reflux.



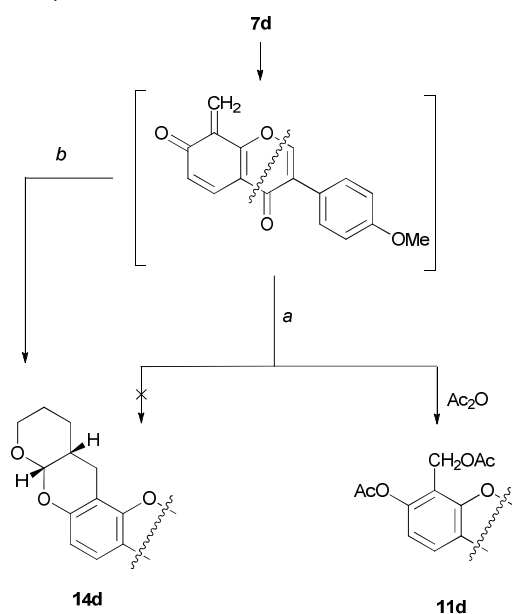
Scheme 2. Conversion of *N,N*-dimethylaminomethyl derivatives **6** and **7** to acetoxymethyl, hydroxymethyl and methoxymethyl derivatives. Legend: *a*, Ac_2O , KOAc; *b*, 0.2M H_2SO_4 , aq. 1,4-dioxane; *c*, HCl, MeOH; *d*, NaOH, MeOH.



Journal Name

ARTICLE

Direct conversion of the N,N-dimethylaminomethyl derivatives **6** and **7** to the corresponding C-6 or C-8 hydroxymethyl derivatives **9** and **12** respectively in basic media or direct transformation **6** and **7** to the corresponding C-6 or C-8 methoxymethyl derivatives in refluxing methanol⁴¹ proceeded in poor yields. An alternate route to these derivatives involved heating **6** or **7** with acetic anhydride in presence of potassium acetate to afford the corresponding diacetates **8** and **11** in excellent yield (Scheme 2). Hydrolysis of the diacetates **8** or **11** using a 2:1 ratio of 0.2M aqueous sulfuric acid in 1,4-dioxane furnished the corresponding hydroxymethyl derivatives **9** or **12**, respectively, and hydrolysis of **8** or **11** using hydrochloric acid in methanol led directly to the C-6 or C-8-methoxymethyl derivatives **10** and **13**, respectively. The substitution of ethanol or isopropanol for methanol led to other alkoxy-methyl-substituted analogs (see Supplementary Material). Treatment of 8-hydroxymethyl derivatives with hydrochloric acid in methanol furnished to the 8-methoxymethyl analogs and treatment of 8-acetoxymethyl derivatives **11** with sodium hydroxide in methanol led to the 8-methoxymethyl analogs **13** (Scheme 2).



Scheme 3. Mechanistic considerations for the conversion of Mannich base **7d** to diacetate **11d**. Legend: *a*, 3,4-dihydro-2H-pyran, Ac₂O, KOAc; *b*, 3,4-dihydro-2H-pyran, DMF, heat.

It should be notice, the developed procedures allow us to synthesize naturally occurring isoflavone **9a** (Cristatein) which is known as a host-specific attractant towards the zoospores of

Aphanomyces cochlioides,¹² and could be useful for the synthesis for various hydroxymethyl and alkoxy-methyl derivatives of natural phenolic compounds.

Table 1. Percent inhibition of prostate cancer PC-3 cell proliferation by isoflavonoids.

Isoflavonoid	C-6 or C-8 Substituent	Inhibition at 10 μ M (%)
8a	6-acetoxymethyl	17.1 \pm 5.9
8b	6-acetoxymethyl	51.7 \pm 5.7
9a	6-hydroxymethyl	14.6 \pm 3.2
9b	6-hydroxymethyl	0 \pm 16.0
10a	6-methoxymethyl	53.4 \pm 8.0
10b	6-methoxymethyl	0 \pm 27.0
11b	8-acetoxymethyl	20.2 \pm 2.0
11c	8-acetoxymethyl	20.6 \pm 3.8
11d	8-acetoxymethyl	99.2 \pm 0.4
11e	8-acetoxymethyl	98.2 \pm 1.5
12b	8-hydroxymethyl	79.5 \pm 3.6
12c	8-hydroxymethyl	0 \pm 16.0
12d	8-hydroxymethyl	99.5 \pm 0.2
12e	8-hydroxymethyl	99.5 \pm 0.5
13a	8-methoxymethyl	0 \pm 1.3
13b	8-methoxymethyl	96.8 \pm 0.8
13c	8-methoxymethyl	0 \pm 7.4
13d	8-methoxymethyl	34.9 \pm 3.3

Conversion of Mannich bases to the acetoxymethyl derivatives could occur either by S_N2 substitutions by acetate on protonated Mannich bases or by elimination-addition sequences *via* an intermediate *ortho*- or *para*-quinone methides. To test the latter suggestion, we treated the Mannich base **7d** with acetic anhydride and potassium acetate in the presence of 3,4-dihydro-2H-pyran under conditions where **7d** could afford either the diacetate **11d** or the inverse

electron-demand Diels-Alder adduct **14d**. We did not observe any of the Diels-Alder adduct **14**, an authentic sample of which was synthesized independently heating the Mannich base **7d** with 3,4-dihydro-2H-pyran in refluxing N,N-dimethylformamide. We excluded possible, competitive conversion of the Diels-Alder adduct **14d** to the diacetate **11d** by demonstrating that treatment of an authentic sample of the Diels-Alder adduct **14d** with acetic anhydride and potassium acetate led to none of the diacetate **11d**, and we also demonstrated that treatment of the diacetate **11d** with 3,4-dihydro-2H-pyran led to none of the Diels-Alder adduct **14d** (Scheme 3). The success of the Diels-Alder reaction supported the intermediacy of an *ortho*-quinone methide, but evidence for this intermediate in the conversion of the Mannich base to the acetoxymethyl derivative in this isoflavonoid system was equivocal.

A screening program using PC-3 prostate cancer cells revealed that several 7-hydroxyisoflavonoids **1** with C-8 acetoxymethyl, hydroxymethyl or alkoxymethyl substituents exhibited antineoplastic activity in the 1-10 micromolar range (Table 1). We observed that C-8 substituted analogs **11**, **12** and **13** were more potent at 10 μ M concentrations than the C-6 substituted analogs **8**, **9** and **10**, respectively. Within the C-8 series, the acetoxymethyl- and hydroxymethyl- isoflavonoids were more potent than the corresponding alkoxymethyl-substituted isoflavonoids.

Also within the C-8 series, the isoflavonoids that possessed a 4-methoxyphenyl group were in general preferable to those with a 2-methoxyphenyl group. For example, isoflavonoids **11d** and **11e** were more potent than **11c**; isoflavonoids **12b**, **12d** and **12e** were more potent than **12c**; and isoflavonoid **13b** was more potent than **13c**. Other substituents than methoxy groups on the 3-phenyl group were also explored (data not shown) but produced inactive isoflavonoids with few exceptions. Finally, within the C-8 series, those isoflavonoids with 7-hydroxy substituents as well as either 8-acetoxymethyl or 8-hydroxymethyl groups (e.g., **11d** and **11e**, **12d** and **12e**) were in general more potent than isoflavonoids with 5-hydroxy-7-methoxy groups (e.g., **8b** and **9b**). The isoflavonoid **11d** with a C-8 acetoxymethyl emerged as a promising lead structure since it retained potency even at 1 mM concentration.

Conclusions

In summary, the Mannich reaction of C-5 or C-7 hydroxylated isoflavonoids provided N,N-(dimethylamino)methyl derivatives that were readily converted to acetoxymethyl, hydroxymethyl, or alkoxymethyl-substituted isoflavonoids. The 5-hydroxyisoflavonoids **5a,5b** afforded C-6 Mannich bases **6a,6b** regioselectively that led *via* the diacetates **8a,8b** to the C-6 hydroxymethyl and methoxymethyl derivatives **9-10a,b** presumably *via* intermediate *ortho*-quinone methides. Analogous reactions of the 7-hydroxyisoflavonoids **5c-5e** afforded the C-8 Mannich bases **7c-7e** regiospecifically, and the diacetates **11c-11e** derived from these Mannich bases underwent substitutions leading to the desired C-8

hydroxymethyl and methoxymethyl derivatives **12,13c-e** *via* intermediate *ortho*-quinone methides. Several C-8 acetoxymethyl, hydroxymethyl or methoxymethyl-substituted isoflavonoids possessed promising potency in the low micromolar range in a PC-3 cell proliferation assay. The synthesis and application of biotinylated analogs for biological target identification will be reported in due course.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Varian 400 spectrometer (at 500 MHz or at 125 MHz, respectively) or on a Varian 400 spectrometer (at 400 MHz or at 100 MHz, respectively) in CDCl_3 or DMSO-d_6 . Structures were also confirmed with HMBC techniques. IR spectra were recorded on a Bruker Vertex 70 FT/IR spectrometer. Melting points were determined in open capillarity tubes with a Buchi B-535 apparatus and were uncorrected. Mass spectra were obtained with an Agilent 1100 spectrometer under chemical ionization conditions. Column chromatography was performed using Macherey-Nagel Silica 60, 0.04-0.063 mm silica gel.

General procedure for the synthesis of isoflavones 5a and 5b. The procedure of Kim³⁶ was repeated using 5 mmol of 5,7-dihydroxy-3-(2-methoxyphenyl)-4H-chromen-4-one³⁹ or 5,7-dihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one,³⁹ 2.07 g (15 mmol) of anhydrous potassium carbonate and 0.5 mL (5.2 mmol) of dimethyl sulfate in 10 mL of acetone for 6 h to afford **5a** or **5b**, respectively.

5-Hydroxy-7-methoxy-3-(2-methoxyphenyl)-4H-chromen-4-one (5a). Pale yellow solid (89% yield); mp 153-154°C; IR (KBr): ν_{max} 2993, 2942, 2839, 1662, 1583, 1495, 1439, 1260, 1181, 748 cm^{-1} ; ^1H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, 2'- OCH_3), 3.88 (s, 3H, 7- OCH_3), 6.33 (s, 1H, 6-H), 6.49 (s, 1H, 8-H), 6.94-7.07 (m, 2H, 3', 5'-H), 7.20-7.28 (m, 1H, 6'-H), 7.32-7.41 (m, 1H, 4'-H), 8.06 (s, 1H, 2-H), 12.76 ppm (s, 1H, 5-OH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.54, 56.06, 92.50, 98.06, 105.25, 111.29, 119.56, 120.13, 120.73, 129.99, 131.55, 155.59, 157.42, 157.52, 161.59, 165.24, 179.90 ppm; MS (CI): m/z 299.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{17}\text{H}_{14}\text{O}_5$: C, 68.45; H, 4.73. Found: C, 68.12; H, 4.97.

5-Hydroxy-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (5b). Pale yellow solid (73% yield); mp 142-143°C (lit⁴ mp 141-142°C); IR (KBr): ν_{max} 2964, 2936, 2833, 1658, 1618, 1579, 1516, 1244, 1192, 1151, 1051 cm^{-1} ; ^1H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, 4'- OCH_3), 3.86 (s, 3H, 7- OCH_3), 6.41 (d, 1H, J = 2.2 Hz, 8-H), 6.65 (d, 1H, J = 2.2 Hz, 6-H), 7.00 (d, 2H, J = 8.8 Hz, 3', 5'-H), 7.51 (d, 2H, J = 8.7 Hz, 2', 6'-H), 8.44 (s, 1H, 2-H), 12.92 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 55.14, 56.06, 92.40, 98.03, 105.37, 113.68, 122.13, 122.73, 130.11, 154.61, 157.45, 159.17, 161.70, 165.21, 180.25 ppm; MS (CI): m/z 299.1 (MH^+ , 100). Anal. Calcd. for $\text{C}_{17}\text{H}_{14}\text{O}_5$: C, 68.45; H, 4.73. Found: C, 68.73; H, 4.94.

General procedure for the synthesis of Mannich bases 6a-6b and 7a-7b. To a suspension of 2 mmol of **5a** or **5b** in 10 mL of 1,4-dioxane was added 1.36 mL (10 mmol) of bis(*N,N*-dimethylamino)methane. The mixture was refluxed for 24-30 h, cooled and concentrated. The mixture of isomeric Mannich

bases **6a-6b** and **7a-7b** was separated by chromatography using 1:50 methanol-dichloromethane.

6-[(Dimethylamino)methyl]-5-hydroxy-7-methoxy-3-(2-methoxyphenyl)-4H-chromen-4-one (6a). Pale yellow solid (48% yield); mp 130-131°C; IR (KBr): ν_{\max} 2937, 2809, 2759, 1659, 1585, 1457, 1282, 1222, 1120, 1078 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.31 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.52 (s, 2H, 6- CH_2), 3.81 (s, 3H, 2'- OCH_3), 3.92 (s, 3H, 7- OCH_3), 6.43 (s, 1H, 8-H), 6.97-7.06 (m, 2H, 3', 5'-H), 7.28-7.32 (m, 1H, 6'-H), 7.35-7.42 (m, 1H, 4'-H), 7.87 (s, 1H, 2-H), 13.12 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, CDCl_3): δ 45.42, 49.70, 55.71, 56.17, 89.45, 106.06, 109.83, 111.25, 119.66, 120.57, 121.37, 130.05, 131.58, 154.11, 157.37, 157.45, 160.53, 164.22, 180.42 ppm; MS (CI): m/z 356.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{20}\text{H}_{21}\text{NO}_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.87; H, 6.17; N, 4.17.

6-[(Dimethylamino)methyl]-5-hydroxy-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (6b). Pale yellow solid (69% yield); mp 140-142°C; IR (KBr): ν_{\max} 2933, 2817, 2757, 1653, 1610, 1514, 1254, 1221, 1123, 832 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.31 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.53 (s, 2H, 6- CH_2), 3.85 (s, 3H, 4'- OCH_3), 3.92 (s, 3H, 7- OCH_3), 6.42 (s, 1H, 8-H), 6.98 (d, 2H, $J = 8.8$ Hz, 3', 5'-H), 7.46 (d, 2H, $J = 8.8$ Hz, 2', 6'-H), 7.88 (s, 1H, 2-H), 13.10 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, CDCl_3): δ 45.38, 49.65, 55.27, 56.15, 89.41, 105.96, 109.81, 114.00, 122.95, 123.77, 130.04, 152.32, 157.30, 159.67, 160.55, 164.29, 180.69 ppm; MS (CI): m/z 356.3 (MH^+ , 100). Anal. Calcd. for $\text{C}_{20}\text{H}_{21}\text{NO}_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.42; H, 6.14; N, 4.23.

8-[(Dimethylamino)methyl]-5-hydroxy-7-methoxy-3-(2-methoxyphenyl)-4H-chromen-4-one (7a). Pale yellow solid (25% yield); mp 92-93°C; IR (KBr): ν_{\max} 2924, 2853, 1654, 1583, 1460, 1312, 1200, 1083, 1017 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.32 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.62 (s, 2H, 8- CH_2), 3.82 (s, 3H, 2'- OCH_3), 3.92 (s, 3H, 7- OCH_3), 6.45 (s, 1H, 6-H), 6.98-7.06 (m, 2H, 3', 5'-H), 7.29-7.32 (m, 1H, 6'-H), 7.36-7.42 (m, 1H, 4'-H), 7.94 (s, 1H, 2-H), 13.12 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, CDCl_3): δ 43.66, 49.05, 55.70, 56.45, 95.23, 105.73, 111.21, 119.04, 120.57, 121.19, 130.23, 131.54, 154.62, 156.14, 157.40, 163.89, 164.02, 180.67 ppm; MS (CI): m/z 356.3 (MH^+ , 100). Anal. Calcd. for $\text{C}_{20}\text{H}_{21}\text{NO}_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.83; H, 6.21; N, 4.13.

8-[(Dimethylamino)methyl]-5-hydroxy-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (7b). Pale yellow solid (28% yield); mp 126-127°C; IR (KBr): ν_{\max} 2934, 2832, 1653, 1578, 1513, 1298, 1248, 1200, 1178, 1039 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.30 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.58 (s, 2H, 8- CH_2), 3.84 (s, 3H, 4'- OCH_3), 3.92 (s, 3H, 7- OCH_3), 6.44 (s, 1H, 6-H), 6.98 (d, 2H, $J = 8.8$ Hz, 3', 5'-H), 7.46 (d, 2H, $J = 8.8$ Hz, 2', 6'-H), 7.95 (s, 1H, 2-H), 13.13 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, CDCl_3): δ 45.21, 49.78, 55.23, 56.14, 95.06, 104.76, 105.48, 113.96, 122.86, 122.88, 129.97, 152.94, 155.73, 159.62, 162.35, 163.94, 181.16 ppm; MS (CI): m/z 356.3 (MH^+ , 100). Anal. Calcd. for $\text{C}_{20}\text{H}_{21}\text{NO}_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.65; H, 5.77; N, 3.75.

General procedure for the synthesis of Mannich bases 7c-7e. To a stirred suspension of 2 mmol of **5c-5e** ^{43,44} in 10 mL of isopropyl alcohol was added 0.3 mL (2.2 mmol) of bis(*N,N*-

dimethylamino)methane. The mixture was heated at 80°C for 4-6 h and was either cooled to induce crystallization or concentrated and then triturated with hexane to induce crystallization of **7c-7e** that were recrystallized from isopropanol-hexane.

8-[(Dimethylamino)methyl]-7-hydroxy-3-(2-methoxyphenyl)-4H-chromen-4-one (7c). Pale yellow solid (91% yield); mp 120-121°C; ^1H NMR (400 MHz, CDCl_3): δ 2.43 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.81 (s, 3H, 2'- OCH_3), 3.99 (s, 2H, 8- CH_2), 6.89 (d, 1H, $^3J = 8.8$ Hz, 6-H), 6.96-7.06 (m, 2H, 3', 5'-H), 7.29-7.40 (m, 2H, 4', 6'-H), 7.88 (s, 1H, H-2), 8.19 (d, 1H, $^3J = 8.8$ Hz, 5-H), 12 ppm (br. s, 1H, 7-OH); ^{13}C NMR (125 MHz, CDCl_3): δ 44.41, 54.87, 55.20, 107.27, 113.80, 115.44, 116.89, 124.22, 124.32, 126.67, 130.00, 151.25, 154.96, 159.41, 163.97, 175.74 ppm; IR (KBr): ν_{\max} 3448, 2951, 1626, 1427, 1246, 1178, 1028 cm^{-1} ; MS (CI): m/z 326.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{19}\text{H}_{19}\text{NO}_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.27; H, 5.77; N, 4.17.

8-[(Dimethylamino)methyl]-7-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (7d). Pale yellow solid (83% yield); mp 174-176°C; IR (KBr): ν_{\max} 3448, 2951, 1626, 1427, 1246, 1178, 1028 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.44 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.85 (s, 3H, 4'- OCH_3), 3.99 (s, 2H, 8- CH_2), 6.90 (d, 1H, $^3J = 8.8$ Hz, 6-H), 6.97 (d, 2H, $^3J = 8.8$ Hz, 3', 5'-H), 7.50 (d, 2H, $^3J = 8.8$ Hz, 2', 6'-H), 7.89 (s, 1H, 2-H), 8.14 (d, 1H, $^3J = 8.8$ Hz, 5-H), 10.21 ppm (br. s, 1H, 7-OH); ^{13}C NMR (125 MHz, CDCl_3): δ 44.41, 54.87, 55.20, 107.27, 113.80, 115.44, 116.89, 124.22, 124.32, 126.67, 130.00, 151.25, 154.96, 159.41, 163.97, 175.74 ppm; MS (CI): m/z 326.1 (MH^+ , 100). Anal. Calcd. for $\text{C}_{19}\text{H}_{19}\text{NO}_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 69.88; H, 5.97; N, 4.39.

8-[(Dimethylamino)methyl]-7-hydroxy-3-(4-methoxyphenyl)-2-methyl-4H-chromen-4-one (7e). Pale yellow solid (91% yield); mp 185-187°C (decomp); IR (KBr): ν_{\max} 3450, 2958, 1626, 1603, 1255, 1176, 1016 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.30 (s, 3H, 2- CH_3), 2.44 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.84 (s, 3H, 4'- OCH_3), 3.98 (s, 2H, 8- CH_2), 6.85 (d, 1H, $^3J = 8.8$ Hz, 6-H), 6.97 (d, 2H, $^3J = 8.7$ Hz, 3', 5'-H), 7.20 (d, 2H, $^3J = 8.7$ Hz, 2', 6'-H), 8.04 (d, 1H, $^3J = 8.8$ Hz, 5-H), 11.30 ppm (br. s, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ 19.28, 44.44, 54.85, 55.21, 106.83, 113.78, 115.03, 115.89, 122.51, 125.33, 126.74, 131.51, 154.68, 158.99, 161.76, 163.69, 176.47 ppm; MS (CI): m/z 340.1 (MH^+ , 100). Anal. Calcd. for $\text{C}_{20}\text{H}_{21}\text{NO}_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.91; H, 5.95; N, 4.33.

General procedure for the synthesis of diacetates 8a-8b or 11a-11e. A mixture of a Mannich base **6a-6b** or **7a-7e** (2 mmol) and 200 mg (2 mmol) of potassium acetate in 5 mL of acetic anhydride was refluxed for 5 min and cooled to room temperature. The mixture was diluted with water to afford a precipitate of **8a-8b** or **11a-11e**, respectively, that was recrystallized from acetonitrile-water.

5-Acetoxy-6-(acetoxymethyl)-7-methoxy-3-(2-methoxyphenyl)-4H-chromen-4-one (8a). White solid (96% yield); mp 143-145°C; IR (KBr): ν_{\max} 2945, 2836, 1767, 1738, 1650, 1617, 1451, 1280, 1235, 1127 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.04 (s, 3H, C-6 $\text{CH}_2\text{OCOCH}_3$), 2.41 (s, 3H, C-5 OCOCH_3), 3.78 (s, 3H, 2'- OCH_3), 3.96 (s, 3H, 7- OCH_3), 5.21 (br. s, 2H, 6- CH_2), 6.80 (s, 1H, 8-H), 6.93-7.03 (m, 2H, 3', 5'-H), 7.24-7.29 (m, 1H, 6'-H), 7.32-7.38 (m, 1H, 4'-H), 7.80 ppm (s, 1H, 2-

H); ^{13}C NMR (101 MHz, CDCl_3): δ 20.85, 21.10, 54.69, 55.68, 56.35, 97.13, 111.34, 111.69, 115.90, 120.52, 120.52, 123.43, 129.85, 131.67, 150.12, 152.45, 157.40, 158.93, 161.92, 169.32, 170.80, 174.02 ppm; MS (CI): m/z 413.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_8$: C, 64.08; H, 4.89. Found: C, 64.27; H, 5.11.

5-Acetoxy-6-(acetoxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (8b). White solid (97% yield); mp 167-169°C; IR (KBr): ν_{max} 2962, 2834, 1734, 1629, 1513, 1453, 1248, 1182, 1123 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.04 (s, 3H, C-6 $\text{CH}_2\text{OCOCH}_3$), 2.44 (s, 3H, C-5 OCOCH_3), 3.83 (s, 3H, 4'- OCH_3), 3.96 (s, 3H, 7- OCH_3), 5.20 (s, 2H, 6- CH_2), 6.80 (s, 1H, 8-H), 6.94 (d, 2H, $J = 8.8$ Hz, 3', 5'-H), 7.39 (d, 2H, $J = 8.8$ Hz, 2', 6'-H), 7.81 ppm (s, 1H, 2-H); ^{13}C NMR (101 MHz, CDCl_3): δ 20.83, 21.08, 54.62, 55.25, 56.35, 97.06, 111.53, 113.91, 116.01, 123.58, 125.95, 130.25, 150.18, 150.85, 158.88, 159.58, 162.00, 169.38, 170.77, 174.40 ppm; MS (CI): m/z 413.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_8$: C, 64.08; H, 4.89. Found: C, 63.89; H, 5.17.

5-Acetoxy-8-(acetoxymethyl)-7-methoxy-3-(2-methoxyphenyl)-4H-chromen-4-one (11a). White solid (98% yield); mp 116-118°C; IR (KBr): ν_{max} 2946, 1759, 1652, 1537, 1389, 1254, 1157, 1022 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.09 (s, 3H, C-8 $\text{CH}_2\text{OCOCH}_3$), 2.40 (s, 3H, C-5 OCOCH_3), 3.79 (s, 3H, 2'- OCH_3), 3.96 (s, 3H, 7- OCH_3), 5.36 (s, 2H, 8- CH_2), 6.67 (s, 1H, 6-H), 6.94-7.10 (m, 2H, 3', 5'-H), 7.24-7.29 (m, 1H, 6'-H), 7.24-7.28 (m, 1H, 4'-H), 7.86 ppm (s, 1H, 2-H); ^{13}C NMR (101 MHz, CDCl_3): δ 20.74, 20.98, 54.38, 55.39, 56.12, 103.49, 109.19, 110.93, 111.38, 119.87, 120.18, 122.50, 129.53, 131.34, 151.37, 152.36, 156.56, 156.99, 161.36, 169.18, 170.72, 173.95 ppm; MS (CI): m/z 413.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_8$: C, 64.08; H, 4.89. Found: C, 64.32; H, 5.07.

5-Acetoxy-8-(acetoxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (11b). White solid (88% yield); mp 124-126°C; IR (KBr): ν_{max} 2943, 2840, 1763, 1740, 1645, 1515, 1411, 1304, 1247, 1182, 1026 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.09 (s, 3H, C-8 $\text{CH}_2\text{OCOCH}_3$), 2.44 (s, 3H, C-5 OCOCH_3), 3.84 (s, 3H, 4'- OCH_3), 3.97 (s, 3H, 7- OCH_3), 5.37 (s, 2H, 8- CH_2), 6.68 (s, 1H, 6-H), 6.96 (d, 2H, $J = 8.8$ Hz, 3', 5'-H), 7.41 (d, 2H, $J = 8.8$ Hz, 2', 6'-H), 7.87 ppm (s, 1H, 2-H); ^{13}C NMR (101 MHz, CDCl_3): δ 20.95, 21.21, 54.57, 55.28, 56.41, 103.95, 109.54, 111.61, 113.97, 123.49, 125.46, 130.27, 151.07, 151.86, 156.91, 159.63, 161.83, 169.59, 171.02, 174.73 ppm; MS (CI): m/z 413.3 (MH^+ , 100). Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_8$: C, 64.08; H, 4.89. Found: C, 63.85; H, 4.61.

7-(Acetoxy)-8-(acetoxymethyl)-3-(2-methoxyphenyl)-4H-chromen-4-one (11c). White solid (98% yield); mp 122-124°C; IR (KBr): ν_{max} 3076, 1759, 1741, 1660, 1255, 1236 and 1178 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.08 (s, 3H, C-8 $\text{CH}_2\text{OCOCH}_3$), 2.40 (s, 3H, C-7 OCOCH_3), 3.82 (s, 3H, 2'- OCH_3), 5.40 (s, 2H, 8- CH_2), 6.97-7.07 (m, 2H, 3', 5'-H), 7.21 (d, 1H, $^3J = 8.8$ Hz, 6-H), 7.30-7.35 (m, 1H, 6'-H), 7.37-7.42 (m, 1H, 4'-H), 8.04 (s, 1H, H-2), 8.35 ppm (d, 1H, $^3J = 8.8$ Hz, 5-H); ^{13}C NMR (125 MHz, CDCl_3): δ 20.70, 20.79, 54.46, 55.29, 114.03, 117.52, 120.31, 122.54, 123.47, 125.16, 128.06, 130.03, 152.28, 153.65, 155.38, 159.77, 168.63, 170.52, 175.59 ppm; MS (CI): m/z

383.1 (MH^+ , 100). Anal. Calcd. for $\text{C}_{21}\text{H}_{18}\text{O}_7$: C, 65.97; H, 4.75. Found: C, 65.83; H, 4.95.

7-(Acetoxy)-8-(acetoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (11d). Pale yellow solid (84% yield); mp 141-143°C; IR (KBr): ν_{max} 3076, 1759, 1741, 1660, 1255, 1236 and 1178 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.07 (s, 3H, C-8 $\text{CH}_2\text{OCOCH}_3$), 2.40 (s, 3H, C-7 OCOCH_3), 3.85 (s, 3H, 4'- OCH_3), 5.39 (s, 2H, 8- CH_2), 6.99 (d, 2H, $^3J = 8.8$ Hz, 3', 5'-H), 7.21 (d, 1H, $^3J = 8.8$ Hz, 6-H), 7.51 (d, 2H, $^3J = 8.8$ Hz, 2', 6'-H), 8.06 (s, 1H, 2-H), 8.36 ppm (d, 1H, $^3J = 8.8$ Hz, 5-H); ^{13}C NMR (125 MHz, CDCl_3): δ 20.69, 20.78, 54.45, 55.29, 114.02, 117.51, 120.30, 122.53, 123.46, 125.15, 128.05, 130.02, 152.27, 153.64, 155.37, 159.77, 168.62, 170.51, 175.59 ppm; MS (CI): m/z 383.1 (MH^+ , 100). Anal. Calcd. for $\text{C}_{21}\text{H}_{18}\text{O}_7$: C, 65.97; H, 4.75. Found: C, 66.21; H, 4.51.

7-(Acetoxy)-8-(acetoxymethyl)-3-(4-methoxyphenyl)-2-methyl-4H-chromen-4-one (11e). White crystals (77% yield); mp 142-144°C; IR (KBr): ν_{max} 2922, 1765, 1737, 1645, 1223, 1199, 1180 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 2.08 (s, 3H, C-8 $\text{CH}_2\text{OCOCH}_3$), 2.36 (s, 3H, 2- CH_3), 2.39 (s, 3H, C-7 OCOCH_3), 3.85 (s, 3H, 4'- OCH_3), 5.40 (s, 2H, 8- CH_2), 6.98 (d, 2H, $^3J = 8.3$ Hz, 3', 5'-H), 7.16 (d, 1H, $^3J = 8.8$ Hz, 6-H), 7.21 (d, 2H, $^3J = 8.3$ Hz, 2', 6'-H), 8.28 ppm (d, 1H, $^3J = 8.8$ Hz, 5-H); ^{13}C NMR (125 MHz, CDCl_3): δ 19.45, 20.72, 20.78, 54.55, 55.24, 113.93, 117.08, 119.84, 121.51, 123.30, 124.57, 127.94, 131.42, 153.46, 154.92, 159.22, 163.21, 168.66, 170.50, 176.11 ppm; MS (CI): m/z 397.2 (MH^+ , 100). Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_7$: C, 66.66; H, 5.09. Found: C, 66.41; H, 5.27.

General procedures for the synthesis of hydroxymethyl derivatives 9 and 12. A solution of **8** or **11** (1 mmol) in 10 mL of 1,4-dioxane and 20 mL of 0.2 M aqueous sulfuric acid was heated at 50-60°C for 6-8 h. The mixture was cooled and diluted with water, and the resulting precipitate was collected by filtration. The crude product was chromatographed using 1-20 methanol-dichloromethane to afford **9** or **12** that was recrystallized from acetonitrile.

5-Hydroxy-6-(hydroxymethyl)-7-methoxy-3-(2-methoxyphenyl)-4H-chromen-4-one (9a). White solid (25%); mp 100-101°C; IR (KBr): ν_{max} 2938, 2837, 1654, 1583, 1494, 1283, 1220, 1129, 1076 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 3.81 (s, 3H, 2'- OCH_3), 3.93 (s, 3H, 7- OCH_3), 4.81 (s, 2H, 6- CH_2OH), 6.43 (s, 1H, 8-H), 6.94-7.08 (m, 2H, 3', 5'-H), 7.27-7.45 (m, 2H, 4', 6'-H), 7.88 (s, 1H, 2-H), 13.19 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, CDCl_3): δ 53.71, 55.70, 56.07, 89.67, 106.18, 111.24 (111.92), 119.37, 120.57, 121.38, 130.15, 131.54, 154.40, 157.40, 157.57, 159.90, 163.20, 180.53 ppm; MS (CI): m/z 329.1 (MH^+ , 100). Anal. Calcd. for $\text{C}_{18}\text{H}_{16}\text{O}_6$: C, 65.85; H, 4.91. Found: C, 65.93; H, 5.07.

5-Hydroxy-6-(hydroxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (9b). White solid (37% yield); mp 138-139°C; IR (KBr): ν_{max} 2930, 2833, 1645, 1611, 1513, 1253, 1223, 1179, 836 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 3.80 (s, 3H, 4'- OCH_3), 3.92 (s, 3H, 7- OCH_3), 4.48 (s, 2H, 6- CH_2OH), 6.74 (s, 1H, 8-H), 6.96-7.05 (m, 2H, 3', 5'-H), 7.47-7.58 (m, 2H, 2', 6'-H), 8.48 (s, 1H, 2-H), 13.23 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, CDCl_3): δ 53.68, 55.31, 56.08, 89.65, 106.12, 111.99, 114.07, 122.73, 123.84, 130.04, 152.57, 157.53,

159.80, 159.98, 163.34, 180.83 ppm; MS (CI): m/z (%): 329.2 (MH^+ , 100). Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.56; H, 4.87.

5-Hydroxy-8-(hydroxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (12b). White solid (48% yield); mp 139-141°C; IR (KBr): ν_{max} 2938, 2835, 1656, 1610, 1582, 1514, 1250, 1178, 1040, 831 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 3.85 (s, 3H, 4'-OCH₃), 3.96 (s, 3H, 7-OCH₃), 4.86 (s, 2H, 8-CH₂OH), 6.44 (s, 1H, 6-H), 6.99 (d, 2H, $^3J = 8.8$ Hz, 3', 5'-H), 7.46 (d, 2H, $^3J = 8.8$ Hz, 2', 6'-H), 7.94 (s, 1H, 2-H), 13.14 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 56.35, 55.36, 56.25, 95.22, 105.56, 106.97, 114.08, 122.70, 123.29, 130.03, 152.66, 154.91, 159.76, 163.03, 163.56, 181.11 ppm; MS (CI): m/z 329.2 (MH^+ , 100). Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.68; H, 5.11.

7-Hydroxy-8-(hydroxymethyl)-3-(2-methoxyphenyl)-4H-chromen-4-one (12c). White crystals (63% yield); mp 163-165°C; IR (KBr): ν_{max} 2953, 1627, 1603, 1441, 1267, 1237 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 3.71 (s, 3H, 2'-OCH₃), 4.72 (s, 2H, 8-CH₂OH), 6.82 (d, 1H, $^3J = 8.8$ Hz, 6-H), 6.95-7.02 (m, 1H, 5'-H), 7.04-7.10 (m, 1H, 3'-H), 7.19-7.25 (m, 1H, 6'-H), 7.32-7.39 (m, 1H, 4'-H), 7.75 (d, 1H, $^3J = 8.8$ Hz, 5-H), 8.12 ppm (s, 1H, H-2); ^{13}C NMR (125 MHz, DMSO- d_6): δ 51.39, 55.13, 113.60, 114.59, 114.87, 116.60, 122.88, 124.25, 125.91, 130.06, 153.10, 155.85, 158.94, 160.51, 174.86 ppm; MS (CI): m/z (%) 299.2 (MH^+ , 100). Anal. Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.55; H, 4.93.

7-Hydroxy-8-(hydroxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (12d). White crystals (69% yield); mp 150-152°C (decomp); IR (KBr): ν_{max} 2953, 1627, 1603, 1441, 1267, 1237 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 3.79 (s, 3H, 4'-OCH₃), 4.70 (s, 2H, 8-CH₂), 4.91 (br s, 1H, 8-CH₂OH), 6.95-7.05 (m, 3H, 6, 3', 5'-H), 7.52 (d, 2H, $^3J = 8.8$ Hz, 2', 6'-H), 7.93 (d, 1H, $^3J = 8.8$ Hz, 5-H), 8.41 (s, 1H, 2-H), 10.77 ppm (s, 1H, 7-OH); ^{13}C NMR (125 MHz, DMSO- d_6): δ 51.39, 55.13, 113.60, 114.59, 114.87, 116.60, 122.88, 124.25, 125.91, 130.06, 153.10, 155.85, 158.94, 160.51, 174.86 ppm; MS (CI): m/z (%) 299.1 (MH^+ , 100). Anal. Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.72; H, 4.58.

7-Hydroxy-8-(hydroxymethyl)-3-(4-methoxyphenyl)-2-methyl-4H-chromen-4-one (12e). White crystals (58% yield); mp 212-214°C (decomp); IR (KBr): ν_{max} 2958, 1633, 1589, 1438, 1246, 1066 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 2.28 (s, 3H, 2-CH₃), 3.79 (s, 3H, 4'-OCH₃), 4.71 (s, 2H, 8-CH₂), 4.89 (br. s, 1H, 8-CH₂OH), 6.92-7.05 (m, 3H, 6, 3', 5'-H), 7.19 (d, 2H, $^3J = 8.8$ Hz, 2', 6'-H), 7.82 (d, 1H, $^3J = 8.8$ Hz, 5-H), 10.68 ppm (s, 1H, 7-OH); ^{13}C NMR (125 MHz, DMSO- d_6): δ 19.18, 51.48, 55.02, 113.43, 114.13, 114.55, 115.51, 121.43, 125.33, 125.64, 131.62, 155.27, 158.47, 160.30, 162.37, 175.19 ppm; MS (CI): m/z (%) 313.1 (MH^+ , 100). Anal. Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.95; H, 5.31.

General procedures for the synthesis of alkoxymethyl derivatives 10 and 13. A mixture of diacetate **8** or **11** (2 mmol) and 0.1 mL of concentrated hydrochloric acid in 10 mL of methanol was refluxed for 16-24 h. The mixture was cooled and diluted with water, and the resulting precipitate was

collected by filtration. The products **10** and **13** were purified by chromatography using 1:20 methanol-dichloromethane.

5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4H-chromen-4-one (10a). White solid (53% yield); mp 123-124°C; IR (KBr): ν_{max} 2934, 2880, 1656, 1585, 1494, 1450, 1284, 1220, 1137, 1078 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 3.23 (s, 3H, 6-CH₂OCH₃), 3.75 (s, 3H, 2'-OCH₃), 3.93 (s, 3H, 7-OCH₃), 4.41 (s, 2H, 6-CH₂OCH₃), 6.78 (s, 1H, 8-H), 6.94-7.09 (m, 2H, 3', 5'-H), 7.28-7.34 (m, 1H, 6'-H), 7.35-7.43 (m, 1H, 4'-H), 8.37 (s, 1H, 2-H), 13.24 (s, 1H, 5-OH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 55.70, 56.17, 58.14, 61.58, 89.56, 106.10, 109.02, 111.26, 119.49, 120.57, 121.46, 130.12, 131.53, 154.23, 157.43, 157.99, 160.95, 164.22, 180.45; MS (CI): m/z 343.3 (MH^+ , 100). Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.58; H, 5.17.

5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (10b). White solid (95% yield); mp 180-181°C; IR (KBr): ν_{max} 2969, 2835, 1655, 1622, 1579, 1516, 1264, 1225, 1138, 1097 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 3.24 (s, 3H, 6-CH₂OCH₃), 3.81 (s, 3H, 4'-OCH₃), 3.93 (s, 3H, 7-OCH₃), 4.43 (s, 2H, 6-CH₂OCH₃), 6.74 (s, 1H, 8-H), 7.01 (d, 2H, $^3J = 8.8$ Hz, 3', 5'-H), 7.53 (d, 2H, $^3J = 8.8$ Hz, 2', 6'-H), 8.46 (s, 1H, 2-H), 13.29 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, DMSO- d_6): δ 55.16, 56.50, 57.28, 60.84, 90.31, 105.06, 108.41, 113.72, 122.33, 122.68, 130.17, 154.67, 157.45, 159.22, 159.97, 164.11, 180.40 ppm; MS (CI): m/z 343.2 (MH^+ , 100). Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.87; H, 5.56.

5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(2-methoxyphenyl)-4H-chromen-4-one (13a). White solid (28% yield); mp 154-155°C; IR (KBr): ν_{max} 2922, 2835, 1664, 1558, 1377, 1311, 1285, 1239, 1095, 1031 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 3.25 (s, 3H, 8-CH₂OCH₃), 3.74 (s, 3H, 2'-OCH₃), 3.93 (s, 3H, 7-OCH₃), 4.51 (s, 2H, 8-CH₂OCH₃), 6.61 (s, 1H, 6-H), 6.98-7.05 (m, 1H, 5'-H), 7.07-7.15 (m, 1H, 3'-H), 7.24-7.31 (m, 1H, 6'-H), 7.36-7.44 (m, 1H, 4'-H), 8.37 (s, 1H, 2-H), 13.14 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, DMSO- d_6): δ 55.51, 56.49, 57.23, 60.89, 95.23, 104.03, 104.45, 111.23, 119.37, 120.04, 120.28, 129.88, 131.45, 155.31, 155.56, 157.29, 162.04, 163.65, 180.10 ppm; MS (CI): m/z 343.3 (MH^+ , 100). Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.93; H, 5.12.

5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (13b). White solid (41% yield); mp 126-128°C; IR (KBr): ν_{max} 2938, 2838, 1662, 1610, 1585, 1541, 1246, 1204, 1072, 1042 cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6): δ 3.25 (s, 3H, 8-CH₂OCH₃), 3.79 (s, 3H, 4'-OCH₃), 3.92 (s, 3H, 7-OCH₃), 4.50 (s, 2H, 8-CH₂OCH₃), 6.60 (s, 1H, 6-H), 7.01 (d, 2H, $J = 8.8$ Hz, 3', 5'-H), 7.52 (d, 2H, $J = 8.8$ Hz, 2', 6'-H), 8.51 (s, 1H, 2-H), 13.24 ppm (s, 1H, 5-OH); ^{13}C NMR (101 MHz, DMSO- d_6): δ 55.16, 56.53, 57.28, 60.92, 95.30, 103.96, 104.65, 113.72, 121.85, 122.66, 130.17, 154.76, 155.38, 159.19, 162.36, 163.81, 180.64 ppm; MS (CI): m/z 343.3 (MH^+ , 100). Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.43; H, 5.22.

7-Hydroxy-8-(methoxymethyl)-3-(2-methoxyphenyl)-4H-chromen-4-one (13c). White crystals (78% yield); mp 164-166°C; IR (KBr): ν_{max} 2937, 1624, 1579, 1427, 1284, 1259 cm^{-1} ; 1H

NMR (400 MHz, DMSO- d_6): δ 3.29 (s, 3H, 8-CH₂OCH₃), 3.71 (s, 3H, 2'-OCH₃), 4.62 (s, 2H, 8-CH₂OCH₃), 6.91-7.02 (m, 2H, 6, 5'-H), 7.04-7.10 (m, 1H, 3'-H), 7.20-7.24 (m, 1H, 6'-H), 7.33-7.40 (m, 1H, 4'-H), 7.82 (d, 1H, ³J = 8.8 Hz, 5-H), 8.17 ppm (s, 1H, H-2); ¹³C NMR (125 MHz, DMSO- d_6): δ 55.07, 57.45, 61.37, 111.24, 113.55, 114.45, 116.53, 122.95, 124.13, 126.70, 130.01, 153.03, 156.16, 158.92, 161.10, 174.71 ppm; MS (CI): m/z 313.2 (MH⁺, 100). Anal. Calcd. for C₁₈H₁₆O₅: C, 69.22; H, 5.16. Found: C, 68.88; H, 5.27.

7-Hydroxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (13d). White crystals (89% yield); mp 167-169°C decomp; IR (KBr): ν_{\max} 2937, 1624, 1579, 1427, 1284, 1259 cm⁻¹; ¹H NMR (400 MHz, DMSO- d_6): δ 3.30 (s, 3H, 8-CH₂OCH₃), 3.79 (s, 3H, 4'-OCH₃), 4.62 (s, 2H, 8-CH₂OCH₃), 6.99 (d, 2H, ³J = 8.8 Hz, 3', 5'-H), 7.05 (d, 1H, ³J = 8.8 Hz, 6-H), 7.53 (d, 2H, ³J = 8.8 Hz, 2', 6'-H), 7.98 (d, 1H, ³J = 8.8 Hz, 5-H), 8.40 (s, 1H, 2-H), 10.83 ppm (s, 1H, 2-H) ppm; ¹³C NMR (125 MHz, DMSO- d_6): δ 55.07, 57.45, 61.37, 111.24, 113.55, 114.45, 116.53, 122.95, 124.13, 126.70, 130.01, 153.03, 156.16, 158.92, 161.10, 174.71 ppm; MS (CI): m/z 313.1 (MH⁺, 100). Anal. Calcd. for C₁₈H₁₆O₅: C, 69.22; H, 5.16. Found: C, 69.01; H, 4.92.

7-Hydroxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-2-methyl-4H-chromen-4-one (13e). White crystals (89% yield); mp 215-217°C decomp; IR (KBr): ν_{\max} 2927, 1614, 1585, 1406, 1294, 1246, 1065 cm⁻¹; ¹H NMR (400 MHz, DMSO- d_6): δ 2.27 (s, 3H, 2-CH₃), 3.29 (s, 3H, 8-CH₂OCH₃), 3.79 (s, 3H, 4'-OCH₃), 4.61 (s, 2H, 8-CH₂OCH₃), 6.98 (d, 2H, ³J = 8.8 Hz, 3', 5'-H), 7.00 (d, 1H, ³J = 8.8 Hz, 6-H), 7.20 (d, 2H, ³J = 8.8 Hz, 2', 6'-H), 7.87 (d, 1H, ³J = 8.8 Hz, 5-H), 10.85 ppm (s, 1H) ppm; ¹³C NMR (125 MHz, DMSO- d_6): δ 19.13, 55.01, 57.48, 61.40, 110.99, 113.41, 114.03, 115.43, 121.52, 125.21, 126.48, 131.60, 155.70, 158.48, 160.89, 162.25, 175.08 ppm; MS (CI): m/z 327.1 (MH⁺, 100). Anal. Calcd. for C₁₉H₁₈O₅: C, 69.93; H, 5.56. Found: C, 70.23; H, 5.28.

3-(4-Methoxyphenyl)-10,11,11a,12-tetrahydro-4H,7aH,9H-dipyran[2,3-b:2',3'-f]chromen-4-one (14d). To a solution of 2 mmol of **7d** in 10 mL of DMF was added 2 mL (22 mmol, 11 eq) of 2H,3,4-dihydropyran. The solution was refluxed for 24-40 h. The solvent and excess 2H,3,4-dihydropyran were evaporated *in vacuo*, and the residue was purified by chromatography with 1:50 methanol-dichloromethane. Pale yellow solid (30% yield); mp 182-183°C; IR (KBr) ν_{\max} 2966, 2933, 1636, 1598, 1511, 1437, 1248, 1205, 1178, 1090, 1029 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.47-1.92 (m, 4H, 10, 11-CH₂), 2.24-2.40 (m, 1H, 11a-CH), 2.90 (dd, 1H, ²J = 17.4 Hz, ³J = 4.2 Hz, 12 α -CH), 3.01 (dd, 1H, ²J = 17.4, ³J = 6.1 Hz, 12 β -CH), 3.75-3.82 (m, 1H, 9 α -CH), 3.85 (s, 3H, 4'-OMe), 3.95-4.09 (m, 1H, 9 β -CH), 5.44 (d, 1H, ³J = 2.0 Hz, 7a-CH), 6.93-7.02 (m, 3H, 6, 3', 5'-H), 7.50 (d, 2H, ³J = 8.7 Hz, 2', 6'-H), 7.96 (s, 1H, 2-H), 8.10 ppm (d, 1H, ³J = 8.8 Hz, 5-H); ¹³C NMR (400 MHz, CDCl₃) δ 23.17, 23.57, 24.02, 30.62, 55.33, 62.58, 96.94, 107.86, 113.94, 115.27, 118.45, 124.19, 124.79, 125.29, 130.13, 151.79, 155.37, 157.15, 159.55, 176.07 ppm; MS (CI): m/z 365.1 (MH⁺, 100). Anal. Calcd for C₂₂H₂₀O₅: C, 72.51; H, 5.53. Found: C, 72.38; H, 5.67.

Cell Proliferation Assay

PC-3 prostate cancer cells were cultured in DMEM/F-12 HAM Mixture (Sigma D8437), 10% Fetal Bovine Serum (Atlanta Biological S11150). Before the treatment, 3.5x10⁴ cells per well were split into 12-well plates. After 24 h, 10 μ M of each compound was added to each well. DMSO was used as a control. This experiment was done in triplicate. Cell viability and number were analyzed using Vi-Cell XR Cell Viability Analyzer (Beckman Coulter).

Acknowledgements

DSW and CL were supported by R21 CA139359 and CA172379 from the NIH, by the Office of the Dean of the College of Medicine, and by NIH Grant Number P20 RR020171 from the National Institute of General Medical Sciences to L. Hersh, PI. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or the NIGMS.

Notes and references

DSW and CL own shares in two limited liability corporations committed to developing new antineoplastic agents patented by the University of Kentucky. The other authors declare no competing financial interests.

1. I. C. Munro, M. Harwood, J. J. Hlywka, A. M. Stephen, J. Doull, W. G. Flamm and H. Adlercreutz, *Nutr. Rev.*, 2003, **61**, 1-33.
2. H. Adlercreutz, *Scand. J. Clin. Lab. Invest.*, 1990, **50**, 3-23.
3. S. Andres, K. Abraham, K. E. Appel and A. Lampen, *Crit. Rev. Toxicol.*, 2011, **41**, 463-506.
4. E. Miadoková, *Interdiscip. Toxicol.*, 2009, **2**, 211-218.
5. J. M. Hamilton-Reeves, S. A. Rebello, W. Thomas, J. W. Slaton and M. S. Kurzer, *J. Nutr.*, 2007, **137**, 1769-1775.
6. J. L. Mohler, C. W. Gregory, O. H. Ford, D. Kim, C. M. Weaver, P. Petrusz, E. M. Wilson and F. S. French, *Clin. Cancer Res.*, 2004, **10**, 440-448.
7. M. A. Titus, M. J. Schell, F. B. Lih, K. B. Tomer and J. L. Mohler, *Clin. Cancer Res.*, 2005, **11**, 4653-4657.
8. M. V. Fiandalo, J. Wilton and J. L. Mohler, *Int. J. Biol. Sci.*, 2014, **10**, 596-601.
9. S. Tai, Y. Sun, J. M. Squires, H. Zhang, W. K. Oh, C.-Z. Liang and J. Huang, *Prostate*, 2011, **71**, 1668-1679.
10. W. Zhang, V. Sviripa, X. Chen, J. Shi, T. Yu, A. Hamza, N. D. Ward, L. M. Kril, C. W. Vander Kooi, C.-G. Zhan, B. M. Evers, D. S. Watt and C. Liu, *ACS Chem. Biol.*, 2013, **8**, 796-803.
11. V. M. Sviripa, W. Zhang, A. G. Balia, O. V. Tsodikov, J. R. Nickell, F. Gizard, T. Yu, E. Y. Lee, L. P. Dwoskin, C. Liu and D. S. Watt, *J. Med. Chem.*, 2014, **57**, 6083-6091.
12. Y. Wen, M. T. Islam and S. Tahara, *Biosci. Biotechnol. Biochem.*, 2006, **70**, 2567-2570.
13. N. Tanaka, T. Murakami, H. Wada, A. B. Gutierrez, Y. Saiki and C. Chen, *Chem. Pharm. Bull.*, 1985, **33**, 5231-5238.
14. L. E. C. Suarez and F. D. Monache, *Phytochemistry*, 1992, **31**, 2481-2482.
15. C. Mahidol, H. Prawat, W. Kaweetripob and S. Ruchirawat, *Heterocycles*, 2002, **57**, 1287-1292.
16. Y. Feng, J. W. Blunt, A. L. J. Cole and M. H. G. Munro, *J. Nat. Prod.*, 2002, **65**, 1681-1682.

17. Y. Chen, M. Cheng, F.-Q. Liu, P. Xia, K. Qian, D. Yu, Y. Xia, Z.-Y. Yang, C.-H. Chen, S. L. Morris-Natschke and K.-H. Lee, *Eur. J. Med. Chem.*, 2011, **46**, 4924-4936.
18. J. P. Phillips and E. M. Barrall, *J. Org. Chem.*, 1956, **21**, 692-694.
19. F. F. Blicke and F. J. McCarty, *J. Org. Chem.*, 1959, **24**, 1061-1069.
20. J. H. Burckhalter, J. N. Wells and W. J. Mayer, *Tetrahedron Lett.*, 1964, **5**, 1353-1359.
21. A. R. Katritzky, X. Lan and J. N. Lam, *Chem. Ber.*, 1991, **124**, 1809-1817.
22. Y. Omura, Y. Taruno, Y. Iriya, M. Morimoto, H. Saimoto and Y. Shigemasa, *Tetrahedron Lett.*, 2001, **42**, 7273-7275.
23. J.-J. Lin and S.-F. Lin, *J. Coll. Interf. Sci.*, 2003, **258**, 155-162.
24. J. R. Farrell, J. Niconchuk, C. S. Higham and B. W. Bergeron, *Tetrahedron Lett.*, 2007, **48**, 8034-8036.
25. M. Minakawa, H.-M. Guo and F. Tanaka, *J. Org. Chem.*, 2008, **73**, 8669-8672.
26. M. Betti, *Gazz. Chim. Ital.*, 1900, **30**, 301-309.
27. M. Betti, *Gazz. Chim. Ital.*, 1900, **30**, 310-316.
28. M. Betti, *Gazz. Chim. Ital.*, 1906, **36**, 392-394.
29. L. O. Paqett, *Encyclopedia of Reagents for Organic Synthesis*, Wiley, UK, 1995.
30. M. Arend, B. Westermann and N. Risch, *Angew. Chem. Int. Ed.*, 1998, **37**, 1044-1070.
31. C. Cardellicchio, G. Ciccarella, F. Naso, F. Perna and P. Tortorella, *Tetrahedron*, 1999, **55**, 14685-14692.
32. C. Cardellicchio, M. A. M. Capozzi and F. Naso, *Tetrahedron Asymm.*, 2010, **21**, 507-517.
33. I. Szatmari and F. Fulop, *Curr. Org. Syn.*, 2004, **1**, 155-165.
34. C. de Graaff, E. Ruijter and R. V. A. Orru, *Chem. Soc. Rev.*, 2012, **41**, 3969-4009.
35. A. C. Talukdar, N. Jain, S. De and H. G. Krishnamurty, *Phytochemistry*, 2000, **53**, 155-157.
36. U. B. Kim, D. P. Furkert and M. A. Brimble, *Org. Lett.*, 2013, **15**, 658-661.
37. S. P. Bondarenko, M. S. Frasinuk and V. P. Khilya, *Chem. Nat. Compd.*, 2012, **48**, 26-29.
38. S. P. Bondarenko and M. S. Frasinuk, *Chem. Nat. Compd.*, 2013, **49**, 841-844.
39. S. P. Bondarenko, A. V. Levenets, M. S. Frasinuk and V. P. Khilya, *Chem. Nat. Compd.*, 2003, **39**, 271-275.
40. P. J. Houghton and Y. Hairong, *Planta Med.*, 1985, **51**, 23-27.
41. J. Li, G. Wang, M. Dong and Q. Zhang, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 2324-2326.
42. F. E. King, M. F. Grundon and K. G. Neill, *J. Chem. Soc.*, 1952, **36**, 4580-4584.
43. M. S. Frasinuk, S. P. Bondarenko, V. P. Khilya, C. Liu, D. S. Watt and V. M. Sviripa, *Org. Biomol. Chem.*, 2015, **13**, 1053-1067.
44. M. S. Frasinuk, S. P. Bondarenko and V. P. Khilya, *Chem. Nat. Compd.*, 2006, **42**, 142-147.