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ARTICLE

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

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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 acetoxymethyl derivatives that were subsequently converted to hydroxymethyl- and methoxymethyl-substituted 5-hydroxy- or 7-hydroxyisoflavonoids related to naturally occurring flavonoids. The C-8 acetoxymethyl, hydroxymethyl or methoxymethyl-substituted isoflavonoids possessed promising inhibitory potency in the low micromolar range in a prostate cancer PC-3 cell proliferation assay.

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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 5α-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-3α,17β-diol, testosterone (T) and DHT. Second, the primary "backdoor" pathway utilizes the conversion of pregnenolone, in several steps, to androsterone (AND) and 5α-androstan-3α,17β-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, 5α-androstane-3α,17β-dione (5α-DIONE) and finally DHT.[6-8]

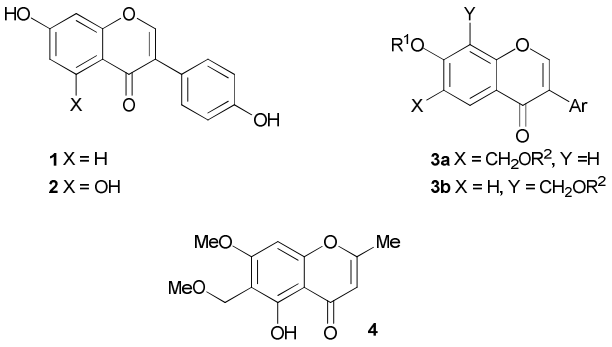

Figure 1. Naturally occurring 7-hydroxyisoflavones, daidzein (**1**) and genistein (**2**), synthetic C-6 or C-8 substituted isoflavonoids **3**, and 6-(methoxymethyl)eugenin (**4**).

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

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

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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.

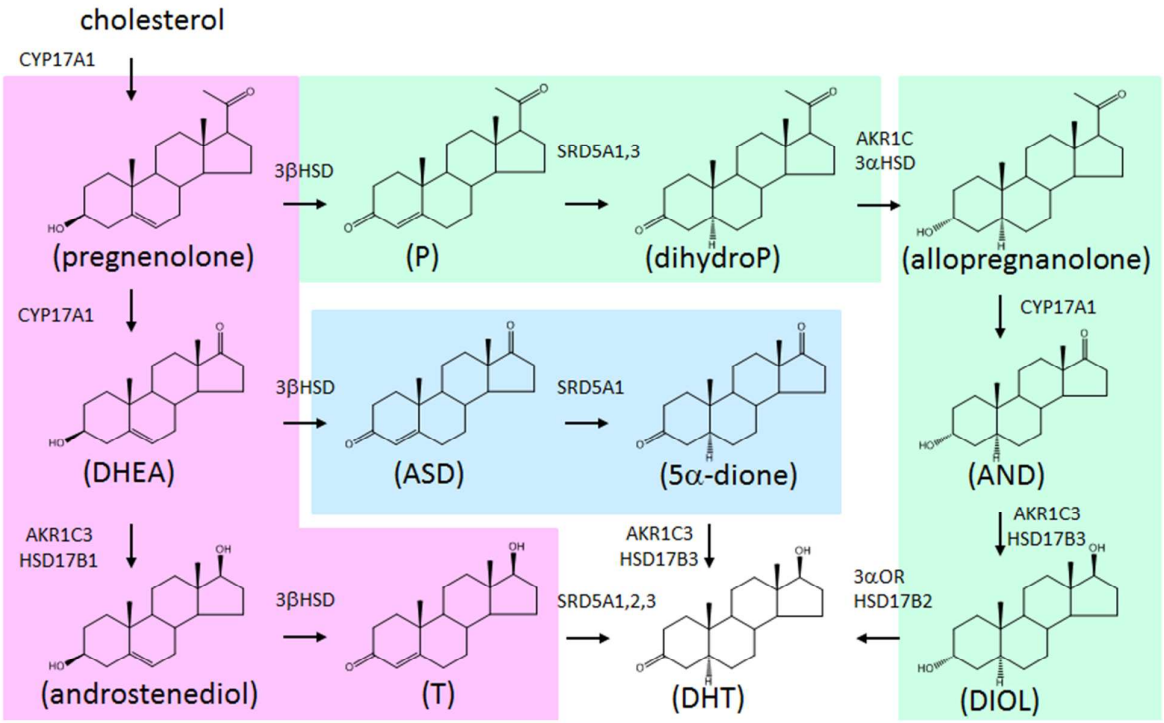

Figure 2. Frontdoor (pink), primary backdoor (green) and secondary backdoor (blue) pathways to 5α-dihydrotestosterone (DHT). Enzymes: Cytochrome P450 17A1 or steroid 17α-monooxygenase (CYP17A1); aldo-keto reductase-3 (AKR1C3), 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase (3βHSD); 17β-hydroxysteroid dehydrogenase (HSD17B2; HSD17B3); steroid 5α-reductase or 3-oxo-5α-steroid Δ^4-dehydrogenase alpha (SRD5A1, 2, 3); 3α-hydroxysteroid dehydrogenase (3αOR). 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-resistant[9] 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 suitable 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 reports[1-4] on the toxicity of isoflavones, we noted that genistein (**2**) displayed IC_{50} values in the 15-100 μM 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.

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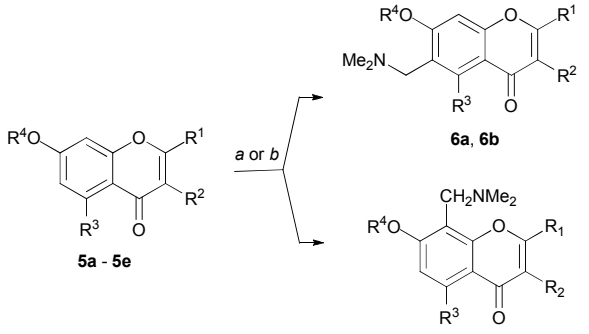
Results and discussion

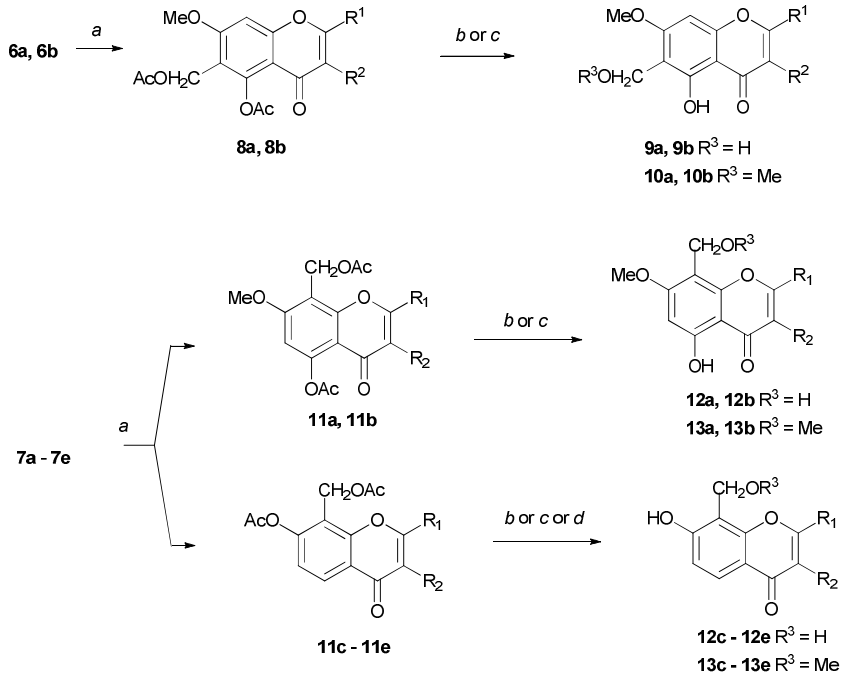
In accord with reported applications of the aminomethylation reaction to phenols[18-25] and β-naphthols,[26-34] heating hydroxylated isoflavonoids with bis(*N,N*-dimethylamino)methane in either 1,4-dioxane or isopropanol furnished the desired N,N-dialkylaminomethyl-substituted derivatives. Synthesis 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*-methylgenistein[35] (**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 hydroxyated 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-hydroxyated 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**.



a R^1 = H, R^2 = C_6H_4OMe-2, R^3 = OH, R^4 = Me
b R^1 = H, R^2 = C_6H_4OMe-4, R^3 = OH, R^4 = Me
c R^1 = H, R^2 = C_6H_4OMe-2, R^3 = R^4 = H
d R^1 = H, R^2 = C_6H_4OMe-4, R^3 = R^4 = H
e R^1 = Me, R^2 = C_6H_4OMe-4, R^3 = R^4 = H

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



a R^1 = H, R^2 = C_6H_4OMe-2
b R^1 = H, R^2 = C_6H_4OMe-4
c R^1 = H, R^2 = C_6H_4OMe-2
d R^1 = H, R^2 = C_6H_4OMe-4
e R^1 = Me, R^2 = C_6H_4OMe-4

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.

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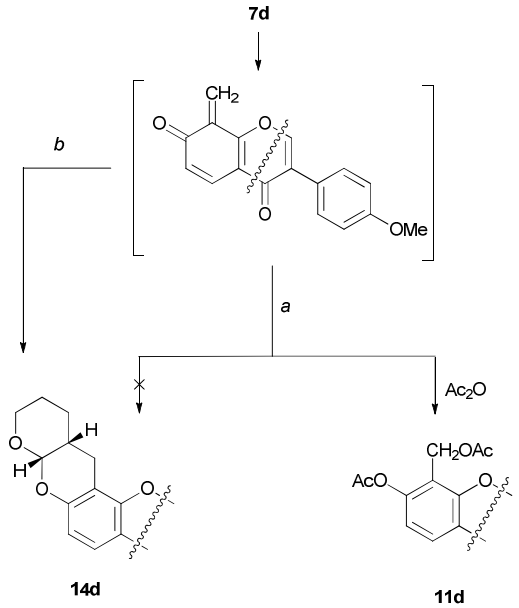
Direct conversion of the N,N-dimethylaminomethyl derivatives **6** and **7** to the corresponding C-6 or C-8 hydroxymethyl derivatives 9 and 12 respectively in basic media or direct transformation **6** and **7** to the corresponding C-6 or C-8 methoxymethyl derivatives in refluxing methanol[41] proceeded 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 alkoxymethyl-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-2*H*-pyran, Ac_2O, KOAc*; b*, 3,4-dihydro-2*H*-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 cochiolides*,[12] and could be useful for the synthesis for various hydroxymethyl and alkoxymethyl 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 μM (%)
8a	6-acetoxymethyl	17.1 ± 5.9
8b	6-acetoxymethyl	51.7 ± 5.7
9a	6-hydroxymethyl	14.6 ± 3.2
9b	6-hydroxymethyl	0 ± 16.0
10a	6-methoxymethyl	53.4 ± 8.0
10b	6-methoxymethyl	0 ± 27.0
11b	8-acetoxymethyl	20.2 ± 2.0
11c	8-acetoxymethyl	20.6 ± 3.8
11d	8-acetoxymethyl	99.2 ± 0.4
11e	8-acetoxymethyl	98.2 ± 1.5
12b	8-hydroxymethyl	79.5 ± 3.6
12c	8-hydroxymethyl	0 ± 16.0
12d	8-hydroxymethyl	99.5 ± 0.2
12e	8-hydroxymethyl	99.5 ± 0.5
13a	8-methoxymethyl	0 ± 1.3
13b	8-methoxymethyl	96.8 ± 0.8
13c	8-methoxymethyl	0 ± 7.4
13d	8-methoxymethyl	34.9 ± 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-2*H*-pyran under conditions where **7d** could afford either the diacetate **11d** or the inverse

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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-2*H*-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-2*H*-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 μM 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** presumabley *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

^{1}H 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 Kim[36] was repeated using 5 mmol of 5,7-dihydroxy-3-(2-methoxyphenyl)-4*H*-chromen-4-one[39] or 5,7-dihydroxy-3-(4-methoxyphenyl)-4*H*-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)-4*H*-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}; ^{1}H 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 $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.12; H, 4.97.

5-Hydroxy-7-methoxy-3-(4-methoxyphenyl)-4*H*-chromen-4-one (5b). Pale yellow solid (73% yield); mp 142-143°C (lit[4] mp 141-142°C); IR (KBr): ν_{max} 2964, 2936, 2833, 1658, 1618, 1579, 1516, 1244, 1192, 1151, 1051 cm^{-1}; ^{1}H 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 $C_{17}H_{14}O_5$: 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

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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)-4*H*-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, $N(CH_3)_2$), 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 $C_{20}H_{21}NO_5$: 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)-4*H*-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, $N(CH_3)_2$), 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 $C_{20}H_{21}NO_5$; 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)-4*H*-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, $N(CH_3)_2$), 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,): 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 $C_{20}H_{21}NO_5$: 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)-4*H*-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, $N(CH_3)_2$), 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 $C_{20}H_{21}NO_5$: 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)-4*H*-chromen-4-one (7c). Pale yellow solid (91% yield); mp 120 - 121°C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 6H, $N(CH_3)_2$), 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 $C_{19}H_{19}NO_4$: 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)-4*H*-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, $N(CH_3)_2$), 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 $C_{19}H_{19}NO_4$: 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-4*H*-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, $N(CH_3)_2$), 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 $C_{20}H_{21}NO_4$: 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)-4*H*-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 CH_2OCOCH_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-

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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 $C_{22}H_{20}O_8$: C, 64.08; H, 4.89. Found: C, 64.27; H, 5.11.

5-Acetoxy-6-(acetoxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4*H*-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}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04 (s, 3H, C-6 $CH_2OCOC\underline{H}_3$), 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 $C_{22}H_{20}O_8$: C, 64.08; H, 4.89. Found: C, 63.89; H, 5.17.

5-Acetoxy-8-(acetoxymethyl)-7-methoxy-3-(2-methoxyphenyl)-4*H*-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}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 3H, C-8 $CH_2OCOC\underline{H}_3$), 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 $C_{22}H_{20}O_8$: C, 64.08; H, 4.89. Found: C, 64.32; H, 5.07.

5-Acetoxy-8-(acetoxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4*H*-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}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 3H, C-8 $CH_2OCOC\underline{H}_3$), 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 $C_{22}H_{20}O_8$: C, 64.08; H, 4.89. Found: C, 63.85; H, 4.61.

7-(Acetoxy)-8-(acetoxymethyl)-3-(2-methoxyphenyl)-4*H*-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}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3H, C-8 $CH_2OCOC\underline{H}_3$), 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 $C_{21}H_{18}O_7$: C, 65.97; H, 4.75. Found: C, 65.83; H, 4.95.

7-(Acetoxy)-8-(acetoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one (11d). Pale yellow soldi (84% yield); mp 141-143°C; IR (KBr): ν_{max} 3076, 1759, 1741, 1660, 1255, 1236 and 1178 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07 (s, 3H, C-8 $CH_2OCOC\underline{H}_3$), 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 $C_{21}H_{18}O_7$: C, 65.97; H, 4.75. Found: C, 66.21; H, 4.51.

7-(Acetoxy)-8-(acetoxymethyl)-3-(4-methoxyphenyl)-2-methyl-4*H*-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}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3H, C-8 $CH_2OCOC\underline{H}_3$), 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 = 8.3Hz, 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 $C_{22}H_{20}O_7$: 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)-4*H*-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}; ^{1}H 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-$C\underline{H}_2OH$), 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 $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.93; H, 5.07.

5-Hydroxy-6-(hydroxymethyl)-7-methoxy-3-(4-methoxyphenyl)-4*H*-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}; ^{1}H 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-$C\underline{H}_2OH$), 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,

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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)-4*H*-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'-OCH_3), 3.96 (s, 3H, 7-OCH_3), 4.86 (s, 2H, 8-$C\underline{H}_2OH$), 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)-4*H*-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'-OCH_3), 4.72 (s, 2H, 8-$C\underline{H}_2OH$), 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)-4*H*-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'-OCH_3), 4.70 (s, 2H, 8-CH_2), 4.91 (br s, 1H, 8-$CH_2O\underline{H}$), 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-4*H*-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-CH_3), 3.79 (s, 3H, 4'-OCH_3), 4.71 (s, 2H, 8-CH_2), 4.89 (br. s, 1H, 8-$CH_2O\underline{H}$), 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)-4*H*-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-$CH_2OC\underline{H}_3$), 3.75 (s, 3H, 2'-OCH_3), 3.93 (s, 3H, 7-OCH_3), 4.41 (s, 2H, 6-$C\underline{H}_2OCH_3$), 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)-4*H*-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-$CH_2OC\underline{H}_3$), 3.81 (s, 3H, 4'-OCH_3), 3.93 (s, 3H, 7-OCH_3), 4.43 (s, 2H, 6-$C\underline{H}_2OCH_3$), 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)-4*H*-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-$CH_2OC\underline{H}_3$), 3.74 (s, 3H, 2'-OCH_3), 3.93 (s, 3H, 7-OCH_3), 4.51 (s, 2H, 8-$C\underline{H}_2OCH_3$), 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)-4*H*-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-$CH_2OC\underline{H}_3$), 3.79 (s, 3H, 4'-OCH_3), 3.92 (s, 3H, 7-OCH_3), 4.50 (s, 2H, 8-$C\underline{H}_2OCH_3$), 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)-4*H*-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

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NMR (400 MHz, DMSO-d_6): δ 3.29 (s, 3H, 8-$CH_2OC\underline{H}_3$), 3.71 (s, 3H, 2'-OCH_3), 4.62 (s, 2H, 8-$C\underline{H}_2OCH_3$), 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); ^{13}C 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 $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.88; H, 5.27.

7-Hydroxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-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}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (s, 3H, 8-$CH_2OC\underline{H}_3$), 3.79 (s, 3H, 4'-OCH_3), 4.62 (s, 2H, 8-$C\underline{H}_2OCH_3$), 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; ^{13}C 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 $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.01; H, 4.92.

7-Hydroxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-2-methyl-4*H*-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}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.27 (s, 3H, 2-CH_3), 3.29 (s, 3H, 8-$CH_2OC\underline{H}_3$), 3.79 (s, 3H, 4'-OCH_3), 4.61 (s, 2H, 8-$C\underline{H}_2OCH_3$), 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; ^{13}C 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 $C_{19}H_{18}O_5$: C, 69.93; H, 5.56. Found: C, 70.23; H, 5.28.

3-(4-Methoxyphenyl)-10,11,11a,12-tetrahydro-4*H*,7a*H*,9*H*-dipyrano[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 2*H*,3,4-dihydropyran. The solution was refluxed for 24-40 h. The solvent and excess 2*H*,3,4-dihydropyran were evaporated *in vacuo*, and the residue was purified by chromatography with1:50 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}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47-1.92 (m, 4H, 10, 11-CH_2), 2.24-2.40 (m, 1H, 11a-CH), 2.90 (dd, 1H, ^2J = 17.4 Hz, ^3J = 4.2 Hz, 12α-CH), 3.01 (dd, 1H, ^2J = 17.4, ^3J = 6.1 Hz, 12β-CH), 3.75-3.82 (m, 1H, 9α-CH), 3.85 (s, 3H, 4'-OMe), 3.95-4.09 (m, 1H, 9β-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); ^{13}C NMR (400 MHz, $CDCl_3$) 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 $C_{22}H_{20}O_5$: 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, $3.5x10^4$ cells per well were split into 12-well plates. After 24 h, 10μM 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).

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Notes and references

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