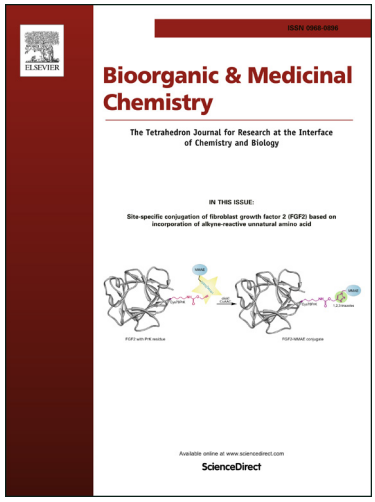


3-*O*-Substituted-3',4',5'-trimethoxyflavonols: synthesis and cell-based evaluation as anti-prostate cancer agents

Xiang Li, Maizie Lee, Guanglin Chen, Qiang Zhang, Shilong Zheng, Guangdi Wang, Qiao-Hong Chen

PII: S0968-0896(17)31178-1
DOI: http://dx.doi.org/10.1016/j.bmc.2017.07.022
Reference: BMC 13859

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 1 June 2017
Revised Date: 8 July 2017
Accepted Date: 11 July 2017

Please cite this article as: Li, X., Lee, M., Chen, G., Zhang, Q., Zheng, S., Wang, G., Chen, Q-H., 3-*O*-Substituted-3',4',5'-trimethoxyflavonols: synthesis and cell-based evaluation as anti-prostate cancer agents, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.07.022

3-*O*-Substituted-3',4',5'-trimethoxyflavonols: synthesis and cell-based evaluation as anti-prostate cancer agents

Xiang Li[a], Maizie Lee[a], Guanglin Chen[a], Qiang Zhang[b,c], Shilong Zheng[b,c], Guangdi Wang[b,c], Qiao-Hong Chen[a,*]

[a]*Department of Chemistry, California State University, Fresno, 2555 E. San Ramon Avenue, M/S SB70, Fresno, CA 93740, USA.*

[b]*RCMI Cancer Research Center, Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA*

[c]*Department of Chemistry, Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA*

ABSTRACT — Twenty-two 3-*O*-substituted-3',4',5'-trimethoxyflavonols have been designed and synthesized for their anti-proliferative activity towards three human prostate cancer cell lines. Our results indicate that most of them are significantly more potent than the parent 3',4',5'-trimethoxyflavonol in inhibiting the cell proliferation in PC-3 and LNCaP prostate cancer cell models. 3-*O*-Substituted-3',4',5'- trimethoxyflavonols have generally higher potency towards PC-3 and LNCaP cell lines than the DU145 cell line. Incorporation of an ethyl group to 3-OH of 3',4',5'-trimethoxyflavonol leads to 3-*O*-ethyl-3',4',5'-trimethoxyflavonol as the optimal derivative with up to 36-fold enhanced potency as compared with the corresponding lead compound 3',4',5'-trimethoxyflavonol, but with reversed PC-3 cell apoptotic response. Introduction of a dipentylaminopropyl group to 3-OH increases not only the antiproliferative potency but also the ability in activating PC-3 cell apoptosis. Our findings imply that modification on 3-OH of trimethoxyflavonol can further enhance its *in vitro* anti-proliferative potency and PC-3 cell apoptosis induction.

1. Introduction:

Flavonoids are nutraceuticals with various health and medicinal benefits. Flavonols belong to a large subgroup of flavonoids that are characteristic of a hydroxyl group at C-3 and are ubiquitously distributed in dietary foods.[1-2] The anti-prostate cancer activity of several naturally occurring flavonols, as exemplified by quercetin (**1**) and fisetin (**2**) (Fig. 1), has been explored by *in vitro* and *in vivo* experiments.[3-8] 3',4',5'-Trimethoxyflavonol (**3**, Fig. 1) is a synthetic flavonol and a promising anti-prostate cancer lead compound that exhibits 5- to 16-fold greater potency than the naturally occurring quercetin (**1**) and fisetin (**2**) in suppressing cell growth in four prostate cancer cell models.[3,9] The *in vitro* anti-prostate cancer potency of 3',4',5'-trimethoxyflavonol (**3**) has been translated into its *in vivo* antitumor efficacy in two prostate cancer xenografts (the murine TRAMP C2 and human-derived 22Rv1) in nude mice, but the efficacy is very moderate even at the highest dose (0.731 mmol/kg).[9] Additionally, oral administration of a single dose of 3',4',5'-trimethoxyflavonol (**3**) at 240 mg/kg yielded a C_{max} of only 18.4 nM in the mice prostate at 6 hours after administration.[10] These data suggest that either potency, bioavailability, or both of 3',4',5'-trimethoxyflavonol (**3**) need to be further improved prior to being considered as a drug candidate for the treatment of prostate cancer. Our earlier WST-1 cell proliferation assay indicates that incorporation of a dibutylamino group to the 3-OH group of 3',4'-dimethoxyflavonol (**4**, Fig. 1) through a three- to five-carbon linker leads to the optimal derivatives (e.g. **5**, Fig. 1) with up to 292-fold enhanced potency as compared with the parent 3',4'-dimethoxyflavonol (**4**) in human prostate cancer cell models.[11] Encouraged by these promising results, the present study aims to explore the possibility of further enhancing the anti-proliferative potency of 3',4',5'-trimethoxyflavonol (**3**) as anti-prostate cancer agents through chemical manipulations on its 3-OH group. Consequently, ten 3-*O*-alkyl-3',4',5'-trimethoxyflavonols (one known and nine new) and twelve new 3-*O*-dialkylaminoalkyl-3',4',5'-trimethoxyflavonols have been synthesized and evaluated for their ability in suppressing prostate cancer cell proliferation.

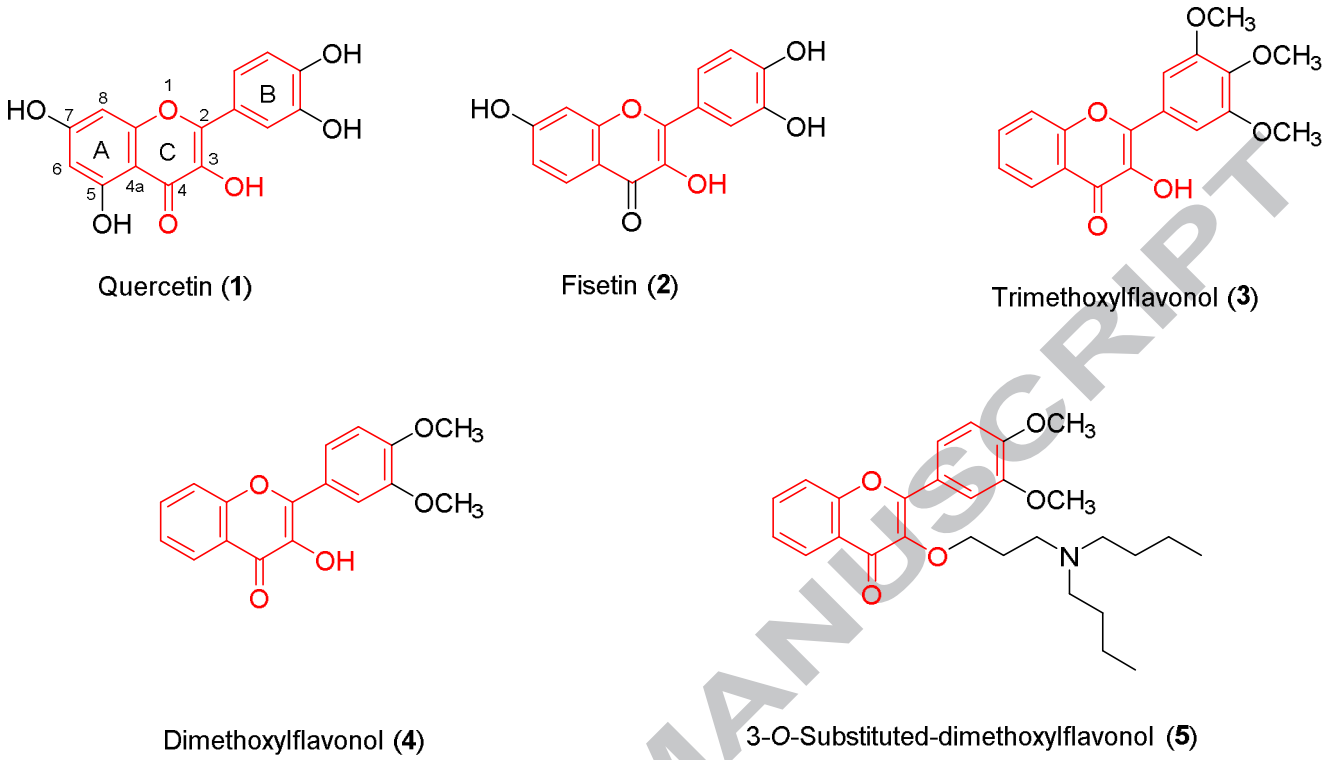


Figure 1. Flavonols as anti-prostate cancer agents

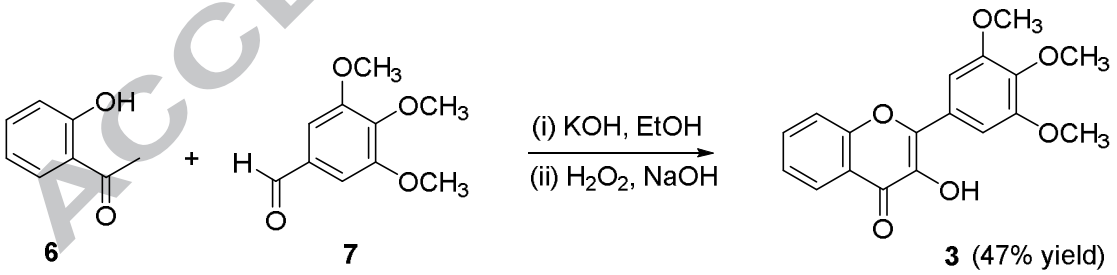
2. Results and Discussion:

2.1. Chemistry

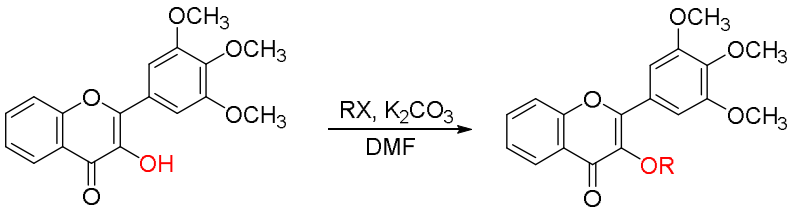
The parent compound, 3',4',5'-trimethoxyflavonol (**3**), has been synthesized through a well-known two-step procedure for the synthesis of flavonols,[12,13] including formation of chalcone via a Claisen-Schmidt condensation of 2'-hydroxylacetophenone (**6**) with 3,4,5-trimethoxylbenzaldehyde (**7**)[14] and cyclization of chalcone through Algar-Flynn-Oyamada (AFO) reaction.[15] This two-step procedure has been widely applied to the synthesis of flavonols, but with tedious workup and purification processes, as well as moderate overall yields. To overcome these limitations, Ozturk and co-workers have developed a two-step one-pot procedure that has been successfully applied to the efficient syntheses of

several other flavonols in 35-79% yields.[16] We envisioned the yield could be further improved by lowering reaction temperature from refluxing to room temperature (or below) but prolonging reaction time. Consequently, we synthesized 3',4',5'-trimethoxyflavonol (**3**), from 2'-hydroxylacetophenone (**6**) and 3,4,5-tryimethoxylbenzaldehyde (**7**), in 47% overall yield (Scheme 1) through a four-day one-pot procedure with facile work-up at room temperature as described in the experimental section.

As shown in Scheme 2, ten 3-*O*-alkyl-3',4',5'-trimethoxyflavonols (**8**-**17**) have been prepared in 54-99 % yields by *O*-alkylation of 3',4',5'-trimethoxyflavonol (**3**) with the appropriate alkyl halide using potassium carbonate as the base and DMF as the aprotic solvent. Twelve new 3-*O*-aminoalkyl-3',4',5'-trimethoxyflavonols (**19**-**30**) have been synthesized from 3',4',5'-trimethoxyflavonol (**3**) in 32-88% yield via a two-step transformation as illustrated in Scheme 3. Potassium carbonate was used as the base and DMF was used as the polar aprotic solvent in both *O*-alkylation and *N*-alkylation reactions. Three crude 3-*O*-bromoalkyl-3',4',5'-trimethoxyflavonols (**18**) were directly used, without further purification, for the follow-up *N*-alkylation reaction. The pure 3-*O*-dialkylaminoalkyl-3',4',5'-trimethoxyflavonols (**19**-**30**) from the *N*-alkylation reaction were obtained by preparative thin layer chromatography (PTLC) over silica gel. It is worth noting that the mixture of dichloromethane:diethylamine (100:3, v/v) is the optimal solvent to retrieve the desired nitrogen-containing products from the preparative thin layer silica gel.



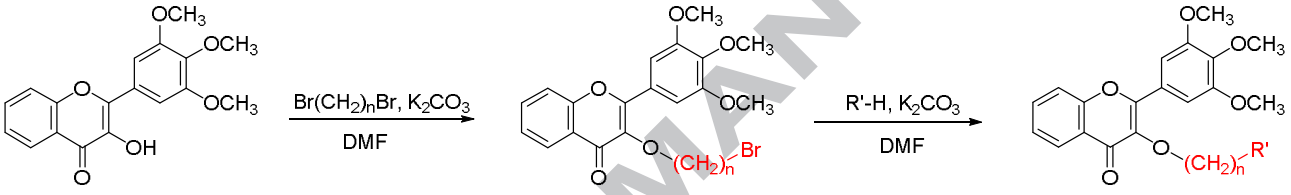
Scheme 1. Synthesis of 3',4',5'-trimethoxyflavonol (**3**)



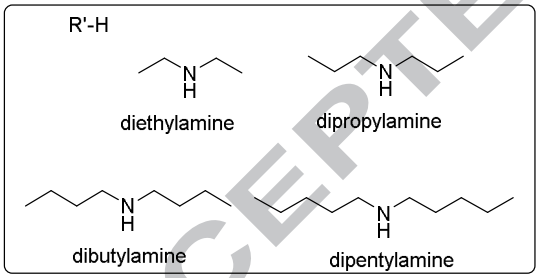
3 3-*O*-alkyl-3',4',5'-trimethoxyflavonols

compound	R	yield
8	methyl	99%
9	ethyl	81%
10	propyl	98%
11	butyl	92%
12	pentyl	89%
13	hexyl	89%
14	heptyl	96%
15	isopropyl	54%
16	*sec*-butyl	84%
17	pentan-2-yl	78%

Scheme 2. Synthesis of 3-*O*-alkyl-3',4',5'-trimethoxyflavonols (**8**-**17**)



3 **18** (n=3,4,5) **19-30**



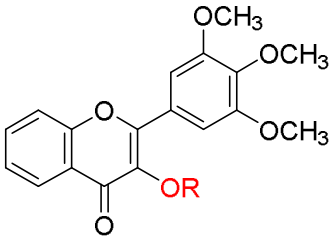
compound	n	R'	Overall Yield for two steps
19	3	*N*,*N*-diethylamino	84%
20	4	*N*,*N*-diethylamino	69%
21	5	*N*,*N*-diethylamino	88%
22	3	*N*,*N*-dipropylamino	69%
23	4	*N*,*N*-dipropylamino	68%
24	5	*N*,*N*-dipropylamino	72%
25	3	*N*,*N*-dibutylamino	69%
26	4	*N*,*N*-dibutylamino	60%
27	5	*N*,*N*-dibutylamino	56%
28	3	*N*,*N*-dipentylamino	32%
29	4	*N*,*N*-dipentylamino	43%
30	5	*N*,*N*-dipentylamino	47%

Scheme 3. Synthesis of 3-*O*-aminoalkyl-3',4',5'-trimethoxyflavonols (**19**-**30**)

2.2. Anti-proliferative effects toward three prostate cancer cell lines

To determine the *in vitro* ability of the synthesized 3-*O*-substituted-3',4',5'-trimethoxyflavonols in suppressing prostate cancer cell proliferation, the anti-proliferative potency of the twenty-two 3-*O*-substituted-3',4',5'-trimethoxyflavonols (**8**-**17**, **19**-**30**) towards both androgen-sensitive (LNCaP) and androgen-insensitive (PC-3 and DU145) human prostate cancer cell lines has been assessed by WST-1cell proliferation assay. Quercetin (**1**), fisetin (**2**), and 3',4',5'-trimethoxyflavonols (**3**) were used as positive controls. The IC_{50} values were calculated from the dose-response curves and summarized in Table 1. Our data show that 3',4',5'-trimethoxyflavonol (**3**), with IC_{50} values of 32.1 μM, 27.2 μM, and 14.7 μM against PC-3, DU145, and LNCaP cells, respectively, is significantly more potent than quercetin and fisetin. These results are consistent with those reported in the literature.[9] It is worth noting that the IC_{50} values in this study for 3',4',5'-trimethoxyflavonol (**3**) are higher than those reported in the literature, which can be probably attributed to the different days of drug exposure (we used three days; while the literature used six days). The PC-3 and LNCaP cancer cells are generally more sensitive than DU145 cells to the 3-*O*-substituted-3',4',5'-trimethoxyflavonols (**8**-**17**, **19**-**30**). Most of the synthetic 3-*O*-substituted-3',4',5'-trimethoxyflavonols are more potent in inhibiting PC-3 and LNCaP prostate cancer cell proliferation than the parent flavonol, 3',4',5'-trimethoxyflavonol (**3**) and the naturally occurring flavonols, quercetin (**1**) and fisetin (**2**), as determined by the IC_{50} values acquired in this study. Incorporation of an ethyl group to 3-OH of 3',4',5'-trimethoxyflavonol (**3**) results in the optimal derivative **9** with 36 times enhanced potency in suppressing PC-3 prostate cancer cell proliferation. 3-*O*-Alkyl-3',4',5'-trimethoxyflavonols with the α-branched alkyl groups (derivatives **16**-**17**) or with relatively lengthy alkyl groups (derivatives **13**-**14**) are similar or less potent than **3**. The introduction of a basic dialkylamino moiety to 3-OH of 3',4',5'-trimethoxyflavonol (**3**) through a 3- to 5-carbon linker can typically bestow 2-12 folds enhancement in anti-proliferative potency toward androgen-insensitive PC-3 prostate cancer cells (derivatives **19**-**30**, Table 1). These 3-*O*-dialkylaminoalkyl-3',4',5'-trimethoxyflavonols (**19**-**30**) have slightly improved potency in inhibiting LNCaP prostate cancer cell proliferation.

Table 1. Anti-proliferative activity of 3-*O*-substituted-3',4',5'-trimethoxyflavonols



Compound	R	IC_{50} (μM) [a]		
		PC-3[b]	DU-145[c]	LNCaP[d]
3	H	32.1 ± 13.1	27.2 ± 8.9	14.7 ± 2.1
8	methyl	19.1 ± 7.2	>50	24.7 ± 8.3
9	ethyl	0.9 ± 0.5	6.2 ± 0.7	5.4 ± 1.1
10	propyl	11.8 ± 3.5	2.8 ± 6.3	5.7 ± 1.9
11	butyl	12.8 ± 3.6	>50	8.9 ± 3.8
12	pentyl	22.2 ± 6.8	>50	15.4 ± 0.3
13	hexyl	29.2 ± 3.5	>50	19.6 ± 7.6
14	heptyl	>50	>50	>50
15	isopropyl	21.2 ± 5.9	> 50	8.8 ± 3.2
16	*sec*-butyl	38.6 ± 21.9	>50	9.3 ± 0.5
17	pentan-2-yl	49.3 ± 17.0	>50	15.1 ± 1.6
19	(*N*,*N*-diethylamino)propyl	13.6 ± 1.7	> 50	37.6 ± 8.8
20	(*N*,*N*-diethylamino)butyl	13.7 ± 4.2	13.3 ± 2.2	13.5 ± 2.2
21	(*N*,*N*-diethylamino)pentyl	6.6 ± 1.5	33.4 ± 3.2	10.0 ± 0.9
22	(*N*,*N*-dipropylamino)propyl	16.8 ± 5.1	>50	17.9 ± 4.9
23	(*N*,*N*-dipropylamino)butyl	11.6 ± 1.3	35.8 ± 3.5	16.8 ± 5.5

24	(*N*,*N*-dipropylamino)pentyl	2.6 ± 0.4	24.2 ± 3.9	7.6 ± 1.5
25	(*N*,*N*-dibutylamino)propyl	7.1 ± 2.2	15.7 ± 4.4	9.0 ± 2.1
26	(*N*,*N*-dibutylamino)butyl	5.3 ± 0.9	15.3 ± 6.3	7.8 ± 1.2
27	(*N*,*N*-dibutylamino)pentyl	6.8 ± 1.7	>50	7.8 ± 0.9
28	(*N*,*N*-dipentylamino)propyl	5.8 ± 1.2	12.3 ± 2.2	5.1 ± 0.4
29	(*N*,*N*-dipentylamino)butyl	7.9 ± 2.7	12.5 ± 2.1	9.7 ± 0.8
30	(*N*,*N*-dipentylamino)pentyl	9.6 ± 2.4	11.7 ± 2.6	7.9 ± 0.4
Quercetin (**1**)	-	> 100	> 100	45.46 ± 1.31
Fisetin (**2**)	-	> 50	> 50	34.1 ± 7.7

[a]IC_{50} is the drug concentration effective in inhibiting 50% of the cell viability measured by WST-1 cell proliferation assay after 3 days exposure. The data were presented as the mean ± standard deviation of the mean from three independent experiments (n = 3).

[b]Human androgen-insensitive prostate cancer cell line derived from bond metastasis of prostate tumor.

[c]Human androgen-insensitive prostate cancer cell line derived from brain metastasis of prostate tumor.

[d]Human androgen-sensitive prostate cancer cell line.

2.3. Anti-proliferative activity of 3 and its derivatives 9 and 28 towards PWR-1E non-neoplastic human prostate epithelial cell line

In this study, we observed the IC_{50} values for **3**, **9**, and **28** are >50, 8.05 ± 0.85, and 14.83 ± 1.83 μM towards PWR-1E non-neoplastic human prostate epithelial cell line. As shown in Figure 2, there are significantly differential responses of these three flavonols to PC-3 androgen-insensitive prostate cancer cells and PWR-1E benign human prostate epithelial cells. Specifically, all three flavonols (**3**, **9**, and **28**) are more effective in inhibiting PC-3 cancer cell proliferation than PWR-1E non-neoplastic human prostate epithelial cell proliferation. It is worth noting that modification at 3-OH of flavonol **3** also improves the antiproliferative potency towards PWR-1E non-neoplastic human prostate epithelial

cell model that is an immortalized cell line and was believed by Rhim and co-workers to represent an early stage in tumor progression.[17]

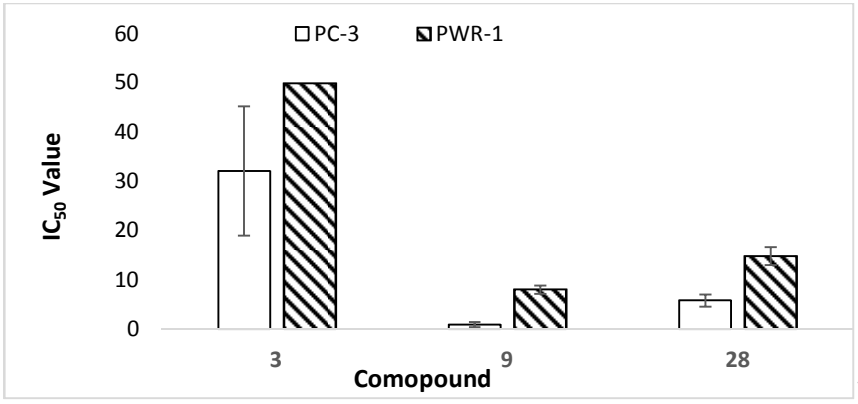


Figure 2: Comparison of IC_{50} values (μM) for **3**, **9**, and **28** in PC-3 cell model with those in the PWR-1E cell model. The data were presented as the mean ± standard deviation of the mean from three independent experiments (n = 3). Statistical significance was established at $P < 0.05$. Note that no error bar for the IC_{50} values for **3** on PC-3 model is expressed because they are greater than 50 μM.

2.4. Effects of 3, 9, and 28 on PC-3 cell apoptosis and cell cycle progression

Quercetin (**1**) and fisetin (**2**), as well as one synthetic 3-*O*-substituted-3,4-dimethyoxyflavonol (**5**), have shown cell cycle perturbation properties in the androgen-insensitive PC-3 human prostate cancer cell line by arresting cell cycle at G_2/M phase.[4,11,18] Trimethoxyflavonol (**3**) has been revealed to arrest cell cycle at the G_2/M phase in murine TRAMP C2 prostate cancer cells, but have no effect on human 22Rv1 cell cycle distribution.[9] No report was available so far on PC-3 cell cycle perturbation activity of trimethoxyflavonol (**3**). Consequently, the effect of parent compound **3**, and derivatives **9** and **28**, at 10 μM and 20 μM on the PC-3 cell cycle was evaluated using flow cytometric analysis with propidium iodide DNA staining. It is worth noting that compound **28** was chosen as the representative aminoalkyl derivative for further investigation on its PC-3 cell cycle regulation and cell apoptosis induction even though it (IC_{50} = 5.8 μM) is slightly less potent than compound **24** (IC_{50} = 2.6 μM) in the PC-3 cell

model. This is because compound **28** shows greater potential to be further developed as an anti-prostate cancer agent due to its slightly greater overall potency towards three human prostate cancer cell lines, as compared with compound **24**. As illustrated in Table 2, modification of 3-OH can change its regulatory effect on PC-3 cell cycle. Specifically, trimethoxyflavonol (**3**) induces PC-3 cell cycle arrest at the *S* phase at 16 h, but at the G_2/M phase at 24 h. 3-*O*-Ethyl-3',4',5'-trimethoxyflavonol (**9**) induces cell cycle arrest at the G_0/G_1 phase at 16 h, and at both G_0/G_1 & S phase at 24 h by increasing PC-3 cell population in the G_0/G_1 and S phase, while fewer cells were observed in the G_2/M phase (Table 2). Interestingly, derivative **28** with a dipentylaminopropyl group at 3-OH of 3',4',5'-trimethoxyflavonol consistently induces PC-3 cell cycle arrest at the G_0/G_1 phase at 10 μM and 20 μM, and at 16 h and 24 h.

The naturally occurring flavonols quercetin (**1**) and fisetin (**2**), and the synthetic flavonol derivative **5** have been demonstrated to promote androgen-insensitive PC-3 human prostate cancer cell apoptosis.[11,19-21] Additionally, trimethoxyflavonol (**3**) has been reported to induce mouse prostate carcinoma TRAMP C2 cell apoptosis *in vitro* as detected by annexin V staining, but not in human prostate carcinoma 22Rv1 cells.[9] No apoptosis activation of trimethoxyflavonol (**3**) in human androgen-insensitive PC-3 prostate cancer cell line has been reported. The F2N12S and SYTOX AADVanced double staining assay in a flow cytometer was used in this study for the discrimination between early apoptotic PC-3 cells and late apoptotic/necrotic PC-3 cells when treated with trimethoxyflavonol (**3**), and derivatives **9** and **28** at the concentrations specified in Figures 3 for 16 h. The data, as listed in Figures 3-4, indicate that trimethoxyflavonol (**3**) and derivative **28** can significantly activate apoptotic cell death in the androgen-insensitive PC-3 prostate cancer cell line in a dose-dependent manner after a 16-hour treatment. The data also suggest that derivative **28** with an amino group at 3-OH has much greater capability in the induction of PC-3 cell apoptosis. Specifically, as shown in Figures 3-4, exposure of PC-3 cells to 5 μM and 10 μM of trimethoxyflavonol (**3**) with its free 3-OH group resulted in 11 ± 1% and 39 ± 13% early apoptotic cells, respectively; while treatment

of PC-3 cells with 5 μM and 10 μM of derivative **28** led to 51 ± 20% and 93 ± 1% early apoptotic cells, respectively. In contrast, derivative **9** with an ethyl group at 3-OH in 3',4',5'-trimethoxyflavonol did not promote significant levels of PC-3 cell apoptotic death even at a high dose of 100 μM after a 16-hour treatment as compared with control cells. These findings imply that modification on 3-OH of trimethoxyflavonol can change not only the *in vitro* anti-proliferative potency but also its associated mechanism of action.

Table 2. Cell cycle analysis of PC-3 prostate cancer cells. PC-3 cancer cells were untreated or treated with **3**, **9**, and **28**, respectively, at 10 μM or 20 μM. Cells were harvested after 16 h, fixed, stained, and analyzed for DNA content.

Cell Cycle	G_0/G_1		S		G_2/M	
Treatment Time	16 hours	24 hours	16 hours	24 hours	16 hours	24 hours
Control (DMSO)	34.1	37.5	**9.9**	8.0	32.3	**27.8**
3 (10 μM)	31.2	34.6	**12.3**	6.0	33.0	**33.3**
3 (20 μM)	33.3	33.8	**11.7**	6.0	26.6	**31.2**
9 (10 μM)	**55.7**	**40.8**	7.7	**18.7**	22.2	19.3
9 (20 μM)	**59.0**	**48.6**	4.6	**11.8**	24.5	17.9
28 (10 μM)	**51.3**	**52.6**	4.5	3.7	28.6	25.4
28 (20 μM)	**40.8**	**59.8**	6.5	1.9	34.8	26.4

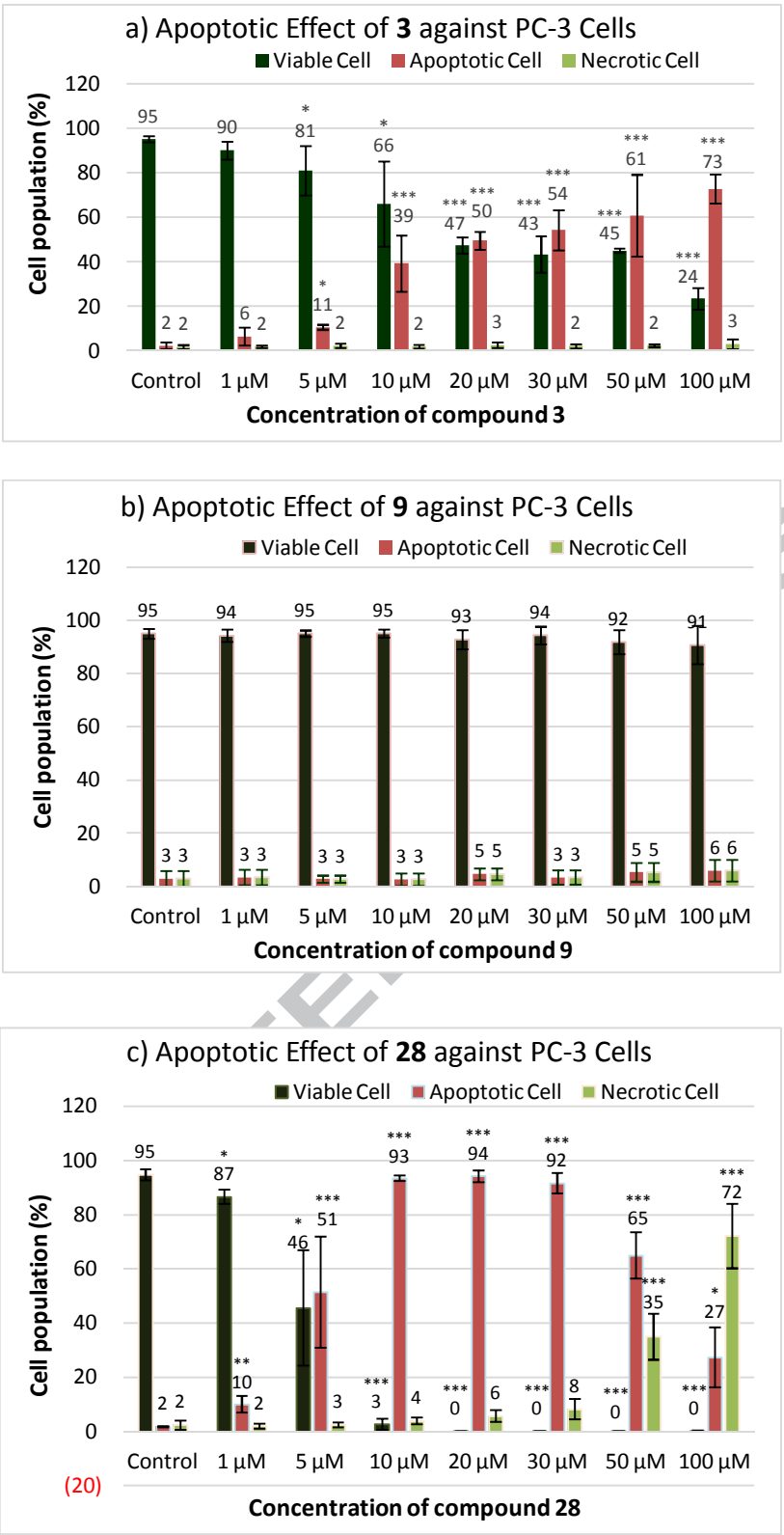
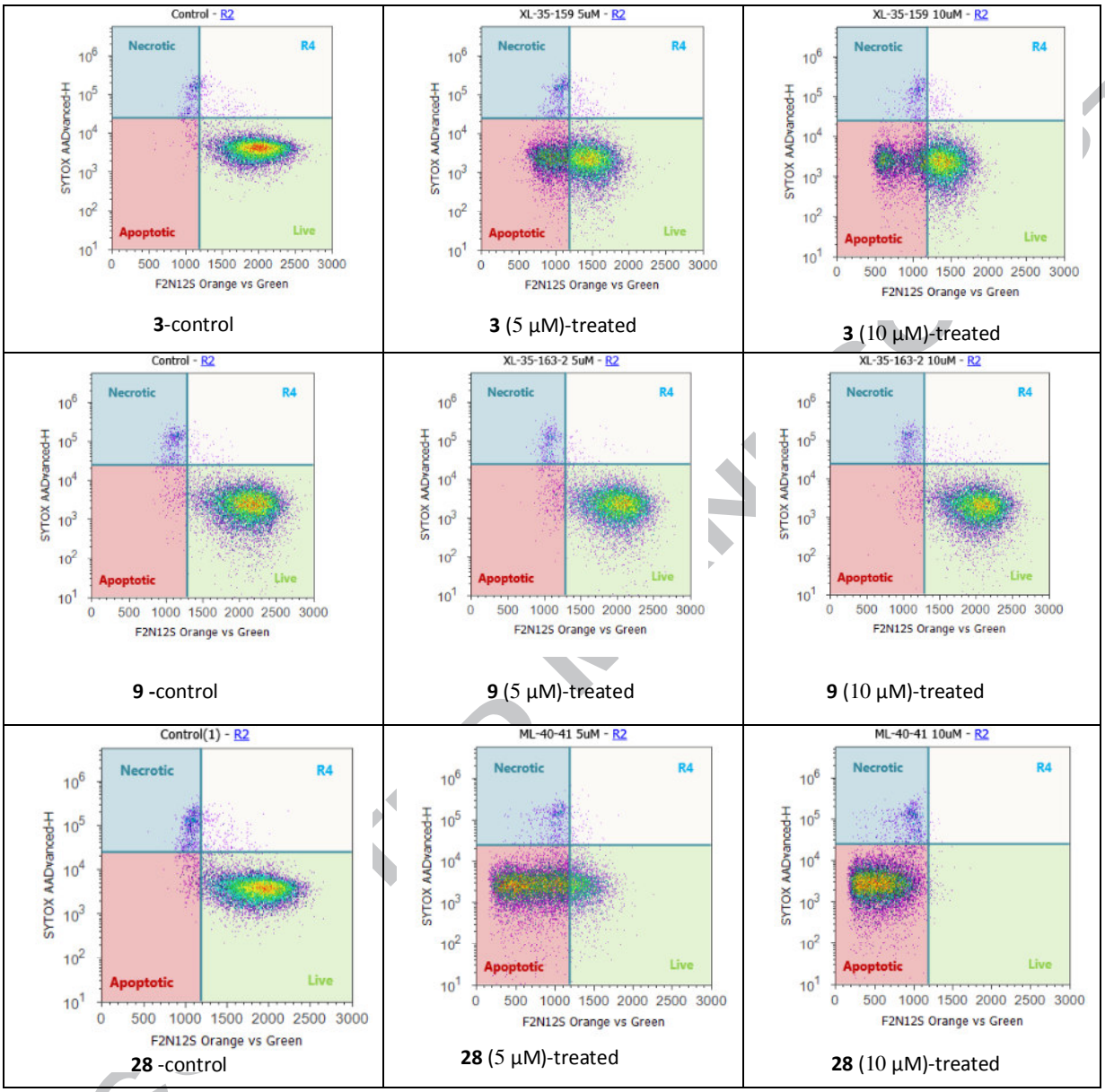


Figure 3. Evolution of viable, apoptotic, and necrotic PC-3 cell populations in response to increasing dosages of flavonols a) **3**, b) **9**; and c) **28**. Shown are the mean ± SD from three independent experiments. Significance is expressed as (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ versus control.



3-control | **3** (5 µM)-treated | **3** (10 µM)-treated

9 -control | **9** (5 µM)-treated | **9** (10 µM)-treated

28 -control | **28** (5 µM)-treated | **28** (10 µM)-treated

Figure 4. Apoptosis in PC-3 cells treated with **3**, **9**, and **28** at 5 µM and 10 µM (by F2N12S and CYTOX AADvanced double staining).

3. Conclusion

Comparing the IC_{50} value, as well as cell cycle regulatory and cell apoptosis inductory ability, of parent compound with those of flavonol derivatives, we can conclude that i) most 3-*O*-alkyl-3',4',5'-trimethoxyflavonols and 3-*O*-dialkylaminoalkyl-3',4',5'-trimethoxyflavonols are more potent than **3** in suppressing cell proliferation of PC-3 and LNCaP prostate cancer cell lines, ii) incorporation of an ethyl group to 3-OH leads to the optimal derivative **9** with up to 36-fold enhanced potency as compared with **3**, but reverse PC-3 cell apoptotic response; iii) introduction of a dipentylaminopropyl group at 3-OH of **3** has appreciably improved antiproliferative potency and capability in inducing PC-3 cell apoptosis, as compared with parent compound **3**, and iv) 3-*O*-substituted-3',4',5'-trimethoxyflavonols have higher potency towards PC-3 and LNCaP cell lines than DU145 cell line. These findings imply that structural modification on 3-OH of trimethoxyflavonol can further enhance its *in vitro* anti-proliferative potency in three human prostate cancer cell lines and its ability to activate PC-3 cell apoptosis.

4. Experiments

4.1. General synthetic procedures: NMR spectra were obtained on a Bruker Fourier 300 spectrometer in $CDCl_3$. The chemical shifts are given in ppm referenced to the solvent peak, and coupling constants are reported in Hz. Anhydrous THF and dichloromethane were purified by PureSolv MD 7 Solvent Purification System from Innovative Technologies (MB-SPS-800). All other reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using silica gel

(32-63 μm). Preparative thin-layer chromatography (PTLC) separations were carried out on thin layer chromatography plates preloaded with silica gel GF254.

4.2. Synthesis of 3',4',5'-trimethoxyflavonol (3). The mixture of 2'-hydroxyacetophenone (0.15 g, 1.08 mmol), 3',4',5'-trimethoxybenzaldehyde (0.20 g, 1.03 mmol), and potassium hydroxide (0.19 g, 3.31 mmol, 3 equiv.) in ethanol (3 mL) was stirred at 0 ^{o}C (ice bath) for 45 min. The reaction was then allowed to proceed at room temperature for 48 hours. The red solution (3',4',5'-trimethoxychalcone) was obtained, to which aqueous solution of sodium hydroxide (1 M, 0.8 mL, 0.8 mmol) and hydrogen peroxide (30%, 0.5 mL, 4.9 mmol) were added at 0 °C (ice bath). After standing in cold room (4 °C) for 48 h, the resulting mixture was stirred at room temperature until the starting material was fully consumed, as detected by TLC. The reaction mixture was acidified with HCl solution (3M) to pH 2 to yield a precipitate, which was filtered off and rinsed with 95% ethanol to give 3',4',5'-trimethoxyflavonol *(***3**) as a yellow solid in 47% yield. M.p. 166-168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (dd, J = 8.1, 1.2 Hz, 1H), 7.69 (ddd, J = 8.6, 6.9, 1.5 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.52 (s, 2H), 7.40 (t, J = 7.5 Hz, 1H), 7.14 (br.s., 1H), 3.95 (s, 6H), 3.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 155.3, 153.3, 144.8, 140.0, 138.3, 133.7, 126.3, 125.5, 124.6, 120.7, 118.3, 105.5, 61.1, 56.4; HRMS (ESI, *m/z*) calcd. for $C_{18}H_{17}O_6$ $[M+H]^+$ 329.1025, found 329.1016; IR (film) 3267, 2929, 2828, 1616, 1606, 1582, 1562, 1505 cm^{-1}.

4.3. The general procedure for the synthesis of 3-*O*-alkyl-3',4',5'-trimethoxyflavonols (8-17). The solution of 3',4',5'-trimethoxyflavonol (**3**, 50 mg, 0.15 mmol) and potassium carbonate (60 mg, 0.43 mmol) in DMF (0.5 mL, 0.3 M) was stirred for 10 min at room temperature. To this solution was added an appropriate alkyl iodide or alkyl bromide (0.75 mmol) through syringe, the subsequent reaction mixture was stirred at room temperature over 12 h until the starting material was completely consumed as detected by TLC. The reaction mixture was poured into ice water (3 mL), and the

subsequent mixture was extracted with ethyl acetate three times. The combined extracts were sequentially rinsed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a crude mass, which was subjected to PTLC purification using hexane/ethyl acetate (100/3, v/v) as eluent to furnish the respective 3-*O*-alkyl-3',4',5'-trimethoxyflavonol (**8**-**17**).

4.3.1. 3-*O*-Methyl-3',4',5'-trimethoxyflavonol (8*).* Yellow solid, 99% yield, mp. 88-90 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 8.1, 1.5 Hz, 1H), 7.68 (ddd, J = 8.6, 7.2, 1.8 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.42 – 7.37 (overlapped, 3H), 3.94 (s, 9H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 155.4, 155.2, 153.2, 141.4, 140.5, 133.6, 126.0, 125.92, 124.8, 124.2, 118.05, 106.2, 61.1, 60.2, 56.4; HRMS (ESI, *m/z*) calcd. for $C_{19}H_{19}O_6$ $[M+H]^+$ 343.1181, found 343.1177; IR (film) 2941, 2836,1644,1601, 1584, 1562, 1504 cm^{-1}.

4.3.2. 3-*O*-Ethyl-3',4',5'-trimethoxyflavonol (9). Yellow solid, 81% yield, mp. 56-58 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 7.8, 1.5 Hz, 1H), 7.68 (ddd, J = 8.6, 7.2, 1.8 Hz, 1H), 7.54 (dd, J = 8.0, 0.5 Hz, 1H), 7.46 (s, 2H), 7.40 (ddd, J = 7.8, 7.2, 0.6 Hz, 1H), 4.13 (q, J = 7.0 Hz, 2H), 3.94 (s, 9H), 1.36 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.4, 155.2, 153.2, 140.4, 140.2, 133.4, 126.2, 125.8, 124.7, 124.1, 118.0, 106.2, 68.4, 61.0, 56.3, 15.8.; HRMS (ESI, *m/z*) calcd. for $C_{20}H_{21}O_6$ $[M+H]^+$ 357.1338, found 357.1331; IR (film) 2982, 2937, 2836, 1627,1615, 1598, 1557, 1505 cm^{-1}.

4.3.3. 3-*O*-Propyl-3',4',5'-trimethoxyflavonol (10). Yellow solid, 98% yield, mp. 84-86 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 8.0, 1.4 Hz, 1H), 7.67 (ddd, J = 8.6, 6.9, 1.4 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.42 (s, 2H), 7.39 (t, J = 7.2 Hz, 1H), 4.00 (t, J = 6.9 Hz, 2H), 3.94 (s, 9H), 1.76 (sextet, J = 7.2 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.2, 153.1, 140.7, 140.3, 133.5, 126.3, 125.9, 124.8, 124.2, 118.0, 106.4, 74.6, 61.1, 56.4, 23.7, 10.5; HRMS (ESI,

m/z) calcd. for $C_{21}H_{23}O_6$ $[M+H]^+$ 371.1494, found 371.1487; IR (film) 2927, 2872, 1627, 1615, 1601, 1583, 1558, 1503 cm^{-1}.

4.3.4. 3-*O*-Butyl-3',4',5'-trimethoxyflavonol (11*)*. Yellow solid, 92% yield, m.p. 89-91 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (dd, J = 8.0, 1.5 Hz, 1H), 7.65 (ddd, J = 8.6, 6.9, 1.5 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.40 (s, 2H), 7.40-7.34 (overlapped, 1H), 4.02 (t, J = 6.7 Hz, 2H), 3.92 (s, 9H), 1.70 (quin, J = 6.9 Hz, 2H), 1.41 (sextet, J = 7.5 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.4, 155.2, 153.0, 140.6, 140.2, 133.4, 126.2, 125.8, 124.7, 124.2, 118.0, 106.3, 72.7, 61.1, 56.3, 32.4, 19.2, 13.9; HRMS (ESI, *m/z*) calcd. for $C_{22}H_{25}O_6$ $[M+H]^+$ 385.1651, found 385.1645; IR (film) 2927, 2872, 1627, 1615, 1601, 1583, 1558, 1503 cm^{-1}.

4.3.5. 3-*O*-Pentyl-3',4',5'-trimethoxyflavonol (12). Yellow solid, 89% yield, m.p. 71-73 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (dd, J = 8.0, 1.5 Hz, 1H), 7.66 (ddd, J = 8.6, 6.9, 1.5 Hz, 1H), 7.52 (dd, J = 8.4, 0.5 Hz, 1H), 7.31 (s, 2H), 7.38 (ddd, J = 8.1, 7.2, 1.2 Hz, 1H), 4.02 (t, J = 6.7 Hz, 2H), 3.930 (s, 6H), 3.926 (s, 3H), 1.72 (quin, J = 7.2 Hz, 2H), 1.40 – 1.21 (m, 4H), 0.84 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.2, 153.0, 140.6, 140.2, 133.4, 126.3, 125.9, 124.7, 124.2, 118.0, 106.3, 73.0, 61.1, 56.3, 30.1, 28.2, 22.5, 14.1; HRMS (ESI, *m/z*) calcd. for $C_{23}H_{27}O_6$ $[M+H]^+$ 399.1807, found 399.1797; IR (film) 3000, 2928, 2872, 2835, 1630, 1611, 1600, 1583, 1562, 1504cm^{-1}.

4.3.6. 3-*O*-Hexyl-3',4',5'-trimethoxyflavonol (13). Yellow solid, 89% yield, m.p. 54-56 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.6 Hz, 1H), 7.66 (ddd, J = 8.7, 7.2, 1.2 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.40 (s, 2H), 7.39 (ddd, J = 8.0, 7.2, 0.9 Hz, 1H), 4.03 (t, J = 6.7 Hz, 2H), 3.934 (s, 6H), 3.932 (s, 3H), 1.71 (quin, J = 7.8 Hz, 2H), 1.40 – 1.30 (m, 2H), 1.30 – 1.19 (m, 4H), 0.84 (t, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.2, 153.1, 140.7, 140.3, 133.5, 126.3, 125.9, 124.8, 124.2, 118.0, 106.4, 73.1, 61.1, 56.4, 31.7, 30.4, 25.8, 22.7, 14.1; HRMS (ESI, *m/z*) calcd. for

$C_{24}H_{29}O_6$ $[M+H]^+$ 413.1964, found 413.1962; IR (film) 2990, 2917, 2837, 1630, 1613, 1599, 1557, 1504 cm^{-1}.

4.3.7. 3-*O*-Heptyl-3',4',5'-trimethoxyflavonol (14). Syrup, 96% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 8.0, 1.1 Hz, 1H), 7.68 (t, J = 6.9 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.41 (s, 2H), 7.39 (t, J = 7.2 Hz, 1H), 4.04 (t, J = 6.7 Hz, 2H), 3.94 (s, 9H), 1.73 (quin, J = 6.6 Hz, 2H), 1.42 – 1.13 (overlapped, 8H), 0.85 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.2, 153.1, 140.7, 140.3, 133.4, 126.3, 125.9, 124.7, 124.2, 118.0, 106.4, 73.1, 61.1, 56.4, 31.8, 30.4, 29.2, 26.0, 22.6, 14.1; HRMS (ESI, *m/z*) calcd.for $C_{25}H_{31}O_6$ $[M+H]^+$ 427.2121, found 427.2113; IR (film) 2927, 2855, 1637, 1614, 1602 cm^{-1}.

4.3.8. 3-*O*-Isopropyl-3',4',5'-trimethoxyflavonol (15). Syrup, 54% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 8.0, 1.4 Hz, 1H), 7.67 (ddd, J = 8.7, 7.2, 1.8 Hz, 1H), 7.54 (dd, J = 8.5, 0.6 Hz, 1H), 7.47 (s, 2H), 7.40 (ddd, J = 8.0, 6.9, 0.9 Hz, 1H), 4.76 (septet, J = 6.3 Hz, 1H), 3.944 (s, 6H), 3.940 (s, 3H), 1.22 (d, J = 6.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 155.9, 155.3, 152.9, 140.2, 139.2, 133.4, 126.7, 126.0, 124.8, 124.1, 118.0, 106.7, 74.7, 61.2, 56.4, 22.7; HRMS (ESI, *m/z*) calcd.for $C_{21}H_{23}O_6$ $[M+H]^+$ 371.1494, found 371.1488; IR (film) 2971, 2934, 2835, 1634, 1614, 1599, 1582, 1558, 1502 cm^{-1}.

4.3.9. 3-*O*-*Sec*-Butyl-3',4',5'-trimethoxyflavonol (16). Syrup, 84% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.6 Hz, 1H), 7.67 (ddd, J = 8.6, 7.1, 1.6 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.45 (s, 2H), 7.39 (ddd, J = 7.8, 7.1, 0.9 Hz, 1H), 4.60 (sextet, J = 6.6 Hz, 1H), 3.93 (s, 9H), 1.79-1.67 (m, 1H), 1.57 – 1.44 (m, 1H), 1.12 (d, J = 6.2 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 155.8, 155.2, 152.9, 140.2, 139.2, 133.4, 126.7, 125.9, 124.7, 124.1, 118.0, 106.8, 79.3, 61.1, 56.4, 29.7, 19.4, 9.9; HRMS (ESI, *m/z*) calcd.for $C_{22}H_{25}O_6$ $[M+H]^+$ 385.1651, found 385.1644; IR (film) 2966, 2934, 2836, 1635, 1613, 1599, 1582, 1559, 1502 cm^{-1}.

4.3.10. 3-*O*-(Pentan-2-yl)-3',4',5'-trimethoxyflavonol (17). Syrup, 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (dd, J = 8.0, 1.6 Hz, 1H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.45 (s, 2H), 7.38 (ddd, J = 8.1, 6.9, 0.9 Hz, 1H), 4.68 (sextet, J = 6.1 Hz, 1H), 3.93 (s, 9H), 1.75 – 1.60 (m, 1H), 1.49-1.35 (m, 3H), 1.10 (d, J = 6.2 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 155.8, 155.2, 152.8, 140.2, 139.1, 133.3, 126.7, 125.9, 124.7, 124.1, 118.0, 106.7, 77.8, 61.1, 56.4, 39.2, 20.0, 18.8, 14.2.; HRMS (ESI, *m/z*) calcd.for $C_{23}H_{27}O_6$ $[M+H]^+$ 399.1807, found 399.1802; IR (film) 2932, 2871, 1635, 1614, 1599, 1582, 1559, 1502 cm^{-1}.

4.4. The general procedure for the synthesis of 3-*O*-dialkylaminoalkyl-3',4',5'-trimethoxyflavonols (19-30): The solution of 3',4',5'-trimethoxyflavonol (50 mg, 0.15 mmol) and potassium carbonate (60 mg, 0.43 mmol, 3 equiv.) in DMF (0.5 mL, 0.3 M) was stirred at room temperature for 10 min before an appropriate alkyl dibromide (0.51 mmol, 3 equiv.) was added through a syringe. The reaction was allowed to proceed at room temperature over 12 h until the starting material was fully consumed as indicated by TLC. The reaction mixture was poured into ice water (3 mL), and the subsequent mixture was extracted with ethyl acetate three times. The combined organic extracts were sequentially rinsed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to generate a crude product. To the solution of this crude product in DMF (0.1 M) was added potassium carbonate (3 equiv.), and the mixture was stirred for 10 min. The appropriate amine (3 equiv.) was added to the reaction mixture through a syringe, and the subsequent reaction mixture was stirred at room temperature over 12 h until no starting material was detected by TLC. The mixture was poured into ice water (3 mL), and the mixture was extracted with ethyl acetate three times. The combined extracts were sequentially rinsed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a crude product, which was subjected to PTLC purification using dichloromethane/methanol (100/3, v/v) as

eluent. The respective 3-*O*-dialkylaminoalkyl-3',4',5'-trimethoxyflavonol was retrieved from PTLC silica gel by washing with dichloromethane/diethylamine (100/3, v/v).

4.4.1. 3-*O*-(3-(Diethylamino)propyl)-3',4',5'-trimethoxyflavonol (19). Syrup, 84% overall yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 7.3 Hz, 1H), 7.67 (t, J = 7.1 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.40-7.38 (overlapped, 1H), 7.38 (s, 2H), 4.09 (t, J = 6.3 Hz, 2H), 3.93 (s, 9H), 2.58-2.46 (overlapped, 6H), 1.96 – 1.81 (m, 2H), 0.97 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 155.7, 155.2, 153.1, 140.5, 140.4, 133.5, 126.2, 125.9, 124.8, 124.2, 118.0, 106.5, 71.4, 61.1, 56.5, 49.9, 46.9, 27.8, 11.2; HRMS (ESI, *m/z*) calcd. for $C_{25}H_{32}NO_6$ $[M+H]^+$ 442.2230, found 442.2224; IR (film) 2922, 2848, 1627, 1614, 1601, 1582, 1559, 1504 cm^{-1}.

4.4.2. 3-*O*-(3-(Diethylamino)butyl)-3',4',5'-trimethoxyflavonol (20). Syrup, 69% overall yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.3 Hz, 1H), 7.67 (ddd, J = 9.9, 8.1, 1.5 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.42-7.37 (overlapped, 1H), 7.41 (s, 2H), 4.06 (t, J = 6.6 Hz, 2H), 3.94 (s, 9H), 2.55-2.44 (overlapped, 6H), 1.74 (quin, J = 7.1 Hz, 2H), 1.56 (quin, J = 7.4 Hz, 2H), 1.00 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.3, 153.1, 140.6, 140.4, 133.5, 126.3, 126.0, 124.8, 124.3, 118.1, 106.4, 72.7, 61.1, 56.4, 52.5, 46.9, 28.4, 23.2, 11.5. HRMS (ESI, *m/z*) calcd. For $C_{26}H_{34}NO_6$ $[M+H]^+$ 456.2386, found 456.2383; IR (film) 2916, 2848, 2793, 1625, 1614, 1600, 1581, 1557, 1504cm^{-1}.

4.4.3. 3-*O*-(3-(Diethylamino)pentyl)-3',4',5'-trimethoxyflavonol (21). Syrup, 88% overall yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 8.0 Hz, 1H), 7.66 (dd, J = 8.3, 7.1 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.39 (s, 2H), 7.39-7.35 (overlapped, 1H), 4.02 (t, J = 6.1 Hz, 2H), 3.92 (s, 9H), 2.51 (q, J = 6.9 Hz, 4H), 2.39 (t, J = 6.1 Hz, 2H), 1.82 – 1.67 (m, 2H), 1.51 – 1.31 (m, 4H), 0.99 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 155.5, 155.2, 153.1, 140.6, 140.4, 133.4, 126.2, 125.9, 124.7, 124.2, 118.0, 106.5, 72.8, 61.1, 56.4, 52.7, 46.9, 30.3, 26.5, 24.1, 11.4; HRMS (ESI, *m/z*)

calcd. for $C_{27}H_{36}NO_6$ $[M+H]^+$470.2542, found 470.2538; IR (film) 2935, 1636, 1614, 1601, 1582, 1561, 1503 cm^{-1}.

4.4.4. 3-*O*-(3-(Dipropylamino)propyl)-3',4',5'-trimethoxyflavonol (22). Syrup, 69% overall yield for two steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, J = 7.9 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.36 (s, 2H), 7.38-7.33 (overlapped, 1H), 4.06 (t, J = 6.5 Hz, 2H), 3.91 (s, 9H), 2.50 (t, J = 6.8 Hz, 2H), 2.29 (t, J = 7.2 Hz, 4H), 1.92 – 1.78 (m, 2H), 1.43 – 1.27 (m, 4H), 0.78 (t, J = 7.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 155.5, 155.2, 153.0, 140.4, 140.3, 133.4, 126.2, 125.8, 124.7, 124.2, 118.0, 106.3, 71.5, 61.0, 56.3, 56.1, 51.0, 28.1, 20.0, 11.9; HRMS (ESI, *m/z*) calcd.for $C_{27}H_{36}NO_6$ $[M+H]^+$ 470.2542, found 470.2535; IR (film) 2945, 2866, 2803, 1634, 1615, 1603, 1583, 1563, 1504 cm^{-1}.

4.4.5. 3-*O*-(3-(Dipropylamino)butyl)-3',4',5'-trimethoxyflavonol (23). Syrup, 68% overall yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (dd, J = 8.0, 1.2 Hz, 1H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.40 (s, 2H), 7.38 (t, J = 7.2 Hz, 1H), 4.04 (t, J = 6.8 Hz, 2H), 3.92 (s, 9H), 2.39 (t, J = 7.3 Hz, 2H), 2.30 (t, J = 7.5 Hz, 4H), 1.73 (quin, J = 7.8 Hz, 2H), 1.58 – 1.45 (m, 2H), 1.45 – 1.31 (m, 4H), 0.81 (t, J = 7.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.4, 155.2, 153.0, 140.6, 140.3, 133.5, 126.2, 125.9, 124.8, 124.2, 118.0, 106.3, 72.9, 61.1, 56.4, 56.1, 53.8, 28.4, 23.4, 20.2, 12.0; HRMS (ESI, *m/z*) calcd. for $C_{28}H_{38}NO_6$ $[M+H]^+$ 484.2699, found 484.2685; IR (film) 2998, 2925, 2872, 2795, 1626, 1601, 1583, 1561, 1503 cm^{-1}.

4.4.6. 3-*O*-(3-(Dipropylamino)pentyl)-3',4',5'-trimethoxyflavonol (24). Syrup, 72% overall yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 7.7 Hz, 1H), 7.65 (t, J = 7.3 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.40 (s, 2H), 7.40-7.35 (overlapped, 1H), 4.02 (t, J = 6.3 Hz, 2H), 3.92 (s, 9H), 2.38-2.26 (overlapped, 6H), 1.81 – 1.66 (m, 2H), 1.46-1.31 (overlapped, 8H), 0.83 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 155.4, 155.2, 153.0, 140.6, 140.3, 133.4, 126.2, 125.9, 124.7, 124.2, 118.0,

106.3, 72.9, 61.1, 56.3, 56.2, 54.1, 30.3, 26.8, 24.0, 20.1, 12.0; HRMS (ESI, *m/z*) calcd. for $C_{29}H_{40}NO_6$ $[M+H]^+$ 498.2856, found 498.2842; IR (film) 2933, 2869, 1638, 1614, 1602, 1582, 1562, 1503 cm^{-1}.

4.4.7. 3-*O*-(3-(Dibutylamino)propyl)-3',4',5'-trimethoxyflavonol (25). Syrup, 69% overall yield for two steps; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.3 Hz, 1H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.40 (s, 2H), 7.40 (t, J = 7.2 Hz, 1H), 4.08 (t, J = 6.5 Hz, 2H), 3.94 (s, 9H), 2.62-2.52 (m, 2H), 2.39 (t, J = 6.9 Hz, 4H), 1.90 (quin, J = 7.5 Hz, 2H), 1.43 – 1.30 (m, 4H), 1.24 (quin, J = 7.1 Hz, 4H), 0.86 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.6, 155.3, 153.1, 140.5, 140.4, 133.5, 126.2, 125.9, 124.8, 124.3, 118.1, 106.4, 71.6, 61.1, 56.4, 53.9, 51.1, 28.9, 28.1, 20.8, 14.1; HRMS (ESI, *m/z*) calcd. for $C_{29}H_{40}NO_6$ $[M+H]^+$ 498.2855, found 498.2840; IR (film) 2930, 2870, 1637, 1614, 1602, 1582, 1562, 1503 cm^{-1}.

4.4.8. 3-*O*-(3-(Dibutylamino)butyl)-3',4',5'-trimethoxyflavonol (26). Syrup, 60% overall yield for two steps; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.4 Hz, 1H), 7.67 (ddd, J = 8.6, 6.9, 1.5 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.42 (s, 2H), 7.39 (t, J = 7.2 Hz, 1H), 4.05 (t, J = 6.8 Hz, 2H), 3.94 (s, 9H), 2.41 (t, J = 6.9 Hz, 2H), 2.36 (t, J = 7.5 Hz, 4H), 1.75 (quin, J = 7.5 Hz, 2H), 1.60 – 1.46 (m, 2H), 1.45 – 1.30 (m, 4H), 1.23 (sextet, J = 7.0 Hz, 4H), 0.87 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 155.4, 155.2, 153.0, 140.6, 140.3, 133.4, 126.2, 125.9, 124.7, 124.2, 118.0, 106.3, 72.9, 61.0, 56.3, 53.8, 53.7, 29.2, 28.4, 23.4, 20.8, 14.1; HRMS (ESI, *m/z*) calcd. for $C_{30}H_{42}NO_6$ $[M+H]^+$ 512.3012, found 512.3007; IR (film) 2930, 2870, 1637, 1614, 1602, 1582, 1562, 1503 cm^{-1}.

4.4.9. 3-*O*-(3-(Dibutylamino)pentyl)-3',4',5'-trimethoxyflavonol (27). Syrup, 56% overall yield for two steps; ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 8.0 Hz, 1H), 7.66 (dd, J = 8.4, 7.1 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.40 (s, 2H), 7.38 (t, J = 7.8 Hz, 1H), 4.03 (t, J = 6.7 Hz, 2H), 3.93 (s, 9H), 2.37 (t, J = 7.2 Hz, 6H), 1.75 (quin, J = 6.9 Hz, 2H), 1.49 – 1.31 (m, 8H), 1.25 (sextet, J = 7.2 Hz, 4H), 0.87 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.2, 153.1, 140.6, 140.4, 133.5, 126.3,

125.9, 124.8, 124.2, 118.0, 106.4, 72.9, 61.1, 56.4, 54.1, 53.9, 30.4, 29.1, 26.8, 24.1, 20.8, 14.2; HRMS (ESI, *m/z*) calcd. for $C_{31}H_{44}NO_6$ $[M+H]^+$ 526.3169, found 526.3165; IR (film) 2931, 2860, 1638, 1614, 1601, 1582, 1562, 1503 cm^{-1}.

4.4.10. 3-*O*-(3-Dipentylamino)propyl)-3',4',5'-trimethoxyflavonol (28). Syrup, 32% overall yield for two steps; ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 7.9 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.40 (s, 2H), 7.40 (t, J = 7.4 Hz, 1H), 4.08 (t, J = 6.4 Hz, 2H), 3.94 (s, 9H), 2.65-2.55 (m, 2H), 2.40 (t, J = 7.2 Hz, 4H), 2.01 – 1.84 (m, 2H), 1.40-1.19 (overlapped, 12H), 0.86 (t, J = 6.5 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.6, 155.3, 153.1, 140.5, 140.5, 133.5, 126.2, 125.9, 124.8, 124.3, 118.1, 106.4, 71.5, 61.1, 56.5, 54.1, 51.1, 29.8, 22.8, 22.7, 14.2; HRMS (ESI, *m/z*) calcd. for $C_{31}H_{44}NO_6$ $[M+H]^+$ 526.3169, found 526.3165; IR (film) 2928, 2858, 1638, 1614, 1602, 1582, 1562, 1504 cm^{-1}.

4.4.11. 3-*O*-(3-(Dipentylamino)butyl)-3',4',5'-trimethoxyflavonol (29). Syrup, 43% overall yield for two steps; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.3 Hz, 1H), 7.66 (ddd, J = 8.7, 7.2, 1.8 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.42 (s, 2H), 7.38 (t, J = 7.2 Hz, 1H), 4.05 (t, J = 6.8 Hz, 2H), 3.93 (s, 9H), 2.40 (t, J = 7.8 Hz, 2H), 2.34 (t, J = 7.8 Hz, 4H), 1.74 (quin, J = 7.5 Hz, 2H), 1.60 – 1.13 (m, 14H), 0.86 (t, J = 6.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.4, 155.2, 153.1, 140.6, 140.4, 133.5, 126.3, 125.9, 124.8, 124.2, 118.0, 106.4, 72.9, 61.1, 56.4, 54.1, 53.8, 29.9, 28.5, 26.7, 26.6, 23.4, 22.9, 22.7, 14.2; HRMS (ESI, *m/z*) calcd. for $C_{32}H_{46}NO_6$ $[M+H]^+$ 540.3325, found 540.3317; IR (film) 2929, 2859, 1639, 1614, 1602, 1582, 1563, 1504 cm^{-1}.

4.4.12. 3-*O*-(3-(Dipentylamino)pentyl)-3',4',5'-trimethoxyflavonol (30). Syrup, 47% overall yield for two steps; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, J = 8.0, 1.3 Hz, 1H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.41 (s, 2H), 7.39 (t, J = 7.2 Hz, 1H), 4.04 (t, J = 6.7 Hz, 2H), 3.93 (s, 9H), 2.37 (t, J = 7.8 Hz, 6H), 1.75 (quin, J = 6.9 Hz, 2H), 1.51 – 1.15 (m, 16H), 0.91 – 0.82

(overlapped, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.5, 155.2, 153.1, 140.6, 140.4, 133.5, 126.3, 125.9, 124.8, 124.3, 118.0, 106.4, 72.9, 61.1, 56.4, 54.2, 54.1, 30.4, 29.9, 26.8, 26.7, 26.6, 24.1, 22.9, 22.7, 14.2; HRMS (ESI, *m/z*) calcd. for $C_{33}H_{48}NO_6$ $[M+H]^+$ 554.3481, found 554.3465; IR (film) 2930, 2859, 1639, 1614, 1602, 1582, 1562, 1504 cm^{-1}.

4.5. Cell culture. All cell lines were initially purchased from American Type Culture Collection (ATCC^{TM}). The PC-3 and LNCaP prostate cancer cell lines were routinely cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. Cultures were maintained in a high humidity environment supplemented with 5% carbon dioxide at a temperature of 37 ^{o}C. The DU145 prostate cancer cells were routinely cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS and 1% penicillin/spreptomycin. The PWR-1E non-neoplastic prostate epithelial cells were routinely cultured in Keratinocyte serum free medium (K-SFM) supplemented with bovine pituitary extract and human recombinant epidermal growth factor.

4.6. WST-1 cell proliferation assay. PC-3, LNCaP, DU145, or PWR-1E cells were plated in 96-well plates at a density of 3,200 each well in 200 µL of culture medium. The cells were then treated with quercetin, or synthesized flavonols at different doses for 3 days, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in a CO_2 incubator at 37 ^{o}C for three days. 10 µL of the premixed WST-1 cell proliferation reagent (Clontech) was added to each well. After mixing gently for one minute on an orbital shaker, the cells were incubated for additional 3 hours at 37 ^{o}C. To ensure homogeneous distribution of color, it is important to mix gently on an orbital shaker for one minute. The absorbance of each well was measured using a microplate-reader (Synergy HT, BioTek) at a wavelength of 430 nm. The IC_{50} value is the concentration of each compound that inhibits cell proliferation by 50% under the experimental conditions and is the average from at least triplicate determinations that were reproducible and statistically significant. For calculating the IC_{50} values, a linear proliferative inhibition was made based on at least five dosages for each compound.

4.7. ***F2N12S and CYTOX AADvanced double staining assay***: PC-3 cells were plated in 24-well plates at a density of 200,000 each well in 400 µL of culture medium. After 3 hours of cell attachment, the cells were then treated with the test compound at different concentrations in CO_2 incubator at 37 °C for 16 hours, while equal treatment volumes of DMSO were used as vehicle control. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf value of 450 g for 5 to 6 minutes. The collected cells were re-suspended with 500 µL HBSS to remove proteins which may affect flow signal. After centrifugation and discarding the supernatant, the collected cells were re-suspended with 300 µl HBSS and stained with 0.3 µL of F2N12S for 3-5 minutes followed by 0.3 µL SytoxAAdvanced for an additional 5 minutes. The fluorescence intensity of the two probes was further measured in individual PC-3 cells using an Attune flow cytometer (Life Technologies) 0.5 to 1 hour after staining.

4.8. ***Cell cycle analysis:*** PC-3 cells were plated in 24-well plates at a density of 200,000 each well in 400 µL of culture medium. After 3 hours of cell attachment, the cells were then treated with the test derivative at 10 µM and 20 µM for 16 hours and 24 hours, respectively, in CO_2 incubator at 37°C, while equal treatment volumes of DMSO were used as vehicle control. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf value 450 g for 5 minutes. After discarding the supernatant, the collected cells were re-suspended with 500 µL 80% cold ethanol to fix for 30 minutes in 4°C. The fixed cells could be stored in -20 °C for one week. After fixation, the ethanol was removed after centrifuging and the cells were washed with PBS. The cells were then re-suspended with 100 µL of 100 mg/mL ribonuclease and were cultured at 37 °C for 30 minutes to degrade all RNA. The cells were stained with 200 µL of 50 µg/mL propidium iodide stock solution for 30 minutes at -20 °C, and then the fluorescence intensity of PI was detected in individual PC-3 cells using an Attune flow cytometer (Life Technologies) within 0.5 to 1 hour after staining.

4.9. Statistical analysis: All data are represented as the mean ± standard deviation (S.D.) for the number of experiments indicated. Other differences between treated and control groups were analyzed using the Student's t-test. A p-value < 0.05 was considered statistically significant.

Acknowledgments

This study was financially supported by California State University (CSU)-Fresno. The HRMS data were supported by NIH RCMI program at Xavier University of Louisiana through Grant 2G12MD007595 (G.W.). We are also grateful to the Graduate Net Initiative and the Division of Graduate Studies at CSU-Fresno for (i) 2015-2016 Graduate Conference Travel Award (to X.L.), (ii) Travel grant award (to X.L.), and (iii) Graduate Student Research and Creative Activities Support Award (to X.L.).

Supplementary data

Supplementary data (comprised of copies of ^{1}H and ^{13}C NMR spectra for the final products) associated with this article can be found, in the online version, at http://dx.doi.org/.

References and notes

1. Mullie, P.; Clarys, P.; Deriemaeker, P.; Hebbelinck, M. *Plant Foods Hum. Nutr.* **2007**, *62*, 93.
2. Mullie, P.; Clarys, P.; Dereimaeker, P.; Hebbelinck, M. *Int. J. Food Sci. Nutr.* **2008**, *59*, 291.
3. Britton, R. G.; Horner-Glister, E.; Pomenya, O. A.; Smith, E. E.; Denton, R.; Jenkins, P. R.; Steward, W. P.; Brown, K.; Gescher, A.; Sale, S. *Eur. J. Med. Chem.* **2012**, *54*, 952.
4. Haddad, A. Q.; Venkateswaran, V.; Viswanathan, L.; Teahan, S. J.; Fleshner, N. E.; Klotz, L. H. *Prostate Cancer Prostatic Dis.* **2006**, *9*, 68.
5. Vue, B.; Zhang, S.; Chen, Q-H. *Anti-Cancer Agents Med Chem.* **2016**, *16*, 1205.

6. Poyil, P.; Budhraja, A.; Son, Y.-O.; Wang, X.; Zhang, Z.; Ding, S.; Wang, L.; Hitron, A.; Lee, J.-C.; Xu, M.; Chen, G.; Luo, J.; Shi, X. *PLoS One* **2012**, *7*, e47516.
7. Firdous, A.; Sharmila, G.; Balakrishnan, S.; RajaSingh, P.; Suganya, S.; Srinivasan, N.; Arunakaran, J. *Food Function* **2014**, *5*, 2632.
8. Khan, N.; Asim, M.; Afaq, F.; Abu, Z. M.; Mukhtar, H. *Cancer Res.* **2008**, *68*, 8555.
9. Hill, C. U.; Saad, S. E.; Britton, R.G.; Gescher, A.J.; Sale, S.; Brown, K.; Howells, L.M. *Cancer Chemother. Pharmacol*. **2015**, *76*, 179.
10. Saad, S. E. A.; Jones, D. J. L.; Norris, L. M.; Horner-Glister, E.; Patel, K. R.; Britton, R. G.; Steward, W. P.; Gescher, A. J.; Brown, K.; Sale, S. *Biomed. Chromatogr.* **2012**, *26*, 1559.
11. Li, X.; Chen, G.; Zhang, X.; Zhang, Q.; Zheng, S.; Wang, G.; Chen, Q.-H. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4241.
12. Burmistrova, O.; Marrero, M.T.; Estévez, S.; Welsch, I.; Brouard, I.; Quintana, J.; Estévez, F. *Eur. J. Med. Chem.* **2014**, *84*, 30.
13. Dias, T. A.; Duarte, C. L.; Lima, C. F.; Proenca, M. F.; Pereira-Wilson, C. *Eur. J. Med. Chem.* **2013**, *65*, 500.
14. Nielsen, A. T.; Houlihan, W. J. *Org Reac.* **1968**, *16*, 1.
15. Dean, F.; Podimuang, V. *J. Chem. Soc.* (Resumed) **1965**, 3978.
16. Gunduz, S.; Goren, A. C.; Ozturk, T. *Org. Lett.* **2012**, *14*, 1576.
17. Rhim, J. S.; Li, H.; Furusato, B. *Adv. Exp. Med. Biol.* **2011**, *720*, 71.
18. Khan, N.; Afaq, F.; Syed, D. N.; Mukhtar, H. *Carcinogenesis* **2008**, *29*, 1049.
19. Meng, F.-M.; Yang, J.-B.; Yang, C.-H.; Jiang, Y.; Zhou, Y.-F.; Yu, B.; Yang, H. *Asian Pac. J. Cancer Prev*. **2012**, *13*, 6369.
20. Vijayababu, M. R.; Kanagaraj, P.; Arunkumar, A.; Ilangovan, R.; Aruldhas, M. M.; Arunakaran, J. *J. Cancer Res. Clin. Oncol.* **2005**, *131*, 765.

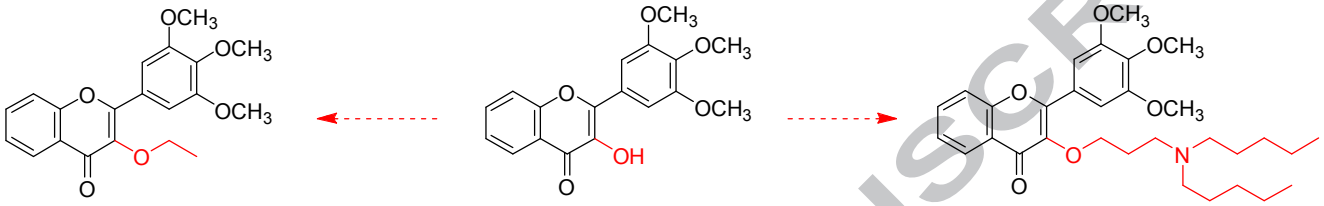
21. Haddad, A. Q.; Fleshner, N.; Nelson, C.; Saour, B.; Musquera, M.; Venkateswaran, V.; Klotz, L. *Nutr. Cancer* **2010**, *62*, 668.

GRAPHICAL ABSTRACT

3-*O*-Substituted-3',4',5'-trimethoxyflavonols: synthesis and cell-based evaluation as anti-prostate cancer agents

Leave this area blank for abstract info.

Xiang Li, Maizie Lee, Guanglin Chen, Qiang Zhang, Shilong Zheng, Guangdi Wang, and Qiao-Hong Chen*



IC_{50} = 0.9 μM (PC-3)
Arrest cell cycle at S and G_0/G_1 phase
Induce 3% apoptotic cells at 10 μM

IC_{50} = 32.1 μM (PC-3)
Arrest cell cycle at S and G_2/M phase
Induce 39% apoptotic cells at 10 μM

IC_{50} = 5.8 μM (PC-3)
Arrest cell cycle at G_0/G_1 phase
Induce 93% apoptotic cells at 10 μM

Key Words: Trimethoxyflavonol derivatives; Prostate cancer; Cell proliferation, Cell apoptosis; Cell cycle regulation.

*Corresponding author: Tel: +1 559 278 2394; fax: +1 559 278 4402; e-mail: qchen@csufresno.edu