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# Nitrogen-containing derivatives of *O*-tetramethylquercetin: Synthesis and biological profiles in prostate cancer cell models

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ABSTRACT

Forty-eight nitrogen-containing quercetin derivatives were synthesized from readily available rutin or quercetin for the *in vitro* evaluation of their biological profiles. The WST-1 cell proliferation assay data indicate that thirty-nine out of the forty-eight derivatives possess significantly improved antiproliferative potency as compared with quercetin and fisetin, as well as the parent 3,3′,4′,7-*O*-tetramethylquercetin toward both androgen-sensitive (LNCaP) and androgen-insensitive (PC-3 and DU145) human prostate cancer cell lines. 5-*O*-Aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins were established as a better scaffold for further development as anti-prostate cancer agents. Among them, 5-*O*-(*N*,*N*-dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin (**44**) was identified as the optimal derivative with $IC_{50}$ values of 0.55–2.82 μM, being over 35–182 times more potent than quercetin. The flow cytometry-based assays further demonstrate that **44** effectively activates PC-3 cell apoptosis.

## 1. Introduction

3,3′,4′,5,7-Pentahydroxyflavone (**1**, Fig. 1), commonly known as quercetin, is a dietary flavonoid ubiquitously distributed in a variety of diets especially in fruits and vegetables [1]. Several epidemiologic studies led to the general conclusion that an inverse correlation exists between the incidence of prostate cancer and the flavonoid-enriched diets in the East Asian [2–6]. This conclusion inspired several research groups to assess the potential of quercetin, a most common and abundant naturally occurring flavonoid, in treating and preventing various cancers including prostate cancer alone or in combination with other dietary natural products [6–9]. Quercetin was revealed to suppress cell proliferation in five prostate cancer cell line models with $IC_{50}$ values ranging from 15.5 to 64 μM [10–13]. As summarized in our review article [7], the underlying mechanism of antiproliferative effects of quercetin in prostate cancer cells was reported to be associated with the regulation of cell cycle, cell apoptosis, and androgen receptor. Quercetin was also demonstrated to have significant *in vivo* anti-prostate tumor efficacy either as a diet supplement or as a pure compound in three different animal models [14–16]. Additionally, the safety profile of quercetin has been confirmed by a rat model and two clinical studies [17–19]. The above-described cell-based and animal-based studies, as well as clinical studies, corroborate the notion that quercetin warrants further exploration as an anti-prostate cancer agent. However, its moderate potency, as evidenced by its $IC_{50}$ values measured in *in vitro* studies and the high dose (200 mg per kg body weight) needed for the *in vivo* animal studies, hinders its further development as a drug candidate for prostate cancer. This study aims to engineer quercetin derivatives with improved potency for the treatment of prostate cancer through chemical manipulations. Our previous studies suggest that introduction of one substituent group, especially lengthy and/or bulky group to a phenolic hydroxyl group might be the best strategy for modification of quercetin as anti-prostate cancer agents [20]. Incorporation of an appropriate amino moiety to the 3-OH of 3′,4′-dimethoxyflavonol through a three-carbon linker led to the optimal derivative **2** with up to 292-fold increase in potency [21] in three prostate cancer cell lines. Also, modification of 5-OH of 2,3-dehydrosilybin (another group of flavonoids) resulted in an optimal derivative **3** that can consistently inhibit prostate cancer cell proliferation [22]. Encouraged by these positive results, this study was planned to explore the possibility of enhancing the antiproliferative potency of quercetin as anti-prostate cancer agents through modification on its 3-OH or 5-OH group. Consequently, twenty-four 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**) and twenty-four 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins

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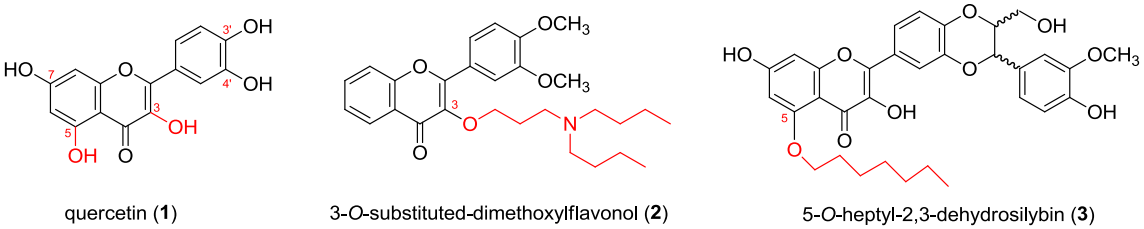



**Fig. 1.** Flavonoids as anti-prostate cancer agents.

(**38–61**) have been designed and synthesized for the evaluation of their biological profiles in prostate cancer cell models. 3′,4′,5,7-*O*-Tetramethylquercetin (**6**) and 3,3′,4′,7-*O*-tetramethylquercetin (**7**) were chosen as the parent compounds because they have grounds to selectively incorporate a linker at either 3-OH or 5-OH and were expected to overcome, to some degree, pharmacokinetic limitations caused by the phenolic hydroxyl groups in quercetin.

## 2. Results and discussion

### 2.1. Chemistry

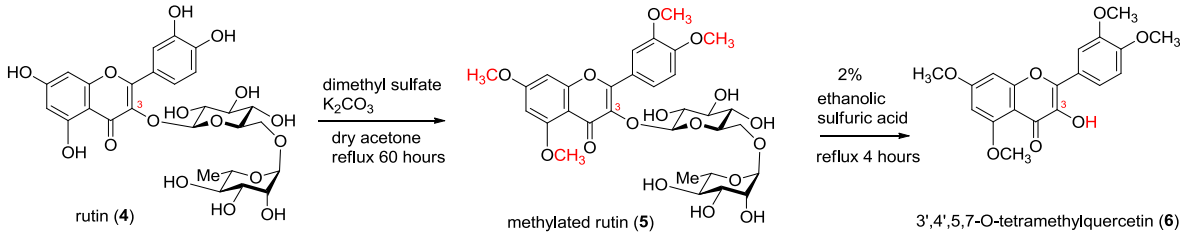
As shown in Scheme 1, 3′,4′,5,7-*O*-tetramethylquercetin (**6**) was synthesized from readily available rutin through global methylation followed by glycoside hydrolysis according to the reported procedure [23]. 3,3′,4′,7-*O*-Tetramethylquercetin (**7**) was prepared from quercetin by selective tetramethylation of quercetin using the procedure as described by Manthey and Guthrie (Scheme 2) [24,25]. As illustrated in Scheme 3, twenty-four 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**) have been prepared by a two-step transformation including *O*-alkylation of 3′,4′,5,7-*O*-tetramethylquercetin (**6**) with the appropriate dibromoalkanes followed by *N*-alkylation of the consequent 3-*O*-bromoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**8–10**) with the suitable amines. In both alkylation reactions, potassium carbonate was used as the base and *N*,*N*-dimethylformamide (DMF) was used as the polar aprotic solvent. Similarly, twenty-four 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**38–61**) have been synthesized from 3,3′,4′,7-*O*-tetramethylquercetin (**7**, Scheme 4). It is worth noting that each of crude 3-*O*-bromoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**8–10**) was pure enough for the follow-up *N*-alkylation reaction. However, the *O*-alkylation of 3,3′,4′,7-*O*-tetramethylquercetin (**7**) cannot reach 100% conversion rate even under harsher reaction conditions including prolonged reaction time (48 h at room temperature) or increased reaction temperature (70 °C). Consequently, each of 5-*O*-bromoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**35–37**) has to be purified by either column chromatography or preparative thin layer chromatography (PTLC) over silica gel prior to being used for the follow-up *N*-alkylation reaction. All pure 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**) and 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**38–61**) were obtained by PTLC purification over silica gel. The mixture of dichloromethane:diethylamine (100:3, v/v) is the optimal solvent to retrieve the desired nitrogen-containing products from the PTLC silica gel.

### 2.2. Antiproliferative activity towards prostate cancer cells

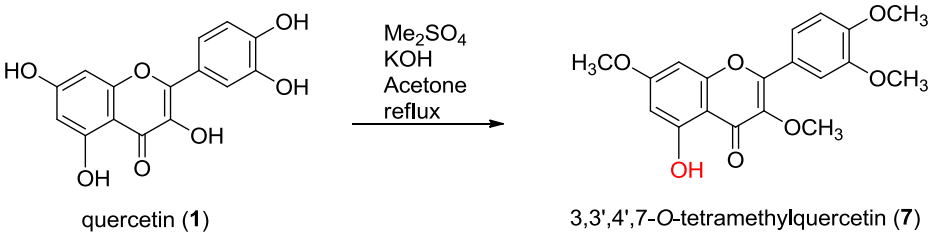
The *in vitro* antiproliferative activity of twenty-four 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**) and twenty-four 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**38–61**) against a panel of prostate cancer cell lines (PC-3, DU145, and LNCaP) was determined using WST-1 cell proliferation assay according to the procedure described in the Experimental Section. Fisetin and quercetin were used as positive controls, and dimethyl sulfoxide (DMSO) was used as negative control. As illustrated in Table 1, all of the twenty-four 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**) have greater antiproliferative potency than quercetin and fisetin as indicated in their $IC_{50}$ values in PC-3 and LNCaP human prostate cancer cell lines. Among them, only fifteen derivatives are significantly more potent than the two positive controls in the DU145 prostate cancer cell line. Derivatives **20** and **21** were identified as two optimal 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins with $IC_{50}$ values of 1.73–6.53 μM. In contrast, all of the twenty-four 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**38–61**, Table 2) are more potent than fisetin, quercetin, and 3,3′,4′,7-*O*-tetramethylquercetin (**7**) against three prostate cancer cell lines. 5-*O*-(*N*,*N*-Dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin (**44**) is established as the optimal derivative with $IC_{50}$ values of 0.55 μM, 2.82 μM, and 1.16 μM, respectively, towards PC-3, DU145, and LNCaP prostate cancer cell lines.

The structure-antiproliferative activity relationship of *O*-aminoalkyl-*O*-tetramethylquercetins can be summarized as below:

- It can be clearly concluded that modification on 3-OH of 3′,4′,5,7-*O*-tetramethylquercetins and 5-OH of 3,3′,4′,7-*O*-tetramethylquercetin did significantly improve their potency.
- 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins are generally more potent than 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins towards prostate cancer cells.
- *N*,*N*-dipentylamino moiety in **20** and **21** is the optimal nitrogen-containing group for the antiproliferative activity of 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins.
- *N*,*N*-dibutylamino moiety in **44**, and *N*,*N*-dipentylamino moiety in **47** & **48** serve as the most preferred nitrogen-containing group for the enhanced *in vitro* antiproliferative potency of 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins.
- All forty-eight derivatives are generally more effective in suppressing PC-3 and LNCaP cell proliferation than in inhibiting the DU145 cell proliferation.




**Scheme 1.** Synthesis of 3′,4′,5,7-*O*-tetramethylquercetin (**6**).




**Scheme 2.** Synthesis of 3,3′,4′,7-*O*-tetramethylquercetin (**7**).

### 2.3. Docetaxel resistance susceptibility

Additionally, the optimal derivative **44** was selected for further investigation on its antiproliferative activity towards docetaxel-resistant prostate cancer cell lines (PC-3/DTX and DU145/DTX) (Table 3). The resistant cells were confirmed to have significantly lost their sensitivity to docetaxel treatment, as evidenced in the 1232-fold (PC-3/DTX) and 7150-fold (DU145/DTX) increase in $IC_{50}$ values. In contrast, compound **44** exhibited comparable antiproliferative activity in both docetaxel-sensitive and docetaxel-resistant cells, and were slightly more potent than docetaxel in PC3/DTX and DU145/DTX cell lines (Table 3).

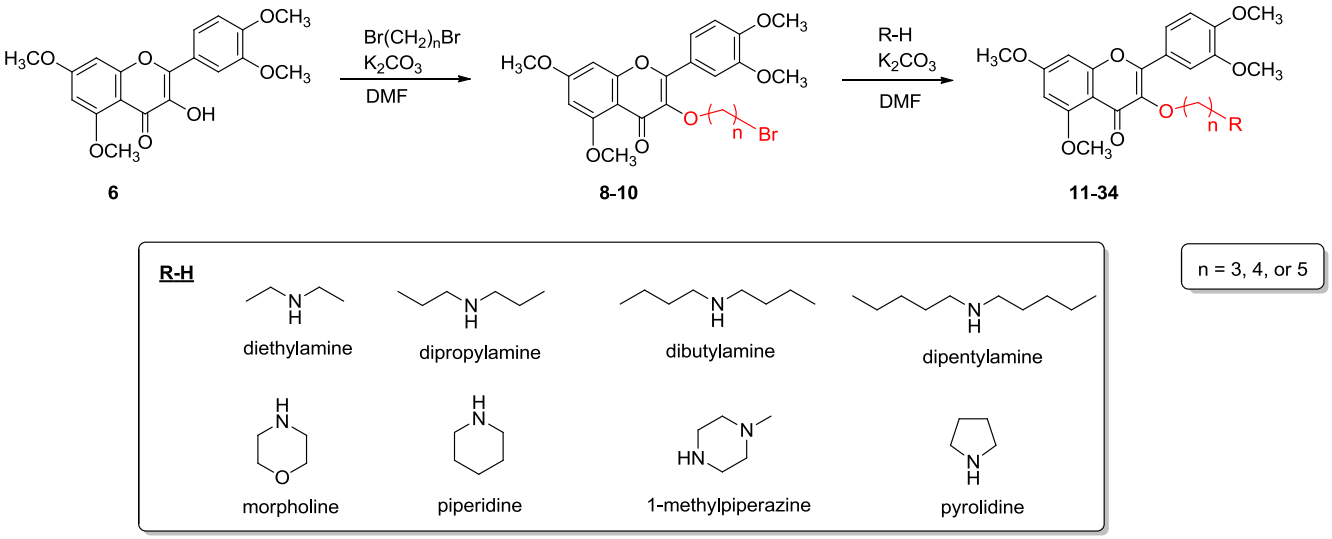
### 2.4. Effects of 5-O-(N,N-dibutylamino)propyl-3,3′,4′,7-O-tetramethylquercetin (44) on PC-3 cell cycle progression and apoptosis

Quercetin was reported to induce PC-3 cell cycle arrest at $G_2$/M and S phase [26]. To evaluate the effect of 5-aminoalkyl group on the PC-3 prostate cancer cell cycle regulation, 5-*O*-(*N*,*N*-dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin (**44**), the optimal derivative, was selected for further assessment using flow cytometry analysis with propidium iodide DNA staining. When PC-3 cells were treated with **44** at 10 and 20 μM, a different cell cycle regulation was observed. Specifically, **44** causes slight accumulation of PC-3 cells in the $G_0/G_1$ phase from 26% for control cells at 16 h to 28% (10 μM) and 30% (20 μM) for the compound-treated cells. The data imply that PC-3 cell proliferation of 5-*O*-(*N*,*N*-dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin may be associated with its cell cycle regulation in the $G_0/G_1$ phase.

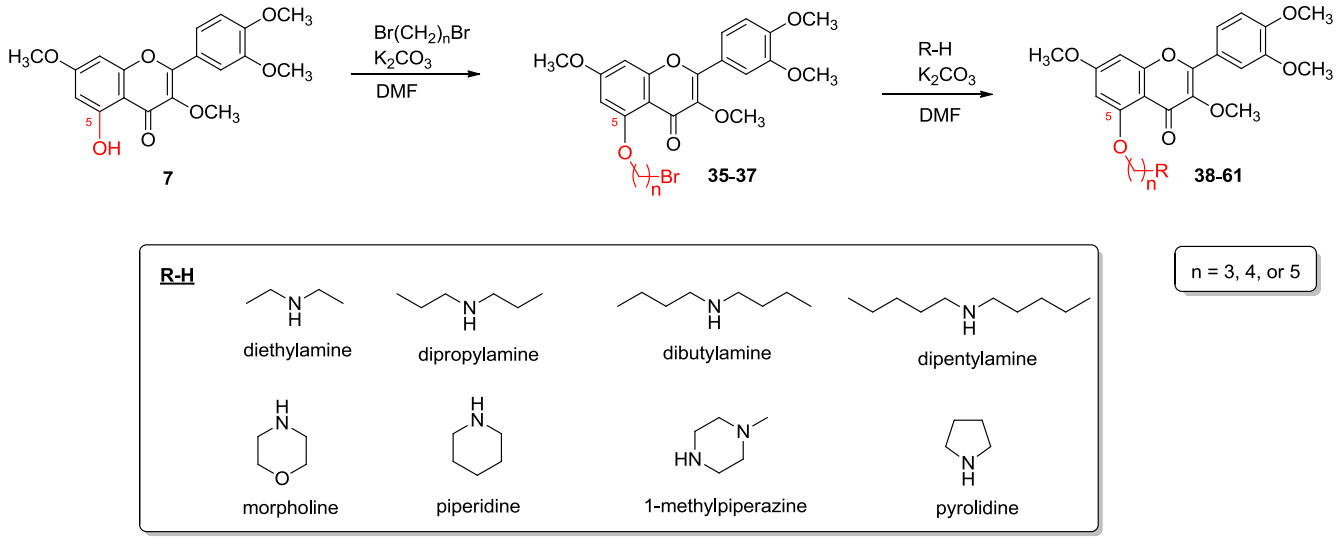
The cell proliferation inhibition of quercetin was revealed to be associated with its cell apoptosis activation in several prostate cancer cell models [12,26–29]. To evaluate the effect of 5-*O*-substitution on the PC-3 cell apoptosis, 5-*O*-(*N*,*N*-dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin as the most promising derivative, was chosen for F2N12S and SYTOX AADvanced double staining flow cytometry-based assay at 0–50 μM dosing range. The results (Figs. 2 and 3) imply that treatment of PC-3 cells with **44** for 16 h led to PC-3 cells dying from apoptosis rather than necrosis in a dose-responsive manner. Specifically, treatment with 10 μM of derivative **44** resulted in 32% early apoptotic cells and only 4% late apoptotic/necrotic cells; 20 μM of derivative **44** significantly activated apoptosis as well, with 50% early apoptotic cells and 11% late apoptotic/necrotic cells. Both apoptotic and necrotic cell populations increased in response to increasing concentration of **44** (0–50 μM concentration range). The apoptotic cell population reached maximum when exposure PC-3 cancer cells to **44** at 30 μM concentration.

## 3. Conclusion

In summary, forty-eight quercetin derivatives containing a nitrogen group at 3-OH of 3′,4′,5,7-*O*-tetramethylquercetin and 5-OH of 3,3′,4′,7-*O*-tetramethylquercetin were synthesized from rutin or quercetin. The WST-1 cell proliferation assay data indicate that thirty-nine out of the forty-eight derivatives possess significantly improved antiproliferative potency as compared with quercetin, fisetin, and 3,3′,4′,7-*O*-tetramethylquercetin against both androgen-sensitive (LNCaP) and androgen-insensitive prostate cancer cell lines (PC-3 and DU145). 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins serve as a better scaffold for further development of anti-prostate cancer agents. Among them, 5-*O*-(*N*,*N*-dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin (**44**) was identified as the optimal derivative with $IC_{50}$ values of 0.55–2.82 μM, being over 35- to 182-fold more potent than quercetin. The flow cytometry-based assays further demonstrate that **44** can significantly induce PC-3 cell apoptosis.



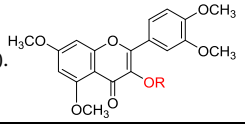

**Scheme 3.** Synthesis of 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**). For the specific structure for each of **11–34**, see Table 1. R-H represents different amines. The structures for all R-H were shown in the box.




**Scheme 4.** Synthesis of 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**38–61**). For the specific structure for each of **38–61**, see Table 2. R-H represents different amines. The structures for all R-H were shown in the box.

**Table 1**

Antiproliferative activity of 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins (**11–34**).



| Compd | R | $IC_{50}$ (μM)[a] | | |
|---|---|---|---|---|
| | | PC-3[b] | DU145[c] | LNCaP[d] |
| Quercetin | – | > 100 | > 100 | 45.5 ± 1.3 |
| Fisetin | – | > 50 | > 50 | 34.1 ± 7.7 |
| **11** | (*N*,*N*-diethylamino)propyl | 16.62 ± 0.24 | > 50 | 17.63 ± 1.04 |
| **12** | (*N*,*N*-diethylamino)butyl | 22.73 ± 7.56 | > 50 | 19.44 ± 3.83 |
| **13** | (*N*,*N*-diethylamino)pentyl | 18.33 ± 6.80 | > 50 | 11.39 ± 2.27 |
| **14** | (*N*,*N*-dipropylamino)propyl | 11.59 ± 1.98 | 25.41 ± 3.68 | 9.57 ± 1.97 |
| **15** | (*N*,*N*-dipropylamino)butyl | 11.05 ± 4.06 | > 50 | 10.99 ± 2.38 |
| **16** | (*N*,*N*-dipropylamino)pentyl | 20.12 ± 3.71 | 34.81 ± 3.25 | 13.80 ± 3.88 |
| **17** | (*N*,*N*-dibutylamino)propyl | 10.70 ± 2.30 | 22.92 ± 4.56 | 7.14 ± 0.78 |
| **18** | (*N*,*N*-dibutylamino)butyl | 7.00 ± 2.11 | 15.12 ± 2.29 | 6.54 ± 1.45 |
| **19** | (*N*,*N*-dibutylamino)pentyl | 7.60 ± 1.87 | 20.13 ± 5.14 | 7.18 ± 0.79 |
| **20** | (*N*,*N*-dipentylamino)propyl | 4.00 ± 0.63 | 4.74 ± 0.51 | 1.73 ± 0.34 |
| **21** | (*N*,*N*-dipentylamino)butyl | 3.81 ± 0.45 | 6.53 ± 0.16 | 3.45 ± 0.23 |
| **22** | (*N*,*N*-dipentylamino)pentyl | 6.97 ± 1.09 | 9.21 ± 0.21 | 6.74 ± 0.79 |
| **23** | morpholinopropyl | 20.38 ± 2.28 | > 50 | 27.34 ± 4.61 |
| **24** | morpholinobutyl | 19.94 ± 2.37 | 28.88 ± 4.26 | 13.29 ± 2.71 |
| **25** | morpholinopentyl | 13.19 ± 3.77 | 18.05 ± 2.13 | 8.48 ± 0.62 |
| **26** | piperidinopropyl | 12.57 ± 4.27 | 41.12 ± 3.70 | 15.06 ± 2.0.48 |
| **27** | piperidinobutyl | 31.28 ± 2.04 | > 50 | 21.49 ± 3.84 |
| **28** | piperidinopentyl | 21.04 ± 2.21 | 27.99 ± 8.32 | 13.49 ± 3.37 |
| **29** | (4-methylpiperazin-1-yl)propyl | 32.01 ± 2.41 | 51.92 ± 4.67 | 22.39 ± 1.96 |
| **30** | (4-methylpiperazin-1-yl)butyl | 12.46 ± 0.84 | > 50 | 12.09 ± 3.24 |
| **31** | (4-methylpiperazin-1-yl)pentyl | 19.89 ± 3.18 | 25.95 ± 0.12 | 14.62 ± 4.10 |
| **32** | pyrrolidinopropyl | 24.01 ± 5.44 | 39.38 ± 7.72 | 18.58 ± 0.81 |
| **33** | pyrrolidinobutyl | 22.51 ± 1.82 | > 50 | 13.32 ± 2.90 |
| **34** | pyrrolidinopentyl | 22.87 ± 2.92 | 22.64 ± 2.37 | 11.37 ± 2.62 |

[a] $IC_{50}$ is the compound 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 ± SD from n = 3.
[b] Human androgen-insensitive prostate cancer cell line derived from bone 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 derived from lymph node metastasis of prostate tumor.

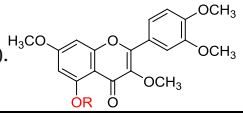
## 4. Experimental section

### 4.1. General procedures

High resolution mass spectrometry (HRMS) was obtained on an Orbitrap mass spectrometer with electrospray ionization (ESI). Nuclear Magnetic Resonance (NMR) spectra were obtained on a Bruker Fourier 300 spectrometer in $CDCl_3$. The chemical shifts are given in ppm referenced to the respective solvent peak, and coupling constants are reported in Hz. All 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 μ).

**Table 2**

Antiproliferative activity of 5-*O*-aminoalkyl-3,3′,4′,7-*O*-tetramethylquercetins (**38–61**).




| Compd | R | $IC_{50}$ (μM)[a] | | |
|---|---|---|---|---|
| | | PC-3[b] | DU145[c] | LNCaP[d] |
| Quercetin | – | > 100 | > 100 | 45.5 ± 1.3 |
| Fisetin | – | > 50 | > 50 | 34.1 ± 7.7 |
| **7** | H | > 50 | > 50 | > 50 |
| **38** | (*N*,*N*-diethylamino)propyl | 16.38 ± 4.23 | 36.19 ± 3.83 | 14.38 ± 2.41 |
| **39** | (*N*,*N*-diethylamino)butyl | 13.94 ± 1.34 | 14.94 ± 1.52 | 8.54 ± 2.64 |
| **40** | (*N*,*N*-diethylamino)pentyl | 17.06 ± 0.68 | 13.58 ± 2.23 | 6.79 ± 0.21 |
| **41** | (*N*,*N*-dipropylamino)propyl | 3.37 ± 1.23 | 8.98 ± 1.57 | 2.94 ± 0.54 |
| **42** | (*N*,*N*-dipropylamino)butyl | 4.71 ± 0.60 | 8.02 ± 2.43 | 2.53 ± 0.73 |
| **43** | (*N*,*N*-dipropylamino)pentyl | 10.20 ± 1.14 | 16.29 ± 4.71 | 5.80 ± 0.80 |
| **44** | (*N*,*N*-dibutylamino)propyl | 0.55 ± 0.16 | 2.82 ± 0.27 | 1.16 ± 0.18 |
| **45** | (*N*,*N*-dibutylamino)butyl | 3.35 ± 0.84 | 9.61 ± 2.03 | 2.50 ± 0.42 |
| **46** | (*N*,*N*-dibutylamino)pentyl | 4.78 ± 0.61 | 12.97 ± 2.46 | 3.14 ± 0.28 |
| **47** | (*N*,*N*-dipentylamino)propyl | 1.85 ± 0.29 | 5.44 ± 0.24 | 2.67 ± 0.38 |
| **48** | (*N*,*N*-dipentylamino)butyl | 2.10 ± 0.38 | 5.33 ± 0.92 | 1.23 ± 0.35 |
| **49** | (*N*,*N*-dipentylamino)pentyl | 4.11 ± 1.11 | 8.48 ± 0.50 | 1.32 ± 0.04 |
| **50** | morpholinopropyl | 12.15 ± 3.84 | 27.95 ± 1.05 | 12.82 ± 0.75 |
| **51** | morpholinobutyl | 12.40 ± 3.17 | 26.02 ± 3.66 | 9.45 ± 2.13 |
| **52** | morpholinopentyl | 11.81 ± 3.81 | 22.93 ± 373 | 6.87 ± 1.13 |
| **53** | piperidinopropyl | 4.07 ± 2.12 | 9.09 ± 0.28 | 4.07 ± 0.33 |
| **54** | piperidinobutyl | 10.31 ± 2.46 | 9.96 ± 1.97 | 6.54 ± 2.39 |
| **55** | piperidinopentyl | 12.61 ± 0.65 | 14.98 ± 1.53 | 7.83 ± 1.77 |
| **56** | (4-methylpiperazin-1-yl)propyl | 4.47 ± 0.92 | 15.70 ± 2.17 | 4.33 ± 0.74 |
| **57** | (4-methylpiperazin-1-yl)butyl | 12.34 ± 1.83 | 17.42 ± 3.54 | 2.97 ± 0.71 |
| **58** | (4-methylpiperazin-1-yl)pentyl | 17.17 ± 2.12 | 20.46 ± 3.35 | 8.46 ± 0.78 |
| **59** | pyrrolidinopropyl | 5.80 ± 2.15 | 14.63 ± 5.95 | 9.42 ± 0.23 |
| **60** | pyrrolidinobutyl | 9.99 ± 2.22 | 13.92 ± 0.62 | 7.86 ± 1.80 |
| **61** | pyrrolidinopentyl | 15.87 ± 5.23 | 18.67 ± 5.52 | 10.28 ± 2.29 |

[a] $IC_{50}$ is the compound 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 ± SD from n = 3.
[b] Human androgen-insensitive prostate cancer cell line derived from bone 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 derived from lymph node metastasis of prostate tumor.

**Table 3**
Antiproliferative activity of **44** against docetaxel-resistant prostate cancer cells.

| Compd | $IC_{50}$: (nM)[a] | | R/S[d] | $IC_{50}$: (nM)[a] | | R/S[c] |
|---|---|---|---|---|---|---|
| | PC-3[b] | PC-3/DTX[c] | | DU145[e] | DU145/DTX [f] | |
| Docetaxel | 1.9 ± 0.6 | 2340 ± 250 | 1232 | 1.2 ± 0.3 | 8580 ± 390 | 7150 |
| **44** | 550 ± 160 | 1700 ± 670 | 3.1 | 2820 ± 270 | 2290 ± 190 | 0.8 |

[a] $IC_{50}$ is the compound 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 ± SD from n = 3.
[b] Human androgen-insensitive prostate cancer cell line derived from bone metastasis of prostate tumor.
[c] Docetaxel-resistant PC-3 prostate cancer cell line.
[d] The relative resistance of the two cell lines obtained by dividing the $IC_{50}$ value of the resistance cell line by the parental cell line.
[e] Human androgen-insensitive prostate cancer cell line derived from brain metastasis of prostate tumor.
[f] Docetaxel-resistant DU145 prostate cancer cell line.

Preparative thin-layer chromatography (PTLC) separations were carried out on thin layer chromatography plates loaded with silica gel 60 GF254 (EMD Millipore Corporation, MA, USA). 3′,4′,5,7-*O*-Tetramethylquercetin (**6**) was synthesized according to the reported procedure [23]. Quercetin was purchased from Fisher Scientific (Alfa Aesar, Cat# A1580722).

### 4.2. Synthesis of 3,3′,4′,7-O-tetramethylquercetin (7) [24,25]

This compound was synthesized by a modified method of Manthey and Guthrie [24]. Quercetin (3.0 g, 10 mmol) was added to a solution of acetone (150 mL), water (75 mL) and 30% aqueous KOH solution (6 mL) in a three-neck round bottom flask. The reaction mixture was refluxed for 10 min. Dimethyl sulfate (2.4 mL) was added, and the mixture was refluxed for 20 min. KOH solution (3 mL) was added, producing a dark brown solution. An additional 2.4 mL of dimethyl sulfate was added, and the solution was again refluxed for 20 min. KOH (3.0 mL) was added, followed by dimethyl sulfate (0.6 mL). An additional aliquot of dimethyl sulfate (3.0 mL) was added and the mixture was refluxed for 1.5 h and allowed to cool down and stand at room temperature overnight. Yellow crystals (1.82 g) were collected and directly used for the next step reaction.

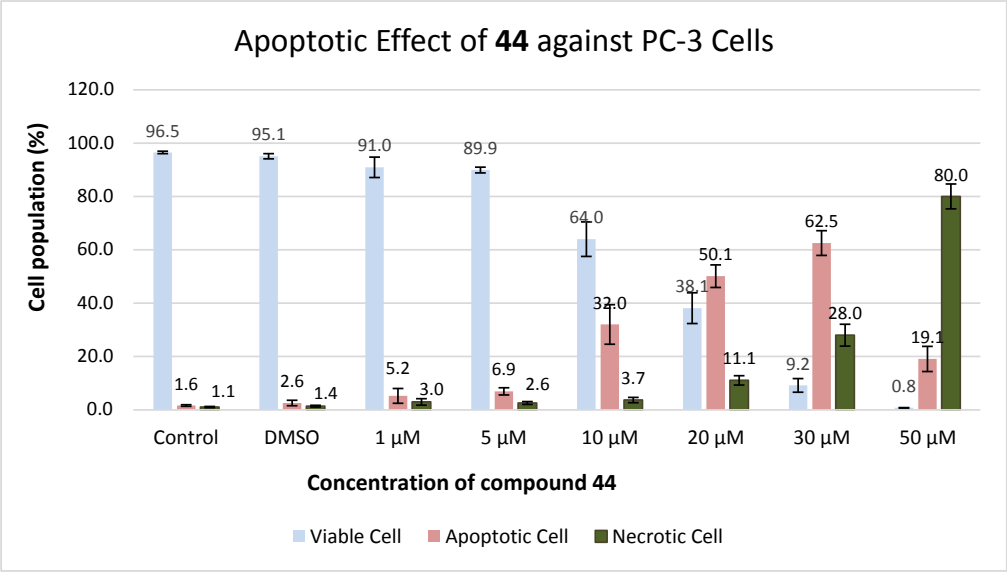



**Fig. 2.** Evolution of viable, apoptotic, and necrotic PC-3 cell populations in response to **44.**

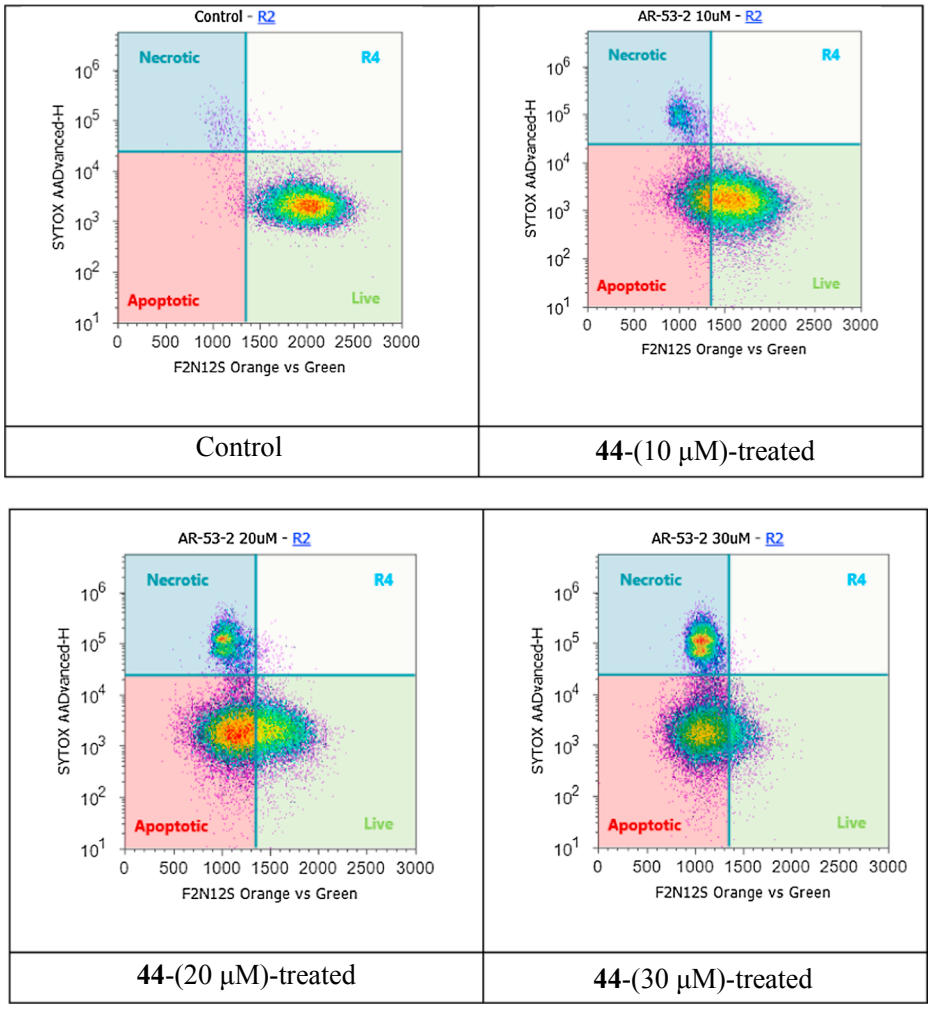



**Fig. 3.** Apoptosis in PC-3 cells treated with **44** at 10, 20, and 40 µM (by F2N12S and SYTOX AADvanced double staining).

*4.3. The general procedure for the synthesis of 3-O-dialkylaminoalkyl-3′,4′,5,7-O-tetramethylquercetins (**11–34**)*

The solution of 3′,4′,5,7-*O*-tetramethylquercetin (**6**, 60 mg, 0.17 mmol) and potassium carbonate (70 mg, 0.51 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 stirred at room temperature for

12 h until the starting material was fully consumed as indicated by TLC. The reaction mixture was diluted with a mixture of ethyl acetate and diethyl ether (100 mL, 1:1, v/v), which was rinsed with water (10 mL × 1) and brine (10 mL × 4). The subsequent organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo* to generate a crude product (**8**, **9**, or **10**). To the solution of this crude product in DMF (0.5 mL, 0.3 M) was added potassium carbonate (70 mg, 0.51 mmol, 3 equiv.), and the mixture was stirred for 10 min. The appropriate amine (0.51 mmol, 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 reaction mixture was diluted with a mixture of ethyl acetate and diethyl ether (100 mL, 1:1, v/v), which was rinsed with water (10 mL × 1) and brine (10 mL × 4). The subsequent organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to generate a crude product, which was subjected to PTLC purification using dichloromethane/methanol (90/10, v/v) as eluent. The respective 3-*O*-dialkylaminoalkyl-3′,4′,5,7-*O*-tetramethylquercetin was retrieved from PTLC silica gel by washing with dichloromethane/diethylamine (100/3, v/v).

#### *4.3.1. 3-O-(N,N-Diethylamino)propyl-3′,4′,5,7-O-tetramethylquercetin (**11**)*

Yield, 69%; yellow wax. IR (film) $\nu_{max}$: 2965, 2836, 1622, 1600, 1513, 1457 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70–7.59 (overlapped, 1H), 7.65 (s, 1H), 6.92 (d, $J$ = 9.0 Hz, 1H), 6.44 (s, 1H), 6.28 (s, 1H), 4.01 (t, $J$ = 6.3 Hz, 2H), 3.91 (s, 9H), 3.85 (s, 3H), 2.56 (t, $J$ = 7.5 Hz, 2H), 2.47 (q, $J$ = 7.2 Hz, 4H), 1.86 (quin, $J$ = 7.8 Hz, 2H), 0.95 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 163.9, 161.0, 158.8, 152.8, 150.8, 148.6, 140.3, 123.5, 121.8, 111.4, 110.8, 109.5, 95.8, 92.5, 71.0, 56.4, 56.1, 56.0, 55.8, 49.8, 46.9, 27.8, 11.5. HR-MS (ESI) *m/z*: calcd for $C_{26}H_{34}NO_7$ [M + H]: 472.2335; found 472.2335.

#### *4.3.2. 3-O-(N,N-Diethylamino)butyl-3′,4′,5,7-O-tetramethylquercetin (**12**)*

Yield, 39%; yellow wax. IR (film) $\nu_{max}$: 2937, 1624, 1602, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.71 (s, 1H), 7.69 (dd, $J$ = 6.9, 2.1 Hz, 1H), 6.97 (d, $J$ = 9.3 Hz, 1H), 6.50 (d, $J$ = 2.1 Hz, 1H), 6.34 (d, $J$ = 2.1 Hz, 1H), 4.03 (t, $J$ = 6.3 Hz, 2H), 3.96 (s, 9H), 3.90 (s, 3H), 2.59–2.49 (overlapped, 6H), 1.73 (quin, $J$ = 6.9 Hz, 2H), 1.65–1.53 (m, 2H), 1.03 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.1, 161.0, 159.0, 153.4, 151.1, 148.8, 139.9, 123.2, 122.0, 111.2, 111.1, 109.4, 96.0, 92.7, 70.8, 56.6, 56.3, 56.2, 56.0, 51.3, 46.6, 27.5, 20.7, 9.0. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{36}NO_7$ [M + H]: 486.2492; found 486.2492.

#### *4.3.3. 3-O-(N,N-Diethylamino)pentyl-3′,4′,5,7-O-tetramethylquercetin (**13**)*

Yield, 61%; yellow oil. IR (film) $\nu_{max}$: 2937, 1622, 1601, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (s, 1H), 7.66 (d, $J$ = 11.1 Hz, 1H), 6.72 (d, $J$ = 8.4 Hz, 1H), 6.46 (d, $J$ = 1.8 Hz, 1H), 6.30 (d, $J$ = 1.8 Hz, 1H), 3.98 (t, $J$ = 6.6 Hz, 2H), 3.92 (s, 9H), 3.86 (s, 3H), 2.55 (q, $J$ = 6.9 Hz, 4H), 2.42 (t, $J$ = 7.2 Hz, 2H), 1.72 (quin, $J$ = 7.5 Hz, 2H), 1.50–1.35 (m, 4H), 1.01 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 163.9, 161.1, 158.9, 152.9, 150.8, 148.6, 140.4, 123.6, 121.8, 111.5, 110.8, 109.5, 95.8, 92.5, 72.3, 56.4, 56.1, 56.0, 55.9, 52.6, 46.8, 30.2, 26.1, 24.1, 11.2. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{38}NO_7$ [M + H]: 500.2648; found 500.2645.

#### *4.3.4. 3-O-(N,N-Dipropylamino)propyl-3′,4′,5,7-O-tetramethylquercetin (**14**)*

Overall yield for two steps, 22%; yellow syrup. IR (film) $\nu_{max}$: 2957, 2872, 2361, 1601 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.68 (dd, $J$ = 7.5, 1.8 Hz, 1H), 7.66 (s, 1H), 6.96 (d, $J$ = 9.0 Hz, 1H), 6.49 (d, $J$ = 2.1 Hz, 1H), 6.33 (d, $J$ = 2.1 Hz, 1H), 4.03 (t, $J$ = 6.3 Hz, 2H), 3.95 (s, 3H), 3.94 (s, 6H), 3.89 (s, 3H), 2.70 (t, $J$ = 6.6 Hz, 2H), 2.43 (t, $J$ = 7.5 Hz, 4H), 1.94 (quin, $J$ = 6.3 Hz, 2H), 1.46 (sextet, $J$ = 7.5 Hz, 4H), 0.83 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 174.2, 164.0, 161.1, 158.9, 153.0, 150.9, 148.7, 140.3, 123.6, 121.9, 111.5, 110.9, 109.5, 95.9, 92.6, 70.8, 56.5, 56.2, 56.1, 56.0, 51.2, 47.7, 27.6, 19.6, 11.9. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{38}NO_7$ [M + H]: 500.2648; found 500.2637.

#### *4.3.5. 3-O-(N,N-Dipropylamino)butyl-3′,4′,5,7-O-tetramethylquercetin (**15**)*

Yield, 92%; yellow oil. IR (film) $\nu_{max}$: 2954, 1622, 1601, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.67 (s, 1H), 7.66 (d, $J$ = 11.1 Hz, 1H), 6.92 (d, $J$ = 8.4 Hz, 1H), 6.44 (s, 1H), 6.27 (s, 1H), 3.99 (t, $J$ = 6.6 Hz, 2H), 3.91 (s, 9H), 3.84 (s, 3H), 2.40 (t, $J$ = 7.2 Hz, 2H), 2.31 (t, $J$ = 7.5 Hz, 4H), 1.69 (quin, $J$ = 7.2 Hz, 2H), 1.52 (quin, $J$ = 6.9 Hz, 2H), 1.38 (sextet, $J$ = 7.5 Hz, 4H), 0.80 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 163.9, 161.0, 158.8, 152.7, 150.7, 148.5, 140.3, 123.6, 121.7, 111.4, 110.7, 109.4, 95.7, 92.4, 72.4, 56.4, 56.1, 56.0, 56.0, 55.8, 53.8, 28.4, 23.3, 20.0, 11.9. HR-MS (ESI) *m/z*: calcd for $C_{29}H_{40}NO_7$ [M + H]: 514.2805; found 514.2801.

#### *4.3.6. 3-O-(N,N-Dipropylamino)pentyl-3′,4′,5,7-O-tetramethylquercetin (**16**)*

Yield, 38%; yellow oil. IR (film) $\nu_{max}$: 2939, 1620, 1600, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (dd, $J$ = 8.7, 2.1 Hz, 1H), 7.65 (d, $J$ = 2.1 Hz, 1H), 6.99 (d, $J$ = 8.4 Hz, 1H), 6.50 (d, $J$ = 2.1 Hz, 1H), 6.34 (d, $J$ = 2.1 Hz, 1H), 3.97 (t, $J$ = 6.3 Hz, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 3.94 (s, 3H), 3.89 (s, 3H), 2.96–2.86 (overlapped, 6H), 1.87–1.74 (m, 8H), 1.53 (quin, $J$ = 7.5 Hz, 2H), 0.96 (t, $J$ = 7.5 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.1, 161.1, 159.0, 153.2, 151.1, 148.8, 140.2, 123.5, 122.0, 111.4, 111.0, 109.5, 96.0, 92.7, 71.5, 56.5, 56.3, 56.2, 56.0, 54.4, 52.6, 29.5, 23.7, 22.8, 17.3, 11.4. HR-MS (ESI) *m/z*: calcd for $C_{30}H_{42}NO_7$ [M + H]: 528.2961; found 528.2961.

#### *4.3.7. 3-O-(N,N-Dibutylamino)propyl-3′,4′,5,7-O-tetramethylquercetin (**17**)*

Overall yield for two steps, 33%; yellow syrup. IR (film) $\nu_{max}$: 3408, 2960, 1601, 1515 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.67 (d, $J$ = 8.7 Hz, 1H), 7.56 (s, 1H), 6.99 (d, $J$ = 8.7 Hz, 1H), 6.50 (d, $J$ = 1.8 Hz, 1H), 6.34 (d, $J$ = 1.5 Hz, 1H), 4.00–3.90 (overlapped, 2H), 3.94 (s, 9H), 3.88 (s, 3H), 3.46–3.41 (m, 2H), 3.04–2.96 (m, 4H), 2.36–2.21 (m, 2H), 1.76 (quin, $J$ = 7.5 Hz, 4H), 1.44–1.32 (m, 4H), 0.94 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.3, 161.0, 159.0, 153.7, 151.3, 149.0, 139.6, 122.9, 122.1, 111.2, 111.0, 109.2, 96.1, 92.7, 69.1, 56.6, 56.4, 56.2, 56.0, 52.8, 51.4, 25.5, 25.3, 20.3, 13.7. HR-MS (ESI) *m/z*: calcd for $C_{30}H_{42}NO_7$ [M + H]: 528.2961; found 528.2963.

#### *4.3.8. 3-O-(N,N-Dibutylamino)butyl-3′,4′,5,7-O-tetramethylquercetin (**18**)*

Yield, 68%; yellow wax. IR (film) $\nu_{max}$: 2932, 2870, 1622, 1601, 1513, 1489, 1457 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (s, 1H), 7.67 (d, $J$ = 9.3 Hz, 1H), 6.94 (d, $J$ = 8.4 Hz, 1H), 6.46 (s, 1H), 6.30 (s, 1H), 3.99 (t, $J$ = 6.6 Hz, 2H), 3.92 (s, 9H), 3.86 (s, 3H), 2.44 (t, $J$ = 7.2 Hz, 2H), 2.38 (t, $J$ = 7.8 Hz, 4H), 1.71 (quin, $J$ = 6.9 Hz, 2H), 1.54 (quin, $J$ = 6.9 Hz, 2H), 1.36 (quin, $J$ = 7.2 Hz, 4H), 1.24 (sextet, $J$ = 6.9 Hz, 4H), 0.86 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 163.9, 161.1, 158.9, 152.8, 150.8, 148.6, 140.4, 123.6, 121.8, 111.5, 110.8, 109.5, 95.8, 92.5, 72.4, 56.4, 56.1, 56.0, 55.8, 53.8, 53.7, 28.8, 28.4, 23.1, 20.8, 14.1. HR-MS (ESI) *m/z*: calcd for $C_{31}H_{44}NO_7$ [M + H]: 542.3118; found 542.3116.

#### *4.3.9. 3-O-(N,N-Dibutylamino)pentyl-3′,4′,5,7-O-tetramethylquercetin (**19**)*

Yield, 51%; yellow oil. IR (film) $\nu_{max}$: 2932, 2860, 1622, 1601,

1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (s, 1H), 7.67 (d, $J$ = 8.7 Hz, 1H), 6.95 (d, $J$ = 8.7 Hz, 1H), 6.47 (d, $J$ = 1.5 Hz, 1H), 6.32 (s, 1H), 3.99 (t, $J$ = 6.6 Hz, 2H), 3.94 (s, 9H), 3.88 (s, 3H), 2.42 (t, $J$ = 7.5 Hz, 6H), 1.71 (quin, $J$ = 6.9 Hz, 2H), 1.78 – 1.24 (m, 12H), 0.88 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 163.9, 161.1, 158.9, 152.8, 150.8, 148.6, 140.5, 123.7, 121.8, 111.6, 110.8, 109.6, 95.8, 92.5, 72.5, 56.5, 56.2, 56.1, 55.9, 54.0, 53.8, 30.3, 28.8, 26.4, 24.1, 20.8, 14.2. HR-MS (ESI) $m/z$: calcd for $C_{32}H_{46}NO_7$ [M + H]: 556.3274 ; found 556.3272.

#### 4.3.10. 3-O-(N,N-Dipentylamino)propyl-3′,4′,5,7-O-tetramethylquercetin (20)

Yield, 70%; yellow oil. IR (film) $\nu_{max}$: 2929, 2855, 1620, 1597, 1514, 1458 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (dd, $J$ = 8.7, 2.1 Hz, 1H), 7.58 (d, $J$ = 1.8 Hz, 1H), 7.02 (d, $J$ = 8.7 Hz, 1H), 6.53 (d, $J$ = 2.4 Hz, 1H), 6.37 (d, $J$ = 2.1 Hz, 1H), 4.04 (t, $J$ = 4.2 Hz, 2H), 3.98 (s, 9H), 3.91 (s, 3H), 3.56–3.45 (m, 2H), 3.08–2.99 (m, 4H), 2.42–2.31 (m, 2H), 1.89–1.77 (m, 4H), 1.41–1.20 (m, 8H), 0.91 (t, $J$ = 6.6 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.0, 161.2, 159.0, 152.9, 150.9, 148.7, 140.4, 123.7, 121.9, 111.5, 110.8, 109.6, 95.9, 92.6, 71.1, 56.6, 56.2, 56.1, 55.9, 54.2, 51.2, 29.9, 28.0, 26.5, 22.8, 14.2. HR-MS (ESI) $m/z$: calcd for $C_{32}H_{46}NO_7$ [M + H]: 556.3274; found 556.3273.

#### 4.3.11. 3-O-(N,N-Dipentylamino)butyl-3′,4′,5,7-O-tetramethylquercetin (21)

Yield, 54%; yellow oil. IR (film) $\nu_{max}$: 2931, 1624, 1603, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (d, $J$ = 1.5 Hz, 1H), 7.65 (dd, $J$ = 8.4, 1.8 Hz, 1H), 6.91 (d, $J$ = 8.4 Hz, 1H), 6.43 (d, $J$ = 1.8 Hz, 1H), 6.27 (d, $J$ = 2.1 Hz, 1H), 3.99 (t, $J$ = 6.9 Hz, 2H), 3.91 (s, 6H), 3.89 (s, 3H), 3.84 (s, 3H), 2.36 (t, $J$ = 7.2 Hz, 2H), 2.31 (t, $J$ = 7.8 Hz, 4H), 1.69 (quin, $J$ = 7.2 Hz, 2H), 1.48 (quin, $J$ = 6.0 Hz, 2H), 1.35 (quin, $J$ = 7.5 Hz, 4H), 1.28–1.15 (overlapped, 8H), 0.83 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 163.9, 161.0, 158.8, 152.6, 150.7, 148.6, 140.4, 123.6, 121.7, 111.5, 110.7, 109.4, 95.7, 92.4, 72.5, 56.4, 56.1, 56.0, 55.8, 54.1. 53.9, 29.8, 28.4, 26.6, 23.3, 22.7, 14.2. HR-MS (ESI) $m/z$: calcd for $C_{33}H_{48}NO_7$ [M + H]: 570.3431; found 570.3429.

#### 4.3.12. 3-O-(N,N-Dipentylamino)pentyl-3′,4′,5,7-O-tetramethylquercetin (22)

Yield, 45%; yellow oil. IR (film) $\nu_{max}$: 2931, 1624, 1603, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (s, 1H), 7.69 (dd, $J$ = 8.7, 2.1 Hz, 1H), 6.97 (d, $J$ = 8.7 Hz, 1H), 6.49 (d, $J$ = 2.1 Hz, 1H), 6.34 (d, $J$ = 2.1 Hz, 1H), 4.00 (t, $J$ = 6.3 Hz, 2H), 3.96 (s, 3H), 3.95 (s, 6H), 3.89 (s, 3H), 2.63–2.50 (overlapped, 6H), 1.75 (quin, $J$ = 7.5 Hz, 2H), 1.62–1.38 (overlapped, 6H), 1.34–1.18 (overlapped, 10*H*), 0.88 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.0, 161.2, 159.0, 152.9, 150.9, 148.7, 140.4, 123.7, 121.9, 111.6, 110.9, 109.6, 95.9, 92.6, 72.3, 56.5, 56.3, 56.1, 55.9, 53.7, 53.6, 31.1, 30.2, 29.7, 25.5, 24.0, 22.7, 14.2. HR-MS (ESI) $m/z$: calcd for $C_{34}H_{50}NO_7$ [M + H]: 584.3587; found 584.3586.

#### 4.3.13. 3-O-Morpholinopropyl-3′,4′,5,7-O-tetramethylquercetin (23)

Overall yield for two steps, 36%; yellow solid. IR (film) $\nu_{max}$: 2937, 2847, 1621, 1600, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.68 (dd, $J$ = 8.7, 2.1 Hz, 1H), 7.66 (s, 1H), 6.97 (d, $J$ = 8.4 Hz, 1H), 6.50 (d, $J$ = 2.4 Hz, 1H), 6.35 (d, $J$ = 2.4 Hz, 1H), 4.04 (t, $J$ = 6.6 Hz, 2H), 3.95 (s, 9H), 3.90 (s, 3H), 3.76 (t, $J$ = 3.6 Hz, 4H), 2.77–2.63 (m, 2H), 2.63–2.48 (m, 4H), 2.00 (quin, $J$ = 6.9 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 174.2, 164.1, 161.2, 159.0, 153.2, 151.0, 148.8, 140.2, 123.5, 122.0, 111.5, 110.9, 109.5, 96.0, 92.6, 70.4, 66.4, 56.6, 56.3, 56.2, 56.0, 56.0, 53.5, 27.0. HR-MS (ESI) $m/z$: calcd for $C_{26}H_{32}NO_8$ [M + H]: 486.2128; found 486.2127.

#### 4.3.14. 3-O-Morpholinobutyl-3′,4′,5,7-O-tetramethylquercetin (24)

Yield, 89%; yellow oil. IR (film) $\nu_{max}$: 2943, 1623, 1602, 1514, 1456 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.64 (d, $J$ = 9.0 Hz, 1H), 7.60 (s, 1H), 6.95 (d, $J$ = 8.4 Hz, 1H), 6.46 (d, $J$ = 1.2 Hz, 1H), 6.31 (s, 1H), 4.00–3.85 (overlapped, 6H), 3.91 (s, 9H), 3.86 (s, 3H), 2.99 (t, $J$ = 6.3 Hz, 2H), 2.99–2.83 (m 4H), 1.95 (quin, $J$ = 5.8 Hz, 2H), 1.76 (quin, $J$ = 6.0 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.1, 160.9, 158.9, 153.3, 151.0, 148.7, 139.9, 123.2, 121.9, 111.2, 110.9, 109.3, 95.9, 92.6, 71.0, 64.8, 57.8, 56.5, 56.2, 56.1, 55.9, 52.4, 27.5, 21.3. HR-MS (ESI) $m/z$: calcd for $C_{27}H_{34}NO_8$ [M + H]: 500.2284; found 500.2275.

#### 4.3.15. 3-O-Morpholinopentyl-3′,4′,5,7-O-tetramethylquercetin (25)

Yield, 58%; yellow oil. IR (film) $\nu_{max}$: 2937, 2857, 1621, 1600, 1512 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (s, 1H), 7.66 (d, $J$ = 8.4 Hz, 1H), 6.94 (d, $J$ = 8.4 Hz, 1H), 6.47 (s, 1H), 6.31 (s, 1H), 3.99 (t, $J$ = 6.3 Hz, 2H), 3.93 (s, 9H), 3.87 (s, 3H), 3.75–3.64 (m, 4H), 2.50–2.33 (m, 4H), 2.28 (t, $J$ = 6.3 Hz, 2H), 1.72 (quin, $J$ = 6.9 Hz, 2H), 1.56–1.34 (m, 4H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.0, 161.1, 158.9, 152.9, 150.8, 148.6, 140.4, 123.7, 121.8, 111.6, 110.8, 109.5, 95.8, 92.5, 72.3, 66.9, 59.1, 56.5, 56.2, 56.1, 55.9, 53.8, 30.3, 26.2, 24.0. HR-MS (ESI) $m/z$: calcd for $C_{28}H_{36}NO_8$ [M + H]: 514.2441; found 514.2447.

#### 4.3.16. 3-O-Piperidinopropyl-3′,4′,5,7-O-tetramethylquercetin (26)

Yield for two steps, 80%; yellow/orange solid. IR (film) $\nu_{max}$: 2935, 1624, 1600, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.65 (d, $J$ = 5.7 Hz, 1H), 7.64 (s, 1H), 6.93 (d, $J$ = 9.0 Hz, 1H), 6.45 (s, 1H), 6.29 (s, 1H), 4.03 (t, $J$ = 6.3 Hz, 2H), 3.92 (s, 9H), 3.86 (s, 3H), 2.45 (t, $J$ = 7.2 Hz, 2H), 2.38–2.28 (m, 4H), 1.90 (quin, $J$ = 6.3 Hz, 2H), 1.56 (quin, $J$ = 5.1 Hz, 4H), 1.45–1.31 (m, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 174.1, 163.9, 161.0, 158.9, 152.9, 150.8, 148.6, 140.2, 123.5, 121.9, 111.5, 110.8, 109.5, 95.8, 92.5, 71.0, 56.4, 56.4, 56.2, 56.0, 55.8, 54.6, 27.6, 25.8, 24.3. HR-MS (ESI) $m/z$: calcd for $C_{27}H_{34}NO_7$ [M + H]: 484.2335; found 484.2331.

#### 4.3.17. 3-O-Piperidinobutyl-3′,4′,5,7-O-tetramethylquercetin (27)

Yield, 29%; yellow oil. IR (film) $\nu_{max}$: 2945, 1622, 1601, 1514, 1457 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.66 (d, $J$ = 8.7 Hz, 1H), 7.62 (s, 1H), 6.97 (d, $J$ = 8.7 Hz, 1H), 6.48 (s, 1H), 6.32 (s, 1H), 4.00–3.90 (overlapped, 2H), 3.92 (s, 9H), 3.87 (s, 3H), 2.93 (t, $J$ = 8.1 Hz, 2H), 2.90 (t, $J$ = 6.6 Hz, 4H), 2.04–1.73 (m, 10*H*). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.1, 161.0, 158.9, 153.3, 151.1, 148.8, 140.0, 123.3, 122.0, 111.3, 111.0, 109.4, 96.0, 92.6, 71.2, 57.7, 56.5, 56.3, 56.1, 55.9, 53.5, 27.7, 23.6, 22.9, 21.5. HR-MS (ESI) $m/z$: calcd for $C_{28}H_{36}NO_7$ [M + H]: 498.2492; found 498.2490.

#### 4.3.18. 3-O-Piperidinopentyl-3′,4′,5,7-O-tetramethylquercetin (28)

Yield, 40%; yellow oil. IR (film) $\nu_{max}$: 2934, 1623, 1602, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (s, 1H), 7.66 (d, $J$ = 9.0 Hz, 1H), 6.95 (d, $J$ = 8.1 Hz, 1H), 6.47 (s, 1H), 6.31 (s, 1H), 3.97 (t, $J$ = 6.3 Hz, 2H), 3.93 (s, 9H), 3.87 (s, 3H), 2.56–2.42 (m, 4H), 2.37 (t, $J$ = 7.5 Hz, 2H), 1.74–1.54 (m, 8H), 1.41–1.35 (m, 4H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 163.8, 161.0, 158.8, 152.8, 150.7, 148.5, 140.3, 123.5, 121.7, 111.4, 110.7, 109.4, 95.7, 92.4, 72.1, 58.9, 56.4, 56.1, 56.0, 55.8, 54.2, 30.0, 25.7, 25.1, 23.94, 23.88. HR-MS (ESI) $m/z$: calcd for $C_{29}H_{38}NO_7$ [M + H]: 512.2648; found 512.2643.

#### 4.3.19. 3-O-(4-Methylpiperazin-1-yl)propyl-3′,4′,5,7-O-tetramethylquercetin (29)

52% overall yield for two steps as a yellow wax. IR (film) $\nu_{max}$: 2938, 2796, 2360, 1624, 1602, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.65 (d, $J$ = 6.6 Hz, 1H), 7.64 (s, 1H), 6.94 (d, $J$ = 9.0 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.31 (d, $J$ = 2.1 Hz, 1H), 4.02 (t, $J$ = 6.3 Hz, 2H), 3.93 (s, 9H), 3.87 (s, 3H), 2.68–2.66 (m, 2H), 2.53–2.46 (m, 8H), 1.90 (quin, $J$ = 6.3 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 174.2, 164.0, 161.1, 158.9, 153.0, 150.9, 148.7, 140.3, 123.6, 121.9, 111.5, 110.8, 109.5, 95.9, 92.5, 70.8, 56.5, 56.2, 56.1, 55.9, 55.3, 54.9, 53.0, 45.9,

27.7. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{35}N_2O_7$ [M + H]: 499.2444; found 499.2437.

#### 4.3.20. 3-O-(4-Methylpiperazin-1-yl)butyl-3′,4′,5,7-O-tetramethylquercetin (30)

Yield, 76%; yellow oil. IR (film) $\nu_{max}$: 2936, 2801, 1621, 1601, 1513, 1456, 1421 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.67 (s, 1H), 7.65 (d, $J$ = 9.0 Hz, 1H), 6.93 (d, $J$ = 8.1 Hz, 1H), 6.46 (s, 1H), 6.30 (s, 1H), 3.99 (t, $J$ = 6.3 Hz, 2H), 3.92 (s, 9H), 3.86 (s, 3H), 2.56–2.38 (m, 8H), 2.33 (t, $J$ = 7.5 Hz, 2H), 2.26 (s, 3H), 1.70 (quin, $J$ = 6.9 Hz, 2H), 1.57 (quin, $J$ = 6.3 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.0, 161.1, 158.9, 152.9, 150.8, 148.6, 140.3, 123.6, 121.8, 111.6, 110.8, 109.5, 95.8, 92.5, 72.2, 58.2, 56.5, 56.2, 56.1, 55.9, 54.9, 52.9, 45.9, 28.3, 23.2. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{37}N_2O_7$ [M + H]: 513.2601; found 513.2601.

#### 4.3.21. 3-O-(4-Methylpiperazin-1-yl)pentyl-3′,4′,5,7-O-tetramethylquercetin (31)

Yield, 53%; yellow oil. IR (film) $\nu_{max}$: 2937, 2803, 1622, 1602, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.67 (s, 1H), 7.64 (d, $J$ = 8.7 Hz, 1H), 6.93 (d, $J$ = 8.4 Hz, 1H), 6.45 (d, $J$ = 1.8 Hz, 1H), 6.29 (s, 1H), 3.96 (t, $J$ = 6.6 Hz, 2H), 3.91 (s, 9H), 3.85 (s, 3H), 2.62–2.57 (m, 8H), 2.31 (t, $J$ = 7.5 Hz, 2H), 2.29 (s, 3H), 1.70 (quin, $J$ = 7.2 Hz, 2H), 1.53–1.30 (m, 4H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 163.9, 161.0, 158.9, 152.8, 150.8, 148.6, 140.3, 123.6, 121.7, 111.5, 110.7, 109.5, 95.8, 92.5, 72.2, 58.4, 56.4, 56.1, 55.8, 54.6, 52.7, 45.7, 30.2, 26.4, 24.0. HR-MS (ESI) *m/z*: calcd for $C_{29}H_{39}N_2O_7$ [M + H]: 527.2757; found 527.2757.

#### 4.3.22. 3-O-Pyrrolidinopropyl-3′,4′,5,7-O-tetramethylquercetin (32)

Overall yield for two steps, 27%; yellow wax. IR (film) $v_{max}$: 2935, 2793, 1622, 1600. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.67 (dd, $J$ = 6.9, 2.1 Hz, 1H), 7.65 (s, 1H), 6.95 (d, $J$ = 9.0 Hz, 1H), 6.48 (d, $J$ = 2.4 Hz, 1H), 6.32 (d, $J$ = 2.4 Hz, 1H), 4.02 (t, $J$ = 6.0 Hz, 2H), 3.939 (s, 3H), 3.936 (s, 3H), 3.93 (s, 3H), 3.88 (s, 3H), 2.74 (t, $J$ = 9.0, 2H), 2.66–2.55 (m, 4H), 1.99 (quin, $J$ = 6.6 Hz, 2H), 1.80 (quin, $J$ = 3.6 Hz, 4H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 174.2, 164.0, 161.1, 158.9, 153.1, 150.9, 148.7, 140.2, 123.5, 121.9, 111.5, 110.9, 109.5, 95.9, 92.6, 70.6, 56.5, 56.3, 56.1, 55.9, 54.2, 53.4, 29.4, 23.5. HR-MS (ESI) *m/z*: calcd for $C_{26}H_{32}NO_7$ [M + H]: 470.2179; found 470.2179.

#### 4.3.23. 3-O-Pyrrolidinobutyl-3′,4′,5,7-O-tetramethylquercetin (33)

Yield, 98%; yellow oil. IR (film) $\nu_{max}$: 2937, 2794, 1621, 1600, 1512 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.64 (s, 1H), 7.63 (d, $J$ = 7.8 Hz, 1H), 6.91 (d, $J$ = 8.1 Hz, 1H), 6.42 (s, 1H), 6.26 (s, 1H), 3.97 (t, $J$ = 6.0 Hz, 2H), 3.89 (s, 9H), 3.83 (s, 3H), 2.50–2.34 (m, 6H), 1.79–1.49 (m, 8H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 163.9, 160.9, 158.8, 152.7, 150.7, 148.5, 140.3, 123.5, 121.7, 111.4, 110.7, 109.4, 95.7, 92.4, 72.2, 56.4, 56.2, 56.1, 56.0, 55.8, 54.1, 28.4, 25.3, 23.4. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{34}NO_7$ [M + H]: 484.2335; found 484.2332.

#### 4.3.24. 3-O-Pyrrolidinopentyl-3′,4′,5,7-O-tetramethylquercetin (34)

Yield, 80%; yellow oil. IR (film) $\nu_{max}$: 2938, 1625, 1604, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (dd, $J$ = 9.9, 2.1 Hz, 1H), 7.68 (s, 1H), 6.99 (d, $J$ = 8.1 Hz, 1H), 6.50 (d, $J$ = 2.4 Hz, 1H), 6.34 (d, $J$ = 2.4 Hz, 1H), 3.98 (t, $J$ = 6.0 Hz, 2H), 3.98 (s, 3H), 3.97 (s, 3H), 3.96 (s, 3H), 3.90 (s, 3H), 3.12–2.98 (m, 4H), 2.89–2.74 (m, 2H), 2.02–1.97 (m, 4H), 1.82–1.72 (m, 4H), 1.56–1.45 (m, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 163.9, 160.9, 158.8, 152.9, 150.8, 148.6, 140.2, 123.4, 121.8, 111.4, 110.8, 109.4, 95.8, 92.5, 71.7, 56.4, 56.1, 56.0, 55.8, 55.8, 53.7, 29.7, 26.5, 23.6, 23.4. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{36}NO_7$ [M + H]: 498.2492; found 498.2493.

### 4.4. General procedure for the synthesis of 5-O-bromoalkyl-3,3′,4′,7-tetramethylquercetins (35-37)

The solution of 3,3′,4′,7-*O*-tetramethylquercetin (60 mg, 0.17 mmol) and potassium carbonate (72 mg, 0.52 mmol, 3 equiv.) in DMF (0.5 mL, 0.3 M) was stirred at room temperature for 10 min before an appropriate alkyl dibromide (0.52 mmol, 3 equiv.) was added through a syringe. The reaction was stirred at room temperature for three days at room temperature (or for overnight at 70 °C). The reaction mixture was diluted with a mixture of ethyl acetate and diethyl ether (100 mL, 1:1, v/v), which was rinsed with water (10 mL × 1) and brine (10 mL × 4). The subsequent organic layer was dried over anhydrous sodium sulfate, and concentrated in vacuo to generate a crude product, which was subjected to PTLC purification eluting with 10% methanol in dichloromethane. The desired compound was retrieved from PTLC by washing with ethyl acetate.

#### 4.4.1. 5-O-(3-Bromopropyl)-3,3′,4′,7-O-tetramethylquercetin (35)

Yield, 73%; yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (dd, $J$ = 7.2, 2.1 Hz, 1H), 7.68 (s, 1H), 6.97 (d, $J$ = 9.0 Hz, 1H), 6.50 (d, $J$ = 2.4 Hz, 1H), 6.34 (d, $J$ = 2.4 Hz, 1H), 4.19 (t, $J$ = 5.7 Hz, 2H), 3.952 (s, 3H), 3.949 (s, 3H), 3.89 (s, 3H), 3.82 (s, 3H), 2.43 (t, $J$ = 6.0 Hz, 2H).

#### 4.4.2. 5-O-(4-Bromobutyl)-3,3′,4′,7-O-tetramethylquercetin (36)

Yield, 58%; yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 7.2, 2.1 Hz, 1H), 7.67 (s, 1H), 6.95 (d, $J$ = 9.0 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.29 (d, $J$ = 2.1 Hz, 1H), 4.07 (t, $J$ = 6.0 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.54 (t, $J$ = 6.3 Hz, 2H), 2.25–2.16 (m, 2H), 2.11–2.06 (m, 2H).

#### 4.4.3. 5-O-(5-Bromopentyl)-3,3′,4′,7-O-tetramethylquercetin (37)

Yield, 58%; yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.71 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.70 (d, $J$ = 2.1 Hz, 1H), 6.98 (d, $J$ = 9.0 Hz, 1H), 6.50 (d, $J$ = 2.4 Hz, 1H), 6.32 (d, $J$ = 2.1 Hz, 1H), 4.08 (t, $J$ = 6.3 Hz, 2H), 3.96 (s, 6H), 3.90 (s, 3H), 3.84 (s, 3H), 3.47 (t, $J$ = 6.9 Hz, 2H), 1.98 (quin, $J$ = 7.5 Hz, 4H), 1.74 (quin, $J$ = 6.6 Hz, 2H).

### 4.5. The general procedure for the synthesis of 5-O-dialkylaminoalkyl-3,3′,4′,7-O-tetramethylquercetins (38-61)

To the solution of the appropriate 5-*O*-bromoalkyl-3,3′,4′,7-*O*-tetramethylquercetin (0.17 mmol) in DMF (0.5 mL, 0.3 M) was added potassium carbonate (70 mg, 0.51 mmol, 3 equiv.), and the mixture was stirred for 10 min. The appropriate amine (0.51 mmol, 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 reaction mixture was diluted with a mixture of ethyl acetate and diethyl ether (100 mL, 1:1, v/v), which was rinsed with water (10 mL × 1) and brine (10 mL × 4). The subsequent organic layer was dried over anhydrous sodium sulfate, and concentrated in vacuo to generate a crude product, which was subjected to PTLC purification using dichloromethane/methanol (90/10, v/v) as eluent. The respective 5-*O*-dialkylaminoalkyl-3,3′,4′,7-*O*-tetramethylquercetin was retrieved from PTLC silica gel by washing with dichloromethane/diethylamine (100/3, v/v).

#### 4.5.1. 5-O-(N,N-Diethylamino)propyl-3,3′,4′,7-O-tetramethylquercetin (38)

Yield, 40%; yellow wax. IR (film) $\nu_{max}$: 2939, 2837, 1625, 1603, 1515 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 6.9, 2.1 Hz, 1H), 7.67 (s, 1H), 6.96 (d, $J$ = 9.0 Hz, 1H), 6.49 (d, $J$ = 2.1 Hz, 1H), 6.33 (d, $J$ = 2.1 Hz, 1H), 4.17 (t, $J$ = 5.7 Hz, 2H), 3.93 (s, 6H), 3.87 (s, 3H), 3.79 (s, 3H), 3.22–3.09 (m, 2H), 3.00–2.83 (m, 4H), 2.34–2.21 (m, 2H), 1.22 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.2, 159.9, 158.8, 153.2, 151.1, 148.8, 141.1, 123.3, 121.8, 111.3,

111.0, 109.4, 96.8, 93.0, 67.6, 60.1, 56.2, 56.1, 56.0, 49.9, 47.5, 25.4, 10.4. HR-MS (ESI) *m/z*: calcd for $C_{26}H_{34}NO_7$ [M + H]: 472.2335; found 472.2334.

#### 4.5.2. 5-O-(N,N-Diethylamino)butyl-3,3′,4′,7-O-tetramethylquercetin (39)

Yield, 92%; brown syrup. IR (film) $\nu_{max}$: 2934, 2836, 1623, 1601, 1514, 1438 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.67 (d, $J$ = 2.1 Hz, 1H), 6.95 (d, $J$ = 9.0 Hz, 1H), 6.46 (d, $J$ = 2.1 Hz, 1H), 6.30 (d, $J$ = 2.4 Hz, 1H), 4.06 (t, $J$ = 6.3 Hz, 2H), 3.933 (s, 3H), 3.931 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H), 2.70–2.62 (overlapped, 6H), 1.95 (quin, $J$ = 6.6 Hz, 2H), 1.85–1.76 (m, 2H), 1.08 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 163.9, 160.4, 158.9, 152.7, 150.9, 148.8, 141.2, 123.5, 121.7, 111.3, 110.9, 109.7, 96.8, 92.6, 69.3, 60.1, 56.2, 56.1, 55.9, 52.4, 46.8, 27.0, 23.0, 11.1. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{36}NO_7$ [M + H]: 486.2492; found 486.2491.

#### 4.5.3. 5-O-(N,N-Diethylamino)pentyl-3,3′,4′,7-O-tetramethylquercetin (40)

Yield, 86%; yellow oil. IR (film) $\nu_{max}$: 2924, 2853, 1625, 1602, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.61 (dd, $J$ = 9.0, 1.8 Hz, 1H), 7.67 (s, 1H), 6.95 (d, $J$ = 9.3 Hz, 1H), 6.47 (d, $J$ = 2.4 Hz, 1H), 6.30 (d, $J$ = 2.1 Hz, 1H), 4.05 (t, $J$ = 6.3 Hz, 2H), 3.94 (s, 6H), 3.85 (s, 3H), 3.81 (s, 3H), 2.81–2.67 (overlapped, 6H), 1.98–1.93 (m, 2H), 1.73–1.60 (m, 4H), 1.15 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.5, 158.9, 152.8, 150.9, 148.8, 141.2, 123.5, 121.8, 111.3, 110.9, 109.7, 96.7, 92.6, 69.3, 60.1, 56.2, 56.1, 55.9, 52.3, 46.7, 28.7, 25.2, 24.1, 10.4. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{38}NO_7$ [M + H]: 500.2648; found 500.2642.

#### 4.5.4. 5-O-(N,N-Dipropylamino)propyl-3,3′,4′,7-O-tetramethylquercetin (41)

Yield, 88%; brown syrup. IR (film) $\nu_{max}$: 2957, 2872, 1623, 1601, 1514, 1439 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.69 (d, $J$ = 1.8 Hz, 1H), 6.96 (d, $J$ = 9.3 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 4.13 (t, $J$ = 6.3 Hz, 2H), 3.94 (s, 6H), 3.87 (s, 3H), 3.82 (s, 3H), 2.84 (t, $J$ = 6.9 Hz, 2H), 2.50 (t, $J$ = 6.9 Hz, 4H), 2.13 (quin, $J$ = 6.6 Hz, 2H), 1.53 (quin, $J$ = 7.8 Hz, 4H), 0.85 (t, $J$ = 7.5 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.5, 158.8, 152.7, 150.9, 148.8, 141.2, 123.5, 121.8, 111.3, 110.9, 109.6, 96.7, 92.6, 67.7, 60.1, 56.2, 56.2, 56.1, 55.9, 50.6, 26.4, 19.8, 12.0. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{38}NO_7$ [M + H]: 500.2648; found 500.2636.

#### 4.5.5. 5-O-(N,N-Dipropylamino)butyl-3,3′,4′,7-O-tetramethylquercetin (42)

Yield, 81%; yellow wax. IR (film) $\nu_{max}$: 2933, 2872, 1623, 1601, 1514, 1439 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (d, $J$ = 6.3 Hz, 1H), 7.68 (s, 1H), 6.97 (d, $J$ = 8.7 Hz, 1H), 6.48 (s, 1H), 6.31 (s, 1H), 4.08 (t, $J$ = 5.7 Hz, 2H), 3.95 (s, 6H), 3.88 (s, 3H), 3.83 (s, 3H), 2.79–2.66 (m, 2H), 2.54 (t, $J$ = 6.6 Hz, 4H), 2.00–1.90 (m, 2H), 1.90–1.78 (m, 2H), 1.61–1.45 (m, 4H), 0.88 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.5, 158.9, 152.7, 150.9, 148.8, 141.3, 123.6, 121.8, 111.3, 110.9, 109.8, 96.8, 92.7, 69.3, 60.1, 56.2, 56.1, 55.9, 55.7, 53.5, 26.9, 22.9, 19.4, 12.0. HR-MS (ESI) *m/z*: calcd for $C_{29}H_{40}NO_7$ [M + H]: 514.2805; found 514.2800.

#### 4.5.6. 5-O-(N,N-Dipropylamino)pentyl-3,3′,4′,7-O-tetramethylquercetin (43)

Yield, 84%; yellow oil. IR (film) $\nu_{max}$: 2937, 1622, 1600, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 9.3, 2.1 Hz, 1H), 7.66 (s, 1H), 6.95 (d, $J$ = 9.3 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.30 (d, $J$ = 2.4 Hz, 1H), 4.05 (t, $J$ = 6.3 Hz, 2H), 3.934 (s, 3H), 3.931 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 2.72 (t, $J$ = 7.5 Hz, 2H), 2.63 (t, $J$ = 8.1 Hz, 4H), 1.95 (quin, $J$ = 7.2 Hz, 2H), 1.77–1.55 (m, 8H), 0.89 (t, $J$ = 7.5 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.5, 158.9, 152.8, 150.9, 148.8, 141.2, 123.5, 121.7, 111.3, 110.9, 109.7, 96.8, 92.6, 69.3, 60.1, 56.2, 56.1, 55.9, 55.3, 53.5, 28.6, 25.2, 24.0, 18.8, 11.8. HR-MS (ESI) *m/z*: calcd for $C_{30}H_{42}NO_7$ [M + H]: 528.2961; found 528.2960.

#### 4.5.7. 5-O-(N,N-Dibutylamino)propyl-3,3′,4′,7-O-tetramethylquercetin (44)

Yield, 17%; yellow syrup. IR (film) $\nu_{max}$: 2954, 2930, 2870, 1624, 1601, 1514, 1489 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.70 (d, $J$ = 1.8 Hz, 1H), 6.98 (d, $J$ = 9.3 Hz, 1H), 6.48 (d, $J$ = 2.4 Hz, 1H), 6.35 (d, $J$ = 2.1 Hz, 1H), 4.13 (t, $J$ = 6.6 Hz, 2H), 3.96 (s, 6H), 3.89 (s, 3H), 3.84 (s, 3H), 2.84–2.72 (m, 2H), 2.55–2.43 (m, 4H), 2.17–2.07 (m, 2H), 1.50–1.40 (m, 4H), 1.33–1.21 (m, 4H), 0.87 (t, $J$ = 7.2 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.2, 159.7, 158.8, 153.3, 151.2, 148.9, 141.2, 123.4, 121.9, 111.3, 111.0, 109.4, 96.9, 93.3, 67.0, 60.1, 56.3, 56.2, 56.1, 53.4, 51.2, 25.9, 24.9, 20.5, 13.9. HR-MS (ESI) *m/z*: calcd for $C_{30}H_{42}NO_7$ [M + H]: 528.2961; found 528.2958.

#### 4.5.8. 5-O-(N,N-Dibutylamino)butyl-3,3′,4′,7-O-tetramethylquercetin (45)

Yield, 72%; yellow wax. IR (film) $\nu_{max}$: 2931, 2870, 1624, 1602, 1514, 1438 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70–7.67 (overlapped, 2H), 6.96 (d, $J$ = 9.0 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.31 (d, $J$ = 1.8 Hz, 1H), 4.07 (t, $J$ = 6.6 Hz, 2H), 3.94 (s, 6H), 3.87 (s, 3H), 3.82 (s, 3H), 2.61 (t, $J$ = 7.2 Hz, 2H), 2.49 (t, $J$ = 7.5 Hz, 4H), 1.95 (quin, $J$ = 7.2 Hz, 2H), 1.76 (quin, $J$ = 6.9 Hz, 2H), 1.42 (quin, $J$ = 7.5 Hz, 4H), 1.28 (sextet, $J$ = 7.5 Hz, 4H), 0.88 (t, $J$ = 7.5 Hz, 4H), 0.88 (t, $J$ = 7.5 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 163.9, 160.5, 158.9, 152.7, 150.9, 148.8, 141.2, 123.6, 121.7, 111.3, 110.9, 109.8, 96.8, 92.6, 69.4, 60.1, 56.2, 56.1, 55.9, 53.7, 53.6, 28.7, 27.0, 23.1, 20.8, 14.2. HR-MS (ESI) *m/z*: calcd for $C_{31}H_{44}NO_7$ [M + H]: 542.3118; found 542.3118.

#### 4.5.9. 5-O-(N,N-Dibutylamino)pentyl-3,3′,4′,7-O-tetramethylquercetin (46)

Yield, 80%; yellow oil. IR (film) $\nu_{max}$: 2934, 2872, 1621, 1600, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 6.6, 2.1 Hz, 1H), 7.67 (s, 1H), 6.95 (d, $J$ = 9.0 Hz, 1H), 6.46 (d, $J$ = 2.1 Hz, 1H), 6.30 (d, $J$ = 2.4 Hz, 1H), 4.05 (t, $J$ = 6.6 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 2.64 (t, $J$ = 6.9 Hz, 2H), 2.59 (t, $J$ = 7.5 Hz, 4H), 1.51 (quin, $J$ = 8.4 Hz, 2H), 1.95–1.47 (m, 8H), 1.29 (sextet, $J$ = 7.5 Hz, 4H), 0.89 (t, $J$ = 7.5 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.5, 158.9, 152.7, 150.9, 148.8, 141.2, 123.5, 121.7, 111.3, 110.9, 109.7, 96.7, 92.6, 69.4, 60.1, 56.2, 56.1, 55.9, 53.6, 53.4, 28.7, 27.8, 25.6, 24.1, 20.7, 10.0. HR-MS (ESI) *m/z*: calcd for $C_{32}H_{46}NO_7$ [M + H]: 556.3274; found 556.3269.

#### 4.5.10. 5-O-(N,N-Dipentylamino)propyl-3,3′,4′,7-O-tetramethylquercetin (47)

Yield, 9%; brown wax. IR (film) $\nu_{max}$: 2929, 2858, 1623, 1601, 1515, 1439 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.70 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.70 (s, 1H), 6.98 (d, $J$ = 9.3 Hz, 1H), 6.51 (d, $J$ = 2.4 Hz, 1H), 6.35 (d, $J$ = 2.4 Hz, 1H), 4.18 (t, $J$ = 5.8 Hz, 2H), 3.96 (s, 6H), 3.89 (s, 3H), 3.82 (s, 3H), 2.82–2.66 (m, 4H), 2.35–2.21 (m, 2H), 1.71–1.56 (m, 4H), 1.35–1.24 (m, 12H), 0.89 (t, $J$ = 6.6 Hz, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.2, 160.0, 158.9, 153.1, 151.1, 148.9, 141.2, 123.4, 121.9, 111.4, 111.0, 109.5, 96.8, 93.0, 67.3, 60.1, 56.2, 56.1, 56.0, 53.9, 50.9, 31.1, 29.9, 29.5, 22.6, 14.1. HR-MS (ESI) *m/z*: calcd for $C_{32}H_{46}NO_7$ [M + H]: 556.3274; found 556.3271.

#### 4.5.11. 5-O-(N,N-Dipentylamino)butyl-3,3′,4′,7-O-tetramethylquercetin (48)

Yield, 96%; brown wax. IR (film) $\nu_{max}$: 2929, 2857, 1623, 1601, 1514, 1439 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.68 (d, $J$ = 2.1 Hz, 1H), 6.96 (d, $J$ = 9.0 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.32 (d, $J$ = 2.1 Hz, 1H), 4.07 (t, $J$ = 6.6 Hz, 2H), 3.95

(s, 3H), 3.94 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 2.56 (t, $J$ = 7.2 Hz, 2H), 2.44 (t, $J$ = 7.8 Hz, 4H), 1.95 (quin, $J$ = 6.3 Hz, 2H), 1.73 (quin, $J$ = 6.9 Hz, 2H), 1.44 (quin, $J$ = 7.2 Hz, 4H), 1.33–1.16 (m, 8H), 0.86 (t, $J$ = 6.6 Hz, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.5, 158.9, 152.7, 150.9, 148.8, 141.3, 123.6, 121.8, 111.4, 110.9, 109.8, 96.8, 92.6, 69.4, 60.1, 56.2, 56.1, 55.9, 53.9, 53.5, 29.8, 27.0, 26.7, 22.8, 22.7, 14.2. HR-MS (ESI) *m/z*: calcd for $C_{33}H_{48}NO_7$ [M + H]: 570.3431; found 570.3430.

#### 4.5.12. 5-O-(N,N-Dipentylamino)pentyl-3,3′,4′,7-O-tetramethylquercetin (49)

Yield, 18%; yellow oil. IR (film) $\nu_{max}$: 2930, 2858, 1623, 1602, 1514 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.71 (dd, $J$ = 9.3, 2.1 Hz, 1H), 7.71 (d, $J$ = 2.1 Hz, 1H), 6.99 (d, $J$ = 9.0 Hz, 1H), 6.50 (d, $J$ = 2.4 Hz, 1H), 6.34 (d, $J$ = 2.4 Hz, 1H), 4.08 (t, $J$ = 6.3 Hz, 2H), 3.97 (s, 6H), 3.90 (s, 3H), 3.85 (s, 3H), 2.62–2.53 (overlapped, 6H), 1.98 (quin, $J$ = 6.9 Hz, 2H), 1.72–1.47 (m, 8H), 1.34–1.24 (m, 8H), 0.89 (t, $J$ = 7.2 Hz, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.0, 160.6, 158.9, 152.8, 150.9, 148.8, 141.3, 123.6, 121.8, 111.3, 110.9, 109.8, 96.8, 92.6, 69.5, 60.2, 56.24, 56.17, 56.15, 55.9, 53.8, 29.8, 28.8, 25.9, 25.8, 24.1, 22.7, 14.2. HR-MS (ESI) *m/z*: calcd for $C_{34}H_{50}NO_7$ [M + H]: 584.3587; found 584.3587.

#### 4.5.13. 5-O-Morpholinopropyl-3,3′,4′,7-O-tetramethylquercetin (50)

Yield, 15%; yellow syrup. IR (film) $\nu_{max}$: 2932, 2851, 1623, 1601, 1514, 1489 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.71 (dd, $J$ = 8.7, 2.1 Hz, 1H), 7.70 (d, $J$ = 1.8 Hz, 1H), 6.98 (d, $J$ = 9.0 Hz, 1H), 6.50 (d, $J$ = 2.1 Hz, 1H), 6.36 (d, $J$ = 2.1 Hz, 1H), 4.15 (t, $J$ = 6.3 Hz, 2H), 3.96 (s, 6H), 3.89 (s, 3H), 3.83 (s, 3H), 3.75 (t, $J$ = 4.5 Hz, 4H), 2.73 (t, $J$ = 6.9 Hz, 2H), 2.63–2.51 (m, 4H), 2.16 (quin, $J$ = 5.1 Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.0, 160.5, 159.0, 152.9, 151.0, 148.9, 141.3, 123.6, 121.8, 111.4, 111.0, 109.8, 97.0, 92.7, 67.6, 66.9, 60.2, 56.2, 56.1, 55.9, 55.5, 53.8, 25.9. HR-MS (ESI) *m/z*: calcd for $C_{26}H_{32}NO_8$ [M + H]: 486.2128; found 486.2130.

#### 4.5.14. 5-O-Morpholinobutyl-3,3′,4′,7-O-tetramethylquercetin (51)

Yield, 95%; light yellow solid. IR (film) $\nu_{max}$: 2933, 1625, 1603, 1515, 1438 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.69–7.67 (overlapped, 1H), 7.67 (s, 1H), 6.95 (d, $J$ = 9.0 Hz, 1H), 6.46 (d, $J$ = 1.8 Hz, 1H), 6.30 (d, $J$ = 1.8 Hz, 1H), 4.07 (t, $J$ = 6.0 Hz, 2H), 3.93 (s, 6H), 3.86 (s, 3H), 3.81 (s, 3H), 3.71 (t, $J$ = 4.5 Hz, 4H), 2.51–2.46 (overlapped, 6H), 1.96 (quin, $J$ = 6.9 Hz, 2H), 1.81 (quin, $J$ = 7.5 Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 163.9, 160.5, 158.9, 152.8, 150.9, 148.8, 141.2, 123.5, 121.7, 111.3, 110.9, 109.7, 96.7, 92.5, 69.2, 66.8, 60.1, 58.5, 56.2, 56.1, 55.9, 53.7, 26.9, 22.8. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{34}NO_8$ [M + H]: 500.2285; found 500.2290.

#### 4.5.15. 5-O-Morpholinopentyl-3,3′,4′,7-O-tetramethylquercetin (52)

Yield, 66%; yellow oil. IR (film) $\nu_{max}$: 2937, 1621, 1601, 1514 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.69 (d, $J$ = 2.1 Hz, 1H), 6.96 (d, $J$ = 9.0 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.30 (d, $J$ = 2.1 Hz, 1H), 4.05 (t, $J$ = 6.6 Hz, 2H), 3.950 (s, 3H), 3.948 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H), 3.72 (t, $J$ = 4.8 Hz, 4H), 2.46 (t, $J$ = 4.2 Hz, 4H), 2.39 (t, $J$ = 7.2 Hz, 2H), 1.96 (quin, $J$ = 6.6 Hz, 2H), 1.59 (quin, $J$ = 3.9 Hz, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.1, 160.2, 158.9, 153.1, 151.0, 148.8, 141.1, 123.3, 121.8, 111.2, 111.0, 109.5, 96.8, 92.8, 69.1, 63.8, 60.1, 57.7, 56.2, 56.1, 55.9, 51.8, 27.8, 24.1, 22.9. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{36}NO_8$ [M + H]: 514.2441; found 514.2452.

#### 4.5.16. 5-O-Piperidinopropyl-3,3′,4′,7-O-tetramethylquercetin (53)

Yield, 14%; yellow syrup. IR (film) $\nu_{max}$: 2932, 1622, 1601, 1515 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.72 (dd, $J$ = 8.4, 2.1 Hz, 1H), 7.70 (d, $J$ = 1.8 Hz, 1H), 6.55 (d, $J$ = 2.1 Hz, 1H), 6.35 (d, $J$ = 2.1 Hz, 1H), 4.26–4.18 (m, 2H), 3.972 (s, 3H), 3.967 (s, 3H), 3.91 (s, 3H), 3.79 (s, 3H), 3.68–3.54 (m, 4H), 3.08–2.93 (m, 2H), 2.62–2.53 (m, 2H), 2.37–2.21 (m, 2H), 1.96–1.81 (m, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.3, 164.4, 159.5, 158.8, 153.8, 151.3, 148.9, 141.1, 123.1, 122.0, 111.3, 111.0, 109.2, 97.1, 93.5, 67.1, 60.2, 56.24, 56.16, 56.11, 56.0, 53.8, 23.8, 22.7, 22.2. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{34}NO_7$ [M + H]: 484.2335; found 484.2335.

#### 4.5.17. 5-O-Piperidinobutyl-3,3′,4′,7-O-tetramethylquercetin (54)

Yield, 77%; brown wax. IR (film) $\nu_{max}$: 2937, 1624, 1602, 1515, 1442 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.73–7.63 (overlapped, 2H), 6.95 (d, $J$ = 9.0 Hz, 1H), 6.46 (s, 1H), 6.31 (s, 1H), 4.06 (t, $J$ = 6.6 Hz, 2H), 3.94 (s, 6H), 3.87 (s, 3H), 3.82 (s, 3H), 2.46 (t, $J$ = 7.2 Hz, 6H), 1.95 (quin, $J$ = 7.2 Hz, 2H), 1.79 (quin, $J$ = 7.8 Hz, 2H), 1.66–1.56 (m, 4H), 1.48–1.35 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 163.9, 160.5, 158.9, 152.7, 150.9, 148.8, 141.3, 123.6, 121.7, 111.3, 110.9, 109.8, 96.8, 92.5, 69.4, 60.1, 58.9, 56.2, 56.1, 55.9, 54.6, 27.1, 25.8, 24.4, 23.2. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{36}NO_7$ [M + H]: 498.2492; found 498.2492.

#### 4.5.18. 5-O-Piperidinopentyl-3,3′,4′,7-O-tetramethylquercetin (55)

Yield, 90%; yellow oil. IR (film) $\nu_{max}$: 2937, 1625, 1603, 1514 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.67 (dd, $J$ = 9.6, 2.1 Hz, 1H), 7.66 (s, 1H), 6.94 (d, $J$ = 9.0 Hz, 1H), 6.46 (d, $J$ = 2.1 Hz, 1H), 6.29 (d, $J$ = 2.1 Hz, 1H), 4.03 (t, $J$ = 6.3 Hz, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 2.70–2.62 (overlapped, 6H), 1.94 (quin, $J$ = 6.9 Hz, 2H), 1.84–1.73 (m, 6H), 1.62–1.50 (m, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.4, 158.8, 152.8, 150.9, 148.8, 141.2, 123.4, 121.7, 111.2, 110.9, 109.6, 96.7, 92.6, 69.3, 60.1, 58.4, 56.2, 56.1, 55.9, 53.9, 28.5, 25.0, 24.5, 24.2, 23.5. HR-MS (ESI) *m/z*: calcd for $C_{29}H_{38}NO_7$ [M + H]: 512.2648; found 512.2648.

#### 4.5.19. 5-O-(4-Methylpiperazin-1-yl)propyl-3,3′,4′,7-O-tetramethylquercetin (56)

Yield, 59%; yellow syrup. IR (film) $\nu_{max}$: 2932, 2836, 1623, 1600, 1514 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.69 (d, $J$ = 7.5 Hz, 1H), 7.68 (s, 1H), 6.97 (d, $J$ = 8.7 Hz, 1H), 6.50 (d, $J$ = 1.8 Hz, 1H), 6.32 (d, $J$ = 2.1 Hz, 1H), 4.14 (t, $J$ = 5.4 Hz, 2H), 3.95 (s, 6H), 3.88 (s, 3H), 3.80 (s, 3H), 3.18–2.84 (overlapped, 10*H*), 2.51 (s, 3H), 2.34–2.20 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.1, 164.1, 159.9, 158.9, 153.3, 151.1, 148.9, 141.2, 123.3, 121.9, 111.3, 111.0, 109.5, 97.0, 93.0, 67.1, 60.2, 56.2, 56.1, 56.0, 54.8, 53.0, 51.4, 44.8, 25.2. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{35}N_2O_7$ [M + H]: 499.2444; found 499.2441.

#### 4.5.20. 5-O-(4-Methylpiperazin-1-yl)butyl-3,3′,4′,7-O-tetramethylquercetin (57)

Yield, 85%; yellow wax. IR (film) $\nu_{max}$: 2937, 2804, 1624, 1602, 1515, 1441 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.68 (d, $J$ = 1.8 Hz, 1H), 6.96 (d, $J$ = 9.3 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.31 (d, $J$ = 2.4 Hz, 1H), 4.07 (t, $J$ = 6.3 Hz, 2H), 3.94 (s, 6H), 3.87 (s, 3H), 3.82 (s, 3H), 2.76–2.31 (overlapped, 10*H*), 1.96 (quin, $J$ = 7.5 Hz, 2H), 1.81 (quin, $J$ = 6.9 Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 163.9, 160.5, 158.9, 152.7, 150.9, 148.8, 141.3, 123.6, 121.7, 111.3, 110.9, 109.8, 96.8, 92.6, 69.3, 60.1, 58.0, 56.2, 56.1, 55.9, 54.7, 52.8, 45.9, 26.9, 23.2. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{37}N_2O_7$ [M + H]: 513.2601; found 513.2603.

#### 4.5.21. 5-O-(4-Methylpiperazin-1-yl)pentyl-3,3′,4′,7-O-tetramethylquercetin (58)

Yield, 93%; yellow oil. IR (film) $\nu_{max}$: 2936, 1621, 1600, 1514 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 8.4, 1.5 Hz, 1H), 7.66 (s, 1H), 6.95 (d, $J$ = 9.3 Hz, 1H), 6.46 (d, $J$ = 2.1 Hz, 1H), 6.29 (d, $J$ = 2.1 Hz, 1H), 4.03 (t, $J$ = 6.3 Hz, 2H), 3.93 (s, 6H), 3.86 (s, 3H), 3.81 (s, 3H), 2.70–2.48 (m, 8H), 2.46 (t, $J$ = 6.3 Hz, 2H), 2.33 (s, 3H), 1.94 (quin, $J$ = 6.6 Hz, 2H), 1.65–1.50 (m, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 163.9, 160.5, 158.9, 152.7, 150.9, 148.8, 141.2, 123.5, 121.7, 111.3, 110.9, 109.7, 96.7, 92.5, 69.3, 60.1, 58.2, 56.2, 56.1, 55.9, 54.5, 52.6, 45.7, 28.8, 26.2, 24.0. HR-MS (ESI) *m/z*:

calcd for $C_{29}H_{39}N_2O_7$ [M + H]: 527.2757; found 527.2755.

*4.5.22. 5-O-Pyrrolidinopropyl-3,3′,4′,7-O-tetramethylquercetin (**59**)*

Yield, 90%; brown syrup. IR (film) $\nu_{max}$: 2934, 2796, 1623, 1601, 1514, 1439 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 9.3, 2.1 Hz, 1H), 7.67 (d, $J$ = 1.5 Hz, 1H), 6.96 (d, $J$ = 9.3 Hz, 1H), 6.49 (d, $J$ = 2.1 Hz, 1H), 6.34 (d, $J$ = 2.1 Hz, 1H), 4.16 (t, $J$ = 6.3 Hz, 2H), 3.94 (s, 6H), 3.87 (s, 3H), 3.80 (s, 3H), 3.09 (t, $J$ = 6.6 Hz, 2H), 2.97–2.84 (m, 4H), 2.27 (quin, $J$ = 6.6 Hz, 2H), 1.98–1.89 (m, 4H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.2, 164.1, 160.0, 158.8, 153.1, 151.1, 148.8, 141.2, 123.4, 121.8, 111.3, 110.9, 109.5, 96.9, 92.9, 67.7, 60.1, 56.2, 56.1, 56.0, 54.4, 53.4, 27.4, 23.6. HR-MS (ESI) *m/z*: calcd for $C_{26}H_{32}NO_7$ [M + H]: 470.2179; found 470.2177.

*4.5.23. 5-O-Pyrrolidinobutyl-3,3′,4′,7-O-tetramethylquercetin (**60**)*

Yield, 92%; brown wax. IR (film) $\nu_{max}$: 2934, 2836, 1622, 1600, 1514, 1439 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 9.0, 2.1 Hz, 1H), 7.68 (d, $J$ = 1.8 Hz, 1H), 6.96 (d, $J$ = 9.0 Hz, 1H), 6.47 (d, $J$ = 2.1 Hz, 1H), 6.31 (d, $J$ = 2.1 Hz, 1H), 4.07 (t, $J$ = 6.0 Hz, 2H), 3.94 (s, 6H), 3.87 (s, 3H), 3.82 (s, 3H), 2.78–2.70 (overlapped, 6H), 2.01–1.82 (overlapped, 8H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.4, 158.9, 152.7, 150.9, 148.8, 141.2, 123.5, 121.8, 111.3, 110.9, 109.7, 96.8, 92.6, 69.3, 60.1, 56.2, 56.1, 56.0, 55.9, 54.1, 26.9, 25.1, 23.6. HR-MS (ESI) *m/z*: calcd for $C_{27}H_{34}NO_7$ [M + H]: 484.2335; found 484.2334.

*4.5.24. 5-O-Pyrrolidinopentyl-3,3′,4′,7-O-tetramethylquercetin (**61**)*

Yield, 74%; yellow oil. IR (film) $\nu_{max}$: 2939, 1623, 1601, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 7.68 (dd, $J$ = 8.4, 2.1 Hz, 1H), 7.66 (s, 1H), 6.95 (d, $J$ = 8.4 Hz, 1H), 6.47 (d, $J$ = 2.4 Hz, 1H), 6.30 (d, $J$ = 2.1 Hz, 1H), 4.07 (t, $J$ = 5.7 Hz, 2H), 3.93 (s, 6H), 3.87 (s, 3H), 3.79 (s, 3H), 3.19–3.07 (m, 4H), 3.01 (t, $J$ = 8.4 Hz, 2H), 2.05–1.90 (m, 8H), 1.72–1.62 (m, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 174.0, 164.0, 160.3, 158.9 152.9, 151.0, 148.8, 141.2, 123.4, 121.8, 111.2, 110.9, 109.6, 96.7, 92.7, 69.1, 60.1, 56.2, 56.1, 55.9, 55.5, 53.6, 28.1, 25.9, 24.0, 23.5. HR-MS (ESI) *m/z*: calcd for $C_{28}H_{36}NO_7$ [M + H]: 498.2492; found 498.2494.

### 4.6. Cell culture

All cell lines were initially purchased from American Type Culture Collection (ATCC). The PC-3 and LNCaP prostate cancer cell lines were routinely cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. The DU-145 prostate cancer cells were routinely cultured in Eagle's Minimum Essential Medium (EMEM) 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 °C.

Docetaxel-resistant prostate cancer cell lines were established based on the procedure illustrated in the literature [30,31]. Docetaxel-resistant DU145 and PC-3 cell lines (DU145/DTX and PC-3/DTX) were developed over a period of one year by stepwise increased concentrations of docetaxel. Cells were continuously maintained in docetaxel, with treatments beginning at the initial $IC_{50}$ values of the respective parent cell lines. Media containing docetaxel will be changed every 2–3 days. As cells displayed resistance to treatments of docetaxel the concentration was subsequently increased.

### 4.7. WST-1 cell proliferation assay

PC-3, DU-145, LNCaP, PC-3/DTX, or DU145/DTX cells were plated in 96-well plates at a density of 3200 each well in 200 μL of culture medium. The cells were then treated with quercetin, fisetin, or synthesized derivatives separately 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 °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 h at 37 °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 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.

### 4.8. 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 h of cell attachment, the cells were then treated with derivative **44** at 10 μM and 20 μM, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in $CO_2$ incubator at 37 °C for 16 h. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf value of 450 g for 5 min. After discarding the supernatant, the collected cells were re-suspended with 500 μL 80% cold ethanol to fix for 30 min in 4 °C. The fixed cells could be stored at −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 min to degrade all RNA. The cells were stained with 200 μL of 50 μg/mL propidium iodide (PI) stock solution for 30 min 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 0.5–1 h after staining.

### 4.9. F2N12S and SYTOX 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 h of cell attachment, the cells were then treated with the derivative **44** at different concentrations and cultured in $CO_2$ incubator at 37 °C for 16 h, 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 min. The collected cells were re-suspended with 500 μL HBSS to remove proteins which may affect flow signal and centrifuged again. After discarding the supernatant, the collected cells were re-suspended with 300 μL HBSS and stained with 0.3 μL of F2N12S for 3–5 min followed by 0.3 μL SYTOX AADvanced for an additional 5 min. The fluorescence intensity of the two probes was further measured in individual PC-3 cells using an Attune flow cytometer (Life Technologies) within 0.5–1 h after staining.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.03.047.

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