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Synthesis and Biological Evaluation of C1-*O*-Substituted-3-(3-Butylamino-2-hydroxy-propoxy)-xanthen-9-one as topoisomerase IIα catalytic inhibitors

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Abstract

Topoisomerase II poison blocks the transitorily generated DNA double-strand breaks (DSBs) from religation, thereby causes severe DNA damage and gene toxicity. While topoisomerase II catalytic inhibitor does not form cleavable DNA-enzyme complex because its function attributes to inhibition of the catalytic steps of the enzyme such as before generating DNA DSBs or in the last step of the catalytic cycle after religation. It has been reported that the stabilizing effect of etoposide on transient cleavable DNA-topoisomerase IIβ complex attributes to its secondary malignancy. Therefore, topoisomerase IIα has been considered as more attractive target than topoisomerase IIβ for the development of chemotherapeutic agents. In the previous work, we reported compounds **I** and **II** as novel topoisomerase IIα catalytic inhibitors targeting for ATP binding site of human topoisomerase IIα ATP-binding domain. As a continuous work, we have designed and synthesized 43 compounds of C1-*O*-alkyl and arylalkyl substitiuted compounds with or without methoxy group on ring A. In the topoisomerase IIα inhibitory test, among the tested C1-*O*-**4**-chlorophenethyl substituted compounds **37** and **47** were more active than others, and compound **37** showed strongest topoisomerase IIα inhibitory activity with 94.4% and 23.0% inhibition, respectively, at 100 and 20 μM. Compounds **37** and **47** have also showed much enhanced cytotoxic activity against T47D cells; IC_{50} (μM): 0.63 ± 0.01 and 0.19 ± 0.02, respectively, which are stronger than reference drugs. Band depletion assay and cleavage complex assay results showed compounds **37** and **47** were potential topoisomerase IIα catalytic inhibitor with low DNA damage.

Keywords: C1-alkyl and arylalkyl substitiued xanthones, anticancer agents, Topoisomerase IIα catalytic inhibitor

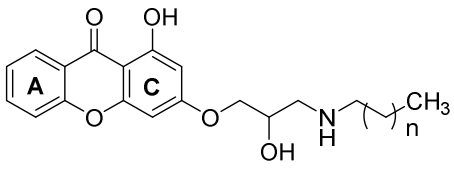
Introduction

Xanthones are hetero-tricyclic planar compounds originally isolated as secondary metabolites from a few higher plant families, fungi and lichen [1] and are named after 'Xanthos' which means yellow in Greek [2]. Diverse pharmacological profiles of xanthone families have been reported including anti-hypertensive [3], anticonvulsant [4], antithrombotic [5], anticholinesterase [6], anticancer activities [7, 8] affected by the substituents. The simplicity of structure and interesting diverse biological activities of xanthones have attracted researchers to put much effort for finding new potential druggable compounds *via* synthetic derivatization or isolation from natural resources.

In the cell proliferation process tangled DNA should overcome the topology problem, which could be solved by topoisomerase enzymes well-known to be taking part in DNA relaxation, supercoiling, catenation/decatenation and knotting/unknotting [9, 10]. Topoisomerases are ubiquitous enzymes and responsible for the topological inter-conversions of DNA by generating transient breaks of DNA strands. Human topoisomerase operates in two different ways. One causes single-strand breaks (SSBs, called type I), and the other induces double-strand breaks (DSBs, type II), during cell proliferation processes [11, 12]. Between these two types of topoisomerase, topoisomerase II is essential to relax supercoiled DNA through catalytic cycle of DSBs [13]. There are two isoforms of topoisomerase II, α and β forms. Despite their similarities, the two enzymes have distinct patterns of expression and physiological functions in vertebrate cells [14-17]. Topoisomerase IIα is essential for the survival of actively growing cells and able to decatenate the replicated chromosomes during chromosome segregation process. Thus the expression level of topoisomerase IIα are up-

regulated dramatically during cell proliferation [18-20]. Furthermore, the expression level of topoisomerase IIα changes over the cell cycle and reaches a maximum point in the late S and G_2/M phase [20-22]. Topoisomerase IIα is found at replication forks and remains tightly associated with chromosomes during mitosis [23, 24]. In contrast, expression of the β isoform is independent of proliferative status of cells and the enzyme dissociates from chromosomes during mitosis [17, 19, 23]. Topoisomerase IIβ has been known to be related with toxicity of topoisomerase II targeting anticancer drugs such as cardiotoxicity and potential development of secondary malignancy during their medication. Therefore, topoisomerase IIα has been considered as more attractive target than topoisomerase IIβ for the development of chemotherapeutic agents [25-27].

Topoisomerase II inhibitors act in two different pathways; (1) the distinct character of topoisomerase II poison is to bind and stabilize transiently and covalently formed DNA-enzyme complex and (2) topoisomerase II catalytic inhibitor does not stabilize the DNA-enzyme complexes but eliminates essential catalytic activities of topoisomerase II. Topoisomerase II poison blocks the transitorily generated DNA DSB from religation, thereby causes severe DNA damage and gene toxicity [28]. While topoisomerase II catalytic inhibitor does not form cleavable DNA-enzyme complex because its function attributes to inhibition of the catalytic steps of the enzyme such as before generating DNA DSBs or in the last step of the catalytic cycle after religation [10, 29-31]. It has been reported that the stabilizing effect of etoposide on transient cleavable DNA-topoisomerase IIβ complex attributes to its secondary malignancy [26, 27].



Compound **I** n = 1
Compound **II** n = 2

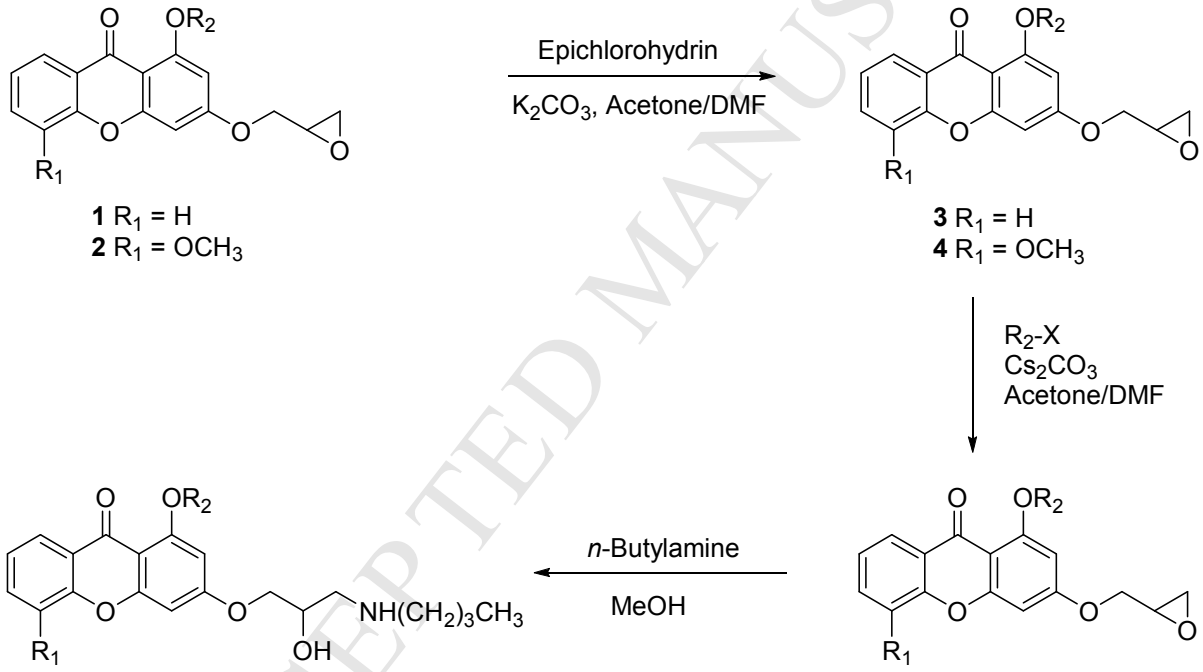
In the previous work, we reported compounds **I** and **II** as novel topoisomerase IIα catalytic inhibitors targeting for ATP binding site of human topoisomerase IIα ATP-binding domain [32, 33]. We extended our interest in the current study to discover human topoisomerase IIα catalytic inhibitor with improvement on catalytic inhibition and reduction of toxicity likely caused by DNA DSBs and to evaluate the mode of action. Based on the previous molecular docking study result of compound **I** with human topoisomerase IIα ATP-binding domain, we have designed and synthesized a series of C1-*O*-alkyl and arylalkyl substitiuted compounds (*n*-butylamine mediated epoxide ring opened derivatives) with or without methoxy group on ring A.

2. Chemistry

Synthesis of Target Compounds: General synthetic methods for the proposed compounds are depicted in Scheme 1. Group I compounds are prepared as two-step process. First, epichlorohydrin coupling reaction at C-3 hydroxy group under basic condition, and then subsequent C-1-*O*-alkylation. Group II compounds are obtained by *n*-butylamine mediated epoxide ring opening reaction in methanol solvent (Scheme 1).

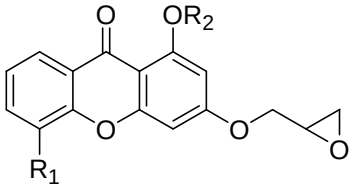
Starting compounds **3** and **4** were synthesized by previous method [32], and then different length of alkyl halide (alkyl = ethyl, isopropyl, butyl, and pentyl) or arylalkyl halide (benzyl, 3-chlorobenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 3-trifluoromethylbenzyl, 3-chlorophenethyl, and 4-chlorophenethyl) were mixed with Cs_2CO_3 or K_2CO_3 in acetone/DMF and then refluxed to give Group I compounds in 5.6-75.2% yields after purification. In the ^{1}H-NMR spectrum, proton peaks of alkyl group in compounds **5-8** and **16-19** were confirmed. Compounds **9-13** and **20-24** showed singlet peak which presents two methylene protons of

benzyl at 5.2 ppm. Compounds **14**, **15**, **25**, and **26** showed two triplet peaks belonging to the ethylene of phenethyl group at 3.2 and 4.2 ppm, respectively. All the Group II compounds have a multiplet peak of methine hydrogen on the epoxide ring at 3.4 ppm. Subsequent epoxide ring opening reaction of Group I with *n*-butylamine (10 equiv.) was accomplished in MeOH solvent to give Group II compounds in 19.1-90.2% yields. In the ^{13}C-NMR spectrum, the methine carbon of opened epoxide ring was down-field shifted from 50.0 to 69.9 ppm. Structures of all the prepared compounds are depicted in Scheme 2.



Scheme 1. General synthetic method of compounds

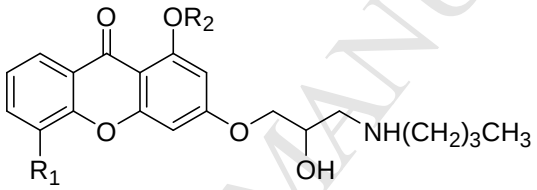
Group I



5 R_1 = H, R_2 = C_2H_5
6 R_1 = H, R_2 = i-C_3H_7
7 R_1 = H, R_2 = C_4H_9
8 R_1 = H, R_2 = C_5H_{11}
9 R_1 = H, R_2 = benzyl
10 R_1 = H, R_2 = 3-chlorobenzyl
11 R_1 = H, R_2 = 3-methoxybenzyl
12 R_1 = H, R_2 = 4-methoxybenzyl
13 R_1 = H, R_2 = 3-trifluoromethylbenzyl
14 R_1 = H, R_2 = 3-chlorophenethyl
15 R_1 = H, R_2 = 4-chlorophenethyl

16 R_1 = OCH_3, R_2 = C_2H_5
17 R_1 = OCH_3, R_2 = i-C_3H_7
18 R_1 = OCH_3, R_2 = C_4H_9
19 R_1 = OCH_3, R_2 = C_5H_{11}
20 R_1 = OCH_3, R_2 = benzyl
21 R_1 = OCH_3, R_2 = 3-chlorobenzyl
22 R_1 = OCH_3, R_2 = 3-methoxybenzyl
23 R_1 = OCH_3, R_2 = 4-methoxybenzyl
24 R_1 = OCH_3, R_2 = 3-trifluoromethylbenzyl
25 R_1 = OCH_3, R_2 = 3-chlorophenethyl
26 R_1 = OCH_3, R_2 = 4-chlorophenethyl

Group II



27 R_1 = H, R_2 = C_2H_5
28 R_1 = H, R_2 = i-C_3H_7
29 R_1 = H, R_2 = C_4H_9
30 R_1 = H, R_2 = C_5H_{11}
31 R_1 = H, R_2 = benzyl
32 R_1 = H, R_2 = 3-chlorobenzyl
33 R_1 = H, R_2 = 3-methoxybenzyl
34 R_1 = H, R_2 = 4-methoxybenzyl
35 R_1 = H, R_2 = 3-trifluoromethylbenzyl
36 R_1 = H, R_2 = 3-chlorophenethyl
37 R_1 = H, R_2 = 4-chlorophenethyl

38 R_1 = OCH_3, R_2 = C_2H_5
39 R_1 = OCH_3, R_2 = i-C_3H_7
40 R_1 = OCH_3, R_2 = C_4H_9
41 R_1 = OCH_3, R_2 = C_5H_{11}
42 R_1 = OCH_3, R_2 = benzyl
43 R_1 = OCH_3, R_2 = 3-chlorobenzyl
44 R_1 = OCH_3, R_2 = 3-methoxybenzyl
45 R_1 = OCH_3, R_2 = 4-methoxybenzyl
46 R_1 = OCH_3, R_2 = 3-trifluoromethylbenzyl
47 R_1 = OCH_3, R_2 = 4-chlorophenethyl

Scheme 2. Structures of the prepared compounds

3. Results and Discussion

3.1. Human Topoisomerase IIα Inhibitory Activities of Compounds

In the topoisomerase IIα inhibitory activity test, Group II compounds (epoxide ring opened compounds) showed stronger activity than Group I at 100 μM. In the group II series, C1-*O*-

arylalkyl substituted compounds (**31**-**37** and **42**-**47**) were better than corresponding alkyl substituted ones (**27**-**30** and **38**-**40**). Compounds without 5-methoxy group also showed more efficient topoisomerase IIα inhibitory activity than 5-methoxy analogues except C1-*O*-(3-chloropheneyl) substituted compounds. Overall, C1-*O*-arylalkyl substituents enhanced enzyme inhibitory activity than alkyl substituted analogues. But the length difference between oxygen and aryl group, methylene and ethylene, did not show clear information. Although the effect of substituents on inhibitory activity was not consistent to make a solid structure and activity relationship, C1-*O*-**4**-chlorophenethyl substituted compounds **37** and **47** were more active than others, H, OCH_3, CF_3 in each series (Table 1 & Figure 1). In this test, among the tested compounds compound **37** showed strongest topoisomerase IIα inhibitory activity with 94.4% and 23.0% inhibition, respectively, at 100 and 20 μM treatment.

Insert Figure 1 and Table 1

3.2. Cytotoxicity of the Compounds

The cytotoxicity assay for compounds was conducted with a range of human tumor cell lines, HCT15 (colon) T47D (breast), HeLa (cervical) and NCI-N87 (stomach). The inhibitory activities are presented as IC_{50} (μM) and listed in Table 2.

Insert Table 2

Overall, compounds tested showed good anticancer activity. In Group II, C1-*O*-arylalkyl derivatives efficiently suppressed cell growth in the tested cell lines, which was better than corresponding C1-*O*-alkly ones. 5-Methoxy-3-epoxide substituted compounds **16**-**25** in Group I showed hundreds nanomolar IC_{50} values in HCT15, which are better than etoposide

and similar to adriamycin used as references. Compounds **37** and **47**, strongest topoisomerase IIα inhibitors among C5-H and C5-methoxy series compounds, also showed much enhanced cytotoxic activity against T47D cells; IC_{50} (μM): 0.63 ± 0.01 and 0.19 ± 0.02, respectively, which are stronger than reference drugs.

3.3 Band depletion assay of the selected compounds 36, 37, and 47

With compound **37** possessing the strongest topoisomerase IIα DNA relaxation inhibitory activity and compound **47** showing most efficient cytotoxicity against T47D human breast cancer cells, compound **36** was additionally selected for further experiments to determine their mode of action clearly. The structural difference of **36** vs **37** and **47** is the position of chlorine and that of **36** vs **47** is the addition of methoxy group in R1 position of ring A. First of all, to verify whether compounds **36**, **37** and **47** function as topoisomerase IIα poisons or catalytic inhibitors, T47D cells were treated with 50 μM of each of compounds and etoposide for 2 hours and followed by cell harvest and cell lysis. The covalently bound topoisomerase IIα to DNA was excluded during DNA removal process while the free topoisomerase IIα was detected by Western blot analysis. As shown in Figure 2, the western blot band of free topoisomerase IIα was depleted in the etoposide (a well-known topoisomerase II poison)-treated T47D cells while it was evidently observed in compounds **36**, **37** and **47** -treated cells (Figure 2). This result suggests compounds **36**, **37** and **47** inhibited the catalytic activity of topoisomerase IIα without stabilizing DNA-topoisomerase IIα cleavable complex.

Insert Figure 2

3.4 Cleavable complex assay of the selected compounds 36, 37 and 47

The function of compounds **36**, **37** and **47** as topoisomerase IIα catalytic inhibitors was revalidated with cleavable complex assay. Etoposide evidently generated the linear truncated DNA because it stabilized the transiently formed DNA-topoisomerase IIα cleavable complex then blocked DNA from religation as shown in Figure 3. However the treatment of compounds **36**, **37** and **47** as the same concentration as etoposide did not make the linear DNA (Figure 3**)**. This result is consistent with that of band depletion assay.

Insert Figure 3

3.5. Comet assay of the selected compounds 36, 37 and 47

Based on results from topoisomerase IIα relaxation assay, DNA band depletion assay and cleavable complex assay, compounds **36**, **37** and **47** were characterized as human topoisomerase IIα catalytic inhibitors. The most difference of topoisomerse IIα catalytic inhibitor over topoisomerase IIα poison is no generation of undesired DNA truncation in which catalytic inhibitors can be advantageous over poisons. Topoisomerase IIα poisons produce in general serious DNA damage induced by stabilizing the cleavable complex followed by preventing the religation of DNA double strand breaks [34]. Compounds **36**, **37** and **47** were evaluated with comet assay in T47D cells to further confirm its mode of action as a topoisomerase IIα catalytic inhibitor because selected compounds showed stronger toxicity in T47D cells among tested human cancer cell lines (Table 2). As shown in Figure 4, the image of inverted fluorescence microscope and the result of image analysis using Komet 5.0 software showed that etoposide made serious comet formation due to its mode of action to generate un-religated short DNA but compounds tested did not at the same treatment of 5

and10 μM for 24 h, which further confirms the catalytic inhibitory activity of compounds **36**, **37** and **47**.

3.5. Molecular Docking Study

Docking study was carried out for compound **36**, **37** and **47** into the ATP-binding domain of human topoisomerase IIα. The analysis of the docked compounds to the ATP binding site of topoisomerase IIα showed that compound **37** has lowest binding energy of -8.45 kcal/mol (Ki = 636.14 nM) (Figure 5A) among four compounds. The only difference between compound **36** and **37** is the chloro substitution on the phenylethoxy group. However, comparing the docked structure, the xanthone ring of compound **36** did not overlap well with purine ring, and there was no hydrogen bond interaction with topoisomerase IIα. The docking was further analyzed by comparing with the AMP-PNP, and it showed that the xanthone ring overlaps with purine ring. Residues that are in close contact with compound **37** are Asn91, Asp94, Asn120, Val137, Ile141, Phe142, Thr147, Ser149, Ala167, Lys168, and Thr215 (Figure 5B). There is hydrogen bond interaction between C3-*O* with Lys168. Also, the amide group and Asn91 and the hydroxyl group with Ala167 have hydrogen bond interactions. The chloro group on the C1-*O* substituted benzene ring may be changed with more polar group since it protrudes out to the solvent accessible area.

4. Conclusion

While topoisomerase II poison blocks the transitorily generated DNA DSB from religation, thereby causes severe DNA damage and gene toxicity, topoisomerase II catalytic inhibitor does not form cleavable DNA-enzyme complex because its function attributes to inhibition of the catalytic steps of the enzyme such as before generating DNA DSBs or in the last step of the catalytic cycle after relegation. It has been reported that the stabilizing effect of etoposide

on transient cleavable DNA-topoisomerase IIβ complex attributes to its secondary malignancy. Therefore, topoisomerase IIα has been considered as more attractive target than topoisomerase IIβ for the development of chemotherapeutic agents. As c continuous work to find efficient topoisomerase IIα catalytic inhibitor, we have designed and synthesized 43 compounds of C1-*O*-alkyl and arylalkyl substitiuted compounds based on compound **I** and **II**. In the topoisomerase IIα inhibitory test, although the structure and activity relationship of the series compounds was not consistent, compounds **37** and **47** exhibited superior inhibitory activity among the tested compounds and especially compound **37** showed strongest topoisomerase IIα inhibitory activity with 94.4% and 23.0% inhibition, respectively, at 100 and 20 μM. In the anticancer activity test, C1-*O*-arylalkyl derivatives in Group II efficiently suppressed cell growth in the tested cell lines, which was better than corresponding C1-*O*-alkly ones. Especially compounds **37** and **47** had IC_{50} (μM) of 0.63 ± 0.01 and 0.19 ± 0.02, respectively, against T47D cells. 5-Methoxy-3-epoxide substituted compounds **16**-**25** in Group I showed hundreds nanomolar IC_{50} values in HCT15, which are better than etoposide and similar to adriamycin used as references. Band depletion assay result suggests compounds **36**, **37** and **47** inhibit the catalytic activity of topoisomerase IIα without stabilizing DNA-topoisomerase IIα cleavable complex. Cleavage complex assay results also showed compounds **36**, **37** and **47** were potential topoisomerase IIα catalytic inhibitor, which is consistent with that of band depletion assay. The advantage of topoisomerase IIα catalytic inhibitor over topoisomerase IIα poison is no generation of undesired DNA truncation, which can be confirmed by comet assay. Etoposide made serious comet formation due to its mode of action to generate un-religated short DNA but compounds **36**, **37** and **47** did not. Molecular docking analysis compound **37** to the ATP binding site of topoisomerase IIα showed that compound **37** has low binding energy. Overall C1-*O*- arylalkyl substitiuted compounds were

potential topoisomerase IIα catalytic inhibitor and compound **37** could be starting point for the efficient modification in this scaffold.

5. Experimental

5.1. Chemistry general

Most chemicals and reagents used were obtained from Aldrich Chemical Co. and others were from company like TCI. Melting points were measured without correction in open capillaries with Barnstead Electrothermal melting point apparatus, Manual MEL-TEMP (Model No: 1202D). Chromatographic separations were monitored by thin-layer chromatography using a commercially available pre-coated Merck Kieselgel 60 F_{254} plate (0.25mm) and detected by visualizing under UV at 254 and 365 nm. Silicagel column chromatography was carried out with Merck Kieselgel 60 (0.040 - 0.063 mm). All solvents used for chromatography were directly used without distillation. The purity was assessed by HPLC (Shimadzu LC-20AD) analysis under the following conditions; column, SunFire C18 (4.6 mm × 150 mm, 5 um); mobile phase, **condition A**: A (water) and B (acetonitrile) using a linear gradient of 50 - 70% B in 0 - 15 min, 70% B in 15 - 20 min, 100% B in 20 - 25 min and 50% B in 25 - 30 min, **condition B**: A (0.2% (v/v) TFA in water) and B (acetonitrile) using a linear gradient of 50 - 70% B in 0 - 15 min, 70% B in 15 - 20 min, 100% B in 20 - 25 min and 50% B in 25 - 30 min, flow rate; 1.0 mL/min; detection, diode array detector (Shimadzu Spd-M20A). The purity of compound is described as percent (%) and retention time was given in minutes. NMR spectra were recorded on Varian AS 400 (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz) with tetramethylsilane as an internal standard. Chemical shift (δ) values are expressed in ppm and coupling constant (*J*) values in hertz (Hz). The melting points were measured on Gallenkamp Melting Point Apparatus without correction.

5.2. General synthetic methods for 1,3-dihydroxyxanthone analogues

5.2.1. 1,3-Dihydroxy-xanthen-9-one (1)

The reaction mixture of salicylic acid (2.06 g, 14.89 mmol), phloroglucinol (2.01 g, 15.94 mmol) and $ZnCl_2$ (4.93 g, 39.04 mmol) in $POCl_3$ (30 mL) was refluxed (4 h) at 80 °C, cooled to room temperature and then poured into iced water very slowly. The solid formed was kept for 1 d at 4 °C, collected and washed with water to give a red solid. After drying under vacuum, the crude product was purified by silica gel column chromatography (eluent: ethyl acetate/*n*-hexane = 1:2) to give compound **1** as a pale yellow solid (0.73 g, 26.5%). m.p. 253-255 °C; R_f 0.66 (eluent: ethyl acetate/*n*-hexane = 1:1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.21 (d, *J* = 2.4 Hz, 1H), 6.31 (d, *J* = 2.0 Hz, 1H), 7.30 (ddd, *J* = 1.2, 7.2, 8.0 Hz, 1H), 7.32 (dd, *J* = 0.4, 8.4 Hz, 1H), 7.62 (ddd, *J* = 2.0, 7.2, 6.8 Hz, 1H), 8.13 (dd, *J* = 2.0, 8.0 Hz, 1H), 10.10 (s, 1H), 12.76 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 94.4, 98.6, 103.1, 117.5, 120.6, 123.8, 125.7, 134.8, 155.9, 157.9, 163.6, 165.8, 180.5 ppm.

5.2.2. 1,3-Dihydroxy-5-methoxy-xanthen-9-one (2)

2,3-dimethoxybenzoic acid (2.01 g, 11.0 mmol), phloroglucinol (2.14 g, 17.0 mmol) and $ZnCl_2$ (8.98 g, 65.9 mmol) in $POCl_3$ (30 mL) was refluxed (4 h) at 80 °C, cooled to room temperature and then poured into iced water very slowly. The solid formed was kept for 1 d at 4 °C, collected and washed with water to give a red solid. After drying under vacuum, the crude product was purified by silica gel chromatography (eluent: ethyl acetate/*n*-hexane = 1:2) to give compound **2** as a pale yellow solid (0.78 g, 27.5%). m.p 286-288 °C; R_f 0.55 (eluent: ethyl acetate/*n*-hexane = 1:1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.96 (s, 3H), 6.17 (d, *J* = 2.4 Hz, 1H), 6.37 (d, *J* = 2.4 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.37 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.66 (dd, *J* = 1.2, 8.0 Hz, 1H), 10.82 (s, 1H), 12.76 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 55.9, 93.9,

98.1, 102.1, 115.6, 115.9, 120.6, 123.4, 145.4, 147.8, 157.0, 162.8, 165.6, 179.6 ppm.

5.3. General method for synthesis of epichlorohydrin analogues

A mixture of compound **1** or **2**, epichlorohydrin (1.5 equiv.) and K_2CO_3 (2 equiv.) in acetone/DMF (10 mL/10 mL) was refluxed (20 h). The reaction was quenched with water (30 mL) and extracted with ethyl acetate (× 3). The organic layer was collected and washed with brine, and then dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (eluent: $CHCl_3$).

5.3.1. 1-Hydroxy-3-oxiranylmethoxy-xanthen-9-one (3)

Following the general method, compound **1** (1.01 g, 4.44 mmol), epichlorohydrin (0.83 g, 8.93 mmol) and K_2CO_3 (1.32 g, 9.56 mmol) was used. The purification was conducted with eluent ($CHCl_3$) to give compound **15** (0.73 g, 57.8%) as a pale yellow solid. R_f 0.76 (DCM); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.79 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.96 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.38-3.41 (m, 1H), 4.01 (dd, *J* = 6.0, 11.2 Hz, 1H), 4.34 (dd, *J* = 3.2, 11.2 Hz, 1H), 6.36 (d, *J* = 2.0 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 7.38 (ddd, *J* = 1.2, 7.2, 7.6 Hz, 1H), 7.43 (dd, *J* = 0.4, 7.6 Hz, 1H), 7.72 (ddd, *J* = 1.6, 7.2, 7.6 Hz, 1H), 8.24 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 49.9, 69.5, 93.6, 97.7, 104.5, 117.8, 120.9, 124.3, 126.1, 135.3, 156.3, 157.9, 163.9, 16.6, 181.1 ppm.

5.3.2. 1-Hydroxy-5-methoxy-3-oxiranylmethoxy-xanthen-9-one (4)

Following the general method, compound **2** (0.31 g, 1.21 mmol), epichlorohydrin (0.24g, 2.55 mmol) and K_2CO_3 (0.38 g, 2.75 mmol) was used. The purification was conducted with eluent ($CHCl_3$) to give compound **13** (0.12 g, 32.3%) as a pale yellow solid. R_f 0.30 (DCM); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.78 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.94 (t, *J* = 4.8 Hz, 1H), 3.39-

3.40 (m, 1H), 4.02 (dd, *J* = 2.0, 10.8 Hz, 1H), 4.03 (s, 3H), 4.33 (dd, *J* = 3.2, 10.8 Hz, 1H), 6.38 (d, *J* = 2.0 Hz, 1H), 6.58 (d, *J* = 2.4 Hz, 1H), 7.23-7.26 (m, 1H), 7.31 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.82 (dd, *J* = 1.6, 8.0 Hz, 1H), 12.83 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 49.9, 56.7, 69.5, 93.5, 98.2, 104.4, 116.0, 117.0, 121.7, 123.9, 146.5, 148.5, 157.7, 163.6, 165.6, 181.1 ppm.

5.4. General method for synthesis of C-1-*O*-alkyl or arylalkylated C-3-(oxiran-2-ylmethoxy) analogues

A mixture of compound **3** or **4**, R-X (3 equiv.) and Cs_2CO_3 (3 equiv.) in acetone/DMF (10 mL/10 mL) was stirred at proper temperature (25-80 °C) for 20 h. The reaction was quenched with water (30 mL) and extracted with ethyl acetate (× 3). The organic layer was collected and washed with brine, and then dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure and residue was purified by silica gel column chromatography.

5.4.1. 1-Ethoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (5)

Following the general method, compound **3** (0.21 g, 0.73 mmol), iodoethane (0.35 g, 2.22 mmol) and Cs_2CO_3 (0.52 g, 1.59 mmol) in DMF (20 mL) were used at room temperature. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **5** (0.17 g, 75.2%) as a white solid. m.p. 164 -166 °C; R_f 0.33 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 3.71 min (condition A, purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.59 (t, *J* = 2.8 Hz, 3H), 2.86 (q, *J* = 2.4 Hz, 1H), 2.96 (t, *J* = 4.4 Hz, 1H), 3.40 (m, 1H), 4.02 (dd, *J* = 8.0, 11.2 Hz, 1H), 4.17 (dd, *J* = 2.0, 6.8 Hz, 2H), 4.36 (dd, *J* = 2.4, 10.4 Hz, 1H), 6.40 (d, *J* = 2.4 Hz, 1H), 6.50 (d, *J* = 2.0 Hz, 1H), 7.32 (ddd, *J* = 1.2, 7.2, 8.0 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.63 (ddd, *J* = 1.6, 7.2, 8.4 Hz, 1H), 8.28 (dd, *J* = 2.4, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.8, 44.8, 50.0, 65.3, 69.4, 93.6, 96.4, 108.0, 117.2, 123.4, 124.0, 127.0, 133.9,

155.2, 160.0, 161.8, 163.8, 175.6 ppm.

5.4.2. 1-Isopropoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (6)

Following the general method, compound **3** (0.20 g, 0.71 mmol), 2- iodopropane (0.17 g, 1.03 mmol) and Cs_2CO_3 (0.52 g, 1.60 mmol) in DMF (20 mL) were used at 50 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **6** (0.13 g, 55.6%) as a white solid. m.p. 132-134 °C; R_f 0.33 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 7.67 min (condition A, purity: 95.1%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.49 (d, *J* = 6.0 Hz, 6H), 2.80 (dd, *J* = 4.2, 5.2 Hz, 1H), 2.96 (dd, *J* = 4.0, 4.4 Hz, 1H), 3.39-3.42 (m, 1H), 4.01 (dd, *J* = 6.0, 11.2 Hz, 1H), 4.36 (dd, *J* = 2.8, 11.2 Hz, 1H), 4.66 (heptet, *J* = 2.0 Hz, 1H), 6.41 (d, *J* = 2.4 Hz, 1H), 7.31 (ddd, *J* = 1.2, 7.6, 8.0 Hz, 1H), 7.35 (dd, *J* = 0.8, 8.4 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.1, 44.8, 50.0, 69.4, 42.3, 93.6, 98.2, 108.1, 117.1, 123.4, 124.0, 127.0, 133.8, 155.2, 160.0, 160.9, 163.7, 175.5 ppm.

5.4.2. 1-Butoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (7)

Following the general method, compound **3** (0.20 g, 0.7 2mmol), 1-iodobutane (0.36 g, 2.15 mmol) and K_2CO_3 (0.36 g, 2.15 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **7** (0.09 g, 37.5%) as a white solid. m.p. 124-126 °C; R_f 0.49 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 11.16 min (condition A, purity: 96.3%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.02 (t, *J* = 7.2 Hz, 3H), 1.60-1.66 (m, 2H), 1.91-1.98 (m, 2H), 2.79 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.96 (t, *J* = 4.0 Hz, 1H), 3.38-3.42 (m, 1H), 4.01 (dd, *J* = 7.0, 10.4 Hz, 1H), 4.09 (t, *J* = 6.4 Hz, 2H), 4.35 (dd, *J* = 2.8, 6.8 Hz, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 7.33 (m, 2H), 7.61 (ddd, *J* = 2.0, 4.0, 7.2 Hz, 1H), 8.28 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.1, 19.5, 31.2, 44.8, 50.0, 69.3, 69.4, 93.4, 96.3, 108.0, 117.2, 123.4, 124.0, 127.0, 133.8, 155.2, 159.9,

162.0, 163.7, 175.5 ppm.

5.4.3. 3-(Oxiran-2-ylmethoxy)-1-(pentyloxy)-9*H*-xanthen-9-one (8)

Following the general method, compound **3** (0.20 g, 0.70 mmol), 1-iodopentane (0.43 g, 2.15 mmol) and Cs_2CO_3 (0.48 g, 1.48 mmol) in DMF (20 mL) were used at room temperature. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **8** (0.13 g, 53.8%) as a white solid. m.p. 120-122 °C; R_f 0.63 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 13.93 min (condition A, purity: 99.5%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.39-1.48 (m, *J* = 3.2 Hz, 2H), 1.52-1.58 (m, 2H), 1.94-2.01 (m, *J* = 7.2 Hz, 2H), 2.80 (dd, *J* = 2.4, 5.6 Hz, 1H), 2.96 (t, *J* = 4.4 Hz, 1H), 3.38-3.42 (m, 1H), 4.00 (dd, *J* = 1.6, 6.4 Hz, 1H), 4.08 (t, *J* = 6.8 Hz, 2H), 4.36 (dd, *J* = 2.4, 6.4 Hz, 1H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 7.33-7.36 (m, 2H), 7.62 (ddd, *J* = 1.6, 7.2, 8.8 Hz, 1H), 8.28 (dd, *J* = 2.4, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.3. 22.7, 28.3, 28.9, 44.8, 50.0, 69.4, 69.7, 93.5, 96.4, 108.0, 117.2, 123.4, 124.0, 127.0, 133.8, 155.2, 159.9, 162.0, 163.8, 175.5 ppm.

5.4.4. 1-Benzyloxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (9)

Following the general method, compound **3** (0.20 g, 0.71 mmol), benzylbromide (0.24 g, 1.43 mmol) and K_2CO_3 (0.24 g, 1.74 mmol) were used at 80 °C (4 h). The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **9** (0.17 g, 63.7%) as a white solid. m.p. 194-196 °C; R_f 0.59 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 11.27 min (condition A, purity: 98.4%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.78 (dd, *J* = 2.4, 4.4 Hz, 1H), 2.95 (t, *J* = 4.4 Hz, 1H), 3.37-3.40 (m, 1H), 3.99 (dd, *J* = 5.6, 10.2 Hz, 1H), 4.34 (dd, *J* = 2.8, 11.2 Hz, 1H), 5.27 (s, 2H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 7.31-7.45 (m, 5H), 7.61-7.66 (m, 3H), 8.31 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8,

49.9, 69.4, 71.0, 94.1, 97.2, 108.3, 117.2, 123.4, 124.1, 126.9, 127.0, 128.0, 128,9, 134.0, 136.5, 155.2, 159.9, 161.1, 163.7, 175.5 ppm.

5.4.5. 1-(3-chlorobenzyloxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (10)

Following the general method, compound **3** (0.17 g, 0.59 mmol), 3-chlorobenzylchloride (0.31 g, 1.52 mmol) and K_2CO_3 (0.26 g, 1.87 mmol) were used at 80 °C (20 h). The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **10** (0.15 g, 61.6%) as a white solid. m.p. 170-172 °C; R_f 0.62 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 4.13 min (condition A, purity: 99.6%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.78 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.95 (dd, *J* = 4.0, 4.4 Hz, 1H), 3.37-3.40 (m, 1H), 3.98 (dd, *J* = 7.0, 11.2 Hz, 1H), 4.35 (dd, *J* = 2.8, 10.8 Hz, 1H), 5.22 (s, 2H), 6.41 (d, *J* = 2.0 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 7.28-7.39 (m, 4H), 7.60-7.66 (m, 3H), 7.64 (ddd, *J* = 1.6, 7.2, 7.2 Hz, 1H), 8.31 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 50.0, 69.5, 70.2, 94.3, 97.3, 108.3, 117.2, 123.3, 124.1, 125.1, 126.9, 127.0, 128.2, 130.3, 134.0, 134.7, 138.6, 155.2, 159.9, 160.8, 169.7, 175.5 ppm.

5.4.6. 1-(3-Methoxybenzyloxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (11)

Following the general method, compound **3** (0.20 g, 0.72 mmol), 3-methoxybenzylchloride (0.32 g, 2.07 mmol) and K_2CO_3 (0.35 g, 2.54 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **11** (0.11 g, 38.4%) as a whites solid. m.p. 124-126 °C; R_f 0.49 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 11.50 min (condition A, purity: 96.7%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.78 (dd, *J* = 2.8, 4.8 Hz, 1H), 2.95 (t, *J* = 4.8 Hz, 1H), 3.36-3.40 (m, 1H), 3.87 (s, 3H), 3.99 (dd, *J* = 7.0, 11.2 Hz, 1H), 4.33 (dd, *J* = 2.8, 12.8 Hz, 1H), 5.25 (s, 2H), 6.44 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.19-7.40 (m, 5H), 7.63 (ddd, *J* = 2.0, 4.0, 11.2 Hz,

1H), 8.31 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 49.9, 55.6, 69.4, 70.9, 94.1, 97.2, 108.3, 112.2, 123.9, 127.2, 119.0, 123.4, 124.0, 127.2, 129.9, 133.9, 138.2, 155.2, 159.9, 160.2, 161.0, 163.7, 175.4 ppm.

5.4.7. 1-(4-Methoxybenzyloxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (12)

Following the general method, compound **3** (0.32 g, 1.13 mmol), 4-methoxybenzylchloride (0.32 g, 2.06 mmol) and K_2CO_3 (0.42 g, 3.01 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **12** (0.03 g, 5.6%) as a white solid. m.p. 130-132 °C; R_f 0.55 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 3.77 min (condition A, purity: 99.3%); ^{1}H-NMR ($CDCl_3$,400 MHz) δ 2.78 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.95 (t, *J* = 4.8 Hz, 1H), 3.37-3.41 (m, 1H), 3.82 (s, 3H), 3.99 (dd, *J* = 2.0, 11.2 Hz, 1H), 4.34 (dd, *J* = 2.8, 11.2 Hz, 1H), 5.20 (s, 2H), 6.45 (d, *J* = 2.4 Hz, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 6.94-6.98 (m, 2H), 7.32 (ddd, *J* = 1.2, 3.2, 8.8 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.53-7.57 (m, 2H), 7.63 (ddd, *J* = 1.6, 4.0, 11.6 Hz, 1H), 8.30 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 50.0, 55.5, 69.4, 70.9, 94.0, 97.3, 114.3, 117.2, 123.4, 124.0, 127.0, 128.6, 133.9, 155.2, 159.5, 159.9, 161.2, 163.7, 175.5 ppm.

5.4.8. 3-(Oxiran-2-ylmethoxy)-1-(3-(trifluoromethyl)benzyloxy)-9*H*-xanthen-9-one (13)

Following the general method, compound **3** (0.21 g, 0.73 mmol), 3-(trichloromethyl)benzylchloride (0.30 g, 1.46 mmol) and K_2CO_3 (0.24 g, 1.74 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **13** (0.23 g, 71.0%) as a white solid. m.p. 152-154 °C; R_f 0.59 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 8.62 min (condition A, purity: 96.3%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.79 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.96 (t, *J* = 4.4 Hz, 1H), 3.38-3.42 (m, 1H), 4.00 (dd, *J* = 6.4, 11.2 Hz, 1H), 4.37 (dd, *J* = 2.8, 11.2 Hz, 1H), 5.29 (s, 2H), 6.46 (d, *J* = 2.0 Hz,

1H), 6.56 (d, *J* = 2.4 Hz, 1H), 7.36 (m, 2H), 7.65 (ddd, *J* = 2.0, 7.2, 11.2 Hz, 1H), 7.84 (s, 1H), 8.00-8.02 (m, 1H), 8.32 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 50.0, 69.5, 70.3, 94.5, 97.3, 108.3, 117.3, 123.3, 123.6, 124.1, 124.8, 127.0, 129.6, 130.5, 130.9, 134.1, 137.6, 155.2, 159.9, 160.7, 163.7, 175.4 ppm.

5.4.8. 1-(3-Chlorophenethoxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (14)

Following the general method, compound **3** (0.19 g, 0.06 mmol), 3-chloro-1-phenethyl bromide (0.30 g, 1.37 mmol) and K_2CO_3 (0.26 g, 1.85 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **14** (0.04 g, 15.4 %) as a white solid. m.p. 146-148 °C; R_f 0.63 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 4.05 min (condition A, purity: 98.2%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.78 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.95 (t, *J* = 4.4 Hz, 1H), 3.28 (t, *J* = 7.2 Hz, 2H), 3.36-3.40 (m, 1H), 4.00 (dd, *J* = 7.0, 11.2 Hz, 1H), 4.25 (t, *J* = 7.2 Hz, 2H), 4.35 (dd, *J* = 2.8, 11.2 Hz, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 7.20-7.38 (m, 5H), 7.43 (dd, *J* = 2.0, 2.0 Hz, 1H), 7.64 (ddd, *J* = 1.6, 7.2, 8.8 Hz, 1H), 8.31 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 35.5, 44.8, 50.0, 69.4, 70.1, 94.0, 96.6, 108.1, 117.2, 123.4, 124.9, 127.0, 127.9, 129.6, 130.0, 133.9, 134.4, 140.3, 155.2, 159.2, 161.4, 163.7, 175.3, 175.4 ppm.

5.4.9. 1-(4-Chlorophenethoxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (15)

Following the general method, compound **3** (0.21 g, 0.72 mmol), 4- chloro-1-phenethyl bromide (0.30 g, 1.37 mmol) and K_2CO_3 (0.26 g, 1.85 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/ *n*-hexane = 1:3) to give compound **15** (0.05 g, 16.5%) as a white solid. m.p. 132-134 °C; R_f 0.63 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 4.10 min (condition A, purity: 98.8%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.77 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.95 (t, *J* = 4.8 Hz, 1H), 3.26 (t, *J* = 6.8 Hz, 2H), 3.36-3.40 (m, 1H),

3.98 (dd, *J* = 2.0, 11.2 Hz, 1H), 4.23 (t, *J* = 7.2 Hz, 2H), 4.34 (dd, *J* = 2.4, 10.8 Hz, 1H), 7.26-7.42 (m, 6H), 7.63 (ddd, *J* = 1.6, 6.8, 8.0 Hz, 1H), 8.30 (dd, *J* = 1.2, 2.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 35.2, 44.8, 49.9, 69.4, 70.3, 93.9, 96.5, 108.2,, 117.2, 123.3, 124.1, 126.9, 128.8, 131.0, 132.7, 133.9, 136.9, 155.2, 159.9, 161.4, 163.7, 175.4 ppm.

5.4.10. 1-Ethoxy-5-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (16)

Following the general method, compound **4** (0.17 g, 0.53 mmol), iodoethane (0.35 g, 2.03 mmol) and Cs_2CO_3 (0.42 g, 1.30 mmol) in DMF (20 mL) were used at room temperature. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **16** (0.12 g, 66.9%) as a white solid. m.p. 150-152 °C; R_f 0.23 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 5.20 min (condition A, purity: 99.9%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.59 (t, *J* = 2.8 Hz, 3H), 2.80 (dd, *J* = 2.4, 4.4 Hz, 1H), 2.95 (t, *J* = 4.4 Hz, 1H), 3.38-3.42 (m, 1H), 4.00 (dd, *J* = 8.5, 11.2 Hz, 1H), 4.01 (s, 3H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.35 (dd, *J* = 2.8, 10.8 Hz, 1H), 6.40 (d, *J* = 2.0, 8.0 Hz, 1H), 7.24 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.86 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.8, 44.8, 49.9, 56.6, 65.2, 69.4, 93.6, 96.8, 107.9, 114.7, 118.0, 123.5, 124.3, 148.2, 159.7, 161.6, 163.7, 175.5 ppm.

5.4.11. 1-Isopropoxy-5-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (17)

Following the general method, compound **4** (0.20 g, 0.64 mmol), 2- iodopropane (0.17 g, 1.03 mmol) and Cs_2CO_3 (0.51 g, 1.55 mmol) were used at room temperature. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:3) to give compound **17** (0.14 g, 64.8%) as a white solid. m.p. 162-164 °C; R_f 0.33 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 4.07 min (condition A, purity: 97.8%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.49 (d, *J* = 7.0 Hz, 6H), 2.78 (dd, *J* = 2.4, 4.8 Hz, 1H), 3.38-3.42 (m, 1H), 4.00 (dd, *J* = 5.6, 7.2 Hz, 1H), 4.01 (s, 3H), 4.34 (dd, *J* = 3.2, 11.2 Hz, 1H), 4.65 (heptet, *J* = 2.4 Hz, 1H), 6.41 (d, *J* = 2.4 Hz,

1H), 6.58 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 1.2, 2.0 Hz, 1H), 7.24 (dd, *J* = 4.0, 8.0 Hz, 1H), 7.58 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.1, 44.8, 49.9, 56.6, 69.4, 72.2, 93.6, 98.5, 108.6, 114.7, 118.0, 123.5, 124.4, 145.4, 148.1, 159.8, 160.7, 163.6, 175.3 ppm.

5.4.12. 1-Butoxy-5-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (18)

Following the general method, compound **4** (0.27 g, 0.86 mmol), 1-iodobutane (0.49 g, 2.64 mmol) and K_2CO_3 (0.39 g, 2.79 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **18** (0.01 g, 31.1 %) as a white solid. m.p. 162-164 °C; R_f 0.44 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 3.85 min (condition A, purity: 99.9%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.01 (t, *J* = 3.2 Hz, 3H), 1.58-1.67 (m, *J* = 3.6 Hz, 2H), 1.91-1.98 (m, *J* = 6.4 Hz, 2H), 2.78 (dd, *J* = 2.4, 4.0 Hz, 1H), 2.94 (t, *J* = 4.4 Hz, 1H), 3.37-3.41 (m, 1H), 3.99 (dd, *J* = 6.0, 10.8 Hz, 1H), 4.00 (s, 3H), 4.34 (dd, *J* = 2.8, 10.8 Hz, 1H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.58 (d, *J* = 2.0 Hz, 1H), 7.14 (dd, *J* = 5.6, 8.0 Hz, 1H), 7.24-7.26 (m, 1H), 7.86 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.1, 19.5, 31.2, 44.8, 49.9, 56.6, 69.3, 69.4, 93.5, 96.7, 107.9, 114.7, 118.0, 123.5, 124.3, 145.4, 148.1, 159.7, 161.8, 163.7, 175.4 ppm.

5.4.13. 5-Methoxy-3-(oxiran-2-ylmethoxy)-1-(pentyloxy)-9*H*-xanthen-9-one (19)

Following the general method, compound **4** (0.15 g, 0.48 mmol), 1-iodopentane (0.43 g, 2.14 mmol) and Cs_2CO_3 (0.43 g, 1.31 mmol) in DMF (20 mL) were used at room temperature. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **19** (0.10 g, 52.7%) as a white solid. m.p. 144-146 °C; R_f 0.45 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 12.83 min (condition A, purity: 97.6%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.40-1.46 (m, 2H), 1.52-1.59 (m, 2H), 1.94-2.01 (m, 2H), 2.78 (dd, *J* = 2.4, 5.6 Hz, 1H), 2.95 (t, *J* = 4.4 Hz, 1H), 3.39-3.41 (m, 1H), 4.00 (dd, *J* = 2.4, 7.5 Hz, 1H),

4.00 (s, 3H), 4.07 (t, *J* = 6.8 Hz, 1H), 6.40 (d, *J* = 2.4 Hz, 1H), 6.58 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.22-7.26 (m, 2H), 7.86 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.2, 22.7, 28.3, 28.9, 44.8, 49.9, 56.6, 69.4, 69.7, 93.5, 96.7, 107.9, 114.7, 118.0, 123.5, 124.3, 14.4, 148.1, 159.7, 161.8, 163.7, 175.4 ppm.

5.4.14. 1-Benzyloxy-5-methoxy-3-oxiranylmethoxy-xanthen-9-one (20)

Following the general method, compound **4** (0.22 g, 0.76 mmol), benzylbromide (0.14 g, 0.81 mmol) and K_2CO_3 (0.33 g, 2.40 mmol) were used. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **20** (0.03 g, 12.1%) as a white solid. m.p. 192-194 °C; R_f 0.44 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 11.20 min (condition A, purity: 95.2%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.77 (dd, *J* = 2.8, 4.8 Hz, 1H), 2.94 (t, *J* = 4.4 Hz, 1H), 3.36-3.40 (m, 1H), 3.99 (dd, *J* = 7.0, 10.8 Hz, 1H), 4.02 (s, 3H), 4.33 (dd, *J* = 3.2, 10.8 Hz, 1H), 5.26 (s, 2H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 7.16 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.23-7.34 (m, 2H), 7.40-7.44 (m, 2H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.89 (dd, *J* = 2.8, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 49.8, 56.6, 69.4, 71.0, 94.1, 97.5, 108.3, 117.2, 123.4, 124.1, 126.9, 127.0, 128.0, 128,9, 134.0, 136.5, 155.2, 159.9, 161.1, 163.7, 175.5 ppm.

5.4.15. 1-(3-Chlorobenzyloxy)-5-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (21)

Following the general method, Compound **4** (0.21 g, 0.65 mmol), 3-chlorobenzylchloride (0.47 g, 2.28 mmol) and K_2CO_3 (0.33 g, 2.37 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:1) to give compound **21** (0.14 g, 47.3%) as a white solid. m.p. 170-172 °C; R_f 0.51 (eluent: ethyl acetate/*n*-hexane=1:1); HPLC: R_T 13.71 min (condition A, purity: 99.9%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.77 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.95 (t, *J* = 4.4 Hz, 1H), 2.98 (dd, *J* = 7.0, 11.2 Hz, 1H), 3.37-3.41 (m, 1H), 4.02 (s, 3H),

4.34 (dd, *J* = 2.4, 10.8 Hz, 1H), 5.22 (s, 2H), 6.43 (d, *J* = 2.4 Hz, 1H), 6.63 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.24-7.40 (m, 4H), 7.60-7.62 (m, 2H), 7.89 (dd, *J* = 1.6, 2.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.3, 49.6, 56.3, 69.3, 69.8, 94.1, 97.3, 107.6, 114.6, 117.4, 123.3, 123.8, 124.8, 126.6, 127.8, 129.9, 134.2, 138.3, 145.1, 147.9, 159.4, 160.2, 163.4, 174.9 ppm.

5.4.16. 5-Methoxy-1-(3-methoxybenzyloxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (22)

Following the general method, compound **4** (0.25 g, 0.78 mmol), 3-methoxybenzylchloride (0.38 g, 2.41 mmol) and Cs_2CO_3 (0.39 g, 2.84 mmol) was used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **22** (0.07 g, 20.9%) as a white solid. m.p. 150-151 °C; R_f 0.46 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 3.68 min (condition A, purity: 100.0%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.77 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.94 (t, *J* = 4.8 Hz, 1H), 3.36-3.40 (m, 1H), 3.87 (s, 3H), 3.97 (dd, *J* = 5.6, 11.2 Hz, 1H), 4.01 (s, 3H), 4.32 (dd, *J* = 2.8, 11.2 Hz, 1H), 5,24 (s, 2H), 6.45 (d, *J* = 2.4 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.16 (dd, *J* = 1.6, 2.0 Hz, 1H), 7.17-7.20 (m, 1H), 7.20-7.26 (m, 2H), 7.27-7.30 (m, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.88 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.8, 48.9, 56.6, 69.4, 70.8, 94.1, 97.6, 108.2, 112.1, 113.9, 114.8, 123.5, 124.3, 129.9, 138.2, 145.5, 148.2, 159.7, 160.2, 160.9, 163.6, 175.3 ppm.

5.4.17. 5-Methoxy-1-(4-methoxybenzyloxy)-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (23)

Following the general method, compound **4** (0.21 g, 0.68 mmol), 4-methoxybenzylchloride (0.28 g, 1.77 mmol) and Cs_2CO_3 (0.42 g, 1.29 mmol) was used at 60 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **23** (0.01 g, 31.1%) as

a white solid. m.p. 102-104 °C; R_f 0.21 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 10.9 min (condition A, purity: 96.1%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.75 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.92 (dd, *J* = 4.8, 4.8 Hz, 1H), 3.35-3.38 (m, 1H), 3.81 (s, 3H), 3.99 (s, 3H), 4.11 (dd, *J* = 7.2, 14.4 Hz, 1H), 4.30 (dd, *J* = 2.4, 11.2 Hz, 1H), 5.17 (s, 2H), 6.44 (d, *J* = 2.4 Hz, 1H), 6.58 (d, *J* = 2.4 Hz, 1H), 6.93-6.95 (m, 2H), 7.14 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.23 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.54 (dd, *J* = 2.4, 7.2 Hz, 1H), 7.86 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.7, 49.9, 55.5, 56.6, 69.4, 70.8, 94.0, 97.5, 108.1, 114.3, 114.7, 117.9, 123.5, 124.3, 128.6, 128.8, 145.4, 148.1, 159.4, 159.6 , 161.0, 163.6, 175.3 ppm.

5.4.18. 5-Methoxy-3-(oxiran-2-ylmethoxy)-1-(3-(trifluoromethyl)benzyloxy)-9*H*-xanthen-9-one (24)

Following the general method, compound **4** (0.10 g, 0.31 mmol), 3-(trichloromethyl)benzylchloride (0.14 g, 0.66 mmol) and K_2CO_3 (0.15 g, 1.09 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **24** (0.07 g, 45.7%) as a white solid. m.p. 152-154 °C; R_f 0.47 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 16.47 min (condition A, purity: 95.1%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.77 (dd, *J* = 2.8, 4.8 Hz, 1H), 2.95 (t, *J* = 4.4 Hz, 1H), 3.38-3.41 (m, 1H), 3.97 (dd, *J* = 2.8, 6.0 Hz, 1H), 4.01 (s, 3H), 4.35 (dd, *J* = 2.8, 11.2 Hz, 1H), 5.27 (s, 2H), 6.45 (d, *J* = 2.4 Hz, 1H), 6.64 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.59-7.60 (m, 2H), 7.84 (s, 1H), 7.88 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 44.7, 49.9, 56.6, 69.5, 70.3, 94.5, 97.6, 108.1, 114.9, 118.0, 123.6, 124.3, 124.8, 129.6, 130.4, 137.6, 145.5, 148.2, 159.7, 160.6, 163.6, 175.3 ppm.

5.4.19. 1-(3-Chlorophenethoxy)-5-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (25)

Following the general method, compound **4** (0.20 g, 0.65 mmol), 3-chloro-1-phenethylbromide (0.30 g, 1.37 mmol) and K_2CO_3 (0.26 g, 1.88 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **25** (0.11 g, 18.3%) as a white solid. m.p. 148-150 °C; R_f 0.48 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 14.15 min (condition A, purity: 95.3%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.76 (dd, *J* = 2.4, 4.8 Hz, 1H), 2.93 (t, *J* = 4.8 Hz, 1H), 3.25 (t, *J* = 7.2 Hz, 2H), 3.35-3.40 (m, 1H), 3.96 (dd, *J* = 7.0, 10.8 Hz, 1H), 4.00 (s, 3H), 4.23 (t, *J* = 6.8 Hz, 1H), 4.31 (dd, *J* = 3.2, 15.2 Hz, 1H), 6.33 (d, *J* = 2.0 Hz, 1H), 6.58 (d, *J* = 2.0 Hz, 1H), 7.17 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.20-7.22 (m, 1H), 7.26 (dd, *J* = 8.0, 8.0 Hz, 3H), 7.34-7.43 (m, 2H), 7.87 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 35.5, 44.8, 49.9, 56.6, 69.4, 70.1, 94.0, 97.0, 108.0, 114.8, 118.0, 123.6, 124.3, 127.0, 127.9, 129.6, 130.0, 134.4, 140.3, 145.5, 149.2, 159.7, 161.2, 163.7, 175.3 ppm.

5.4.20. 1-(4-Chlorophenethoxy)-5-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one (26)

Following the general method, compound **4** (0.20 g, 0.65 mmol), 4-chloro-1-phenethylbromide (0.30 g, 1.37 mmol) and K_2CO_3 (0.26 g, 1.88 mmol) were used at 80 °C. The purification was conducted with eluent (ethyl acetate/*n*-hexane = 1:2) to give compound **26** (0.09 g, 29.9%) as a white solid. m.p. 206-208 °C; R_f 0.55 (eluent: ethyl acetate/*n*-hexane = 1:1); HPLC: R_T 14.51 min (condition A, purity: 95.0%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.77 (dd, *J* = 2.8, 4.8 Hz, 1H), 2.94 (t, *J* = 4.0 Hz, 1H), 2.98 (dd, *J* = 2.0, 10.8 Hz, 1H), 3.25 (t, *J* = 3.2 Hz, 1H), 3.36-3.40 (m, 1H), 4.02 (s, 3H), 4.22 (t, *J* = 2.8 Hz, 2H), 4.33 (dd, *J* = 2.4, 10.8 Hz, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.24-7.31 (m, 4H), 7.39-7.41 (m, 2H), 7.88 (dd, *J* = 1.2, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 34.6, 39.7, 44.1, 49.4, 56.1, 69.0, 69.7, 93.6, 96.4, 114.4, 117.2, 123.1, 123.6,

128.2, 130.6, 131.9, 136.6, 144.9, 147.7, 159.1, 160.7, 163.3, 174.7 ppm.

5.5. General method for n-butylamine mediated Epoxide Ring Opened compounds

Group 1 compound and *n*-butylamine (10 equiv.) in MeOH (10 mL) was stirred at 50 °C under N_2 (20 h), and then cooled to room temperature. Solvent was evaporated and H_2O was added. The aqueous mixture was extract with ethyl acetate (× 3). The organic layer was collected and washed with brine, and then dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure and residue was purified by silica gel column chromatography.

5.5.1. 3-(3-Butylamino-2-hydroxypropoxy)-1-ethoxy-9*H*-xanthen-9-one (27)

Following the general method, compound **5** (0.15 g, 0.49 mmol) and *n*-butylamine (0.18 g, 2.43 mmol) were used to give compound **27** (0.05 g, 24.4%) as a pale orange solid. m.p. 164-166 °C; R_f 0.33 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 4.11 min (condition B, purity: 98.7%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.43 (hexet, *J* = 3.6 Hz, 2H), 1.57 (t, *J* = 7.2 Hz, 3H), 1.87 (quint, *J* = 8.0 Hz, 2H), 3.04-3.08 (m, 2H), 3.15-3.20 (m, 1H), 3.30 (dd, *J* = 2.4, 12.8 Hz, 1H), 4.06 (dd, *J* = 7.5, 9.6 Hz, 1H), 4.12-4.17 (m, 4H), 4.63-4.64 (m, 1H), 6.34 (d, *J* = 2.4 Hz, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 7.25-7.28 (m, 2H), 7.56 (ddd, *J* = 1.6, 7.2, 8.0 Hz, 1H), 8.22 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.8, 14.8, 20.2, 28.2, 49.0, 51.4, 65.3, 65.6, 69.9, 93.5, 96.2, 108.0, 117.1, 123.2, 124.0, 126.8, 133.9, 155.0, 159.8, 161.7, 163.4, 175.5 ppm.

5.5.2. 3-(3-Butylamino-2-hydroxypropoxy)-1-isopropoxy-9*H*-xanthen-9-one (28)

Following the general method, compound **6** (16.3 g, 0.06 mmol) and *n*-butylamine (0.04 g, 0.61 mmol) were used to give compound **28** (0.01 g, 49.0%) as a pale yellow solid. m.p. 180-

182 °C; R_f 0.33 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 7.91 min (condition B, purity: 92.3%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J* = 7.6 Hz, 3H), 1.40 (hexet, *J* = 7.6 Hz, 2H), 1.48 (d, *J* = 5.6 Hz, 6H), 1.86 (quint, *J* = 7.6 Hz, 2H), 2.99-3.10 (m, 2H), 3.15 (dd, *J* = 9.6, 12.4 Hz, 1H), 3.29 (dd, *J* = 2.8, 12.0 Hz, 1H), 4.07 (dd, *J* = 5.6, 10.0 Hz, 1H), 4.15 (dd, *J* = 4.8, 9.6 Hz, 1H), 4.60-4.68 (m, 1H), 6.28 (d, *J* = 2.0 Hz, 1H), 6.41 (d, *J* = 2.0 Hz, 1H), 7.25-7.30 (m, 2H), 7.57 (ddd, *J* = 1.6, 6.8, 8.4 Hz, 1H), 8.22 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.8, 20.2, 22.1, 28.7, 49.0, 51.5, 65.9, 70.0, 72.4, 93.6, 98.0, 108.8, 117.1, 123.3, 124.0, 126.9, 133.9, 155.1, 159.9, 160.9, 163.3, 175.4 ppm.

5.5.3. 1-Butoxy-3-(3-butylamino-2-hydroxypropoxy)-9*H*-xanthen-9-one (29)

Following the general method, compound **7** (0.06 g, 0.17 mmol) and *n*-butylamine (0.15 g, 2.02 mmol) were used to give compound **29** (0.06 g, 90.2%) as a white solid. m.p. 80-82 °C; R_f 0.46 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 2.51 min (condition B, purity: 98.0%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.93 (t, *J* = 7.2 Hz, 3H), 1.01 (t, *J* = 7.6 Hz, 3H), 1.37 (hexet, *J* = 6.8 Hz, 2H), 1.52 (quint, *J* = 6.8 Hz, 2H), 1.63 (hexet, *J* = 7.6 Hz, 2H), 1.95 (quint, *J* = 6.4 Hz, 2H), 2.64-2.73 (m, 4H), 2.78 (dd, *J* = 8.0, 12.0 Hz, 1H), 2.91 (dd, *J* = 4.0, 12.0 Hz, 1H), 3.48 (s, 1H), 4.05-4.12 (m, 4H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 7.26-7.32 (m, 1H), 7.35-7.35 (m, 1H), 7.61 (ddd, *J* = 2.0, 4.0, 12.0 Hz, 1H), 8.27 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.1, 14.2, 19.5, 20.5, 31.2, 32.2, 49.7, 51.7, 67.8, 69.3, 71.0, 93.5, 96.3, 107.9, 117.1, 123.4, 123.9, 126.9, 133.8, 155.2, 159.9, 161.9, 164.1, 175.5 ppm.

5.5.4. 3-(3-Butylamino-2-hydroxypropoxy)-1-pentyloxy-9*H*-xanthen-9-one (30)

Following the general method, compound **8** (0.11 g, 0.31 mmol) and *n*-butylamine (0.10 g, 1.42 mmol) were used to give compound **30** (0.06 g, 43.0%) as a pale yellow solid. m.p. 188-

190 °C; R_f 0.59 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 3.14 min (condition B, purity: 95.6%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J* = 7.2 Hz, 6H), 1.28-1.39 (m, 4H), 1.40-1.63 (m, 4H), 1.79 (quint, *J* = 7.6 Hz ,2H), 1.98 (quint, *J* = 7.6 Hz, 2H), 2.79 (hexet, *J* = 4.8 Hz, 2H), 3.09 (t, *J* = 7.6 Hz, 2H), 3.22 (dd, *J* = 4.4, 9.6 Hz, 1H), 4.06 (dd, *J* = 7.3, 7.3 Hz, 2H), 4.13 (dd, *J* = 7.2, 7.2 Hz, 1H), 6.42 (d, *J* = 2.4 Hz, 1H), 7.26-7.31 (m, 2H), 7.58 (ddd, *J* = 2.0, 4.0, 8.0 Hz, 1H), 8.24 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.8, 14.2, 20.2, 22.7, 28.3, 28.5, 28.9, 49.0, 51.5, 65.8, 69.8, 70.0, 93.4, 96.1, 108.1, 117.1, 123.3, 124.0, 126.9, 133.9, 155.1, 159.8, 161.9, 163.4, 175.4 ppm.

5.5.5. 1-Benzyloxy-3-(3-butylamino-2-hydroxypropoxy)-9*H*-xanthen-9-one (31)

Following the general method, compound **9** (0.10 g, 0.26 mmol) and *n*-butylamine (0.19 g, 2.53 mmol) were used to give compound **31** (0.02 g, 19.1%) as an ivory solid. m.p. 94-96 °C; R_f 0.45 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 2.65 min (condition B, purity: 95.7%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.94 (t, *J* = 7.6 Hz, 3H), 1.41 (hexet, *J* = 7.6 Hz, 2H), 1.81 (quint, *J* = 7.6 Hz, 2H), 2.99 (dd, *J* = 7.2, 7.2 Hz, 2H), 3.10 (dd, *J* = 7.6, 12.4 Hz, 1H), 3.20 (dd, *J* = 3.2, 12.4 Hz, 1H), 3.97 (dd, *J* = 5.6, 10.0 Hz, 1H), 4.05 (dd, *J* = 4.8, 9.6 Hz, 1H), 4.53-4.54 (m, 1H), 5.22 (s, 2H), 6.32 (d, *J* = 2.4 Hz, 1H), 6.40 (d, *J* = 2.0 Hz, 1H), 7.23-7.32 (m, 4H), 7.41 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.53 (ddd, *J* = 1.6, 6.8, 8.4 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 2H), 8.21 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$,100 MHz) 13.8, 20.2, 28.8, 48.9, 51.3, 65.9, 70.1, 71.0, 94.0, 96.9, 107.7, 117.2, 123.1, 124.0, 126.8, 127.0, 128.0, 128.9, 133.9, 136.5, 155.0, 159.7, 161.0, 163.4, 175.3 ppm.

5.5.6. 3-(3-Butylamino-2-hydroxypropoxy)-1-((3-chlorobenzyl)oxy)-9*H*-xanthen-9-one (32)

Following the general method, compound **10** (0.10 g, 0.24 mmol) and *n*-butylamine (0.18 g,

2.43 mmol) were used to give compound **32** (0.07 g, 61.2%) as a white solid. m.p. 144-146 °C; R_f 0.34 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 3.79 min (condition B, purity: 97.2%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.80 (t, *J* = 7.2 Hz, 3H), 1.25 (hexet, *J* = 7.2 Hz, 2H), 1.46 (quint, *J* = 7.2 Hz, 2H), 2.61 (td, *J* = 4.8, 12.8 Hz, 2H), 2.72 (dd, *J* = 8.4, 12.4 Hz, 1H), 2.83 (dd, *J* = 3.6, 12.4 Hz, 1H), 3.93-4.00 (m, 2H), 4.05-4.10 (m, 1H), 5.08 (s, 2H), 6.31 (d, *J* = 2.0 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 7.15-7.26 (m, 4H), 7.46-7.48 (m, 1H), 7.52 (ddd, *J* = 2.0, 7.2, 8.4 Hz, 2H), 8.14 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.8, 20.1, 30.9, 49.2, 51.6, 67.1 ,69.8, 70.9, 94.1, 97.0, 107.6, 117.0, 122.9, 123.8, 124.8, 126.5, 126.6, 127.8, 130.0 ,133.8, 134.2, 138.4, 154.9, 159.6, 160.3, 163.8, 175.1 ppm.

5.5.7. 3-(3-Butylamino-2-hydroxypropoxy)-1-(3-methoxybenzyloxy)-9*H*-xanthen-9-one (33)

Following the general method, compound **11** (0.06 g, 0.15 mmol) and *n*-butylamine (0.11 g, 1.52 mmol) were used to give compound **33** (0.03 g, 46.9%) as a white solid. m.p. 136-138 °C; R_f 0.33 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 2.85 min (condition B, purity: 93.2%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.93 (t, *J* = 7.6 Hz, 3H), 1.37 (hexet, *J* = 7.6 Hz, 1H), 1.54 (quint, *J* = 7.2 Hz, 2H), 2.70 (td, *J* = 7.2, 12.0 Hz, 2H), 2.79 (dd, *J* = 8.4, 12.0 Hz, 2H), 2.90 (dd, *J* = 4.0, 12.0 Hz, 1H), 3.87 (s, 3H), 4.04 (d, *J* = 4.8 Hz, 2H), 4.08-4.12 (m, 1H), 5.22 (s, 2H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 2.4, 8.0 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.26-7.35 (m, 4H), 7.61 (ddd, *J* = 1.6, 7.2, 8.0 Hz, 1H), 8.29 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.1, 20.5, 32.0, 49.6, 51.7, 55.5, 67.7, 70.8, 71.0, 94.1, 97.1, 108.1, 112.1, 113.8, 117.2, 118.9, 123.3, 124.0, 127.0, 129.9, 133.9, 138.2, 155.2, 159.9, 160.2, 160.9, 164.0, 175.4 ppm.

5.5.8. 3-(3-Butylamino-2-hydroxypropoxy)-1-(4-methoxybenzyloxy)-9*H*-xanthen-9-one

(34)

Following the general method, compound **12** (0.12 g, 0.29 mmol) and *n*-butylamine (0.22 g, 3.03 mmol) were used to give compound **34** (0.11 g, 79.8%) as a yellow solid. m.p. 104-106 °C; R_f 0.45 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 3.20 min (condition B, purity: 96.0%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.84 (t, *J* = 7.2 Hz, 3H), 1.27 (hexet, *J* = 7.2 Hz, 2H), 1.42 (quint, *J* = 6.8 Hz, 2H), 2.60 (td, *J* = 7.2, 11.6 Hz, 2H), 2.68 (dd, *J* = 7.2, 12.4 Hz, 1H), 2.79 (dd, *J* = 3.2, 12.4 Hz, 1H), 3.72 (s, 3H), 3.97-4.03 (m, 3H), 5.01 (s, 3H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 6.83-6.87 (m, 2H), 7.20-7.28 (m, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.54 (ddd, *J* = 2.4, 7.2, 8.4 Hz, 1H), 8.17 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.0, 20.3, 32.0, 49.5, 51.8, 55.3, 67.7, 70.6, 71.1, 93.9, 97.0, 107.7, 114.0, 117.0, 123.0, 123.7, 126.6, 128.4, 133.7, 154.9, 159.2, 159.6, 160.8, 164.0, 175.1 ppm.

5.5.9. 3-(3-Butylamino-2-hydroxypropoxy)-1-((3-(trifluoromethyl)benzyl)oxy)-9*H*-xanthen-9-one (35)

Following the general method, compound **13** (0.08 g, 0.22 mmol) and *n*-butylamine (0.10 g, 1.30 mmol) were used to give compound **35** (0.03 g, 32.0%) as a white solid. m.p. 198-200 °C; R_f 0.33 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 4.51 min (condition B, purity: 96.2%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.94 (t, *J* = 7.2 Hz, 3H), 1.39 (hexet, *J* = 7.6 Hz, 2H), 1.61 (quint, *J* = 7.6 Hz, 2H), 2.78 (td, *J* = 8.0, 10.0 Hz, 2H), 2.86 (dd, *J* = 4.0, 8.0, 12.4 Hz, 1H), 3.00 (dd, *J* = 3.6, 12.4 Hz, 1H), 4.04-4.11 (m, 2H), 4.21-4.26 (m, 1H), 5.26 (s, 2H), 6.43 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 7.30-7.35 (m, 2H), 7.58-7.60 (m, 2H), 7.61 (ddd, *J* = 1.6, 6.8, 8.0 Hz, 1H), 7.84 (s, 1H), 8.01 (dd, *J* = 4.0, 4.0 Hz, 1H), 8.29 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.0, 20.3, 31.4, 49.4, 51.7, 67.3, 70.1, 71.0, 94.4, 97.2, 107.9, 117.1, 123.1, 123.4, 123.9, 124.6, 126.7, 129.4, 130.3, 133.9, 137.5, 155.1, 159.8, 160.4, 163.9, 175.3 ppm.

5.5.10. 3-(3-Butylamino-2-hydroxypropoxy)-1-(3-chlorophenethoxy)-9*H*-xanthen-9-one (36)

Following the general method, compound **14** (0.06 g, 0.15 mmol) and *n*-butylamine (0.15 g, 2.02 mmol) were used to give compound **36** (0.03 g, 37.4%) as a white solid. m.p. 116-118 °C; R_f 0.34 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 4.07 min (condition B, purity: 96.3%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.93 (t, *J* = 7.2 Hz, 3H), 1.41 (hexet, *J* = 7.2 Hz, 2H), 1.84 (quint, *J* = 8.0 Hz, 2H), 3.00-3.04 (m, 2H), 3.12 (dd, *J* = 10.0, 12.0 Hz, 1H), 3.23 (dd, *J* = 10.0, 12.0 Hz, 1H), 3.25 (t, *J* = 7.2 Hz, 2H), 4.01 (t, *J* = 7.2 Hz, 2H), 4.56-4.60 (m, 1H), 6.29 (d, *J* = 2.4 Hz, 1H), 6.37 (d, *J* = 2.4 Hz, 1H), 7.19 (dt, *J* = 1.6, 8.4 Hz, 1H), 7.22-7.28 (m, 3H), 7.34-7.37 (m, 1H), 7.44 (dd, *J* = 1.6, 1.6 Hz, 1H), 7.55 (ddd, *J* = 1.6, 7.2, 8.4 Hz, 1H), 8.22 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.8, 20.2, 28.4, 35.5, 48.9, 51.3, 65.7, 70.0, 70.2, 94.0, 96.3, 108.0, 117.2, 123.2, 124.1, 126.8, 127.0, 128.0, 129.6, 130.0, 133.9, 134.4, 140.4, 155.0, 159.7, 161.3 163.3, 175.2 ppm.

5.5.11. 3-(3-Butylamino-2-hydroxypropoxy)-1-(4-chlorophenethoxy)-9*H*-xanthen-9-one (37)

Following the general method, compound **15** (0.10 g, 0.24 mmol) and *n*-butylamine (0.22 g, 3.03 mmol) were used to give compound **37** (0.04g, 32.0%) as a white solid. m.p. 118-120 °C; R_f 0.35 (eluent: MeOH:CH_2Cl_2 = 1:5).; HPLC: R_T 4.22 min (condition B, purity: 96.7%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, *J* = 7.6 Hz, 3H), 1.33 (hexet, *J* = 7.6 Hz, 2H), 1.72 (quint, *J* = 8.0 Hz, 2H), 2.89-2.92 (m, 2H), 3.00 (dd, *J* = 9.6, 12.4 Hz, 1H), 3.14 (dd, *J* = 2.4, 12.4 Hz, 1H), 3.16 (t, *J* = 6.4 Hz, 2H), 3.96-4.04 (m, 1H), 4.07 (dd, *J* = 4.8, 14.0 Hz, 1H), 4.14 (t, *J* = 2.4, 12.4 Hz, 1H), 4.45-4.47 (m, 1H), 6.25 (d, *J* = 2.0 Hz, 1H), 6.40 (d, *J* = 2.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.21-7.28 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.55 (ddd, *J* = 1.6, 7.2, 8.4

Hz, 1H), 8.18 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.6, 20.0 ,28.1, 34.9, 48.6, 51.1, 65.3, 70.1, 70.1, 93.9, 96.2, 107.7, 117.1, 123.0, 123.9, 126.6, 128.5, 130.9, 132.3, 133.8, 136.8, 154.9, 159.6, 161.1, 163.4, 175.2 ppm.

5.5.12. 3-(3-Butylamino-2-hydroxypropoxy)-1-ethoxy-5-methoxy-9*H*-xanthen-9-one (38)

Following the general method, compound **16** (0.11 g, 0.31 mmol) and *n*-butylamine (0.22 g, 3.03 mmol) were used to give compound **37** (0.05 g, 34.2%) as a white solid. m.p. 162-164 °C; R_f 0.34 (eluent: MeOH:CH_2Cl_2 = 1:5).; HPLC: R_T 10.52 min (condition B, purity: 99.8%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.93 (t, *J* = 7.6 Hz, 3H), 1.34-1.42 (m, 2H), 1.48-1.55 (m, 2H), 1.58 (t, *J* = 7.2 Hz, 3H), 2.63-2.71 (m, 2H), 2.78 (dd, *J* = 8.0, 12.0 Hz, 1H), 2.89 (dd, *J* = 7.2, 8.4 Hz, 1H), 4.00 (s, 3H), 4.05-4.12 (m, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 6.38 (d, *J* = 2.4 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 7.15 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.21-7.26 (m, 1H), 7.85 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.2, 14.8, 20.5, 32.1, 49.6, 51.6, 56.6, 65.2, 67.7, 71.1, 93.6, 96.8, 107.8, 114.7, 118.0, 123.5, 124.3, 145.5, 148.2, 159.7, 161.5, 164.1, 175.5 ppm.

5.5.13. 3-(3-Butylamino-2-hydroxypropoxy)-1-isopropoxy-5-methoxy-9*H*-xanthen-9-one (39)

Following the general method, compound **17** (0.08 g, 0.25 mmol) and *n*-butylamine (0.18 g, 2.45 mmol) were used to give compound **39** (0.06 g, 57.1%) as a pale yellow solid. m.p. 198-200 °C; R_f 0.33 (eluent: MeOH: CH_2Cl_2 = 1:5); HPLC: R_T 14.36 min (condition B, purity: 94.4%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.93 (t, *J* = 7.2 Hz, 3H), 1.36 (hexet, *J* = 6.8 Hz, 2H), 1.49 (d, *J* = 6.0 Hz, 6H), 1.50 (quint, *J* = 6.8 Hz, 2H), 2.63-2.69 (m, 2H), 2.75 (dd, *J* = 8.0, 12.0 Hz, 2H), 2.87 (dd, *J* = 3.2, 12.0 Hz, 1H), 4.00 (s, 3H), 4.06-4.09 (m, 2H), 4.60-4.66 (m, 1H), 6.39 (d, *J* = 2.0 Hz, 1H), 6.59 (d, *J* = 2.4 Hz, 1H), 7.13 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.22

(dd, *J* = 8.0, 16.0 Hz, 1H), 7.84 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.4, 19.7, 21.3, 31.0, 48.8, 51.6, 55.7, 67.1 70.0, 71.9, 71.1, 93.1, 97.8, 106.9, 114.1, 116.6, 122.6, 144.5, 147.4, 158.9, 159.5, 162.6, 163.5, 174.0 ppm.

5.5.14. 1-Butoxy-3-(3-butylamino-2-hydroxypropoxy)-5-methoxy-9*H*-xanthen-9-one (40)

Following the general method, compound **18** (0.07 g, 0.19 mmol) and *n*-butylamine (0.15 g, 2.02 mmol) were used to give compound **40** (0.04 g, 42.9%) as a white solid. m.p. 80-82 °C; R_f 0.44 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 2.70 min (condition B, purity: 95.8%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.94 (t, *J* = 7.2 Hz, 3H), 1.01 (t, *J* = 7.2 Hz, 3H), 1.38 (hexet, *J* = 7.2 Hz, 2H), 1.49 (quint, *J* = 7.2 Hz, 2H), 1.62 (hexet, *J* = 7.6 Hz, 2H), 1.96 (quint, *J* = 7.2 Hz, 2H), 2.70 (td, *J* = 4.4, 11.2 Hz, 2H), 2.73-2.79 (m, 1H), 2.86-2.90 (m, 1H), 4.00 (s, 3H), 4.04-4.13 (m, 5H), 6.38 (d, *J* = 2.4 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 7.14 (d, *J* = 2.4 Hz, 1H), 7.21-7.26 (m, 1H), 7.86 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.1, 14.2, 19.5, 20.6, 31.2, 32.5, 49.7, 51.7, 56.6, 68.0, 69.3, 71.2, 93.5, 96.7, 107.8, 114.6, 118.0, 123.4, 124.3, 145.4, 148.2, 159.7, 161.7, 164.1, 175.4 ppm.

5.5.15. 3-(3-Butylamino-2-hydroxypropoxy)- 5-methoxy-1-pentyloxy-9*H*-xanthen-9-one (41)

Following the general method, compound **19** (0.08 g, 0.22 mmol) and *n*-butylamine (0.10 g, 1.30 mmol) were used to give compound **41** (0.03 g, 32.0 %) as a white solid. m.p. 198-200 °C; R_f 0.33 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 3.05 min (condition B, purity: 98.4%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.44 (hexet, *J* = 4.0 Hz, 4H), 1.55 (quint, *J* = 7.0 Hz, 3H), 1.88 (quint, *J* = 8.0 Hz, 4H), 1.93 (quint, *J* = 7.0 Hz, 4H), 3.06-3.10 (m, 2H), 3.17-3.19 (m, 1H), 3.31 (dd, *J* = 2.4, 12.8 Hz, 1H), 3.94 (s, 3H), 4.14 (dd, *J* = 4.4, 9.0 Hz, 1H), 4.64-4.66 (m, 1H), 6.35 (d, *J* = 2.0 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 7.07

(dd, *J* = 1.6, 8.0 Hz, 1H), 7.17 (dd, *J* = 8.4, 8.4 Hz, 1H), 7.78 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.7, 14.2, 20.2, 22.7, 28.2, 23.3, 29.0, 48.9, 51.3, 56.5, 65.6, 69.8, 70.0, 93.6, 96.4, 107.9, 114.6, 117.8, 123.5, 124.2, 145.3, 148.1, 159.6, 161.8, 163.4, 175.3 ppm.

5.5.16. 1-Benzyloxy-3-(3-(butylamino)-2-hydroxypropoxy)-5-methoxy-9*H*-xanthen-9-one (42)

Following the general method, compound **20** (0.06 g, 0.20 mmol) and *n*-butylamine (0.15 g, 2.01 mmol) were used to give compound **42** (0.04 g, 37.1%) as a white solid. m.p. 150-152 °C; R_f 0.53 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 21.11 min (condition B, purity: 94.4%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.95 (t, *J* = 7.6 Hz, 3H), 1.39 (hexet, *J* = 7.6 Hz, 2H), 1.64 (quint, *J* = 7.6 Hz, 2H), 2.77-2.85 (m, 2H), 2.91 (dd, *J* = 8.8, 12.0 Hz, 1H), 3.01 (dd, *J* = 3.2, 12.4 Hz, 1H), 3.94 (s, 3H), 4.01 (dd, *J* = 5.2, 9.2 Hz, 1H), 4.09 (dd, *J* = 6.8, 14.0 Hz, 1H), 4.27-4.30 (m, 1H), 5.20 (s, 2H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 7.09 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.17 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.21-7.32 (m, 1H), 7.40 (dd, *J* = 8.0, 8.0 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.82 (dd, *J* = 1.6, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.0, 20.4, 30.4 49.2, 51.5, 56.5, 66.7, 70.7, 70.8, 94.0, 97.3, 107.9, 114.7, 117.8, 123.4, 124.1, 126.9, 127.9, 128.8, 136.5, 145.3, 148.1, 159.6, 160.8, 163.7, 175.3 ppm.

5.5.17. 3-(3-Butylamino-2-hydroxypropoxy)-1-(3-chlorobenzyloxy)-5-methoxy-9*H*-xanthen-9-one (43)

Following the general method, compound **21** (0.08 g, 0.18 mmol) and *n*-butylamine (0.13 g, 1.80 mmol) were used to give compound **43** (0.04 g, 43.5 %) as a white solid. m.p. 146-148 °C; R_f 0.52 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 3.62 min (condition B, purity: 97.4%); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.32 (hexet, *J* = 7.2 Hz,

2H), 1.50 (quint, *J* = 7.6 Hz, 2H), 2.76 (dd, *J* = 7.2, 7.2 Hz, 2H), 3.97 (s, 3H), 4.03-4.07 (m, 1H), 4.10 (dd, *J* = 6.0, 10.0 Hz, 1H), 4.18 (dd, *J* = 2.4 Hz, 1H), 6.63 (d, *J* = 2.4 Hz, 1H), 6.79 (d, *J* = 2.4 Hz, 1H), 7.34 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.42 (ddd, *J* = 1.6, 8.4, 10.0 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.67 (dd, *J* = 1.2, 7.6 Hz, 1H), 7.80 (dd, *J* = 1.6, 7.6 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.8, 19.7, 30.0, 48.3, 51.0, 56.2, 66.7, 69.1, 71.3, 94.1, 97.2, 106.5, 115.5, 116.5, 123.3, 123.7, 125.2, 126.4, 127.4, 130.2, 133.1, 139.4, 144.5, 147.8, 158.9, 159.8, 164.0, 173.6 ppm.

5.5.18. 3-(3-Butylamino-2-hydroxypropoxy)-5-methoxy-1-(3-methoxybenzyloxy)-9*H*-xanthen-9-one (44)

Following the general method, compound **22** (0.22 g, 0.50 mmol) and *n*-butylamine (0.37 g, 5.06 mmol) were used to give compound **44** (0.11 g, 43.4%) as a pale yellow solid. m.p. 86-88 °C; R_f 0.33 (eluent: MeOH: CH_2Cl_2 = 1:5); HPLC: R_T 2.90 min (condition B, purity: 91.0%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.83 (t, *J* = 6.0 Hz, 3H), 1.25 (hexet, *J* = 7.2 Hz, 2H), 1.39 (quint, *J* = 6.8 Hz, 2H), 2.55 (td, *J* = 5.2, 13.2 Hz, 2H), 2.60 (dd, *J* = 3.6, 12.0 Hz, 1H), 2.74 (dd, *J* = 3.6, 12.0 Hz, 1H), 3.76 (s, 3H), 3.91 (s, 3H), 3.96-4.00 (m, 2H), 4.31-4.35 (m, 1H), 5.12 (s, 2H), 6.35 (d, *J* = 2.4 Hz, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 6.73 (dd, *J* = 2.4, 8.0 Hz, 1H), 7.07 (dd, *J* = 1.2, 8.0 Hz, 2H), 7.12-7.26 (m, 3H), 7.74 (dd, *J* = 1.2, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 14.2, 20.6, 32.4, 49.7, 51.7, 55.5, 56.6, 67.9, 70.8, 71.2, 94.1, 97.5, 108.0, 112.1, 113.8, 114.7, 118.0, 119.0, 123.5, 124.3, 129.9, 138.2, 145.5, 148.2, 159.7, 160.1, 160.8, 164.0, 175.3 ppm.

5.5.19. 3-(3-Butylamino-2-hydroxypropoxy)-5-methoxy-1-(4-methoxybenzyloxy)-9*H*-xanthen-9-one (45)

Following the general method, compound **23** (0.05 g, 0.12 mmol) and *n*-butylamine (0.07 g,

1.01 mmol) were used to give compound **45** (0.05 g, 79.6%) as a white solid. m.p. °C; R_f 0.48 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 2.46 min (condition B, purity: 89.4%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.94 (t, *J* = 7.2 Hz, 3H), 1.26 (hexet, *J* = 7.2 Hz, 2H), 1.40 (quint, *J* = 7.2 Hz, 1H), 2.64-2.71 (m, 2H), 2.72-2.76 (m, 1H), 3.33 (dd, *J* = 3.6, 12.0 Hz, 1H), 3.82 (s, 3H), 4.01 (s, 3H), 4.02-4.08 (m, 2H), 5.18 (s, 2H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 6.94-6.79 (m, 2H), 7.16 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.22-7.26 (m, 1H), 7.54-7.57 (m, 2H), 7.88 (dd, *J* = 1.6, 8.0 Hz, 1H).

5.5.20. 3-(3-(butylamino)-2-hydroxypropoxy)-5-methoxy-1-(3-(trifluoromethyl)benzyloxy)-9*H*-xanthen-9-one (46)

Following the general method, compound **24** (0.05 g, 0.11 mmol) and *n*-butylamine (0.07 g, 0.91 mmol) were used to give compound **46** (0.04 g, 73.2%) as a pale yellow solid. m.p. 106-108 °C; R_f 0.25 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 4.09 min (condition B, purity: 90.8%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.26 (t, *J* = 7.2 Hz, 3H), 1.40 (hexet, *J* = 7.6 Hz, 2H), 1.78 (quint, *J* = 7.6 Hz, 2H), 2.95-3.02 (m, 2H), 3.09 (dd, *J* = 9.6, 12.4 Hz, 1H), 3.21 (dd, *J* = 2.8, 12.4 Hz, 1H), 3.94 (s, 3H), 4.09-4.15 (m, 3H), 4.51-4.54 (m, 1H), 5.25 (s, 2H), 6.43 (d, *J* = 2.0 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 7.08 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.18 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.57-7.59 (m, 3H), 7.80 (dd, *J* = 1.6, 8.0 Hz, 1H), 8.00-8.02 (m, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz) 13.7, 20.0, 29.4, 48.7, 51.3, 56.3, 66.2, 69.9, 70.7, 94.5, 97.3, 107.5, 114.8, 117.3, 123.4, 123.4, 123.8, 124.4, 129.2, 130.3, 137.6, 145.1, 148.0, 159.4, 160.1, 163.8, 174.8 ppm.

5.5.21. 3-(3-Butylamino-2-hydroxypropoxy)-1-(3-chlorophenethoxy)-5-methoxy-9*H*-xanthen-9-one (47)

Following the general method, compound **25** (0.08 g, 0.25 mmol) and *n*-butylamine (0.18 g,

2.45 mmol) were used to give compound **47** (0.06 g, 57.1%) as a white solid. m.p. 198-200 °C; R_f 0.23 (eluent: MeOH:CH_2Cl_2 = 1:5); HPLC: R_T 3.81 min (condition B, purity: 97.3%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.94 (t, *J* = 7.6 Hz, 3H), 1.40-1.45 (m, 2H), 1.81-1.85 (m, 2H), 2.30-3.05 (m, 2H), 3. 11 (dd, *J* = 10.0, 12.8 Hz, 1H), 3.22 (t, *J* = 6.4 Hz, 2H), 3.25 (dd, *J* = 2.4, 12.8 Hz, 1H), 3.95 (s, 3H), 4.02 (dd, *J* = 2.0, 9.6 Hz, 1H), 4.08 (dd, *J* = 4.8, 10.0 Hz, 1H), 4.20 (t, *J* = 6.8 Hz, 2H), 4.56-4.58 (m, 1H), 6.30 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 7.09 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.20 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.26-7.29 (m, 2H), 7.39-7.41 (m, 2H), 7.81 (dd, *J* = 1.2, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 13.4, 19.8, 28.3, 32.0, 34.7, 48.4, 51.0, 56.2, 65.4, 69.8, 93.7, 96.4, 107.3, 109.7, 114.5, 117.3, 123.2, 123.8, 128.3, 130.7, 132.0, 136.6, 145.0, 147.8, 159.2, 160.7, 163.3, 174.8 ppm.

5.6. Human topoisomerase IIα relaxation assay. All the test compounds were dissolved in DMSO at a concentration of 20 mM as a stock solution and stored under -20°C until needed. The DNA topoisomerase IIα inhibitory activity of each compound was measured as follows. A mixture containing 200 ng of supercoiled pBR322 plasmid DNA (Thermo Scientific, USA) and 1 unit of human DNA topoisomerase IIα (Usb Corp., USA) was incubated with and without the prepared compounds in the assay buffer (10 mM Tris-HCl (pH 7.9) containing 50 mM NaCl, 50 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin) for 30 min at 30 °C. The reaction in a final volume of 20 μL was quenched by adding 3μL of 7 mM EDTA. The reaction products were analyzed on 1% agarose gel at 25 V for 4 h with TAE as the running buffer. The gels were stained and the DNA bands were visualized for 15 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by trans-illumination with UV light and were quantitated using Alpha Tech Imager (Alpha Innotech Corporation).

5.7. Cytotoxicity assay. Four different cancer cell lines were used to evaluate the cytotoxicity; human ductal breast epithelial tumor cell line (T47D), human colorectal carcinoma cell line (HCT15), human gastric tumor cell line (NCI-N87) and human cervix adenocarcinoma cell line (HeLa). The experiments were performed as described previously [35]. The cancer cells were cultured according to the supplier's instructions. In brief, cells (2~4 x 10^4 cells per well) were seeded overnight in 0.1 mL of the media supplied with 10% Fetal Bovine Serum (Hyclone, USA) in a 5% CO_2 incubator at 37 °C. Cells were then treated with compounds in graded concentrations for 72 h. 5 μL of the cell counting kit-8 solution (Dojindo, Japan) was added to each well and incubated for an additional 4 h under the same conditions. The absorbance of each well was determined using an Automatic Elisa Reader System (Bio-Rad 3550) at a wavelength of 450 nm. To determine the IC_{50} values, the absorbance readings at 450 nm were fitted to a four-parameter logistic equation. Adriamycin, etoposide and camptothecin, which were used as the positive controls, were purchased from Sigma.

5.8. Band depletion assay. This assay was performed as described previously with a minor modification [36]. T47D cells were seeded overnight at a density of 2 x 10^5 cells per well. The cells were treated for 2 hours at 37 °C with each of etoposide and compounds **36**, **37**, **47** and **48**, and co-treated with etoposide and each compound in only RPMI 1960 (serum free media) followed by cell harvesting. The cell pellet after centrifugation (4 °C, 3200 rpm, and 3 minutes) was washed with 1 mL of PBS and then ice-incubated for 1.5 hours, followed by lysis with denaturing agent (62.5 mM Tris-HCl (pH 6.8), 1 mM EDTA and 2% SDS) and sonication (10~20 bursts, 2 seconds). The samples were ice-incubated for 20 minutes and centrifuged for 20 minutes (4 °C, 12,000 rpm and 30 minutes) again. Finally the supernatant

of sample was taken to perform Western blot analysis on 10% SDS-PAGE gels. All primary antibodies used were purchased from Cell Signaling Technology Inc. (USA).

5.9. Cleavable complex assay. The mixture of 250 ng of supercoiled DNA pBR322 and 3 units of human topoisomerase IIα with or without compound at the designated concentrations in the figure legend was incubated at 37 °C for 30 minutes in the reaction buffer (10 mM tris-HCl (pH 7.9) containing 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL BSA and 1 mM ATP). The reaction was then terminated by the addition of 2.5 μL of stop solution (5% SDS, 25% ficoll and 0.05% bromophenol blue) followed by treatment of 2 μL of 0.25 mg/mL proteinase K (Sigma Aldrich, USA) with continuous incubation at 45 °C for 30 minutes to eliminate the protein. Samples were electrophoresed on a 1 % (w/v) agarose gel containing 0.5 μg/mL ethidium bromide at 30 V for 6 hours in TAE running buffer. DNA bands on the gel were detected by UV and visualized by AlphaImager™.

5.10. Comet Assay To evaluate DNA damage, comet assay was performed using single-cell gel electrophoresis with a Trevigen kit (Gaithersburg, MD) according to the method previously reported [34,37] Briefly, T47D cells, seeded in a density of 1 x 10^5 cells per well in six-well plates were treated with 10 μM of each compound and etoposide for 24 h and harvested by trypsinization followed by resuspending cells in 1 mL of ice-cold PBS. Then, 8 μL of resuspened cells were mixed with 80 μL of low-melting agarose at 37 ˚C, spread on slides and solidified in the dark for 40 min at 4 ˚C. Slides were lysed in ice-cold lysis solution in the dark for 30 min at 4 ˚C and then submerged in a fresh alkaline solution (pH>13) at room temperature for 30 min to allow alkaline unwinding. Electrophoresis was performed under alkaline conditions for 20 min for 15 V. Slides were rinsed twice with distilled water, once with 70 % ethanol and stained with SYBR Green (Trevigen, Gaithersburg) in a TE

buffer for 5 min in the dark at 4 ˚C. Comet images were obtained using an inverted fluorescence microscope (Zeiss, Axiovert 200) at 10X magnification and percent DNA in tail was analyzed by Komet 5.0 software (kinetic imaging Ltd, UK). Data were represented both by imaging and graphically by randomly selecting comet lengths of T47D cells.

5.11. Molecular docking studies. The coordinates for the ATP binding domain of human topoisomerase IIα were retrieved from the Protein Data Bank (PDB code 1ZXM) [38]. All the water molecules, and ligands and were removed and the hydrogen atoms were added [39]. The structures of compound **48** was constructed by SYBYL X-2.1 and minimized energetically using a Tripos force field with Gasteiger-Huckel charges. The receptor and ligand file were prepared according to the original publication protocols [40,41]. Docking was carried out with Autodock using the Lamarckian genetic algorithm search parameters. Default search parameters were used except for population size of 270,000 and 50 docking runs.

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Scheme 1. General synthetic method of compounds

Scheme 2. Structures of the prepared compounds

Table 1. Topoisomerase IIα inhibitory activities of compounds

Table 2. Cytotoxicity test results of compounds

Figure 1. Topoisomerase IIα inhibitory activities of all the prepared compounds. All the synthesized compounds were examined in a final concentration of 20 and 100 μM, respectively, as designated. Lane D: pBR322 only, Lane T: pBR322 + topoisomerase IIα, Lane E: pBR322 + topoisomerase IIα + etoposide, Lanes for compounds: pBR322 + topoisomerase IIα + each compound in designated concentrations.

Figure 2. Band depletion assay of compounds **36**, **37** and **47**. The cells seeded in a density of 2×10^5 were treated with 50 μM of each of etoposide, compounds **36**, **37** and **47** for 2 hours at 37 °C and then were harvested. The harvested cells were lysed by denaturing agent of 62.5 mM Tris-HCl (pH 6.8) containing 1mM EDTA and 2% SDS. The prepared lysates in according to the experimental method were detected by Western blotting. The etoposide treatment abolished the free topoisomerase IIα, however treatment of compounds **36**, **37** and **47** remained the free topoisomerase IIα band reflecting that compounds **36**, **37** and **47** function as a topoisomerase IIα catalytic inhibitor not poison.

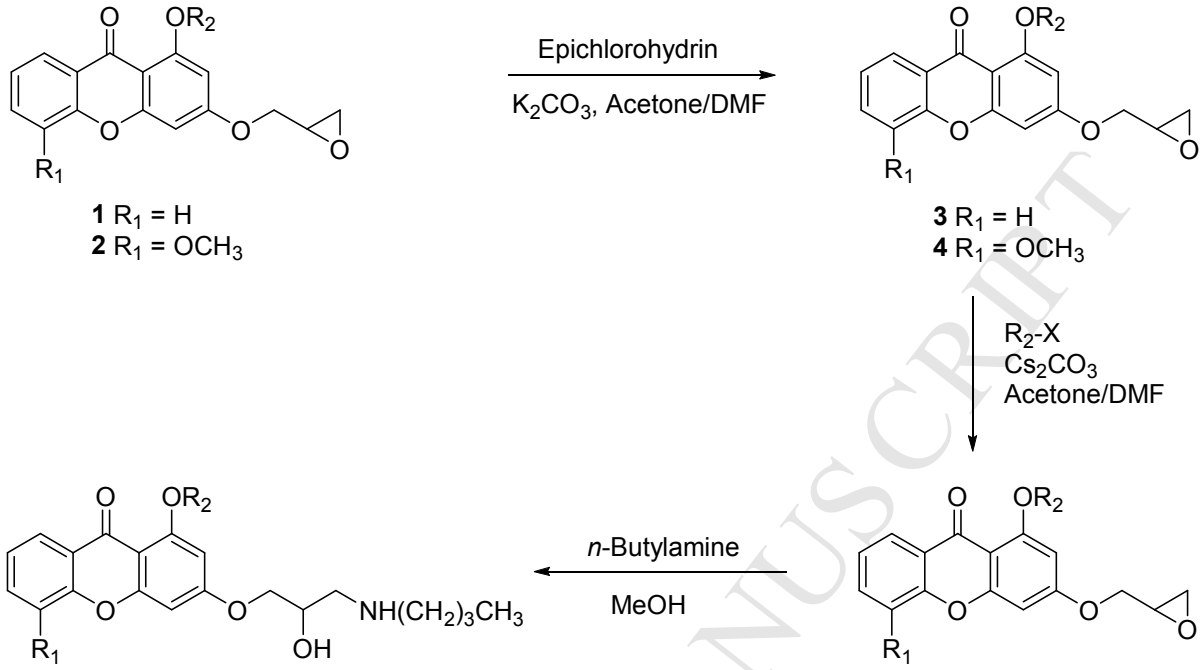
Figure 3. Cleavable complex assay of compounds **36**, **37** and **47**. To revalidate topoisomerase IIα catalytic inhibitor, 250 ng of pBR322 DNA was treated with 3 units of topoisomerase IIα for 10 minutes prior to treatment of etoposide (100 μM), each compound (100 μM) or co-treatment. After incubation for additional 30 minutes at 37 °C, the agarose gel electrophoresis were performed. A linear band on the gel for compounds **36**, **37** and **47** was not observed reflecting that they are topoisomerase IIα catalytic inhibitors.

Figure 4. Comet assay to assess compound-induced DNA damage. Cells were seeded in 6-well tissue-culture plates, reached 70% confluence and treated with 5 μM and 10 μM of each compound for 24 h followed by processing comet assay. (A) Images of control (non-treated), etoposide (topo poison) and compounds **36**, **37** and **47** treated cells showing comet formation.

(B) Graphical representation of the selected comet lengths of untreated- and treated-T47D cells in pixels with corresponding to concentration in (A).

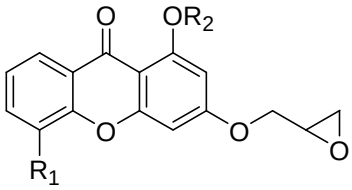
Figure 5. Interaction of compound **37** and ATP binding site of ATPase domain of topoisomerase IIα. The compound and the protein binding site are represented in sticks. The atoms are colored by atom types and the carbons of compound **37**, AMP-PNP, and topoisomerase IIα in pink, light blue, and gray, respectively. Magnesium is represented as green sphere and the hydrogen bonds are shown in green dotted lines. (A) Interaction between compound **37** and topoisomerase IIα. (B) Overlay of compound **37** and AMP-PNP.

Scheme 1.



Scheme 2.

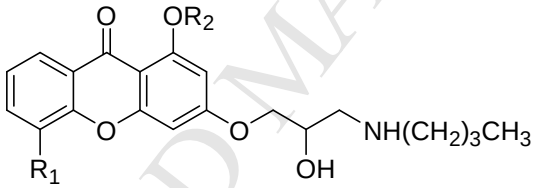
Group I



5 R_1 = H, R_2 = C_2H_5
6 R_1 = H, R_2 = i-C_3H_7
7 R_1 = H, R_2 = C_4H_9
8 R_1 = H, R_2 = C_5H_{11}
9 R_1 = H, R_2 = benzyl
10 R_1 = H, R_2 = 3-chlorobenzyl
11 R_1 = H, R_2 = 3-methoxybenzyl
12 R_1 = H, R_2 = 4-methoxybenzyl
13 R_1 = H, R_2 = 3-trifluoromethylbenzyl
14 R_1 = H, R_2 = 3-chlorophenethyl
15 R_1 = H, R_2 = 4-chlorophenethyl

16 R_1 = OCH_3, R_2 = C_2H_5
17 R_1 = OCH_3, R_2 = i-C_3H_7
18 R_1 = OCH_3, R_2 = C_4H_9
19 R_1 = OCH_3, R_2 = C_5H_{11}
20 R_1 = OCH_3, R_2 = benzyl
21 R_1 = OCH_3, R_2 = 3-chlorobenzyl
22 R_1 = OCH_3, R_2 = 3-methoxybenzyl
23 R_1 = OCH_3, R_2 = 4-methoxybenzyl
24 R_1 = OCH_3, R_2 = 3-trifluoromethylbenzyl
25 R_1 = OCH_3, R_2 = 3-chlorophenethyl
26 R_1 = OCH_3, R_2 = 4-chlorophenethyl

Group II



27 R_1 = H, R_2 = C_2H_5
28 R_1 = H, R_2 = i-C_3H_7
29 R_1 = H, R_2 = C_4H_9
30 R_1 = H, R_2 = C_5H_{11}
31 R_1 = H, R_2 = benzyl
32 R_1 = H, R_2 = 3-chlorobenzyl
33 R_1 = H, R_2 = 3-methoxybenzyl
34 R_1 = H, R_2 = 4-methoxybenzyl
35 R_1 = H, R_2 = 3-trifluoromethylbenzyl
36 R_1 = H, R_2 = 3-chlorophenethyl
37 R_1 = H, R_2 = 4-chlorophenethyl

38 R_1 = OCH_3, R_2 = C_2H_5
39 R_1 = OCH_3, R_2 = i-C_3H_7
40 R_1 = OCH_3, R_2 = C_4H_9
41 R_1 = OCH_3, R_2 = C_5H_{11}
42 R_1 = OCH_3, R_2 = benzyl
43 R_1 = OCH_3, R_2 = 3-chlorobenzyl
44 R_1 = OCH_3, R_2 = 3-methoxybenzyl
45 R_1 = OCH_3, R_2 = 4-methoxybenzyl
46 R_1 = OCH_3, R_2 = 3-trifluoromethylbenzyl
47 R_1 = OCH_3, R_2 = 4-chlorophenethyl

Table 1. Topoisomerase IIα inhibitory activities of compounds

	% Inhibition of Topoisomerase IIα at the concentration of			
Compound/Conc.	100 μM	Compound	100 μM	20 μM*
Etoposide	74.9	**Etoposide**	74.9	45.6
5	0	**27**	4.8	-
6	0.1	**28**	1.5	-
7	0	**29**	27.1	-
8	0	**30**	29.4	-
9	0	**31**	65.4	22.2
10	0	**32**	56.3	22.4
11	0	**33**	58.7	19.5
12	0	**34**	55.9	21.1
13	0	**35**	64.6	9.3
14	0	**36**	46.2	16.6
15	0	**37**	94.4	23.3
16	2.5	**38**	10.7	-
17	1.5	**39**	7.5	-
18	14.1	**40**	24.2	-
19	5	**41**	36.3	9.5
20	3.3	**42**	51.6	11.1
21	1.7	**43**	45.4	6.3
22	0	**44**	41.6	12.4
23	0	**45**	44.8	13.9
24	0	**46**	58.1	14.2
25	0	**47**	62.8	20.4
26	0			

*****Compounds of which % inhibition against topoisomerase IIα were more than 30% at 100 μM were further examined at 20 μM treatment. '-' means not texted.

Table 2. Cytotoxicity test results of compounds

	IC_{50} (μM)			
	HCT15	T47D	HeLa	NCI-N87
Adriamycin	0.47±0.00	1.42±0.02	1.70±0.06	0.71±0.01
Etoposide	1.06±0.11	2.8±0.17	10.03±0.33	1.29±0.03
Camptothecin	0.0014±0.0001	0.08±0.00	0.13±0.00	0.60±0.04
5	3.96±0.05	3.60±0.09	0.80±0.02	3.91±0.23
6	1.63±0.36	1.94±0.06	1.62±0.05	0.29±0.05
7	2.45±0.06	3.75±0.08	1.81±0.02	4.55±0.32
8	3.28±0.17	>50	1.79±0.01	10.95±0.13
9	1.50±0.01	3.06±0.13	1.92±0.07	5.07±0.06
10	1.96±0.01	3.30±0.16	3.57±0.19	1.89±0.04
11	1.49±0.02	3.73±0.05	1.57±0.03	3.26±0.04
12	1.72±0.05	2.96±0.15	1.58±0.02	3.73±0.14
13	5.08±0.09	2.30±0.05	4.28±0.07	1.36±0.03
14	4.13±0.04	1.36±0.04	1.46±0.05	3.18±0.16
15	5.27±0.06	0.97±0.00	4.91±0.30	2.02±0.07
16	0.82±0.01	0.83±0.01	1.17±0.06	1.19±0.09
17	0.31±0.01	1.75±0.12	1.87±0.07	3.12±0.63
18	0.22±0.00	1.63±0.04	1.08±0.02	0.31±0.03
19	0.37±0.01	4.57±0.53	4.06±0.58	1.69±0.11
20	0.34±0.01	2.02±0.08	1.66±0.05	1.75±0.06
21	0.17±0.01	1.55±0.03	1.70±0.12	0.95±0.01
22	0.35±0.01	4.05±0.26	2.43±0.80	1.32±0.04
23	0.42±0.02	7.68±0.92	1.74±0.32	1.41±0.04
24	0.36±0.01	2.87±0.20	4.27±0.07	2.24±0.21
25	1.08±0.10	2.74±0.26	0.47±0.03	1.11±0.12
26	45.22±1.25	>50	>50	1.49±0.10
27	28.17±1.47	7.02±0.23	>50	1.77±0.52
28	43.4±0.80	4.71±0.47	36.62±1.61	2.05±0.04
29	8.61±0.55	1.54±0.05	2.07±0.03	1.63±0.03

30	4.58±0.08	1.27±0.17	2.56±0.12	0.06±0.01
31	3.09±0.34	2.81±0.01	7.05±0.15	1.44±0.04
32	2.24±0.06	1.17±0.05	2.83±0.12	0.17±0.03
33	1.91±0.03	2.57±0.03	5.86±0.05	0.07±0.01
34	2.17±0.01	1.41±0.00	4.81±0.11	1.80±0.20
35	2.45±0.02	0.14±0.01	2.26±0.21	0.24±0.06
36	2.28±0.03	1.72±0.07	2.56±0.04	0.38±0.03
37	2.13±0.02	0.63±0.01	1.37±0.03	3.63±0.42
38	10.6±0.03	4.28±0.20	23.48±0.36	2.24±0.17
39	9.60±0.13	2.67±0.01	14.39±0.35	0.08±0.01
40	6.02±0.04	1.13±0.17	7.65±0.06	1.64±0.15
41	1.81±0.01	0.78±0.01	3.84±0.06	0.48±0.15
42	2.08±0.01	2.40±0.01	2.25±0.07	1.23±0.03
43	1.59±0.09	0.67±0.02	2.40±0.01	1.16±0.04
44	1.74±0.02	1.36±0.06	2.30±0.06	1.32±0.05
45	2.29±0.01	1.52±0.03	1.82±0.14	0.25±0.02
46	2.62±0.02	2.00±0.02	2.62±0.05	1.98±0.06
47	1.49±0.02	0.19±0.02	2.60±0.14	0.40±0.00

Figure 1.

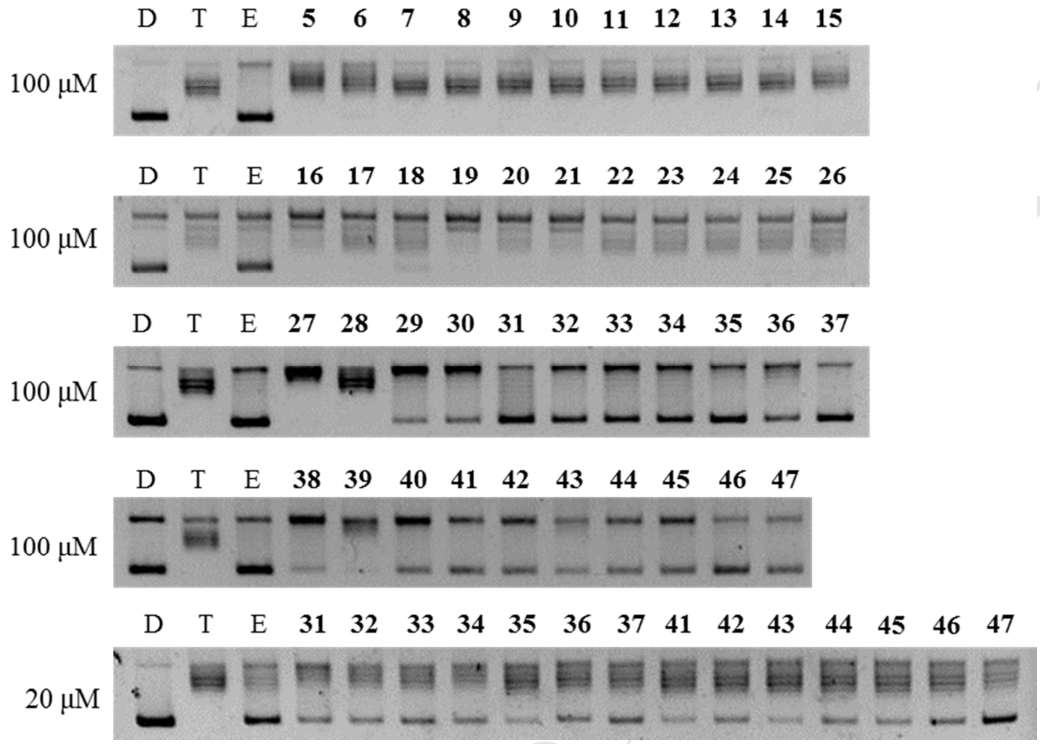


Figure 2.

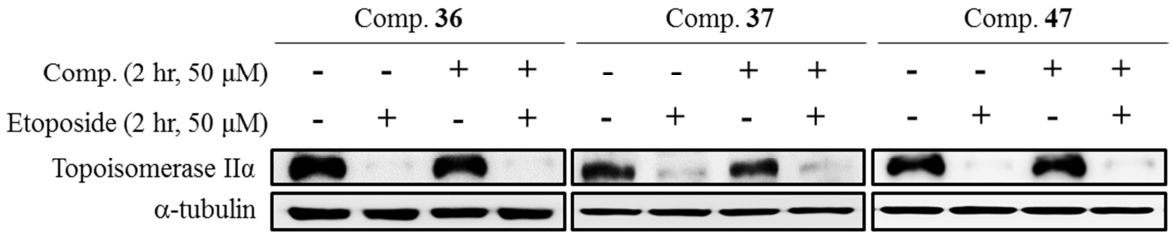


Figure 3.

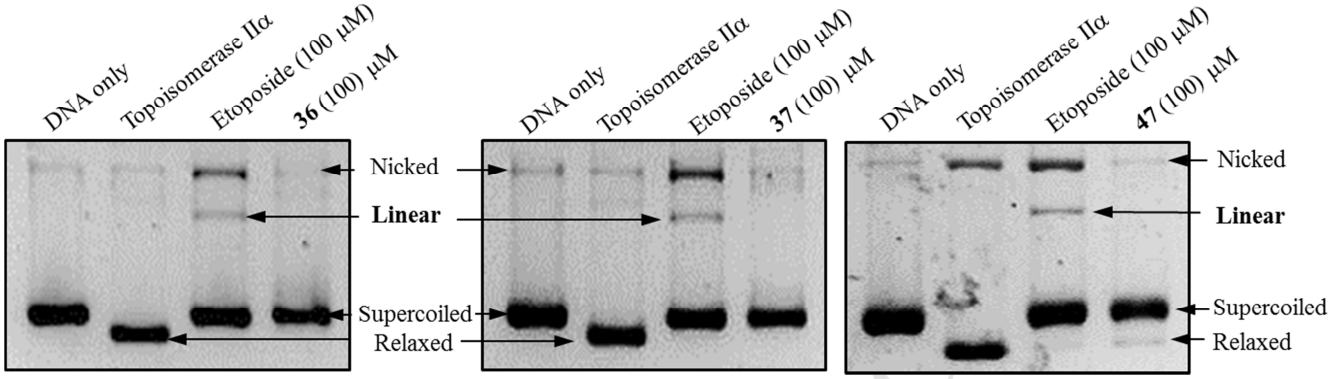


Figure 4.

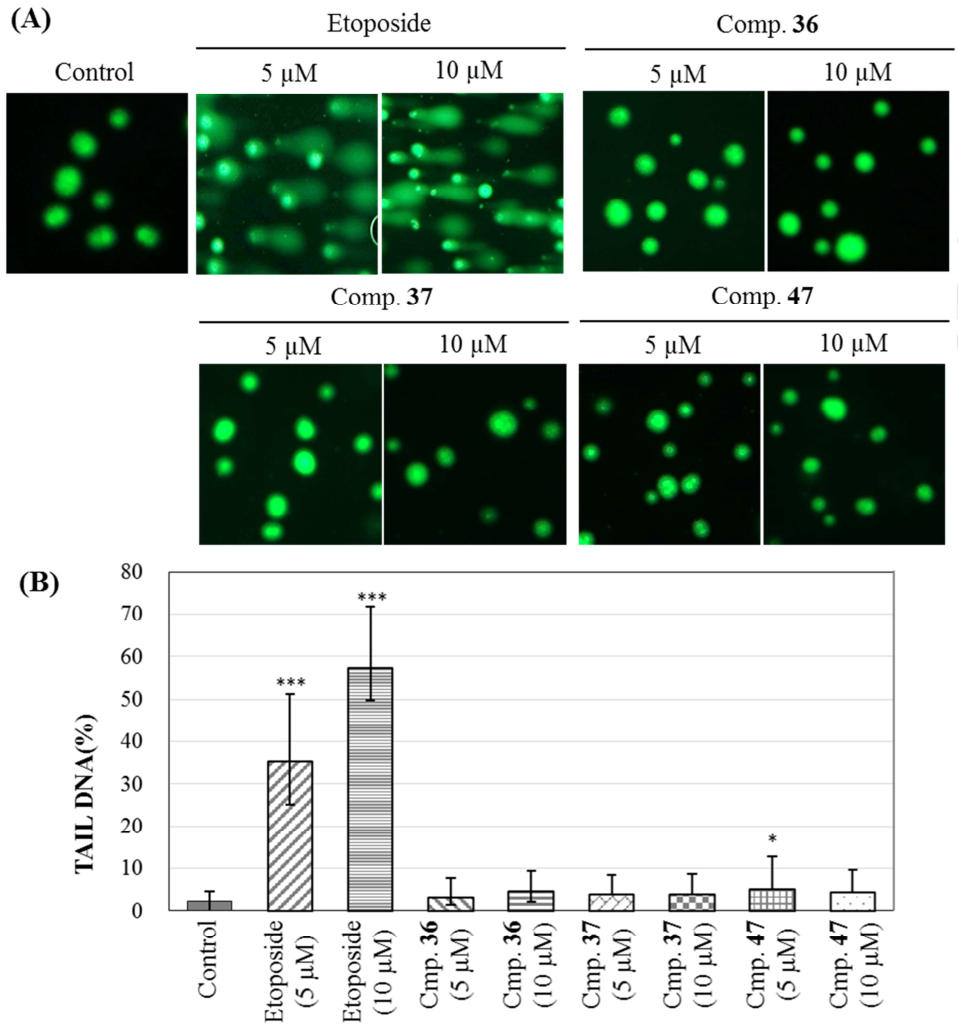
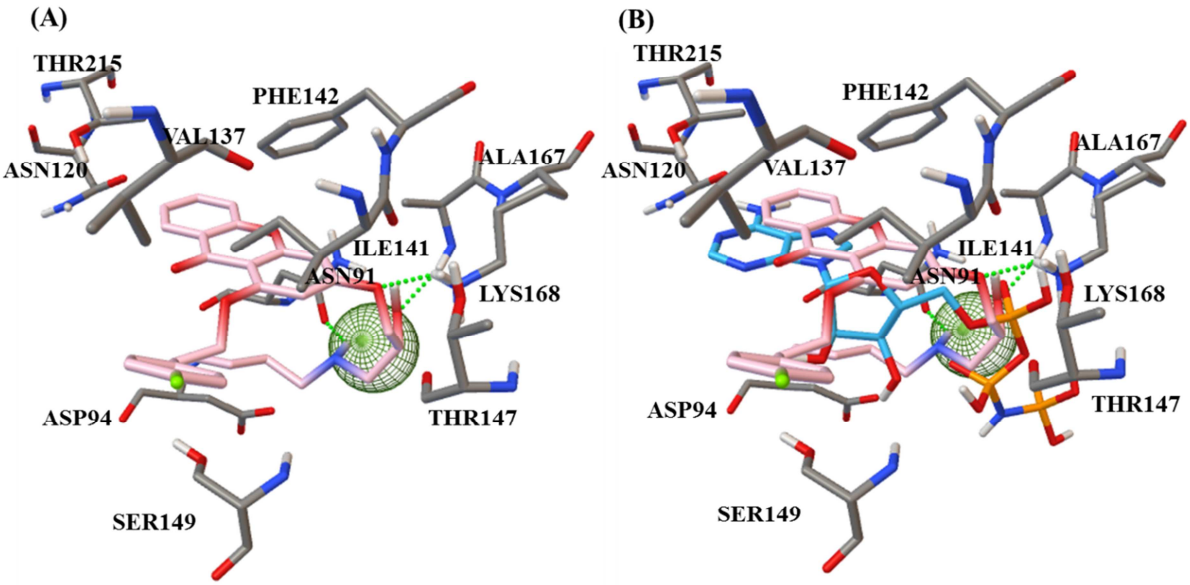
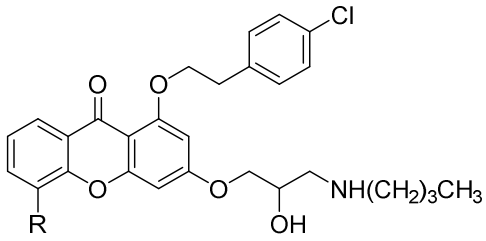

(A)
Control
Etoposide
5 μM
10 μM
Comp. 36
5 μM
10 μM
Comp. 37
5 μM
10 μM
Comp. 47
5 μM
10 μM
(B)
TAIL DNA(%)
0
10
20
30
40
50
60
70
80
***
***
*
Control
Etoposide (5 μM)
Etoposide (10 μM)
Cmp. 36 (5 μM)
Cmp. 36 (10 μM)
Cmp. 37 (5 μM)
Cmp. 37 (10 μM)
Cmp. 47 (5 μM)
Cmp. 47 (10 μM)

Figure 5.



Graphical Abstract

Compound **37** and **47** were potential topoisomerase IIα catalytic inhibitors with low DNA damage.



37 R = H
47 R = OCH_3

	Topoisomerase IIα inhibition	**Cytotoxicity: T47D (IC_{50})**
Compound 37	**23.3% at 20 μM**	**0.63 ± 0.01**
Compound 47	**20.4% at 20 μM**	**0.19 ± 0.02**

Research Highlights

- C1-*O*- arylalkyl xanthones were potential topoisomerase IIα catalytic inhibitors.
- Compound **37** showed efficient cytotoxicity against T47D cell.
- Compound **37** induced much less DNA damage than etoposide in T47D cell.
- Compound **37** was confirmed as a potential topoisomerase IIα catalytic inhibitor

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