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Synthesis of novel naphthoquinone aliphatic amides and esters and their anticancer evaluation

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ABSTRACT

Fourteen new naphthoquinone aliphatic amides and seventeen naphthoquinone aliphatic esters were synthesized in nine to ten steps from 1-hydroxy-2-naphthoic acid with 9–25% overall yield for the amides, and 16–21% overall yield for the esters. The key step of the amide synthesis is a coupling reaction between amine and various aliphatic acids using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as a coupling agent while for the ester synthesis, DCC/DMAP or CDI was used as the coupling reagent between aliphatic acids and naphthoquinone alcohol. Both naphthoquinone amides and esters were evaluated for their anticancer activity against KB cells. It was found that naphthoquinone aliphatic amides showed stronger anticancer activity than those of the esters when the chains are longer than 7-carbon atoms. The optimum chain of amides is expected to be 16-carbon atoms. In addition, naphthoquinone aliphatic esters with α-methyl on the ester moiety possessed much stronger anticancer activity than the straight chains. Decatenation assay revealed that naphthoquinone amide with 16-carbon atoms chain at 15 μM and 20 μM can completely inhibit hTopoIIα activity while at 10 μM the enzyme activity was moderately inhibited. Molecular docking result also showed the same trend as the cytotoxicity and decatenation assay.

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1. Introduction

Rhinacanthins (1,4-naphthoquinone) and Rhinacanthone (1,2-naphthoquinone) isolated from *Rhinacanthus nasutus* (Thai name: Thong Pan Chang) (Acanthaceae) showed biological activities [1–5], especially anticancer activity. Studies on apoptosis mechanism of rhinacanthins and rhinacanthone were investigated [5]. Because of very interesting anticancer activity of rhinacanthins and rhinacanthone, we have synthesized rhinacanthins [6], rhinacanthone, 1,2-pyrano, 1,4-pyrano and furanonaphthoquinones [7], naphthoquinone aromatic and aliphatic esters [6,8] and naphthoquinone 2′-cyclopentyl and 2′-cyclohexyl esters [9]. Most of them exhibited anticancer activity especially naphthoquinone aromatic esters, whereas some aliphatic esters showed very good antimalarial activity with lesser or no toxicity against Vero cells [8]. In addition, it was found that 2′,2′-dimethyl substituents on the propyl chain showed more potent activity than those of other 2′,2′-substituents [9]. This was the reason why novel naphthoquinone aromatic amides with 2′,2′-dimethyl groups were synthesized and evaluated for anticancer activity [10]. Although it was revealed that naphthoquinone aromatic amides exhibited less anticancer activity than those of aromatic esters with 2′,2′-dimethyl substituents [8], it is still worthwhile to further synthesize novel naphthoquinone aliphatic amides, as well as to evaluate their anticancer activity in comparison with those of the aromatic amides and the aliphatic esters, and to compare them with those of the naphthoquinone ester and amide series.

Therefore this paper describes the synthesis of fourteen novel naphthoquinone aliphatic amides and seventeen naphthoquinone aliphatic esters starting from commercially available 1-hydroxy-2-naphthoic acid in nine to ten steps. All thirty-one synthetic aliphatic amides and esters were tested for cytotoxicity against KB cells and normal Vero cells in comparison with the aromatic ones. According to our previous work on naphthoquinone esters and amides [6,10], the compound exhibited the TopoII inhibition activity in corresponding with β-lapachone (1,2-naphthoquinone) and related naphthoquinones [11]. Therefore, in this paper, the decatenation assay for human Topoisomerase IIα and molecular

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docking analysis of some selected naphthoquinone compounds were also carried out.

2. Results and discussion

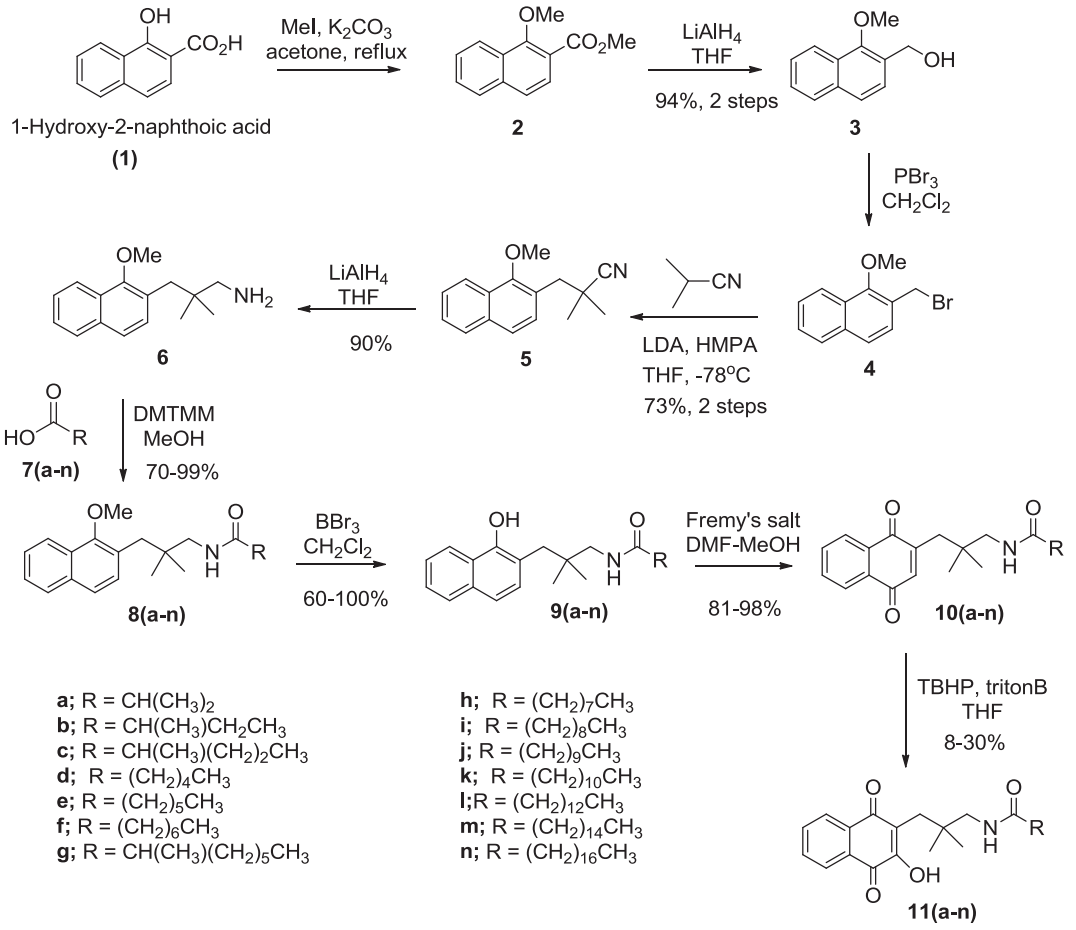
2.1. Synthesis

A series of naphthoquinone aliphatic amides was synthesized and investigated for their anticancer activity. Methylation of 1-hydroxy-2-naphthoic acid (**1**) followed by reduction, bromination, alkylation and then reduction provided amine **6** [10]. Coupling reactions between **6** and various aliphatic acids **7a**–**n** were done using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) in methanol to give various amides **8a**–**n** with good to excellent yields (70–99% yield). Then demethylation using boron tribromide gave naphthol amides **9a**–**n** with various acyl groups in moderate to quantitative yield. The target naphthoquinone aliphatic amides **11a**–**n** (Scheme 1), were obtained by oxidation of **9a**–**n** with Fremy's salt followed by hydroxylation with *tert*-butyl hydroperoxide (TBHP). It was found that the hydroxylation step of naphthoquinone aliphatic amides gave lower yields than those of aromatic amides [10]. This may be accounted for by the easier hydrolysis of the aliphatic amide group than the aromatic amide group (less electrophilicity) under basic condition. In comparison to the aliphatic amides, seventeen naphthoquinone aliphatic esters were also synthesized by the same method as used in our previous report [8] (Scheme 2) and their anticancer activity was also determined.

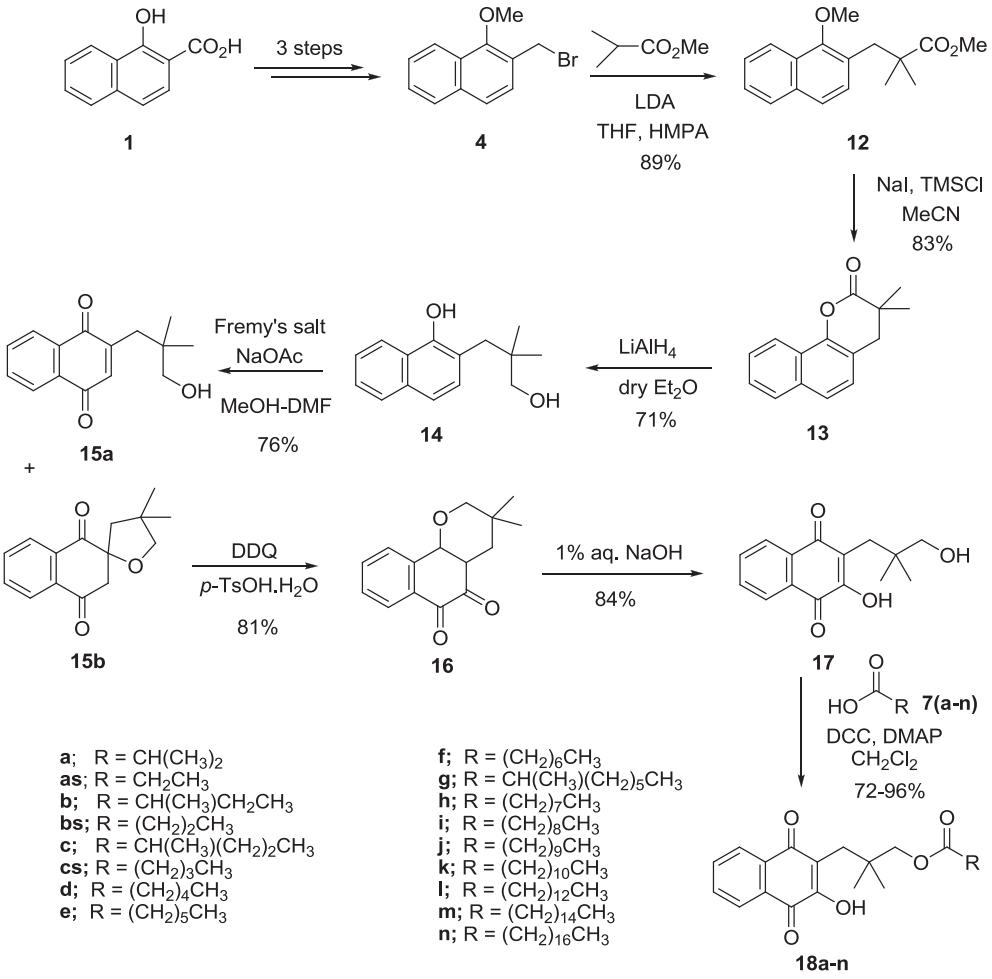
2.2. Biological activity

Fourteen novel synthetic naphthoquinone aliphatic amides and seventeen naphthoquinone aliphatic esters were evaluated for their cytotoxicity against human cancer cell lines KB (oral cavity cancer) by the REsazurin Microplate Assay (REMA) [12] and normal Vero cell lines by green fluorescent protein (GFP)-based assay [13]. The results are shown in Tables 1 and 2.

Tables 1 and 2 demonstrate anticancer activity against KB cells of naphthoquinone aliphatic amides and esters, respectively. The results showed that chain lengths of amide and ester moieties affected the anticancer activities. It can be seen that when the chain lengths are between 3- to 7-carbon atoms, the ester series (**18a**–**e**, IC_{50} = 1.76–41.01 μM) inhibited KB cells stronger than the amide series (**11a**–**e**, IC_{50} = 28.21–101.86 μM). With the exception of amide **11d** and ester **18d** which showed similar results (IC_{50} values of 40.23 and 41.01 μM, respectively). With respect to the aliphatic amides, in contrast to those with shorter chain lengths, the longer chain aliphatic amides with more than 7-carbon atoms showed more potent anticancer activities. For example, amide **11f** (IC_{50} = 24.64 μM) with an 8-carbon atoms showed stronger activity than ester **18f** (IC_{50} = 38.29 μM) and amides **11m** (16-carbon atoms) and **11n** (18-carbon atoms) exhibited very strong activity with IC_{50} values of 5.12 and 6.35 μM, respectively, whereas esters **18m** (16-carbon atoms) and **18n** (18-carbon atoms) showed much lesser anticancer activities. However, it is interesting to note that these two esters (**18m** and **18n**) showed very good antimalarial activity, which has been described in our previous paper [8].



Scheme 1. Synthesis of naphthoquinone aliphatic amides **11a**–**n**.



Scheme 2. Synthesis of naphthoquinone aliphatic esters **18a**–**n**.

Regarding to the results, amides having 16- and 18-carbon atoms chains could be the optimal chain length for the anticancer activity.

Interestingly, the esters containing α-methyl on the chain inhibited cancer cells stronger than those of the ones without α-methyl group whereas no difference of the amide series was detected. In the same manner, most of naturally occurring naphthoquinone aliphatic esters containing α-methyl isolated from *R. nasutus* such as rhinacanthin-C, G, H, and K (Fig. 1) showed potent anticancer activities [3]. Therefore, it would be very useful for further comparison with and without α-methyl group on the aliphatic chain of the ester series.

Table 2 shows that naphthoquinone aliphatic esters with α-methyl on the chain (**18a**, **18b**, **18c** and **18g**, IC_{50} = 14.38, 4.65, 1.76, 16.78 μM, respectively) exhibited more potent cytotoxicity than the naphthoquinone esters without α-methyl group given with the same chain lengths (**18as**, **18bs**, **18cs** and **18f**, IC_{50} = 84.40, 42.98, 27.79, 38.29 μM, respectively).

According to our findings, it is evident that naphthoquinone aliphatic amides showed stronger anticancer activity than the aromatic amides [10] and aliphatic esters. In order to gain further understanding on structure–activity relationship (SAR), some naphthoquinone aliphatic amides and esters (i.e. **11c**, **11m**, **18c** and **18m**) were selected for the evaluation of human Topoisomerase IIα (hTopoIIα) inhibition and for molecular docking analysis.

2.3. Human Topoisomerase IIα inhibitory activity

The decatenation assay was used to determine the inhibitory activity of novel naphthoquinone aromatic amides and esters versus hTopoIIα. Enzyme hTopoIIα catalyzes the ATP-dependent decatenation of long-chain, catenated DNA molecules into free relaxed and supercoiled forms [14]. Fig. 2 shows hTopoIIα inhibitory activity against four selected compounds (**11c**, **11m**, **18c** and **18m**) at final concentration of 50 μM. It can be clearly seen that compound **11m**, which is a naphthoquinone aliphatic amide with 16-carbon atoms side chain long, exhibited strong hTopoIIα inhibitory activity while compound **18m**, a naphthoquinone aliphatic ester with 16-carbon atoms side chain long, can moderately inhibit the hTopoIIα activity. This result correlated well with the cytotoxicity test in that aliphatic amides with long carbon side chains showed better cytotoxicity compared to the aliphatic esters. In contrast to the naphthoquinone aliphatic ester or amide containing a long side chain, those with shorter side chains, such as compounds **11c** and **18c**, displayed low hTopoIIα inhibitory activity. However, by comparing between the naphthoquinone aliphatic ester and amide with short side chains, the naphthoquinone aliphatic ester can inhibit hTopoIIα activity better than the naphthoquinone aliphatic amide. Therefore, the trend of hTopoIIα inhibition activity is altered when the aliphatic side chain grows. Taken together, it can be concluded that with the short side chain, the naphthoquinone aliphatic ester is a better hTopoIIα inhibitor

Table 1
Cytotoxicity of the synthetic naphthoquinone aliphatic amides **11a**–**n** against KB cells and normal Vero cells.

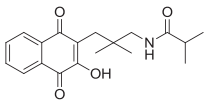
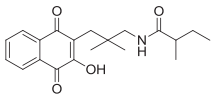
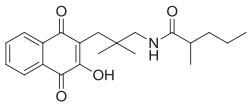
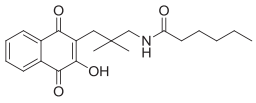
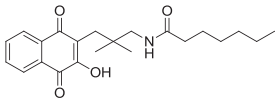
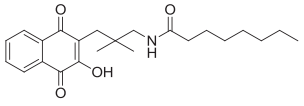
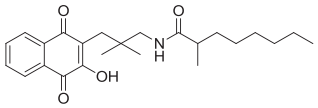
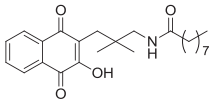
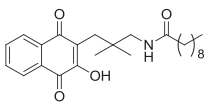
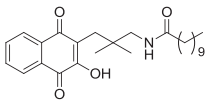
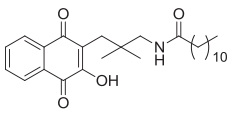
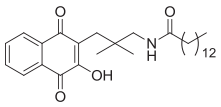
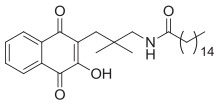
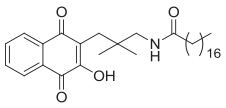
Compounds	IC_{50} (μM)[a]	
	Anticancer (KB cells)	Cytotoxicity to primary cells (Vero cells)
 **11a**	94.63	Non-cytotoxic
 **11b**	101.86	Non-cytotoxic
 **11c**	47.08	48.09
 **11d**	40.23	46.58
 **11e**	28.21	24.82
 **11f**	24.64	18.42
 **11g**	23.73	15.54
 **11h**	19.65	16.67
 **11i**	18.50	15.04
 **11j**	17.63	14.92

Table 1 (*continued*)

Compounds	IC_{50} (μM)[a]	
	Anticancer (KB cells)	Cytotoxicity to primary cells (Vero cells)
 **11k**	12.82	13.86
 **11l**	25.10	9.81
 **11m**	5.12	5.60
 **11n**	6.35[b]	5.76[b]
Doxorubicin[c]	0.96	23.94

KB, human epidermoid carcinoma.
[a] Data are typical values from six replicate experiments.
[b] Partially soluble in 100% DMSO.
[c] Used as a reference.

Table 2
Cytotoxicity of the synthetic naphthoquinone aliphatic esters **18a**–**n** against KB cells and normal Vero cells.

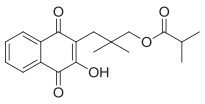
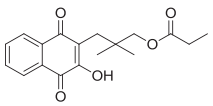
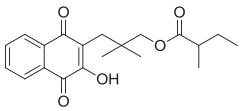
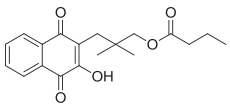
Compounds	IC_{50} (μM)[a]	
	Anticancer (KB cells)	Cytotoxicity to primary cells (Vero cells)
 **18a**	14.38	123.10
 **18as**	84.40	60.06
 **18b**	4.65	0.87
 **18bs**	42.98	73.16

Table 2 (*continued*)

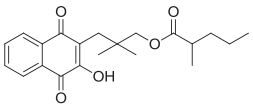
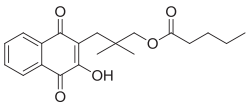
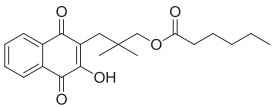
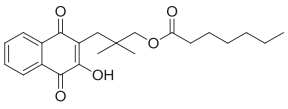
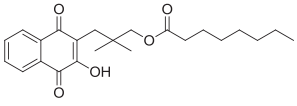
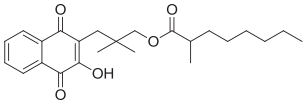
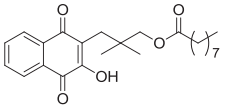
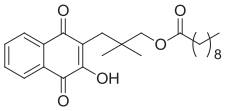
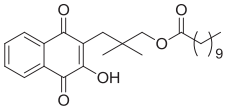
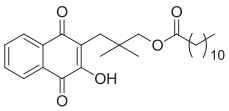
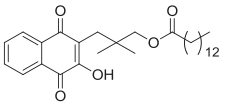
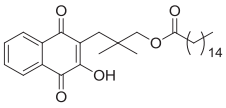
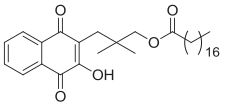
Compounds	IC_{50} (μM)[a]	
	Anticancer (KB cells)	Cytotoxicity to primary cells (Vero cells)
 **18c**	1.76	0.12
 **18cs**	27.79	7.84
 **18d**	41.01	169.07
 **18e**	14.42	99.34
 **18f**	38.29	>258.75
 **18g**	16.78	0.45
 **18h**	38.70	149.81
 **18i**	55.48	202.64
 **18j**	54.83	>233.34
 **18k**	35.70	248.54

Table 2 (*continued*)

Compounds	IC_{50} (μM)[a]	
	Anticancer (KB cells)	Cytotoxicity to primary cells (Vero cells)
 **18l**	51.36	58.43
 **18m**	27.07	4.41
 **18n**	76.66	15.51
Doxorubicin[b]	0.96	23.94

KB, human epidermoid carcinoma.
[a] Data are typical values from six replicate experiments.
[b] Used as a reference.

than the naphthoquinone aliphatic amide. However, when the side chain is extended, the naphthoquinone aliphatic amide becomes a potent hTopoIIα inhibitor.

To gain better understanding in structure–activity relationship, compound **11m** was further evaluated for hTopoIIα inhibitory activity at lower concentrations (5, 10, 15 and 20 μM) as shown in Fig. 3. The compound can completely inhibit hTopoIIα activity at final concentrations of 15 μM and 20 μM while moderately reducing hTopoIIα activity at final concentration of 10 μM. However, the compound did not show any hTopoIIα inhibitory activity at final concentration of 5 μM compared to doxorubicin at the same concentration. In comparison with our previous naphthoquinone aromatic amides [10], it can be clearly seen that naphthoquinone aliphatic amide **11m** is a better hTopoIIα inhibitor than naphthoquinone aromatic amides. Therefore, molecular docking of some selected naphthoquinone aliphatic amides and esters was performed in order to obtain an insight into the binding of the compounds to DNA binding domain of hTopoIIα.

2.4. Molecular docking analysis

According to our previous work [6,10], naphthoquinone aromatic amides and esters were found to bind at the DNA binding domain of hTopoIIα. The different spatial arrangement between naphthoquinone aromatic amides containing phenyl and naphthyl groups was observed while for naphthoquinone aromatic esters the quinone ring was found lying on the right-hand side and the propyl chain pointing down. With difference in structural rigidity between naphthoquinone aromatic amides/esters and the aliphatic ones, molecular docking analysis of hTopoIIα with selected naphthoquinone aromatic amides and esters was then carried out.

According to Fig. 4, in compounds **11c** and **18c**, the carbonyl groups of the amide and ester form hydrogen bonds with R804 located near the active site Y805. The distance between hydrogen bond donor and acceptor for compound **11c** is slightly longer than compound **18c**, indicating a weaker hydrogen bonding interaction. The amino acid R804 is conserved among TopoII family and the

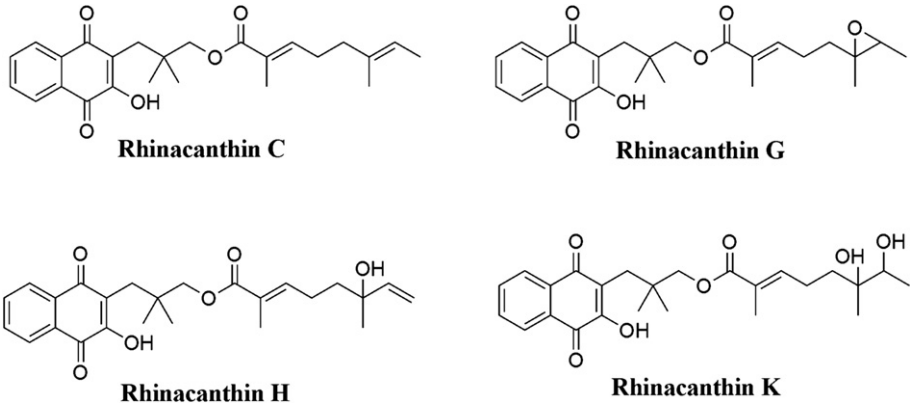


Fig. 1. Structures of rhinacanthin-C, -G,-H, and -K.

R804A mutation decreased decatenation and DNA cleavage activity about 5 and 10 folds, respectively [15]. Unlike compounds **11c** and **18c**, compounds **11m** and **18m** bound to hTopoIIα with slightly different orientation. Compound **11m** forms hydrogen bond with K798 while no hydrogen bonding is observed in compound **18m** which may relate to the weaker hTopoIIα inhibition compared to compound **11m**. Residues K798 and R804 are solvent-accessible positively-charged residues located at a loop near DNA recognition helix α4 of the HTH motif [16]. Collectively, our docking result correlated well with that of cytotoxicity assay and hTopoIIα inhibitory activity in that naphthoquinone aliphatic amide with longer chain is a better inhibitor than the ester while with the shorter chain length, the naphthoquinone aliphatic ester is a more potent inhibitor than the amide.

3. Conclusion

Fourteen new naphthoquinone amides and seventeen naphthoquinone aliphatic esters were synthesized and evaluated for anticancer activity. Naphthoquinone aliphatic amides were synthesized from 1-hydroxy-2-naphthoic acid in 9 steps whereas naphthoquinone aliphatic esters were synthesized as described in the previous report [8]. Naphthoquinone aliphatic amides with the shorter length of aliphatic chain (3- to 7-carbon atoms) demonstrated less anticancer activity than those of aliphatic ones with the longer chains than 7-carbon atoms. Naphthoquinone aliphatic amides with the chain length, longer than 7-carbon atoms showed stronger anticancer activity than aliphatic esters containing linear chains of the same number of carbons. The optimum aliphatic chain of amides, 16- and 18-carbon atoms, displayed very good anticancer activity. In comparison with the aromatic amides, the aliphatic amides showed stronger activity. However, among these, naphthoquinone aromatic esters [6] showed the strongest activity. For the ester series, α-methyl substituent of the aliphatic chain had a pronounced effect on anticancer activity than those without α-methyl group. The decatenation assay revealed that naphthoquinone amide **11m** can inhibit hTopoIIα activity at 10 μM, which was much better than previously reported naphthoquinone aromatic amides. Therefore, further development of this compound can lead to a potent hTopoIIα inhibitor.

4. Experimental section

4.1. Chemistry

The starting material, 1-hydroxy-2-naphthoic acid, was purchased from FLUKA. All reagents were obtained from FLUKA, MERCK, ACROSS and ALDRICH. Solvents were dried by distillation from the appropriate drying reagents immediately prior to use. Tetrahydrofuran and ether were distilled from sodium and benzophenone under nitrogen atmosphere. Dichloromethane was distilled from calcium hydride under nitrogen. Dimethylformamide was distilled under reduced pressure and stored over Type 3 Å molecular sieves. Moisture- and air-sensitive reactions were carried out under an atmosphere of nitrogen. Analytical thin-layer chromatography (TLC) was conducted using Merck TLC aluminum sheet (silica gel 60 F_{254}). Compounds were visualized by ultraviolet light and/or by heating the plate after dipping in a 1% solution of vanillin

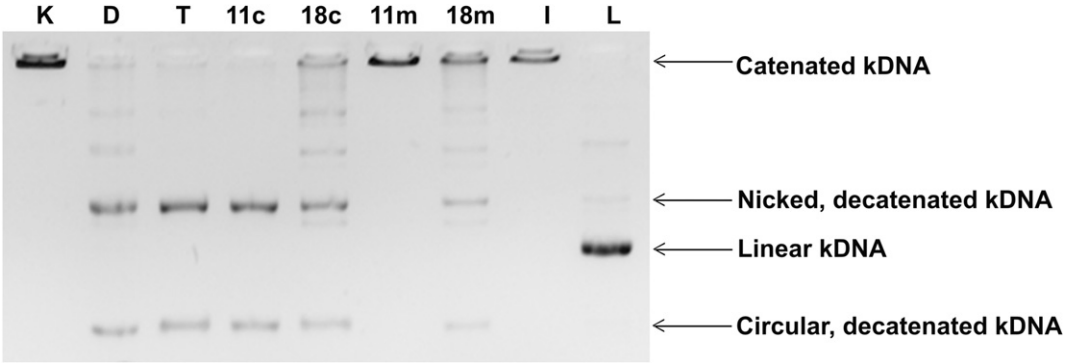


Fig. 2. Topoisomerase IIα inhibitory activity of naphthoquinone aliphatic amides (**11c**, **11m**) and naphthoquinone aliphatic esters (**18c**, **18m**). The compounds were examined in final concentration of 50 μM. Lanes K: kDNA only, lane D: decatenated kDNA only, lane T: kDNA + hTopoIIα, lanes **11c**, **18c**, **11m** and **18m**: kDNA + hTopoIIα + compounds **11c**, **18c**, **11m** and **18m**, respectively, lane I: kDNA + hTopoIIα + 50 μM doxorubicin and lane L: linearized kDNA marker. Catenated kDNA does not migrate out of the loading wells.

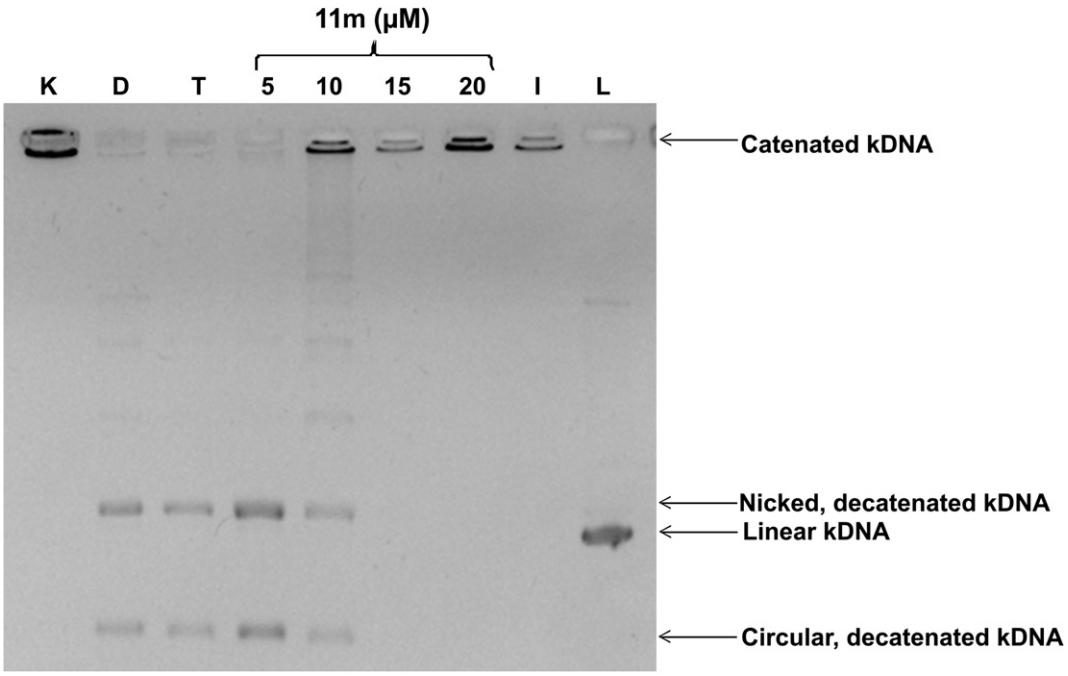


Fig. 3. Topoisomerase IIα inhibitory activity of compound **11m** at various concentrations. The compound was examined in final concentration of 5, 10, 15 and 20 μM, respectively, as designated. Lanes K: kDNA only, lane D: decatenated kDNA only, lane T: kDNA + hTopoIIα, Lane 5, 10, 15, and 20: kDNA + hTopoIIα + compounds **11m** at 5, 10, 15 and 20 μM, respectively, lane I: kDNA + hTopoIIα + 5 μM doxorubicin and lane L: linearized kDNA marker. Catenated kDNA does not migrate out of the loading wells.

in 0.1 M sulfuric acid in ethanol. Flash column chromatography was performed on silica gel (230–400 mesh, Merck 9385). Infrared (IR) spectra were recorded on a Perkin–Elmer 2000 Fourier transform infrared spectrophotometer. Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AVANCE 300 MHz spectrometer and VARIAN^{UNITY} INOVA 400 MHz spectrometer. Amine **6** and naphthoquinone aliphatic esters **18a**–**n** were prepared as described in previous reports [8,10].

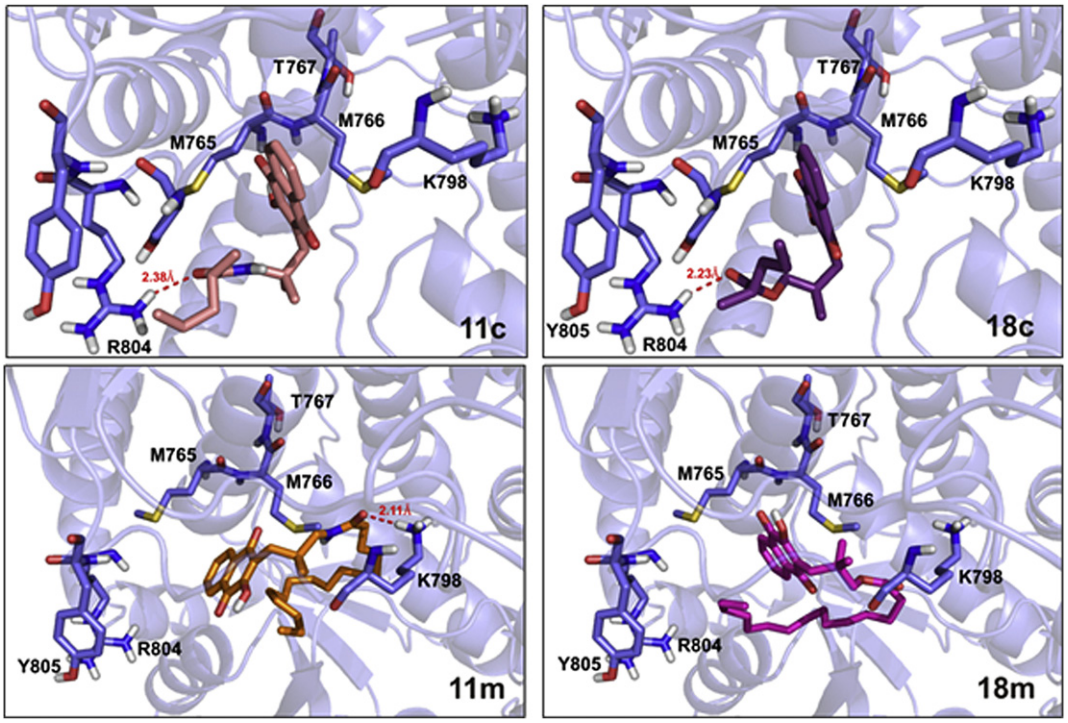


Fig. 4. H-bond interaction observed in DNA-binding domain of hTopoIIα. The amino acids of hTopoIIα are depicted in stick representation and colored by the atom type (carbon, blue slate; oxygen, red; hydrogen, white and nitrogen, blue). The dotted red lines represent the hydrogen bonding interaction. Compounds **11c**, **18c**, **11m** and **18m** are shown in stick and colored by the atom type (carbon, salmon, deep purple, orange, magenta respectively; oxygen, red; hydrogen, white and nitrogen, blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Synthetic procedures

4.2.1. General procedure for amide coupling

To a mixture of amine **6** (1 mmol) and carboxylic acids **7a**–**n** (1.1 mmol) in methanol (10 mL) was added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) (1.1 mmol). The reaction mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and then the residue was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography to give *N*-[3-(1-methoxy-2-naphthyl)-2,2-dimethylpropyl]amide (**8a**–**n**). All spectroscopic data of compounds **8a**–**n** are shown in the Supplementary data.

4.2.2. General procedure for demethylation reaction of N-[3-(1-methoxy-2-naphthyl)-2,2-dimethylpropyl] amide (*8a*–*n*)

To a solution of *N*-[3-(1-methoxy-2-naphthyl)-2,2-dimethylpropyl] amide (**8a**–**n**) (1 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C was slowly added BBr_3 (2 mmol). The reaction mixture was warmed to room temperature and stirred under nitrogen atmosphere for 3 h. Water was added. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give *N*-[3-(1-hydroxy-2-naphthyl)-2,2-dimethylpropyl] amide (**9a**–**n**). The crude product was used in the next step without further purification.

4.2.3. General procedure for Fremy's salt oxidation reaction of N-[3-(1-hydroxy-2-naphthyl)-2,2-dimethylpropyl] amide (*9a*–*n*)

To a solution of *N*-[3-(1-hydroxy-2-naphthyl)-2,2-dimethylpropyl] amide (**9a**–**n**) (1 mmol) in MeOH (10 mL) and DMF (30 mL) was added NaOAc (1 M, 10 mL) followed by Fremy's salt (6 mmol) in water (20 mL). After stirring at room temperature for 12 h, the reaction mixture was extracted with diethyl ether. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, ethyl acetate:hexane, 1:2) to give the *N*-[3-(1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] amide (**10a**–**n**). All spectroscopic data of compounds **10a**–**n** are shown in the Supplementary data.

4.2.4. General procedure for hydroxylation reaction of N-[3-(1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] amide (*10a*–*n*)

To a solution of *N*-[3-(1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] amide (**10a**–**n**) (1 mmol) in THF (13 mL), was added TBHP (12 mmol) followed by triton B (3 mmol) until the solution turned red. The reaction mixture was stirred at room temperature for 20 min and then 10% HCl was added until the solution turned yellow. The mixture was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, ethyl acetate:hexane, 1:2) to give *N*-[3-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] amide (**11a**–**n**).

*4.2.4.1. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]-2-methylpropanamide (**11a**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11a** (9%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 127–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.08 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.76 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.69 (td, J = 7.6, 1.4 Hz, 1H, ArH), 6.92 (t, J = 6.7 Hz, 1H, NH), 2.91 (d, J = 6.7 Hz, 2H, CH_2N), 2.53 (s, 2H, CH_2Ar), 2.50 (heptet, J = 6.9 Hz, 1H, CH), 1.24 (d, J = 6.9 Hz, 6H, 2× CH_3), 0.95 (s, 6H, 2× CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 177.2 (C═O), 155.0 (Ar), 135.1 (ArH), 133.3 (ArH), 132.8 (Ar), 129.3 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 46.8 (CH_2), 38.2 (C), 36.1 (CH), 31.9 (CH_2), 26.2 (2× CH_3), 19.8 (2× CH_3); IR (KBr) = 3291 (NH), 2968, 2930 (CH), 1668, 1641, 1624 (C═O), 1595, 1577 (C═C), 1272 (C–N), 1216 (C–O); HRMS (ESI$^+$): m/z [M + Na] calcd for $C_{19}H_{23}NNaO_4$: 352.1519, found: 352.1517.

*4.2.4.2. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]-2-methylbutanamide (**11b**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11b** (11%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 120–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.07 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.75 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.68 (td, J = 7.6, 1.4 Hz, 1H, ArH), 6.95 (t, J = 6.7 Hz, 1H, NH), 2.99–2.87 (m, 2H, CH_2N), 2.52 (s, 2H, CH_2Ar), 2.32–2.22 (m, 1H, CH), 1.80–1.68 (m, 1H, CH_2), 1.56–1.43, (m, 1H, CH_2), 1.21 (d, J = 6.9 Hz, 3H, CH_3), 0.95–0.94 (overlapping, 9H, 3× CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 176.9 (C═O), 155.1 (Ar), 135.1 (ArH), 133.2 (ArH), 132.7 (Ar), 129.3 (Ar), 127.1 (ArH), 126.3 (ArH), 121.5 (Ar), 47.0 (CH_2), 43.6 (CH), 38.1 (C), 31.8 (CH_2), 27.3 (CH_2), 26.2 (2× CH_3), 17.6 (CH_3), 12.0 (CH_3); IR (KBr) = 3294 (NH), 2967, 2920, 2873 (CH), 1668, 1641, 1623 (C═O), 1596, 1566 (C═C), 1272 (C–N), 1215 (C–O); HRMS (ESI$^+$): m/z [M + Na] calcd for $C_{20}H_{25}NNaO_4$: 366.1676, found: 366.1692.

*4.2.4.3. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]-2-methyl pentanamide (**11c**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11c** (21%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (ddd, J = 7.6, 1.4, 0.4 Hz, 1H, ArH), 8.03 (ddd, J = 7.6, 1.4, 0.4 Hz, 1H, ArH), 7.71 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.64 (td, J = 7.6, 1.4 Hz, 1H, ArH), 6.84 (t, J = 6.7 Hz, 1H, NH), 2.93–2.82 (m, 2H, CH_2N), 2.48 (s, 2H, CH_2Ar), 2.33–2.25 (m, 1H, CH), 1.70–1.63 (m, 1H, CH_2), 1.39–3.28 (m, 1H + 2H, CH_2), 1.16 (d, J = 6.9 Hz, 3H, CH_3), 0.90 (s, 6H, 2× CH_3), 0.87 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 176.8 (C═O), 155.1 (Ar), 135.0 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 46.9 (CH_2), 41.8 (CH), 38.1 (C), 36.5 (CH_2), 31.8 (CH_2), 26.1 (2× CH_3), 20.7 (CH_2), 18.0 (CH_3), 14.0 (CH_3); IR (KBr) = 3295 (NH), 2963, 2930, 2871 (CH), 1669, 1642, 1623 (C═O), 1596, 1566 (C═C), 1271 (C–N), 1215 (C–O); HRMS (ESI$^+$): m/z [M + Na] calcd for $C_{21}H_{27}NNaO_4$: 380.1832, found: 380.1836.

*4.2.4.4. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] hexanamide (**11d**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11d** (22%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 79–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.04 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.72 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.65 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.53 (s, 1H, OH), 6.79 (t, J = 6.8 Hz, 1H, NH), 2.87 (d, J = 6.8 Hz, 2H, CH_2N), 2.48 (s, 2H, CH_2Ar), 2.24 (t, J = 7.5 Hz, 2H, CH_2CO), 1.66 (quintet, J = 7.5 Hz, 2H, CH_2), 1.35–1.27 (m, 4H, 2× CH_2), 0.91 (s, 6H, 2× CH_3), 0.86 (t, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.4 (C═O), 155.0 (Ar), 135.1 (ArH), 133.2 (ArH), 132.8 (Ar), 129.3 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 47.1 (CH_2), 38.1 (C), 37.2 (CH_2), 31.9 (CH_2), 31.6 (CH_2), 26.2 (2× CH_3), 25.6 (CH_2), 22.4 (CH_2), 14.0 (CH_3); IR (KBr) = 3357 (NH), 2960, 2924, 2855 (CH), 1673, 1660, 1632 (C═O), 1594, 1578, 1550 (C═C), 1273 (C–N), 1216 (C–O); HRMS (ESI$^+$): m/z [M + Na] calcd for $C_{21}H_{27}NNaO_4$: 380.1832 found 380.1850.

*4.2.4.5. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] heptanamide (**11e**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11e** (10%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 85–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.13 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.81 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.74 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.63 (s, 1H, OH), 6.87 (t, J = 6.8 Hz, 1H, NH), 2.96 (d, J = 6.8 Hz, 2H, CH_2N), 2.57 (s, 2H, CH_2Ar), 2.32 (t, J = 7.8 Hz, 2H, CH_2CO), 1.74 (quintet, J = 7.8 Hz, 2H, CH_2), 1.47–1.31 (m, 6H, 3× CH_2), 0.99 (s, 6H, 2× CH_3), 0.92 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.3 (C═O), 155.0 (Ar), 135.1 (ArH), 133.2 (ArH), 132.8 (Ar), 129.3 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 47.1 (CH_2), 38.1 (C), 37.3 (CH_2), 31.9 (CH_2), 31.6 (CH_2), 29.4 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.5 (CH_2), 14.0 (CH_3); IR (KBr) = 3358 (NH), 2928, 2870 (CH), 1673, 1632 (C═O), 1594, 1578, 1549 (C═C), 1274 (C–N), 1216 (C–O); HRMS (ESI^+): m/z [M + Na] calcd for $C_{22}H_{29}NNaO_4$: 394.1989, found: 394.2008.

*4.2.4.6. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl] octanamide (**11f**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11f** (13%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 113–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.06 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.74 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.67 (td, J = 7.6, 1.4 Hz, 1H, ArH), 6.85 (t, J = 6.3 Hz, 1H, NH), 2.92 (d, J = 6.3 Hz, 2H, CH_2N), 2.53 (s, 2H, CH_2Ar), 2.27 (t, J = 7.7 Hz, 2H, CH_2CO), 1.70 (quintet, J = 7.7 Hz, 2H, CH_2), 1.40–1.21 (m, 8H, 4× CH_2), 0.95 (s, 6H, 2× CH_3), 0.86 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.4 (C═O), 155.1 (Ar), 135.1 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 47.1 (CH_2), 38.0 (C), 37.3 (CH_2), 31.9 (CH_2), 31.7 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); IR (KBr) = 3355 (NH), 2957, 2925, 2853 (CH), 1671, 1632 (C═O), 1594, 1578, 1551 (C═C), 1274 (C–N), 1216 (C–O); HRMS (ESI^+): m/z [M + Na] calcd for $C_{23}H_{31}NNaO_4$: 408.2145, found: 408.2152.

*4.2.4.7. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]-2-methyl octanamide (**11g**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11g** (19%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 102–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12 (dd, J = 7.8, 1.4 Hz, 1H, ArH), 8.09 (dd, J = 7.8, 1.4 Hz, 1H, ArH), 7.77 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.70 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.58 (s, 1H, OH), 6.86 (t, J = 6.6 Hz, 1H, NH), 2.94 (dd, J = 6.6, 4.5 Hz, 2H, CH_2N), 2.54 (s, 2H, CH_2Ar), 2.39–2.30 (m, 1H, CH), 1.79–1.67 (m, 1H, CH_2), 1.37–1.22 (m, 1H + 8H, CH_2), 1.21 (d, J = 6.4 Hz, 3H, CH_3), 0.96 (s, 6H, 2× CH_3), 0.86 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 178.8 (C═O), 155.0 (Ar), 135.1 (ArH), 133.3 (ArH), 132.9 (Ar), 129.3 (Ar), 127.2 (ArH), 126.3 (ArH), 121.6 (Ar), 47.0 (CH_2), 42.1 (CH), 38.2 (C), 34.5 (CH_2), 31.9 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 27.6 (CH_2), 26.3 (CH_3), 26.2 (CH_3), 22.6 (CH_2), 18.1 (CH_3), 14.1 (CH_3); IR (KBr) = 3295 (NH), 2965, 2922, 2855 (CH), 1668, 1642, 1623 (C═O), 1597, 1560 (C═C), 1271 (C–N), 1214 (C–O); HRMS (ESI^+): m/z [M + Na] calcd for $C_{24}H_{33}NNaO_4$: 422.2302, found 422.2321.

*4.2.4.8. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]nonanamide (**11h**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11h** (25%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 111–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (dd, J = 7.6, 1.1 Hz, 1H, ArH), 8.08 (dd, J = 7.6, 1.1 Hz, 1H, ArH), 7.76 (td, J = 7.6, 1.3 Hz, 1H, ArH), 7.69 (td, J = 7.6, 1.3 Hz, 1H, ArH), 6.90 (t, J = 6.3 Hz, 1H, NH), 2.93 (d, J = 6.3 Hz, 2H, CH_2N), 2.54 (s, 2H, CH_2Ar), 2.30 (t, J = 7.7 Hz, 2H, CH_2CO), 1.71 (quintet, J = 7.7 Hz, 2H, CH_2), 1.40–1.24 (m, 10H, 5× CH_2), 0.96 (s, 6H, 2× CH_3), 0.86 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.4 (C═O), 155.1 (Ar), 135.0 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 47.1 (CH_2), 38.0 (C), 37.3 (CH_2), 31.8 (2× CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); IR (KBr) = 3357 (NH), 2959, 2923, 2853 (CH), 1665, 1633 (C═O), 1594, 1578, 1550 (C═C), 1273 (C–N), 1215 (C–O); HRMS (ESI^+): m/z [M + Na] calcd for $C_{24}H_{33}NNaO_4$: 422.2302, found: 422.2320.

*4.2.4.9. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]decanamide (**11i**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11i** (17%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 107–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.16 (dd, J = 7.6, 1.1 Hz, 1H, ArH), 8.13 (dd, J = 7.6, 1.1 Hz, 1H, ArH), 7.81 (td, J = 7.6, 1.6 Hz, 1H, ArH), 7.74 (td, J = 7.6, 1.6 Hz, 1H, ArH), 6.88 (t, J = 6.8 Hz, 1H, NH), 2.96 (d, J = 6.8 Hz, 2H, CH_2N), 2.57 (s, 2H, CH_2Ar), 2.32 (t, J = 7.8 Hz, 2H, CH_2CO), 1.74 (quintet, J = 7.8 Hz, 2H, CH_2), 1.44–1.24 (m, 12H, 6× CH_2), 0.99 (s, 6H, 2× CH_3), 0.89 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.5 (C═O), 155.1 (Ar), 135.0 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.5 (Ar), 47.0 (CH_2), 38.0 (C), 37.2 (CH_2), 31.8 (CH_2), 29.6 (2× CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.6 (CH_2), 14.1 (CH_3); IR (KBr) = 3358 (NH), 2921, 2853 (CH), 1664, 1631 (C═O), 1594, 1578, 1551 (C═C), 1273 (C–N), 1216 (C–O); HRMS (ESI^+): m/z [M + H]$^+$ calcd for $C_{25}H_{36}NO_4$: 414.2644, found 414.2628.

*4.2.4.10. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]undecanamide (**11j**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11j** (14%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 93–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (dd, J = 7.6, 1.1 Hz, 1H, ArH), 8.07 (dd, J = 7.6, 1.1 Hz, 1H, ArH), 7.75 (td, J = 7.6, 1.1 Hz, 1H, ArH), 7.68 (td, J = 7.6, 1.1 Hz, 1H, ArH), 6.89 (t, J = 6.3 Hz, 1H, NH), 2.92 (d, J = 6.3 Hz, 2H, CH_2N), 2.53 (s, 2H, CH_2Ar), 2.29 (t, J = 7.7 Hz, 2H, CH_2CO), 1.69 (quintet, J = 7.7 Hz, 2H, CH_2), 1.40–1.19 (m, 14H, 7× CH_2), 0.95 (s, 6H, 2× CH_3), 0.84 (t, J = 6.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.5 (C═O), 155.1 (Ar), 135.0 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.5 (Ar), 47.0 (CH_2), 38.0 (C), 37.2 (CH_2), 31.8 (2× CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (2× CH_2), 29.3 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.6 (CH_2), 14.1 (CH_3); IR (KBr) = 3361 (NH), 2921, 2853 (CH), 1665, 1632 (C═O), 1594, 1578, 1550 (C═C), 1273 (C–N), 1215 (C–O); HRMS (ESI^+): m/z [M + Na] calcd for $C_{26}H_{37}NNaO_4$: 450.2615, found: 450.2618.

*4.2.4.11. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]dodecanamide (**11k**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11k** (14%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 97–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, J = 7.6 Hz, 1H, ArH), 8.06 (d, J = 7.6 Hz, 1H, ArH), 7.73 (td, J = 7.6, 1.1 Hz, 1H, ArH), 7.67 (td, J = 7.6, 1.1 Hz, 1H, ArH), 6.89 (t, J = 6.6 Hz, 1H, NH), 2.92 (d, J = 6.6 Hz, 2H, CH_2N), 2.52 (s, 2H, CH_2Ar), 2.28 (t, J = 7.4 Hz, 2H, CH_2CO), 1.68 (quintet, J = 7.4 Hz, 2H, CH_2), 1.40–1.15 (m, 16H, 8× CH_2), 0.94 (s, 6H, 2× CH_3), 0.83 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz,

$CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.5 (C═O), 155.3 (Ar), 135.0 (ArH), 133.2 (ArH), 132.7 (Ar), 129.4 (Ar), 127.0 (ArH), 126.2 (ArH), 121.6 (Ar), 47.1 (CH_2), 38.0 (C), 37.2 (CH_2), 31.8 (CH_2), 29.6 (2× CH_2), 29.5 (CH_2), 29.4 (3× CH_2), 29.3 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.6 (CH_2), 14.1 (CH_3); IR (KBr) = 3355 (NH), 2917, 2853 (CH), 1673, 1662, 1633 (C═O), 1594, 1578, 1551 (C═C), 1273 (C–N), 1216 (C–O); HRMS (ESI^+): *m*/*z* [M + Na] calcd for $C_{27}H_{39}NNaO_4$: 464.2771, found: 464.2775.

*4.2.4.12. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]tetradecanamide (**11l**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11l** (12%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 94–95 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.13 (ddd, *J* = 7.6, 1.4, 0.4 Hz, 1H, ArH), 8.10 (ddd, *J* = 7.6, 1.4, 0.4 Hz, 1H, ArH), 7.77 (td, *J* = 7.6, 1.4 Hz, 1H, ArH), 7.71 (td, *J* = 7.6, 1.4 Hz, 1H, ArH), 6.86 (t, *J* = 6.5 Hz, 1H, NH), 2.94 (d, *J* = 6.5 Hz, 2H, CH_2N), 2.55 (s, 2H, CH_2Ar), 2.30 (t, *J* = 7.5 Hz, 2H, CH_2CO), 1.71 (quintet, *J* = 7.5 Hz, 2H, CH_2), 1.43–1.17 (m, 20H, 10× CH_2), 0.96 (s, 6H, 2× CH_3), 0.87 (t, *J* = 6.5 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.4 (C═O), 155.0 (Ar), 135.1 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.6 (Ar), 47.1 (CH_2), 38.1 (C), 37.3 (CH_2), 31.9 (CH_2), 29.7 (2× CH_2), 29.6 (2× CH_2) 29.5 (CH_2), 29.4 (3× CH_2), 29.3 (CH_2), 26.4 (2× CH_3), 25.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3); IR (KBr) = 3354 (NH), 2918, 2851 (CH), 1673, 1661, 1631 (C═O), 1594, 1578, 1552 (C═C), 1273 (C–N), 1216 (C–O); HRMS (ESI^+): *m*/*z* [M + Na] calcd for $C_{29}H_{43}NNaO_4$: 492.3084, found: 492.3072.

*4.2.4.13. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]hexadecanamide (**11m**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11m** (19%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 94–95 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.11 (ddd, *J* = 7.6, 1.3, 0.4 Hz, 1H, ArH), 8.03 (ddd, *J* = 7.6, 1.3, 0.4 Hz, 1H, ArH), 7.76 (td, *J* = 7.6, 1.4 Hz, 1H, ArH), 7.69 (td, *J* = 7.6, 1.4 Hz, 1H, ArH), 6.89 (t, *J* = 6.4 Hz, 1H, NH), 2.93 (d, *J* = 6.4 Hz, 2H, CH_2N), 2.54 (s, 2H, CH_2Ar), 2.30 (t, *J* = 7.7 Hz, 2H, CH_2CO), 1.71 (quintet, *J* = 7.7 Hz, 2H, CH_2), 1.42–1.21 (m, 24H, 12× CH_2), 0.96 (s, 6H, 2× CH_3), 0.86 (t, *J* = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.4 (C═O), 155.1 (Ar), 135.0 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.5 (Ar), 47.1 (CH_2), 38.0 (C), 37.3 (CH_2), 31.9 (2× CH_2), 29.7 (3× CH_2), 29.6 (3× CH_2), 29.5 (CH_2), 29.4 (2× CH_2), 29.3 (CH_2), 26.2 (2× CH_3), 25.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); IR (KBr) = 3355 (NH), 2918, 2850 (CH), 1673, 1662, 1631 (C═O), 1594, 1578, 1551 (C═C), 1273 (C–N), 1216 (C–O); HRMS (ESI^+): *m*/*z* [M + Na] calcd for $C_{31}H_{47}NNaO_4$: 520.3397, found: 520.3393.

*4.2.4.14. N-[3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl]octadecanamide (**11n**).* Flash column chromatography, eluting with 2:1 hexane:ethyl acetate afforded the product **11n** (25%) as a yellow amorphous solid, which was recrystallized from hexane to give yellow crystals, mp 95–96 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.06 (ddd, *J* = 7.6, 1.4, 0.4 Hz, 1H, ArH), 8.04 (ddd, *J* = 7.6, 1.4, 0.4 Hz, 1H, ArH), 7.71 (td, *J* = 7.6, 1.4 Hz, 1H, ArH), 7.65 (td, *J* = 7.6, 1.4 Hz, 1H, ArH), 6.84 (t, *J* = 6.7 Hz, 1H, NH), 2.88 (d, *J* = 6.7 Hz, 2H, CH_2N), 2.49 (s, 2H, CH_2Ar), 2.25 (t, *J* = 7.7 Hz, 2H, CH_2CO), 1.66 (quintet, *J* = 7.7 Hz, 2H, CH_2), 1.37–1.11 (m, 28H, 14× CH_2), 0.91 (s, 6H, 2× CH_3), 0.81 (t, *J* = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.0 (C═O), 180.9 (C═O), 173.5 (C═O), 155.1 (Ar), 135.1 (ArH), 133.2 (ArH), 132.8 (Ar), 129.4 (Ar), 127.1 (ArH), 126.3 (ArH), 121.5 (Ar), 47.1 (CH_2), 38.1 (C), 37.3 (CH_2), 31.9 (2× CH_2), 29.7 (8× CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 26.2 (2× CH_3), 26.0 (CH_2), 22.7 (CH_2), 14.1 (CH_3); IR (KBr) = 3317 (NH), 2918, 2849 (CH), 1671, 1645 (C═O), 1594, 1579, 1554 (C═C), 1274 (C–N), 1214 (C–O); HRMS (ESI^+): *m*/*z* [M + Na] calcd for $C_{33}H_{51}NNaO_4$: 548.3710, found: 548.3700.

*4.2.5. Compounds **12**–**17** were prepared as described in the previous report [6,7]*

Their spectral data are shown in Supplementary data.

*4.2.6. General procedure for coupling of naphthoquinone alcohol **17** with fatty acids to naphthoquinone esters **18a**–**n** [8]*

To a solution of carboxylic acid (1.3 mmol) and 4-dimethylaminopyridine (DMAP) (0.3 mmol) in dry dichloromethane (10 mL) was added naphthoquinone alcohol **17** (1 mmol) in dry dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 5 min when a solution of 1,3-dicyclohexylcarbodiimide (DCC) (1.3 mmol) in dry dichloromethane (15 mL) was added. Stirring was continued overnight at room temperature. The precipitate of dicyclohexylurea was filtered off and the filtrate washed with saturated ammonium chloride solution then water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel) to give the target product, naphthoquinone aliphatic esters.

*4.2.6.1. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl-2-methyl propanoate (**18a**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18a** (86%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 70.5–71.5 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, *J* = 7.5, 1.2 Hz, 1H, ArH), 8.10 (dd, *J* = 7.5, 1.2 Hz, 1H, ArH), 7.77 (td, *J* = 7.5, 1.2 Hz, 1H, ArH), 7.70 (td, *J* = 7.5, 1.2 Hz, 1H, ArH), 7.44 (s, 1H, OH), 3.85 (s, 2H, OCH_2), 2.69 (s, 2H, CH_2Ar), 2.54 (heptet, *J* = 7.0 Hz, 1H, CH), 1.17 (d, *J* = 7.0 Hz, 6H, 2× CH_3), 0.99 (s, 6H, 2× CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.8 (C═O), 181.2 (C═O), 177.0 (C═O), 154.3 (Ar), 134.9 (ArH), 132.9 (ArH, Ar), 129.3 (Ar), 127.0 (ArH), 126.1 (ArH), 121.6 (Ar), 72.4 (CH_2), 37.0 (C), 34.2 (CH), 31.8 (CH_2), 25.0 (2× CH_3), 19.0 (2× CH_3); IR (KBr) = 3362 (OH), 2969, 2932, 2873 (CH), 1729, 1666, 1644 (C═O), 1594, 1466, 1371, 1275 (C═C), 1216, 1151 (C–O); MS (EI), *m*/*z* (% relative intensity): 331 ($[M + H]^+$, 21), 243 (74), 187 (100), 159 (45), 145 (33); Anal. Calcd for $C_{19}H_{22}O_5$: C 69.07, H 6.71. Found: C 69.26, H 6.31.

*4.2.6.2. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl propanoate (**18as**).* Flash column chromatography, eluting with 49:1 hexane:ethyl acetate afforded the product **18as** (90%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 89–90 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, *J* = 7.6, 1.2 Hz, 1H, ArH), 8.10 (dd, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.77 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.70 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.43 (s, 1H, OH), 3.86 (s, 2H, OCH_2), 2.69 (s, 2H, CH_2Ar), 2.31 (q, *J* = 7.6 Hz, 2H, CH_2), 1.13 (t, *J* = 7.6 Hz, 3H, CH_3), 0.91 (s, 6H, 2× CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.8 (C═O), 181.2 (C═O), 174.5 (C═O), 154.2 (Ar), 135.0 (ArH), 132.9 (ArH, Ar), 129.3 (Ar), 127.0 (ArH), 126.1 (ArH), 121.7 (Ar), 72.5 (CH_2), 36.8 (C), 31.9 (CH_2), 27.6 (CH_2), 25.0 (2× CH_3), 9.2 (CH_3); IR (KBr) = 3323 (OH), 2985, 2953, 2925 (CH), 1708, 1668, 1646 (C═O), 1592, 1460, 1368, 1271 (C═C), 1213, 1022 (C–O); MS (EI), *m*/*z* (% relative intensity): 316 (M^+, 5), 257 (5), 244 (11), 243 (100), 187 (2); Anal. Calcd for $C_{18}H_{20}O_5$: C 68.34, H 6.37. Found: C 68.46, H 6.49.

*4.2.6.3. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl-2-methyl butanoate (**18b**).* Flash column chromatography, eluting with 25:1 hexane:ethyl acetate afforded the product **18b** (81%) as a yellow gum, which was recrystallized from

hexane–dichloromethane to give yellow crystals, mp 70.5–71.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (ddd, J = 7.5, 1.2, 0.6 Hz, 1H, ArH), 8.10 (ddd, J = 7.5, 1.2, 0.6 Hz, 1H, ArH), 7.77 (td, J = 7.5, 1.2 Hz, 1H, ArH), 7.70 (td, J = 7.5, 1.2 Hz, 1H, ArH), 7.45 (s, 1H, OH), 3.87 (s, 2H, OCH_2), 2.69 (s, 2H, CH_2Ar), 2.35 (hextet, J = 7.0 Hz, 1H, CH), 1.73–1.60 (m, 1H, CH_2), 1.52–1.38 (m, 1H, CH_2), 1.14 (d, J = 7.0 Hz, 3H, CH_3), 0.99 (s, 6H, 2× CH_3), 0.89 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.9 (C=O), 177.3 (C=O), 154.9 (Ar), 135.6 (ArH), 133.6 (ArH, Ar), 130.0 (Ar), 127.7 (ArH), 126.7 (ArH), 122.3 (Ar), 73.1 (CH_2), 41.9 (CH), 37.6 (C), 32.6 (CH_2), 27.3 (CH_2), 25.6 (2× CH_3), 17.2 (CH_3), 12.2 (CH_3); IR (KBr) = 3371 (OH), 2967, 2932, 2873 (CH), 1731, 1667, 1650 (C=O), 1594, 1461, 1368, 1274 (C=C), 1217, 1049 (C–O); MS (EI), m/z (% relative intensity): 344 (M^+, 10), 243 (100), 225 (43), 187 (97), 159 (71); Anal. Calcd for $C_{20}H_{24}O_5$: C 69.75, H 7.02. Found: C 96.64, H 7.20.

*4.2.6.4. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl butanoate (**18bs**).* Flash column chromatography, eluting with 14:1 hexane:ethyl acetate afforded the product **18bs** (87%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 66–67 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16–8.04 (m, 1H, ArH), 8.04–8.01 (m, 1H, ArH), 7.69 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.62 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.37 (s, 1H, OH), 3.79 (s, 2H, OCH_2), 2.61 (s, 2H, CH_2Ar), 2.18 (t, J = 7.4 Hz, 2H, CH_2), 1.59–1.47 (m, 2H, CH_2), 0.92 (s, 6H, 2× CH_3), 0.85 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.8 (C=O), 181.2 (C=O), 173.7 (C=O), 154.2 (Ar), 135.0 (ArH), 132.9 (ArH, Ar), 129.3 (Ar), 127.0 (ArH), 126.1 (ArH), 121.7 (Ar), 72.5 (CH_2), 36.8 (C), 36.3 (CH_2), 31.9 (CH_2), 25.0 (2× CH_3), 18.4 (CH_2), 13.7 (CH_3); IR (KBr) = 3378 (OH), 2968, 2932, 2873 (CH), 1701, 1667, 1646 (C=O), 1593, 1459, 1367, 1271 (C=C), 1214, 1015 (C–O); MS (EI), m/z (% relative intensity): 330 (M^+, 11), 243 (9), 229 (64), 211 (96), 187 (48), 159 (28), 149 (100); Anal. Calcd for $C_{19}H_{22}O_5$: C 69.07, H 6.71. Found: C 69.47, H 6.69.

*4.2.6.5. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl-2-methyl pentanoate (**18c**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18c** (77%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 57–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (dd, J = 7.6, 1.2 Hz, 1H, ArH), 8.10 (dd, J = 7.6, 1.2 Hz, 1H, ArH), 7.77 (td, J = 7.6, 1.2 Hz, 1H, ArH), 7.70 (td, J = 7.6, 1.2 Hz, 1H, ArH), 7.45 (br s, 1H, OH), 3.86 (s, 2H, OCH_2), 2.69 (s, 2H, CH_2Ar), 2.43 (hextet, J = 7.2 Hz, 1H, CH), 1.68–1.59 (m, 1H, CH_2), 1.24–1.42 (m, 1H + 2H, CH_2), 1.14 (d, J = 7.2 Hz, 3H, CH_3), 0.99 (s, 6H, 2× CH_3), 0.88 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.9 (C=O), 177.5 (C=O), 154.9 (Ar), 135.6 (ArH), 133.6 (ArH, Ar), 130.0 (Ar), 127.7 (ArH), 126.7 (ArH), 122.3 (Ar), 73.1 (CH_2), 40.1 (CH), 37.6 (C), 36.5 (CH_2), 32.6 (CH_2), 25.7 (CH_3), 25.6 (CH_3), 21.0 (CH_2), 17.6 (CH_3), 14.6 (CH_3); IR (KBr) = 3381 (OH), 2959, 2932, 2863 (CH), 1730, 1660, 1650 (C=O), 1594, 1456, 1370, 1275 (C=C), 1218, 1048 (C–O); MS (EI), m/z (% relative intensity): 357 ($[M - H]^+$, 29), 243 (100), 225 (46), 187 (99), 159 (69); Anal. Calcd for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.18, H, 7.42.

*4.2.6.6. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl pentanoate (**18cs**).* Flash column chromatography, eluting with 24:1 hexane:ethyl acetate afforded the product **18cs** (45%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 51–52 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.09 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.69 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.67 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.44 (s, 1H, OH), 3.85 (s, 2H, OCH_2), 2.68 (s, 2H, CH_2Ar), 2.27 (t, J = 7.6 Hz, 2H, CH_2), 1.65–1.58 (m, 2H, CH_2), 1.38–1.25 (m, 2H, CH_2), 0.99 (s, 6H, 2× CH_3), 0.88 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.8 (C=O), 181.2 (C=O), 173.8 (C=O), 154.2 (Ar), 135.0 (ArH), 133.0 (ArH), 132.9 (Ar), 129.3 (Ar), 127.0 (ArH), 126.1 (ArH), 121.7 (Ar), 72.5 (CH_2), 36.8 (C), 34.1 (CH_2), 32.0 (CH_2), 27.0 (CH_2), 25.0 (2× CH_3), 22.3 (CH_2), 13.7 (CH_3); IR (KBr) = 3373 (OH), 2957, 2925, 2865 (CH), 1739, 1666, 1647 (C=O), 1596, 1461, 1374, 1275 (C=C), 1217, 1157 (C–O); MS (EI), m/z (% relative intensity): 344 (M^+, 5), 243 (100), 225 (9), 187 (13), 159 (2); Anal. Calcd for $C_{20}H_{24}O_5$: C 69.75, H 7.02. Found: C 69.94, H 7.36.

*4.2.6.7. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl hexanoate (**18d**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18d** (74%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 54.5–55.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.08 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.77 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.69 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.62 (br s, 1H, OH), 3.87 (s, 2H, OCH_2), 2.69 (s, 2H, CH_2Ar), 2.27 (t, 2H, J = 7.5 Hz, CH_2), 1.51 (quintet, J = 7.5, 2H, CH_2), 1.31–1.25 (m, 4H, 2× CH_2), 1.00 (s, 6H, 2× CH_3), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.8 (C=O), 174.6 (C=O), 154.9 (Ar), 135.6 (ArH), 133.5 (ArH, Ar), 130.0 (C), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 31.9 (CH_2), 25.7 (2× CH_3), 25.3 (CH_2), 22.9 (CH_2), 14.5 (CH_3); IR (KBr) = 3366 (OH), 2940, 2865 (CH), 1738, 1644 (C=O), 1372, 1272 (C=C), 1212, 1155 (C–O); MS (EI), m/z (% relative intensity): 358 (M^+, 2), 243 (100), 225 (12), 187 (16), 158 (2), 149 (18); Anal. Calcd for $C_{21}H_{26}O_5$: C 70.37, H 7.31. Found: C 70.49, H 7.57.

*4.2.6.8. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl heptanoate (**18e**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18e** (83%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 57.5–58.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, 1H, J = 7.6, 1.1 Hz, ArH), 8.10 (dd, 1H, J = 7.6, 1.1 Hz, ArH), 7.78 (td, J = 7.6, 1.1 Hz, 1H, ArH), 7.70 (td, J = 7.6, 1.1 Hz, 1H, ArH), 7.58 (br s, 1H, OH), 3.87 (s, 2H, OCH_2), 2.69 (s, 2H, CH_2Ar), 2.28 (t, 2H, J = 7.4 Hz, CH_2), 1.60 (quintet, J = 7.4 Hz, 2H, CH_2), 1.35–1.21 (m, 6H, 3× CH_2), 1.00 (s, 6H, 2× CH_3), 0.88 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.8 (C=O), 174.6 (C=O), 154.9 (Ar), 135.6 (ArH), 133.5 (ArH, Ar), 130.0 (C), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.0 (CH_2), 29.5 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.1 (CH_2), 14.6 (CH_3); IR (KBr) = 3370 (OH), 2928, 2858 (CH), 1739, 1644 (C=O), 1374, 1276 (C=C), 1212, 1156 (C–O); MS (EI), m/z (% relative intensity): 372 (M^+, 9), 242 (26), 227 (11), 188 (28), 159 (12), 113 (100); Anal. Calcd for $C_{22}H_{28}O_5$: C 70.94, H 7.58. Found: C 70.68, H 7.85.

*4.2.6.9. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl octanoate (**18f**).* Flash column chromatography, eluting with 20:1 hexane:ethyl acetate afforded the product **18f** (96%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 56–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 8.09 (dd, J = 7.6, 1.4 Hz, 1H, ArH), 7.76 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.69 (td, J = 7.6, 1.4 Hz, 1H, ArH), 7.44 (s, 1H, OH), 3.85 (s, 2H, OCH_2), 2.68 (s, 2H, CH_2Ar), 2.26 (t, J = 7.6 Hz, 2H, CH_2), 1.63–1.55 (m, 2H, CH_2), 1.27 (br s, 8H, 4× CH_2), 0.99 (s, 6H, 2× CH_3), 0.87 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.8 (C=O), 181.2 (C=O), 173.9 (C=O), 154.2 (Ar), 134.9 (ArH), 132.9 (Ar), 132.9 (ArH), 129.3 (Ar), 127.0 (ArH), 126.1 (ArH), 121.7 (Ar), 72.5 (CH_2), 36.8 (C), 34.4 (CH_2), 32.0 (CH_2), 31.6 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 25.0 (2× CH_3), 24.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); IR (KBr) = 3366 (OH), 2924, 2865 (CH),

1735, 1666, 1645 (C=O), 1595, 1461, 1376, 1275 (C=C), 1218, 1156 (C–O); MS (EI), *m*/*z* (% relative intensity): 385 ([M − H]+, 45), 243 (100); Anal. Calcd for $C_{23}H_{30}O_5$: C 71.48, H 7.82. Found: C 71.72, H 7.71.

*4.2.6.10. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl-2-methyl octanoate (**18g**).* Flash column chromatography, eluting with 25:1 hexane:ethyl acetate afforded the product **18g** (84%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 57.5–58.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15–8.11 (m, 1H, ArH), 8.11–8.05 (m, 1H, ArH), 7.76 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.69 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 3.85 (s, 2H, OCH_2), 2.68 (s, 2H, CH_2Ar), 2.40 (hextet, *J* = 7.0 Hz, 1H, CH_3), 1.68–1.89 (m, 2H, CH_2), 1.40–1.32 (m, 2H, CH_2), 1.31–1.18 (m, 6H, 3× CH_2), 1.13 (d, *J* = 7.0 Hz, 3H, CH_3), 0.99 (s, 6H, 2× CH_3), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.9 (C=O), 177.5 (C=O), 154.9 (Ar), 135.6 (ArH), 133.6 (ArH, Ar), 130.0 (C), 127.7 (ArH), 126.7 (ArH), 122.3 (Ar), 73.1 (CH_2), 40.4 (CH), 37.6 (C), 34.4 (CH_2), 32.6 (CH_2), 32.3 (CH_2), 29.8 (CH_2), 27.8 (CH_2), 25.7 (CH_3), 25.6 (CH_3), 23.2 (CH_2), 17.7 (CH_3), 14.7 (CH_3); IR (KBr) = 3389 (OH), 2959, 2930, 2856 (CH), 1722, 1650 (C=O), 1461, 1373, 1268 (C=C), 1214, 1049 (C–O); MS (EI), *m*/*z* (% relative intensity): 400 ([M − H]$^+$, 29), 243 (100), 225 (46), 187 (99), 159 (69); Anal. Calcd for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.68, H, 8.41.

*4.2.6.11. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl nonanoate (**18h**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18h** (73%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 52.5–53.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 7.6 Hz, 1H, ArH), 8.03 (d, *J* = 7.6 Hz, 1H, ArH), 7.80 (t, *J* = 7.6 Hz, 1H, ArH), 7.73 (t, *J* = 7.6 Hz, 1H, ArH), 7.47 (s, 1H, OH), 3.87 (s, 2H, OCH_2), 2.71 (s, 2H, CH_2Ar), 2.29 (t, *J* = 7.5 Hz, 2H, CH_2), 1.62–1.58 (m, 2H, CH_2), 1.28–1.20 (m, 10H, 5× CH_2), 1.02 (s, 6H, 2× CH_3), 0.90 (t, *J* = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.8 (C=O), 174.5 (C=O), 154.9 (Ar), 135.6 (ArH), 133.5 (ArH, Ar), 130.0 (Ar), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.4 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.2 (CH_2), 14.7 (CH_3); IR (KBr) = 3366 (OH), 2923, 2850 (CH), 1739, 1644 (C=O), 1462, 1373, 1268 (C=C), 1212, 1155 (C–O); MS (EI), *m*/*z* (% relative intensity): 400 (M$^+$, 4), 242 (28), 227 (18), 188 (58), 187 (35), 159 (38), 141 (100); Anal. Calcd for $C_{24}H_{32}O_5$: C 71.97, H 8.05. Found: C 71.82, H 8.35.

*4.2.6.12. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl decanoate (**18i**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18i** (72%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 61–62 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (d, *J* = 7.6 Hz, 1H, ArH), 8.12 (d, *J* = 7.6 Hz, 1H, ArH), 7.80 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.73 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.46 (s, 1H, OH), 3.88 (s, 2H, OCH_2), 2.71 (s, 2H, CH_2Ar), 2.29 (t, *J* = 7.6 Hz, 2H, CH_2), 1.69–1.59 (m, 2H, CH_2), 1.32–1.25 (m, 12H, 6× CH_3), 1.02 (s, 6H, 2× CH_3), 0.91 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.8 (C=O), 174.5 (C=O), 154.8 (Ar), 135.6 (ArH), 133.6 (ArH, Ar), 130.0 (Ar), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.5 (CH_2), 30.0 (CH_2), 29.9 (2× CH_2), 29.8 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.3 (CH_2), 14.7 (CH_3); IR (KBr) = 3364 (OH), 2921, 2850 (CH), 1734, 1660, 1645 (C=O), 1376, 1277 (C=C), 1216, 1156 (C–O); MS (EI), *m*/*z* (% relative intensity): 414 (M$^+$, 5), 243 (10), 242 (28), 227 (43), 188 (61), 187 (64), 159 (65), 155 (79), 71 (100); Anal. Calcd for $C_{25}H_{34}O_5$: C 72.43, H 8.27. Found: C 72.21, H 8.16.

*4.2.6.13. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl undecanoate (**18j**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18j** (83%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 64.5–65.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (dd, *J* = 7.6, 1.1 Hz, 1H, ArH), 8.12 (dd, *J* = 7.6, 1.1 Hz, 1H, ArH), 7.80 (td, *J* = 7.6, 1.1 Hz, 1H, ArH), 7.73 (td, *J* = 7.6, 1.1 Hz, 1H, ArH), 7.46 (s, 1H, OH), 3.88 (s, 2H, OCH_2), 2.71 (s, 2H, CH_2Ar), 2.29 (t, *J* = 7.6 Hz, 2H, CH_2), 1.70–1.57 (m, 2H, CH_2), 1.39–1.22 (m, 14H, 7× CH_2), 1.01 (s, 6H, 2× CH_3), 0.90 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.8 (C=O), 181.2 (C=O), 173.9 (C=O), 154.2 (Ar), 135.0 (ArH), 133.0 (Ar), 132.9 (ArH), 129.3 (Ar), 127.0 (ArH), 126.1 (ArH), 121.7 (Ar), 72.5 (CH_2), 36.8 (C), 34.4 (CH_2), 32.0 (CH_2), 31.9 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 25.1 (2× CH_3), 25.0 (CH_2), 22.7 (CH_2), 14.1 (CH_3); IR (KBr) = 3365 (OH), 2917, 2850 (CH), 1738, 1645 (C=O), 1518, 1462, 1377, 1276 (C=C), 1212, 1157 (C–O); MS (EI), *m*/*z* (% relative intensity): 428 (M$^+$, 9), 243 (28), 241 (56), 227 (22), 188 (82), 187 (50), 159 (56), 71 (100); Anal. Calcd for $C_{26}H_{36}O_5$: C 72.87, H 8.47. Found: C 72.59, H 8.32.

*4.2.6.14. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl dodecanoate (**18k**).* Flash column chromatography, eluting with 30:1 hexane:ethyl acetate afforded the product **18k** (79%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 68–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (dd, *J* = 7.6, 1.3 Hz, 1H, ArH), 8.13 (dd, *J* = 7.6, 1.3 Hz, 1H, ArH), 7.80 (td, *J* = 7.6, 1.3 Hz, 1H, ArH), 7.73 (td, *J* = 7.6, 1.3 Hz, 1H, ArH), 7.46 (s, 1H, OH), 3.88 (s, 2H, OCH_2), 2.71 (s, 2H, CH_2Ar), 2.29 (t, *J* = 7.6 Hz, 2H, CH_2), 1.69–1.59 (m, 2H, CH_2), 1.35–1.22 (m, 16H, 8× CH_2), 1.02 (s, 6H, 2× CH_3), 0.91 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.8 (C=O), 174.5 (C=O), 154.8 (Ar), 135.6 (ArH), 133.6 (Ar), 133.5 (ArH), 130.0 (Ar), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.5 (CH_2), 30.2 (2× CH_2), 30.1 (CH_2), 29.9 (2× CH_2), 29.8 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.3 (CH_2), 14.7 (CH_3); IR (KBr) = 3365 (OH), 2920, 2850 (CH), 1735, 1645 (C=O), 1515, 1462, 1378, 1272 (C=C), 1210, 1153 (C–O); MS (EI), *m*/*z* (% relative intensity): 442 (M$^+$, 6), 243 (21), 242 (50), 229 (7), 227 (24), 188 (81), 187 (88), 159 (91), 71 (100); Anal. Calcd for $C_{27}H_{38}O_5$: C 73.27, H 8.65. Found: C 73.40, H 8.64.

*4.2.6.15. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl tetradecanoate (**18l**).* Flash column chromatography, eluting with 25:1 hexane:ethyl acetate afforded the product **18l** (80%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 79–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, *J* = 7.6, 1.2 Hz, 1H, ArH), 8.10 (dd, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.77 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.70 (td, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.44 (s, 1H, OH), 3.86 (s, 2H, OCH_2), 2.68 (s, 2H, CH_2Ar), 2.26 (t, 2H, *J* = 7.6 Hz, CH_2), 1.63–1.57 (m, 2H, CH_2), 1.33–1.21 (m, 20H, 10× CH_2), 0.99 (s, 6H, 2× CH_3), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C=O), 181.9 (C=O), 174.5 (C=O), 154.8 (Ar), 135.6 (ArH), 133.6 (Ar), 133.5 (ArH), 130.0 (Ar), 127.7 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.5 (CH_2), 30.3 (3× CH_2), 30.2 (CH_2), 30.1 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.3 (CH_2), 14.7 (CH_3); IR (KBr) = 3364 (OH), 2918, 2850 (CH), 1733, 1667, 1645 (C=O), 1595, 1472, 1376, 1276 (C=C), 1218, 1155 (C–O); MS (EI), *m*/*z* (% relative intensity): 470 (M$^+$, 62), 469 (64), 243 (100), 187 (52), 159 (47); Anal. Calcd for $C_{29}H_{42}O_5$: C 74.01, H 8.99. Found: C 74.06, H 8.69.

*4.2.6.16. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl hexadecanoate (**18m**).* Flash column chromatography,

eluting with 25:1 hexane:ethyl acetate afforded the product **18m** (77%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 76–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, J = 7.6, 1.2 Hz, 1H, ArH), 8.10 (dd, J = 7.6, 1.2 Hz, 1H, ArH), 7.77 (td, J = 7.6, 1.2 Hz, 1H, ArH), 7.70 (td, J = 7.6, 1.2 Hz, 1H, ArH), 7.45 (s, 1H, OH), 3.86 (s, 2H, OCH_2), 2.68 (s, 2H, CH_2Ar), 2.26 (t, 2H, J = 7.6 Hz, CH_2), 1.65–1.48 (m, 2H, CH_2), 1.25 (br s, 24H, 12× CH_2), 0.99 (s, 6H, 2× CH_3), 0.88 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C═O), 181.9 (C═O), 174.5 (C═O), 154.8 (Ar), 135.6 (ArH), 133.6 (Ar), 133.5 (ArH), 130.0 (Ar), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.5 (CH_2), 30.3 (6× CH_2), 30.1 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.3 (CH_2), 14.7 (CH_3); IR (KBr) = 3363 (OH), 2917, 2849 (CH), 1733, 1667, 1645 (C═O), 1595, 1471, 1376, 1276 (C═C), 1218, 1154 (C–O); MS (EI), m/z (% relative intensity): 498 (M^+, 31), 497 (69), 243 (100); Anal. Calcd for $C_{31}H_{46}O_5$: C 74.66, H 9.30. Found: C 74.36, H 9.45.

*4.2.6.17. 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl octadecanoate (**18n**).* Flash column chromatography, eluting with 25:1 hexane:ethyl acetate afforded the product **18n** (74%) as a yellow gum, which was recrystallized from hexane–dichloromethane to give yellow crystals, mp 82–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dd, J = 7.6, 1.2 Hz, 1H, ArH), 8.10 (dd, J = 7.6, 1.2 Hz, 1H, ArH), 7.77 (td, J = 7.6, 1.2 Hz, 1H, ArH), 7.70 (td, J = 7.6, 1.2 Hz, 1H, ArH), 7.44 (s, 1H, OH), 3.86 (s, 2H, OCH_2), 2.68 (s, 2H, CH_2Ar), 2.26 (t, 2H, J = 7.2 Hz, CH_2), 1.68–1.58 (m, 2H, CH_2), 1.25 (br s, 28H, 14× CH_2), 0.99 (s, 6H, 2× CH_3), 0.88 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.5 (C═O), 181.9 (C═O), 174.5 (C═O), 154.8 (Ar), 135.6 (ArH), 133.6 (Ar), 133.5 (ArH), 130.0 (Ar), 127.6 (ArH), 126.7 (ArH), 122.4 (Ar), 73.2 (CH_2), 37.5 (C), 35.0 (CH_2), 32.6 (CH_2), 32.5 (CH_2), 30.3 (8× CH_2), 30.1 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 25.7 (2× CH_3), 25.6 (CH_2), 23.3 (CH_2), 14.7 (CH_3); IR (KBr) = 3363 (OH), 2917, 2849 (CH), 1733, 1667, 1645 (C═O), 1595, 1471, 1376, 1276 (C═C), 1217, 1155 (C–O); MS (EI), m/z (% relative intensity): 526 (M^+, 90), 243 (100), 229 (6), 187 (9); Anal. Calcd for $C_{33}H_{50}O_5$: C 75.25, H 9.57. Found: C 75.08, H 9.69.

4.3. Resazurin microplate assay (REMA) [12]

Novel naphthoquinone aliphatic amides and esters were subjected to cytotoxic evaluation against KB human-cell lines (epidermoid carcinoma of oral cavity, ATCC CCL-17). Cells at a logarithmic growth phase were harvested and diluted to 7 × 10^4 cells/mL for KB in fresh medium. Successively, 5 mL of a test sample diluted in 0.5% DMSO and 45 mL of cell suspension were added into 384-well plates. The plates were incubated at 37 °C in 5% CO_2 incubator. After the incubation period (3 days for KB), 12.5 mL of 62.5 mg/mL resazurin solution was added to each well, and the plates were then incubated at 37 °C for 4 h. Fluorescence signals were measured using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 nm and 590 nm, respectively. Does response curves were plotted from 6 concentrations of 2-fold serially diluted test compounds and the 50% inhibition concentration (IC_{50}) was determined by curve fitting using the SOFTMax Pro software (Molecular Devices, USA). Doxorubicin and 0.5% DMSO were used as a positive and a negative control, respectively.

4.4. Green fluorescent protein (GFP)-based assay [13]

The GFP-expressing Vero cell lines were generated in-house by stably transfecting the African green monkey kidney cell lines (Vero, ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). The cell lines were maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate and 0.8 mg/mL geneticin, at 37 °C in a humidified incubator with 5% CO_2. The assay was carried out by adding 45 μL of cell suspension at 3.3 × 10^4 cells/mL to each well of 384-well plates containing 5 μL of test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days in 37 °C incubator with 5% CO_2. Fluorescence signals were measured by using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) in the bottom-reading mode with excitation and emission wavelengths of 485 and 535 nm. Fluorescence signal at day 4 was subtracted with background fluorescence at day 0. IC_{50} values were obtained as previously described in Section 4.3.

4.5. Topoisomerase assay

Human Topoisomerase IIα activity was determined using Topoisomerase II assay kit (TopGEN Inc.). The reaction mixture containing 60 ng of kinetoplast DNA (kDNA) and 2 units of hTopoIIα was incubated with and without naphthoquinone aliphatic amides and esters at 37 °C for 1 h in complete assay buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, and 2 mM ATP). Doxorubicin was used as a positive control while decatenated and linearized kDNA were used as markers. The reaction in a final volume of 20 μL was stopped by adding stop buffer/gel loading dye (1% Sarkosyl, 0.025% bromophenol blue and 5% glycerol). Reaction products were run on 1% agarose gel in 0.5× TBE buffer with 0.5 μg/mL ethidium bromide included in the gel. Electrophoresis was performed at 50 V for 1.30 h. After electrophoresis, the gel was destained with distilled water for 30 min and photographed over a UV transilluminator using DNr Bio-Imaging system. One unit of Topoisomerase II is defined as the amount of enzyme that decatenates 0.2 μg of kDNA in 30 min at 37 °C.

4.6. Molecular docking studies

Three-dimensional structure of human TopoIIα (hTopoIIα) was modeled by SWISS-MODEL using human TopoIIβ (hTopoIIβ) as a template (PDB: 3qx3A) [17]. Amino acid sequence identity between hTopoIIα and TopoIIβ was 74.1%. The quality of the modeled hTopoIIα was evaluated using the program PROCHECK [18]. The model possesses a good geometry with 87% of all residues in the most favored region and 10.8% in the allowed region of the Ramachandran plot. Naphthoquinone aliphatic amides (**11c** and **11m**) and esters (**18c** and **18m**) structures were built and optimized at the calculation level hf/6-31g with Gaussian 03. Gasteiger charges were added by using Auto Dock4.0 [19]. All hydrogen atoms were added and water molecules were removed from the protein structure. Lamarckian genetic algorithm (LGA) was used in this study with default search parameters being used except for 100 docking runs. The grid size was set to 60 × 60 × 60 points with a grid spacing of 0.375 Å. The structures with relative lower binding free energy and the most cluster members were chosen for the optimum docking conformation. Three-dimensional structures with the best-docked conformation were visualized and analyzed by PyMOL v. 0.99 and Discovery Studio 2.5 (Accelrys, Inc., CA, USA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.006.

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