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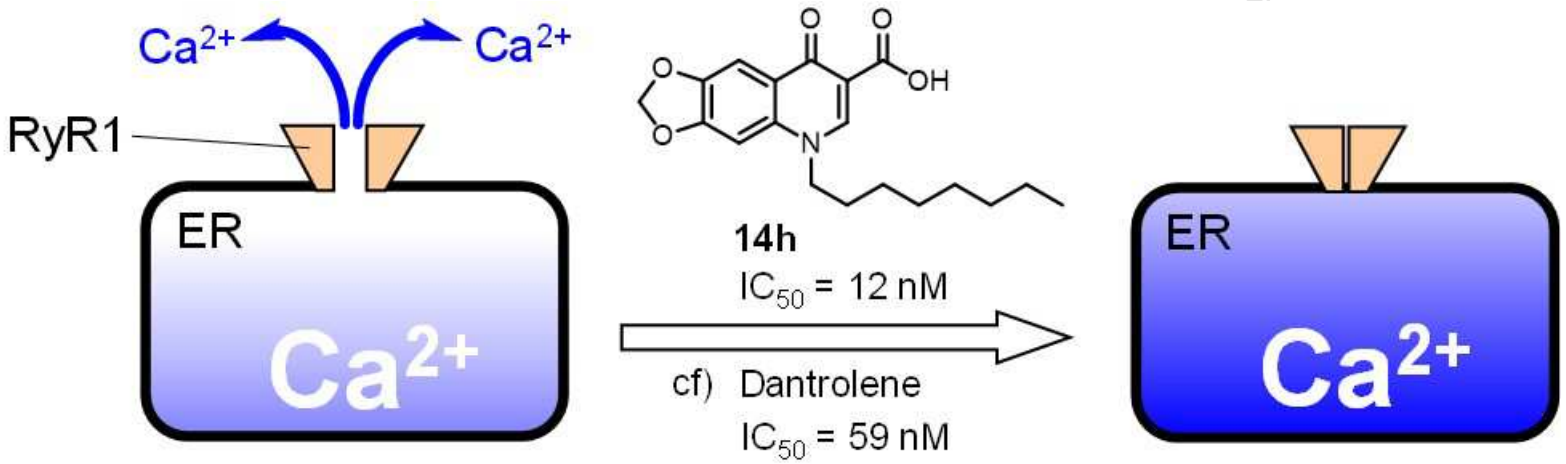
Graphical abstract



Structural development of a type-1 ryanodine receptor (RyR1) Ca^{2+}-release channel inhibitor guided by endoplasmic reticulum Ca^{2+} assay

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Abstract

Type-1 ryanodine receptor (RyR1) is a calcium-release channel localized on sarcoplasmic reticulum (SR) of the skeletal muscle, and mediates muscle contraction by releasing Ca^{2+} from the SR. Genetic mutations of RyR1 are associated with skeletal muscle diseases such as malignant hyperthermia and central core diseases, in which over-activation of RyR1 causes leakage of Ca^{2+} from the SR. We recently developed an efficient high-throughput screening system based on the measurement of Ca^{2+} in endoplasmic reticulum, and used it to identify oxolinic acid (**1**) as a novel RyR1 channel inhibitor. Here, we designed and synthesized a series of quinolone derivatives based on **1** as a lead compound. Derivatives bearing a long alkyl chain at the nitrogen atom of the quinolone ring and having a suitable substituent at the 7-position of quinolone exhibited potent RyR1 channel-inhibitory activity. Among the synthesized compounds, **14h** showed more potent activity than dantrolene, a known RyR1 inhibitor, and exhibited high RyR1 selectivity over RyR2 and RyR3. These compounds may be promising leads for clinically applicable RyR1 channel inhibitors.

Key words: Ryanodine receptor, quinolone, calcium ion channel

1. Introduction

Ryanodine receptors (RyRs) are huge calcium-release channels with molecular masses of >2 MDa, located on the sarcoplasmic reticulum (SR) membrane of skeletal and cardiac muscles. RyR1 and its isoform RyR2 are expressed in skeletal muscles and cardiac muscles, respectively, and play essential roles in muscle contraction.[1–4] In addition, RyR3 is expressed ubiquitously in various tissues and is involved in many biological processes, including neurotransmission. In the case of skeletal muscles, an action potential promotes mechanical coupling of dihydropyridine receptor and RyR1, followed by Ca^{2+} release from the SR store though the channel pore of RyR1. The resulting increase of cytoplasmic Ca^{2+} level induces skeletal muscle contraction. Genetic mutations of RyR1 are known to be associated with several muscle diseases, such as malignant hyperthermia (MH) and central core diseases (CCD),[5, 6] in which overactivation of the mutated RyR1 channel causes leakage of Ca^{2+} from the SR store. In addition, the symptoms of several diseases, such as muscular dystrophy[7] and Alzheimer disease,[8] are thought to be caused, at least in part, by over-activation of non-mutated RyR1 induced by external factors. Therefore, inhibitors of RyR1 channel activity are expected to be candidates for treating RyR1-related diseases.

Dantrolene (Figure 1) is the only RyR1 channel-targeting drug currently in clinical use; it is employed to treat MH,[9] a lethal disease caused by abnormal Ca^{2+} release from mutated RyR1 channels, triggered by administration of volatile anesthetics. Dantrolene inhibits the abnormal Ca^{2+} release by direct interaction with RyR1, ameliorating the symptoms of MH.[10, 11] Unfortunately, it is not applicable to chronic muscle diseases related to RyR1, such as CCD. Although novel RyR1 inhibitors are needed, not much research has been done in this area.[12] This is mainly due to lack of efficient assay method of the RyR1 channel activity that is suitable for high-throughput screening (HTS) and structural development studies.[13]

We have recently developed an efficient HTS system for RyR1 inhibitors based on the measurement of the Ca^{2+} concentration in the endoplasmic reticulum (ER) ($[Ca^{2+}]_{ER}$) of HEK293 cells,[14–16] and we screened oxolinic acid (**1**) as an RyR1-specific inhibitor (Figure 1) from a chemical library of well-characterized drugs (1535 compounds) owned by Tokyo Medical and Dental University (TMDU).[16] Compound **1** not only inhibited mutant RyR1 channels, but also inhibited the activation of WT RyR1 channel by caffeine, a known RyR channel activator. Thus, it could potentially treat not only diseases due to mutated RyR1, but also diseases due to over-activation of WT RyR by other factors or modifications. Compound **1** is a first-generation quinolone antibiotic, which has been used for the treatment of urinary tract infections[17] and is currently using for infection prevention in aquaculture.[18] Its activity is due to the inhibition of bacterial DNA gyrase, a bacteria-specific enzyme.[19] The toxicity of **1** toward humans is low, and indeed, no serious adverse effects have been reported. Thus, **1** seems to be a promising drug candidate for RyR1-related diseases. However, the inhibitory potency of **1** (EC_{50}: 0.81 μM) was lower than that of dantrolene

(EC_{50}: 0.059 μM) by one order of magnitude (Figure 2c). Therefore, in this study, we evaluated the structure-activity relationship of **1**, focusing on structural development to obtain more potent RyR1 channel inhibitors.

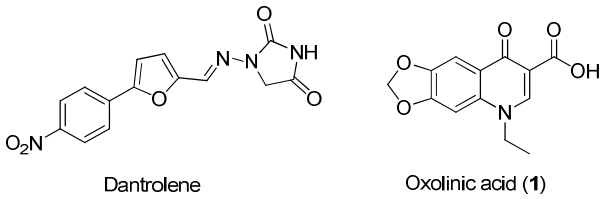


Figure 1. Structures of dantrolene and oxolinic acid (**1**).

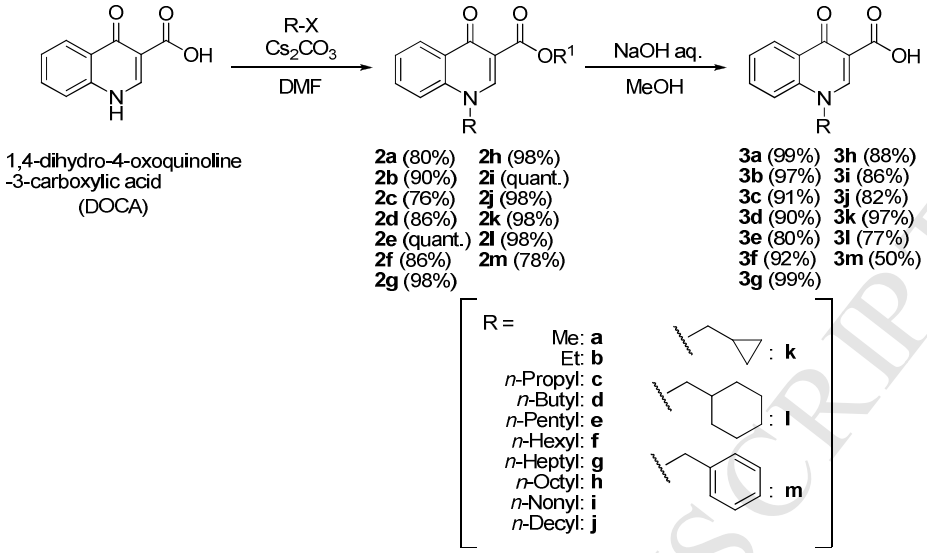
2. Results and Discussion

2.1. Molecular design and synthesis

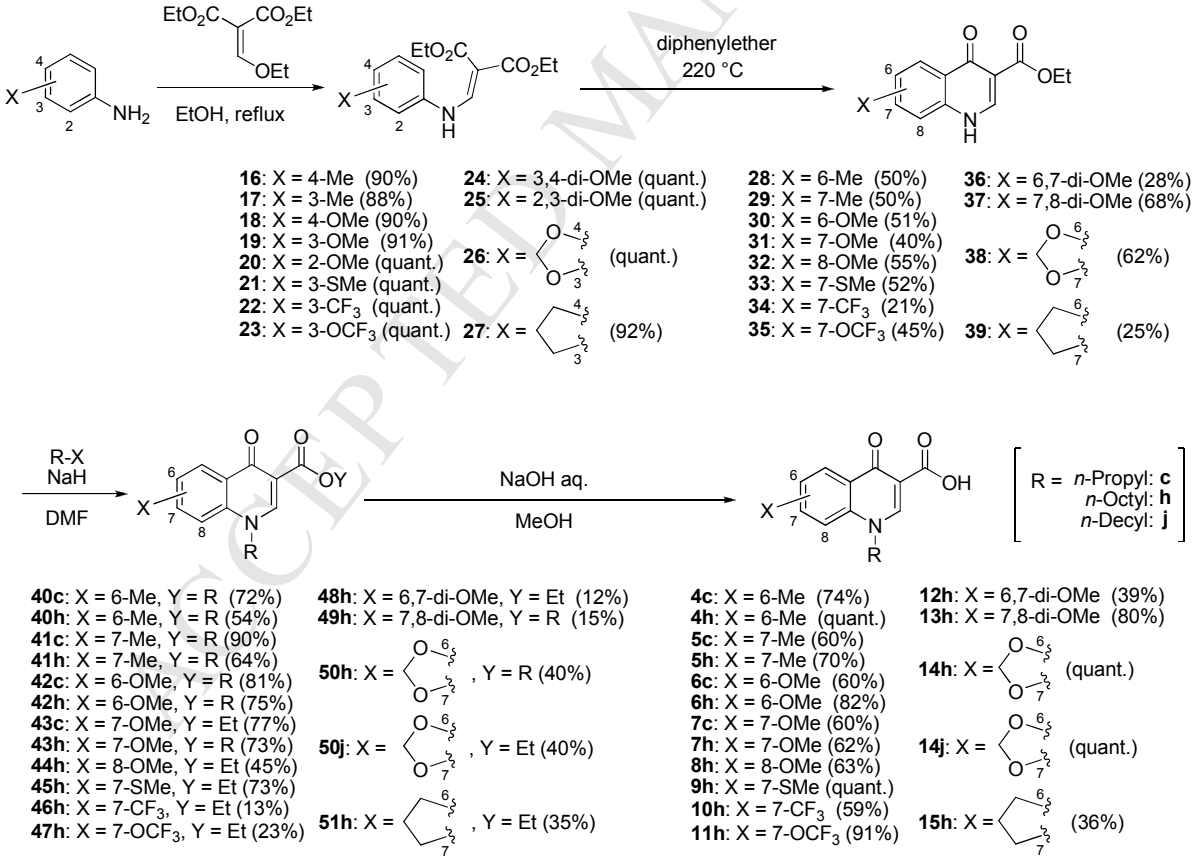
In the previous library screening of RyR1 channel inhibitors, none of 17 quinolone antibiotics, many of which were new quinolones having a fluorine group and a large substituent such as piperadine at 6- and 7-position of quinolone, respectively, exhibited RyR1 channel-inhibitory activity.[16] Based on these results, we designed a novel series of quinolone derivatives having a small substituent at the 6-, 7-, or 8-position of quinolone and different-sized alkyl groups at the 1-nitrogen atom.

First, quinolones without a substituent on the benzene moiety and with various alkyl groups on the nitrogen atom were synthesized to investigate the importance of the *N*-substituent. Alkylation of 1,4-dihydro-4-oxoquinoline-3-carboxylic acid (DOCA) with alkyl halide by using cesium carbonate as a base gave *N*,*O*-dialkylated compounds **2**. The ester group of **2** was hydrolyzed in aqueous sodium hydroxide solution to afford the target carboxylic acids **3** (Scheme 1).

Next, we focused on the effect of substituents on the benzene part of the quinolone ring. A series of substituted quinolone derivatives **4** – **15** was synthesized (Scheme 2). Substituted anilines were coupled with diethyl ethoxymethylenemalonate to afford compounds **16** – **27**. Intramolecular cyclization of **16** – **27** by heating in diphenylether gave the corresponding quinolones **28** – **39**. *N*-Alkylations of **28** – **39** were performed using sodium hydride as a base, since the reactions did not proceed under the conditions used for the synthesis of **2** (i.e., with cesium carbonate). In some cases, the *N*-alkylated products included transesterification products with the alkylating reagents. The ester groups of **40** – **51** were hydrolyzed in aqueous sodium hydroxide solution to afford **4** – **15** (Scheme 2).



Scheme1. Synthesis of the quinolone derivatives **3** with various *N*-substituents.

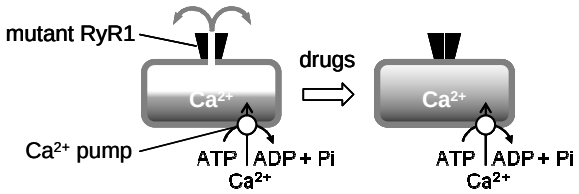


Scheme 2. Synthesis of the quinolone derivatives **4** – **11** substituted on the benzene ring.

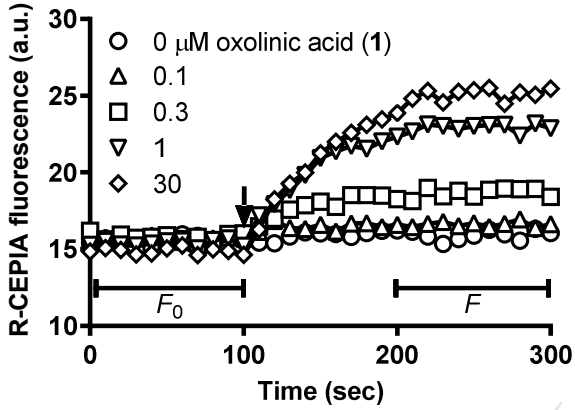
2.2. RyR1 channel-inhibitory activity

The RyR1 channel-inhibitory activities of the synthesized compounds were evaluated by means of our $[Ca^{2+}]_{ER}$ assay,[16] based on the fact that inhibitors suppress Ca^{2+} leakage from the ER in HEK293 cells carrying a disease-associated mutation of RyR1, resulting in an increase of $[Ca^{2+}]_{ER}$ due to Ca^{2+} uptake *via* the Ca^{2+} pump (Figure 2a). $[Ca^{2+}]_{ER}$ was measured with R-CEPIA1er, an ER Ca^{2+}-sensing fluorescent protein,[20] and time-lapse fluorescence measurements were performed using a FlexStation3 fluorometer with 96-well plates. Figure 2b shows typical results of fluorescence measurements in HEK293 cells expressing RyR1 with the R2163C mutation. Addition of 0.1 – 30 μM **1** gradually increased the $[Ca^{2+}]_{ER}$ signal and reached a plateau in a dose-dependent manner, indicating that **1** suppressed the Ca^{2+} leakage *via* RyR1. Figure 2c shows the dose dependency of R2163C RyR1 channel-inhibitory activity. The vertical axis of Figure 2c represents the $[Ca^{2+}]_{ER}$ increase ratio F/F_0 which was obtained by normalizing the average fluorescence for the last 100 seconds (F) to that for the first 100 seconds (F_0) in the time-lapse fluorescent measurement (Figure 2b). The dose-response curve of **1** was fitted by the Michaelis-Menten equation, and the EC_{50} value was determined to be 0.81 μM. The value was one-order higher than that of dantrolene (EC_{50}: 0.059 μM). In contrast, DOCA, a quinolone derivative not alkylated at the 1-nitrogen atom or substituted at the benzene ring, had no effect on the R-CEPIA1er fluorescence at concentrations up to 30 μM.

(a)



(b)



(c)

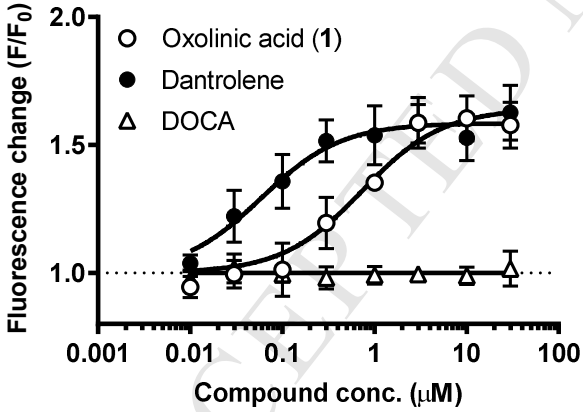
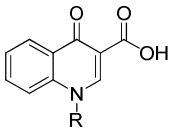


Figure 2. (a) Schematic illustration of the $[Ca^{2+}]_{ER}$ assay principle. (b) Time-lapse R-CEPIA1er fluorescence measurement with HEK293 cells expressing mutant RyR1. Compound **1** (0.1, 0.3, 1, and 30 μM) and DMSO was added at 100 seconds (arrow). (c) Dose-dependence of the effect of **1**, dantrolene, and DOCA on $[Ca^{2+}]_{ER}$ in R2163C RyR1 cells. Data are mean ± SD (n = 7). Dotted line denotes 1 F/F_0, which means no inhibitory activity.

We initially assessed the effects of alkylation at the 1-nitrogen atom of DOCA. Table 1 summarizes the EC_{50} values of RyR1 channel-inhibitory activity of **3a** – **3m**, evaluated by $[Ca^{2+}]_{ER}$ assay. The *N*-alkylated DOCAs **3a** – **3m** showed inhibitory activity, and its potency depended on the length of the alkyl chain. Specifically, the inhibitory potency increased as the *N*-alkyl chain of **3** became longer. Especially, **3h** bearing an *n*-octyl group showed the highest inhibitory activities (EC_{50}: 0.27 μM), being more potent than the lead compound **1** (EC_{50}: 0.81 μM). Further elongation of alkyl chain (*n*-nonyl and *n*-decyl group for **3i** and **3j**, respectively) resulted in the decrease of inhibitory activity. In addition, **3k**, **3l**, and **3m**, bearing a cyclopropylmethyl, cyclohexylmethyl, and benzyl group, respectively, exhibited weak inhibitory activities. The bulkiness of the longer alkyl chains or these cyclic substituents might interfere with the interaction of the compound with the target site.

Table 1 RyR1 channel-inhibitory activities of **3**.

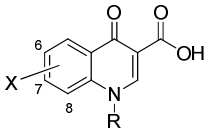


Compound	R	EC_{50} (μM)
DOCA	H	inactive
3a	Me	>10
3b	Et	>10
3c	*n*-Pr	3.2 (±0.3)
3d	*n*-Bu	3.3 (±0.5)
3e	*n*-Pentyl	6.7 (±1.0)
3f	*n*-Hexyl	5.3 (±0.9)
3g	*n*-Heptyl	0.56 (±0.10)
3h	*n*-Octyl	0.27 (±0.05)
3i	*n*-Nonyl	0.69 (±0.05)
3j	*n*-Decyl	2.0 (±0.2)
3k	Cyclopropylmethyl	>10
3l	Cyclohexyllmethyl	5.7 (±1.1)
3m	Benzyl	>10

*EC_{50} values were obtained by fitting the Michaelis-Menten equation, $y = 1 + A_{max}([x]/[x] + EC_{50})$, to the dose-response curve.

Next, we examined the effect of substitution of the quinolone ring at the 6-, 7-, or 8-position (**4** – **15**). Table 2 summarizes the inhibitory activity of *N*-*n*-propyl and *N*-*n*-octyl quinolones **4** – **8**. *N*-*n*-octyl quinolones (**h** series) exhibited more potent RyR1 channel-inhibitory activities than the corresponding *N*-*n*-propyl quinolones (**c** series) for 7-substituted quinolones (**5** and **7**), while **h** and **c** series exhibited comparable activities for 6-substituted quinolones (**4** and **6**). Among the *N*-*n*-octyl quinolones, **5h** and **7h** having a 7-methyl and a 7-methoxy group, respectively, exhibited more potent inhibitory activity than non-substituted quinolone **3h**. However, **4h**, **6h**, and **8h** having a 6-methyl, 6-methoxy, and 8-methoxy group, respectively, exhibited lower inhibitory activity than **3h**. These results indicate that introduction of a 7-substituent on the quinolone ring is favorable for the inhibitory activity.

Table 2. RyR1 channel-inhibitory activities of **3** – **8**.



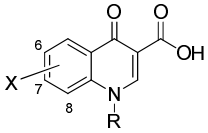
Compound	X	R	EC_{50} (μM)
3c	H	*n*-Pr	3.2 (±0.3)
4c	6-Me	*n*-Pr	1.1 (±0.1)
5c	7-Me	*n*-Pr	1.1 (±0.1)
6c	6-OMe	*n*-Pr	4.4 (±0.7)
7c	7-OMe	*n*-Pr	0.88 (±0.11)
3h	H	*n*-Octyl	0.27 (±0.05)
4h	6-Me	*n*-Octyl	1.2 (±0.1)
5h	7-Me	*n*-Octyl	0.16 (±0.02)
6h	6-OMe	*n*-Octyl	5.5 (±0.8)
7h	7-OMe	*n*-Octyl	0.076 (±0.011)
8h	8-OMe	*n*-Octyl	7.8 (±1.41)

*EC_{50} values were derived from dose-response curve fitted by the Michaelis-Menten equation: $y = 1 + A_{max}([x]/[x] + EC_{50})$.

Next, we examined the RyR1 channel-inhibitory activities of *N*-*n*-octyl quinolones having various substituents at the 7-position (Table 3). Substitution of the methoxy group of **7h** to a methylthio group (**9h**) decreased the inhibitory activity. In addition, substitution of the methoxy group to a trifluoromethyl (**10h**) or a trifluoromethoxy group (**11h**) greatly decreased the inhibitory activity. Furthermore, **12h** having 6,7-dimethoxy substitution was inactive, while **13h** having 7,8-dimethoxy substitution exhibited moderate inhibitory activity. On the other hand, compound **14h** having the 6,7-methylenedioxo structure, corresponding to the cyclic analog of the inactive 6,7-dimethoxy compound **12h**, exhibited more potent inhibitory activity than **7h**. The results suggested that the orientation of methoxy group at the 7-position was important for the activity. Thus, the orientation that the 7-methyoxy group directs to the 6-position would be favorable. In the compound **14h**, the 7-substituent was fixed in the preferable orientation, while the repulsion between two methoxy groups in compound **12h** caused unfavorable conformation. Compound **15h** having 6,7-propano group exhibited much lower activity than **14h**, which indicated the significance of oxygen atoms. Compound **14j** having *N*-*n*-decyl group did not show RyR1 channel-inhibitory activity, which indicated that the *n*-octyl group is the most suitable for the activity as in the case of *N*-alkylated DOCAs (Table 1). Among the synthesized quinolones, **14h** was the most potent RyR1 channel inhibitor, whereas **12h**, **14j**, and **15h** having the similar structural elements to **14h** showed little or no activity, suggesting that structural requirements for potent inhibitory activity are strict.

Figure 3a shows dose dependency of **1**, **5h**, **7h** and **14h** for RyR1 channel-inhibitory activity. The potency was greatly increased by structure development. **14h** (EC_{50}: 0.012 μM) was nearly 70-fold more potent than **1** (EC_{50}: 0.81 μM) and even five-fold more potent than dantrolene (EC_{50}: 0.059 μM) (Figure 2c). We previously reported that **1** does not inhibit RyR2, a cardiac isoform[16]. Compounds **5h**, **7h** and **14h** did not affect $[Ca^{2+}]_{ER}$ of HEK293 cells expressing RyR2 (Figure 3b). On the other hand, these compounds as well as **1** slightly increased $[Ca^{2+}]_{ER}$ of RyR3 cells at 10 μM (Figure 3c), while the activity was very weak.

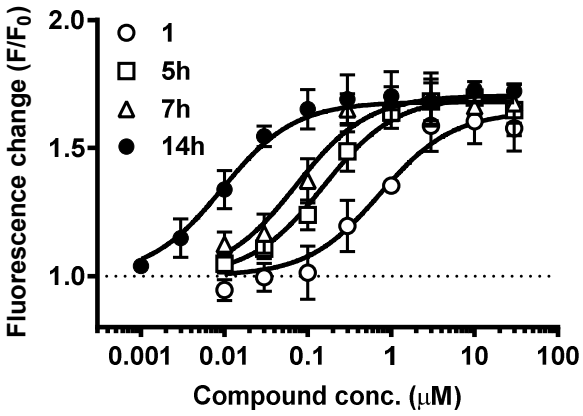
Table 3. RyR1 channel-inhibitory activities of 7-substituted quinolones **7h** and **9h** – **15h**.



Compound	X	R	EC_{50} (μM)
7h	7-OMe	*n*-Octyl	0.076 (±0.011)
9h	7-SMe	*n*-Octyl	0.41 (±0.091)
10h	7-CF_3	*n*-Octyl	> 10
11h	7-OCF_3	*n*-Octyl	7.1 (±1.0)
12h	6,7-$(OMe)_2$	*n*-Octyl	inactive
13h	7,8-$(OMe)_2$	*n*-Octyl	1.3 (±0.27)
14h		*n*-Octyl	0.012 (±0.001)
14j		*n*-Decyl	inactive
15h		*n*-Octyl	9.8 (±0.7)
1	H	Et	0.81 (±0.09)
dantrolene	-	-	0.059 (±0.011)

*EC_{50} values were derived from dose-response curve fitted by the Michaelis-Menten equation: $y = 1 + A_{max}([x]/[x] + EC_{50})$.

(a)



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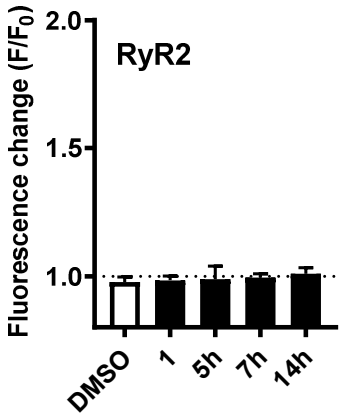

(c)

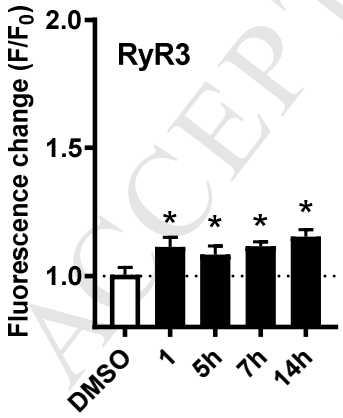

Figure 3 (a) Dose-dependent effects of **1**, **5h**, **7h**, and **14h** on $[Ca^{2+}]_{ER}$ in R2163C RyR1 cells. (b) and (c) Effects of **1**, **5h**, **7h**, and **14h** at 10 μM on $[Ca^{2+}]_{ER}$ in RyR2 (b) and RyR3 (c) cells.
Data are mean ± SD (n = 6). Dotted line denotes 1 F/F_0, which means no inhibitory activity. *P<0.05 compared with DMSO (one-way analysis of variance with Dunnett's test).

2.3. Ryanodine binding-inhibitory activity

To examine whether the synthesized quinolone derivatives inhibit RyR1 directly, their effects on the binding of [^{3}H]ryanodine to RyR1 were examined. Ryanodine, a poisonous alkaloid, binds to RyR1 in the open state.[13b, c] Namely, the binding of [^{3}H]ryanodine to RyR1 could be a good indicator of the channel activity. Microsomes isolated from R2163C RyR1 HEK293 cells were used for the binding assay. The binding of [^{3}H]ryanodine to RyR1 exhibited biphasic Ca^{2+} dependency with a peak at about 1 μM Ca^{2+} (Figure 4a). We tested all the synthesized compounds at 10 μM concentration and found that only three compounds (**5h**, **7h** and **14h**) show inhibitory activity. Upon addition of **5h**, **7h** and **14h**, the binding of [^{3}H]ryanodine decreased with a slight shift of the Ca^{2+} dependency to a higher concentration range of Ca^{2+}. The inhibitory effect of **5h**, **7h** and **14h** on [^{3}H]ryanodine binding was dose-dependent and the IC_{50} values in the presence of 1 μM Ca^{2+} were 1.3 μM, 1.6 μM and 0.73 μM, respectively (Figure 4b). These values were more than one-order higher than EC_{50} values obtained by $[Ca^{2+}]_{ER}$ assay (see Tables 2 and 3). This apparent discrepancy in the inhibitory activity between $[Ca^{2+}]_{ER}$ assay and [^{3}H]ryanodine-binding assay may be explained by the presence of soluble factors that would promote the inhibitory action of quinolone compounds. Since [^{3}H]ryanodine-binding assay was carried out with isolated microsomes, soluble factors would have been largely removed during the preparation of microsomes. Another possibility would be that a conformational change in the quinolone-binding site would occur during the microsome-isolation process, which decreased the binding affinity. These results suggest that quinolone compounds directly inhibit the RyR1 channel activity.

We also tested the effects of the synthetic compounds (**5h**, **7h** and **14h**) as well as **1** on [^{3}H]ryanodine binding to RyR2 and RyR3. Consistent with the $[Ca^{2+}]_{ER}$ assay, no effects were observed on RyR2 (Fig. 4c). The compounds did not inhibit RyR3 (Figure 4d). This appears to be inconsistent with the results of $[Ca^{2+}]_{ER}$ assay. The inhibitory effect on RyR3 might be too weak to be detected by [^{3}H]ryanodine binding assay.

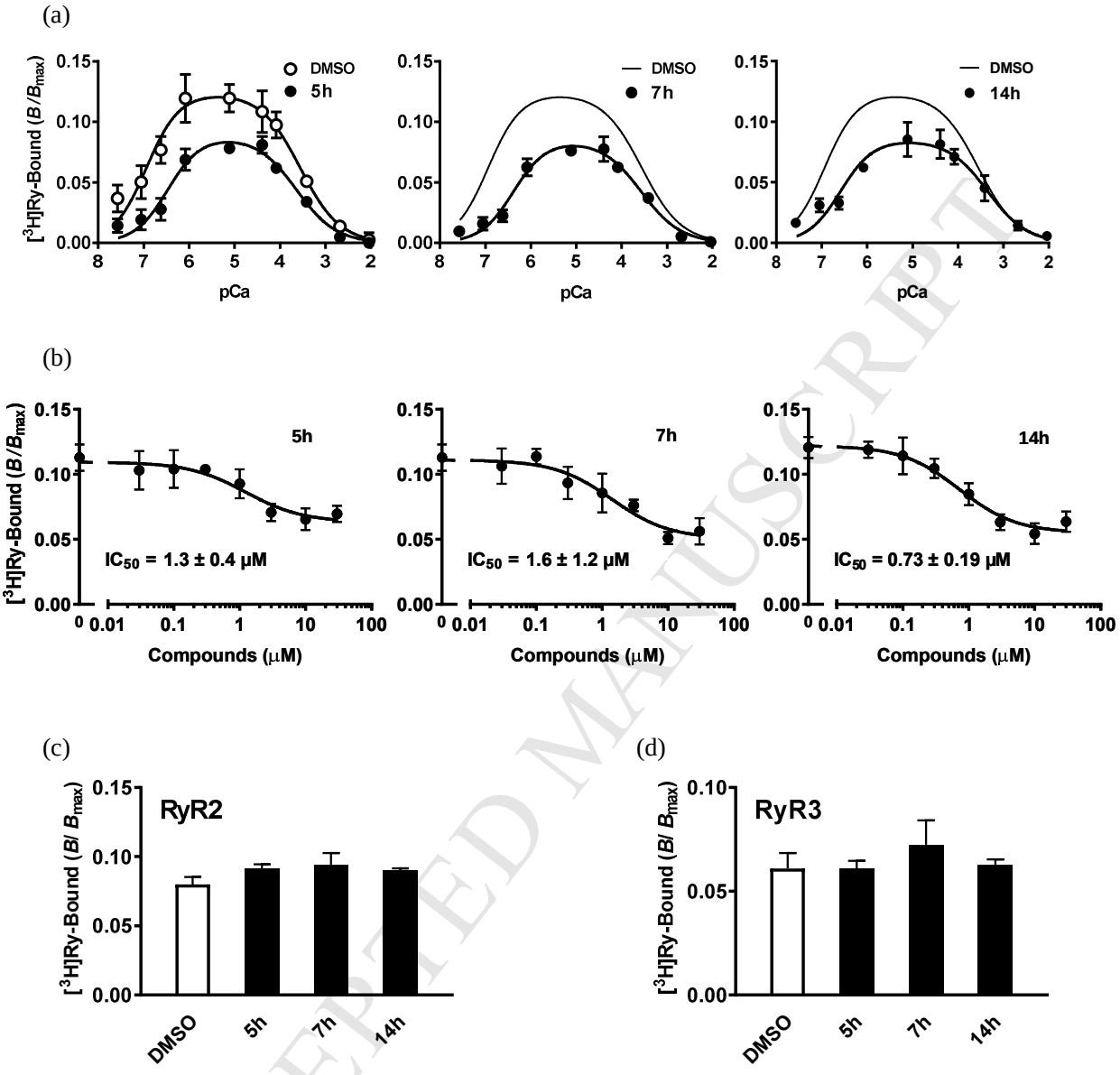

Figure 4. Effects of **5h**, **7h**, and **14h** on the [^{3}H]ryanodine binding. (a) Ca^{2+}-dependent [^{3}H]ryanodine binding to R2163C RyR1 in the presence (closed circles) and absence (open circles) of the compound (10 μM). (b) Dose-dependent [^{3}H]ryanodine-binding inhibition by the compound in the presence of 1 μM Ca^{2+}. (c) and (d) Effects of **1**, **5h**, **7h**, and **14h** at 10 μM on [^{3}H]ryanodine binding to RyR2 (b) and RyR3 (c). Data are mean ± SD (n = 4)

3. Conclusion

We designed and synthesized a series of quinolone derivatives as RyR1 channel inhibitor candidates, based on our previously identified inhibitor, oxolinic acid (**1**), as the lead compound. HEK293 cell-based $[Ca^{2+}]_{ER}$ assay revealed that the *N*-alkyl group of the quinolone structure is important for the RyR1 channel-inhibitory activity, and introduction of a long alkyl chain, such as an *n*-octyl group, is also favorable. In addition, suitable modification of the 7-position of the quinolone ring increased the inhibitory activity. Notably, **14h** exhibited extremely potent activity, much higher than that of dantrolene. The synthesized quinolone derivatives exhibited high selectivity for RyR1 over RyR2 and RyR3. [^{3}H]Ryanodine-binding assay suggested that the potent RyR1 channel-inhibitory activities of the quinolone derivatives involved direct interaction with RyR1. Detailed investigation of the mechanism behind the RyR1-inhibitory activities is in progress.

The lead compound **1** is a first-generation quinolone antibiotic, and its toxicity to humans is quite low.[17] Therefore, **1** and its derivatives may be promising candidates for the treatment of RyR1-related diseases, such as MH and CCD. Although there are several reports for development of RyR inhibitors based on known drugs or natural products,[12] this is the first report on the structural development of RyR channel inhibitors based on a lead compound obtained from an HTS study. Our powerful HTS platform should be applicable for further structural development of RyR modulators, as well as discovery of modulators of RyR2 and RyR3.

4. Experimental

4.1. General

All reagents were purchased from Sigma-Aldrich, Tokyo Kasei, Wako Chemicals, and Kanto Chemicals and were used without further purification. NMR spectra were recorded on Bruker AVANCE 400 or Bruker AVANCE 500 spectrometers. Chemical shifts for NMR are reported as parts of per million (ppm) relative to chloroform (7.26 ppm for ^{1}H NMR and 77.23 ppm for ^{13}C NMR), DMSO-d_6 (2.50 ppm for ^{1}H NMR and 39.51 ppm for ^{13}C NMR), and methanol-*d4* (3.31 ppm for ^{1}H NMR and 49.00 ppm for ^{13}C NMR). Mass spectra were collected on a Bruker Daltonics microTOF-2focus spectrometer in the positive ion modes. Melting points were obtained on a Yanagimoto micro melting point apparatus without correction.

4.2. Synthesis

4.2.1. General procedure for synthesis of **2a**–**k**

A mixture of 1,4-Dihydoro-4-oxoquinoline-3-carboxylic acid (200 mg, 1.05 mmol) and Cs_2CO_3 (1.03 g, 3.17 mmol) in DMF (3 mL) was stirred at room temperature for 10 min. Then, the corresponding alkyl halide (3.17 mmol) was added and the stirring was continued at 55 °C for 9 h. After the solvent was removed *in vacuo*, the residue was extracted with ethyl acetate and water. The

organic layer was washed with brine, and dried over sodium sulfate. The solvent was removed under reduced pressure to give **2**.

4.2.1.1. Methyl 1-methyl-4-quinolone-3-carboxylate (**2a**)

White solid (80% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 8.5, 1.5 Hz, 1 H), 8.53 (s, 1 H), 7.74 (ddd, *J* = 8.5, 7.0, 1.5 Hz, 1 H), 7.49 (ddd, *J* = 8.4, 7.0, 1.1, 1 H), 7.45 (br d, *J* = 8.0 1 H), 3.95 (s, 1 H), 3.91 (s, 1 H).

4.2.1.2. Ethyl 1-ethyl-4-quinolone-3-carboxylate (**2b**)

White solid (90% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 8.2, 1.3 Hz, 1 H), 8.52 (s, 1 H), 7.70 (ddd, *J* = 8.5, 7.2, 1.8 Hz, 1 H), 7.48–7.43 (m, 2 H), 4.42 (q, *J* = 7.2 Hz, 2 H), 4.27 (q, *J* = 7.5 Hz, 2 H), 1.56 (t, *J* = 7.2 Hz, 3 H), 1.43 (t, *J* = 7.2 Hz, 3 H).

4.2.1.3. Propyl 1-propyl-4-quinolone-3-carboxylate (**2c**)

White solid (76% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (s, 1 H), 8.56 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.70 (ddd, *J* = 8.6, 7.0, 1.6 Hz, 1 H), 7.48–7.44 (m, 2 H), 4.31 (t, *J* = 6.9 Hz, 2 H), 4.21 (t, *J* = 7.3 Hz, 2 H), 1.95 (sext, *J* = 7.5 Hz, 2 H), 1.83 (sext, *J* = 7.4 Hz, 2 H), 1.05 (t, *J* = 7.5 Hz, 3 H), 1.04 (t, *J* = 7.4 Hz, 3 H).

4.2.1.4. Butyl 1-butyl-4-quinolone-3-carboxylate (**2d**)

White solid (86% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (dd, *J* = 7.9, 1.5 Hz, 1 H), 8.44 (br s, 1 H), 7.66 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1 H), 7.44–7.41 (m, 2 H), 4.33 (t, *J* = 6.8 Hz, 2 H), 4.17 (t, *J* = 6.6 Hz, 2 H), 1.86 (quin, *J* = 7.4 Hz, 2 H), 1.77 (quin, *J* = 7.1 Hz, 2 H), 1.50 (quin, *J* = 7.3 Hz, 2 H), 1.44 (quin, *J* = 6.9 Hz, 2 H), 1.00–0.95 (m, 6 H).

4.2.1.5. Pentyl 1-pentyl-4-quinolone-3-carboxylate (**2e**)

White solid (quant.): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (dd, *J* = 8.0, 1.7 Hz, 1 H), 8.46 (s, 1 H), 7.67 (ddd, *J* = 8.6, 8.1, 1.3 Hz, 1 H), 7.44–7.40 (m, 2 H), 4.32 (t, *J* = 6.9 Hz, 2 H), 4.17 (t, *J* = 7.4 Hz, 2 H), 1.89 (quin, *J* = 6.8 Hz, 2 H), 1.80 (quin, *J* = 7.1 Hz, 2 H), 1.45–1.38 (m, 8 H), 0.95–0.89 (m, 6 H).

4.2.1.6. Hexyl 1-hexyl-4-quinolone-3-carboxylate (**2f**)

White solid (86% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (dd, *J* = 7.9, 1.7 Hz, 1 H), 8.44 (s, 1 H), 7.66 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1 H), 7.44–7.39 (m, 2 H), 4.31 (t, *J* = 7.0 Hz, 2 H), 4.16 (t, *J* = 7.5 Hz, 2 H), 1.87 (quin, *J* = 7.4 Hz, 2 H), 1.78 (quin, *J* = 7.3 Hz, 2 H), 1.45–1.28 (m, 12 H), 0.89–0.87 (m, 6 H).

4.2.1.7. Heptyl 1-heptyl-4-quinolone-3-carboxylate (**2g**)

White solid (98% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, *J* = 7.2, 1.7 Hz, 1 H) 8.46 (s, 1 H), 7.68 (ddd, *J* = 7.9, 7.9, 1.5 Hz, 1 H), 7.46–7.42 (m, 2 H), 4.32 (t, *J* = 6.9 Hz, 2 H), 4.17 (t, *J* = 7.5 Hz, 2 H), 1.89 (quin, *J* = 7.4 Hz, 2 H), 1.80 (quin, *J* = 7.1 Hz, 2 H), 1.45–1.24 (m, 16 H), 0.92–0.85 (m, 6 H).

4.2.1.8. Octyl 1-octyl-4-quinolone-3-carboxylate (**2h**)

White solid (98% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (dd, *J* = 8.0, 1.3 Hz, 1 H), 8.43 (s, 1 H), 7.66 (ddd, *J* = 8.5, 7.2 1.5 Hz, 1 H), 7.45–7.38 (m, 2 H), 4.30 (t, *J* = 7.3 Hz, 2 H), 4.15 (t, *J* = 7.1 Hz, 2 H), 1.85 (quin, *J* = 7.2 Hz, 2 H), 1.78 (quin, *J* = 7.2 Hz, 2 H), 1.43–1.26 (m, 20 H), 0.88–0.85 (m, 6 H).

4.2.1.9. Nonyl 1-nonyl-4-quinolone-3-carboxylate (**2i**)

White solid (98% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 8.3, 1.7 Hz, 1 H), 8.48 (s, 1 H), 7.69 (ddd, *J* = 8.6, 7.2 1.6 Hz, 1 H), 7.47–7.31 (m, 2 H), 4.34 (t, *J* = 7.0 Hz, 2 H), 4.18 (t, *J* = 7.5 Hz, 2 H), 1.92 (quin, *J* = 6.6 Hz, 2 H), 1.81 (quin, *J* = 6.9 Hz, 2 H), 1.48–1.25 (m, 24 H), 0.90–0.87 (m, 6 H).

4.2.1.10. Decyl 1-decyl-4-quinolone-3-carboxylate (**2i**)

White solid (98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 8.1 Hz, 1 H), 8.48 (s, 1 H), 7.66 (ddd, *J* = 8.5, 7.4 1.0 Hz, 1 H), 7.47–7.42 (m, 2 H), 4.34 (t, *J* = 7.0 Hz, 2 H), 4.18 (t, *J* = 7.4 Hz, 2 H), 1.90 (quin, *J* = 6.6 Hz, 2 H), 1.81 (quin, *J* = 7.0 Hz, 2 H), 1.49–1.23 (m, 28 H), 0.90–0.87 (m, 6 H).

4.2.1.11. Cyclopropylmethyl 1-(cyclopropylmethyl)-4-quinolone-3-carboxylate (**2k**)

White solid (98% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (s, 1 H), 8.56 (br d, *J* = 7.9 Hz, 1 H), 7.69 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1 H), 7.55 (br d, *J* = 8.5 Hz, 1 H), 7.47–7.42 (m, 2 H), 4.17 (d, *J* = 8.1 Hz, 2 H), 4.04 (d, *J* = 7.9 Hz, 2 H), 1.41–1.24 (m, 2 H), 0.76 (ddd, *J* = 7.3, 5.6, 4.3 Hz, 2 H), 0.61 (ddd, *J* = 7.3, 5.8, 4.1 Hz, 2 H), 0.49–0.46 (m, 2 H), 0.41–0.37 (m, 2 H).

4.2.1.12. Cyclohexylmethyl 1-(cyclohexylmethyl)-4-quinolone-3-carboxylate (**2l**)

White solid (98% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 8.1, 1.6 Hz, 1 H), 8.41 (s, 1 H), 7.68 (ddd, *J* = 8.6, 7.9, 1.6 Hz, 1 H), 7.44 (ddd, *J* = 8.4, 8.0, 0.5, 1 H), 7.41 (br d, *J* = 8.5, 1 H), 4.15 (d, *J* = 6.7 Hz, 1 H), 3.99 (d, *J* = 7.3 Hz, 1 H), 1.91–1.69 (m, 11 H), 1.34–1.03 (m, 11 H).

4.2.1.13. Benzyl 1-benzyl-4-quinolone-3-carboxylate (**2m**)

White solid (78% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1 H), 8.56 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.55 (ddd, *J* = 7.8, 7.1, 1.7 Hz, 1 H), 7.54–7.51 (m, 2), 7.41 (ddd, *J* = 7.6, 7.3, 1.8 Hz, 1 H), 7.39–7.29 (m, 7 H), 7.16 (br d, *J* = 8.2 Hz, 2 H), 5.41 (s, 2 H), 5.38 (s, 2 H).

4.2.2. General procedure for synthesis of **16**–**27**

A solution of corresponding aniline (9.32 mmol) and diethyl ethoxymethylenemalonate (9.32 mmol) in ethanol (20 mL) was heated at 90 °C for 18 h. After the solvent was removed under reduced pressure, the residue was extracted with ethyl acetate and water. The organic layer was washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by open column chromatography (eluent: *n*-hexane/ethyl acetate = 4 : 1) to give corresponding compound **16**–**27**.

4.2.2.1. Diethyl 2-[(4-toluidino)methylene]malonate (**16**)

Pale yellow oil (90% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.98 (d, *J* = 13.4 Hz, 1 H), 8.50 (d, *J* = 13.8 Hz, 1 H), 7.15 (d, *J* = 8.3 Hz, 2 H), 7.01 (d, *J* = 8.2 Hz, 2 H), 4.30 (q, *J* = 7.1 Hz, 2 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 2.31 (s, 3 H), 1.37 (t, *J* = 7.1 Hz, 3 H), 1.32 (t, *J* = 7.2 Hz, 3 H).

4.2.2.2. Diethyl 2-[(3-toluidino)methylene]malonate (**17**)

Pale yellow oil (88% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (d, *J* = 13.6 Hz, 1 H), 8.51 (d, *J* = 13.7 Hz, 1 H), 7.23 (t, *J* = 7.4 Hz, 1 H), 6.97–6.90 (m, 3 H), 4.30 (q, *J* = 7.2 Hz, 2 H), 4.24 (q, *J* = 7.2 Hz, 2H), 2.35 (s, 3 H), 1.34 (t, *J* = 7.2 Hz, 3 H), 1.32 (t, *J* = 7.2 Hz, 3 H).

4.2.2.3. Diethyl 2-[(4-methoxyphenylamino)methylene]malonate (**18**)

Pale yellow oil (90% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.98 (d, *J* = 13.7 Hz, 1 H), 8.43 (d, *J* = 13.8 Hz, 1 H), 7.07 (d, *J* = 8.9 Hz, 2 H), 6.90 (d, *J* = 8.9 Hz, 2 H), 4.29 (q, *J* = 7.1 Hz, 2 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 3.80 (s, 3 H), 1.37 (t, *J* = 7.1 Hz, 3 H), 1.31 (t, *J* = 7.1 Hz, 3 H).

4.2.2.4. Diethyl 2-[(3-methoxyphenylamino)methylene]malonate (**19**)

Pale yellow oil (91% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (d, *J* = 13.5 Hz, 1 H), 8.50 (d, *J* = 13.5 Hz, 1 H), 7.26 (t, *J* = 8.1 Hz, 1 H), 6.73–6.64 (m, 3 H), 4.30 (q, *J* = 7.1 Hz, 2 H), 4.24 (q, *J* = 7.1 Hz, 2 H), 3.80 (s, 3 H), 1.37 (t, *J* = 6.4 Hz, 3 H), 1.34 (t, *J* = 6.2 Hz, 3 H).

4.2.2.5. Diethyl 2-[(2-methoxyphenylamino)methylene]malonate (**20**)

Pale yellow oil (quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.10 (d, *J* = 13.6 Hz, 1 H), 8.55 (d, *J* = 14.0 Hz, 1 H), 7.23 (br d, *J* = 8.0 Hz, 1 H), 7.08 (t, *J* = 7.6 Hz, 1 H), 6.97 (t, *J* = 7.6 Hz, 1 H), 6.92 (br d, *J* = 8.4 Hz, 1 H), 4.32 (q, *J* = 7.2 Hz, 2 H), 4.24 (q, *J* = 7.2 Hz, 2 H), 3.92 (s, 3 H), 1.38 (t, *J* = 7.2 Hz,

3 H), 1.32 (t, *J* = 6.8 Hz, 3 H).

4.2.2.6. Diethyl 2-[[(3-methylthio)phenylamino]methylene]malonate (**21**)
Pale yellow oil (quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.97 (d, *J* = 13.6 Hz, 1 H), 8.49 (d, *J* = 13.6 Hz, 1 H), 7.27 (t, *J* = 8.0 Hz, 1 H), 7.01 (dd, *J* = 7.6, 0.8 Hz, 1 H), 6.97 (t, *J* = 2.0 Hz, 1 H), 6.90 (dd, *J* = 8.0, 2.0 Hz, 1 H), 4.30 (q, *J* = 7.2 Hz, 2 H), 4.25 (q, *J* = 6.8 Hz, 2 H), 1.38 (t, *J* = 7.2 Hz, 3 H), 1.33 (t, *J* = 7.2 Hz, 3 H).

4.2.2.7. Diethyl 2-[[(3-trifluoromethyl)phenylamino]methylene]malonate (**22**)
Pale yellow oil (quant.): ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.11 (d, *J* = 13.2 Hz, 1 H), 8.50 (d, *J* = 13.4 Hz, 1 H), 7.50 (t, *J* = 7.9 Hz, 1 H), 7.39 (br d, *J* = 7.8 Hz, 1 H), 7.36 (s, 1 H), 7.31 (br d, *J* = 8.0 Hz, 1 H), 4.31 (q, *J* = 7.1 Hz, 2 H), 4.26 (q, *J* = 7.1 Hz, 2 H), 1.38 (t, *J* = 7.1 Hz, 3 H), 1.34 (t, *J* = 7.2 Hz, 3 H).

4.2.2.8. Diethyl 2-[[(3-trifluoromethyloxy)phenylamino]methylene]malonate (**23**)
Pale yellow oil (quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.03 (d, *J* = 13.4 Hz, 1 H), 8.46 (d, *J* = 13.4 Hz, 1 H), 7.39 (t, *J* = 8.1 Hz, 1 H), 7.06 (ddd, *J* = 8.3, 1.8, 0.8 Hz, 1 H), 7.00 (ddd, *J* = 8.3, 1.2, 0.8 Hz, 1 H), 6.98 (br s, 1 H), 4.31 (q, *J* = 7.1 Hz, 2 H), 4.26 (q, *J* = 7.1 Hz, 2 H), 1.38 (t, *J* = 7.2 Hz, 3 H), 1.33 (t, *J* = 7.1 Hz, 3 H).

4.2.2.9. Diethyl 2-[(3,4-dimethoxyphenylamino)methylene]malonate (**24**)
Pale yellow oil (quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.99 (d, *J* = 13.6 Hz, 1 H), 8.43 (d, *J* = 13.6 Hz, 1 H), 6.84 (d, *J* = 8.8 Hz, 1 H), 6.70 (dd, *J* = 8.8, 2.8 Hz, 1 H), 6.64 (d, *J* = 2.4 Hz, 1 H), 4.29 (q, *J* = 6.8 Hz, 2 H), 4.23 (q, *J* = 7.2 Hz, 2 H), 3.88 (s, 3 H), 3.86 (s, 3 H), 1.37 (t, *J* = 7.2 Hz, 3 H), 1.31 (t, *J* = 7.2 Hz, 3 H).

4.2.2.10. Diethyl 2-[(2,3-dimethoxyphenylamino)methylene]malonate (**25**)
Pale yellow oil (quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.14 (d, *J* = 14.0 Hz, 1 H), 8.53 (d, *J* = 14.0 Hz, 1 H), 7.06 (t, *J* = 8.0 Hz, 1 H), 6.87 (dd, *J* = 8.4, 0.8 Hz, 1 H), 6.68 (dd, *J* = 8.4, 1.2 Hz, 1 H), 4.32 (q, *J* = 7.2 Hz, 2 H), 4.25 (q, *J* = 7.2 Hz, 2 H), 3.92 (s, 3 H), 3.88 (s, 3 H), 1.38 (t, *J* = 7.2 Hz, 3 H), 1.33 (t, *J* = 7.2 Hz, 3 H).

4.2.2.11. Diethyl [[3,4-(methylenedioxy)phenylamino]methylene]malonate (**26**)
White solid (quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (d, *J* = 13.6 Hz, 1 H), 8.38 (d, *J* = 13.6 Hz, 1 H), 6.78 (d, *J* = 8.3 Hz, 1 H), 6.68 (d, *J* = 2.3 Hz, 1 H), 6.58 (dd, *J* = 8.32, 2.3 Hz, 1 H), 5.99 (s, 2 H), 4.29 (q, *J* = 7.1 Hz, 2 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 1.38 (t, *J* = 7.1 Hz, 3 H), 1.32 (t, *J* = 7.1 Hz,

3 H).

4.2.2.12. Diethyl [(5-indanylamino)methylene]malonate (**27**)

White solid (92% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.98 (d, *J* = 13.3 Hz, 1 H), 8.50 (d, *J* = 13.8 Hz, 1 H), 7.19 (d, *J* = 7.9 Hz, 1 H), 7.02 (s, 1 H), 6.90 (d, *J* = 7.8 Hz, 1 H), 4.30 (q, *J* = 7.1 Hz, 2 H), 4.21 (q, *J* = 7.2 Hz, 2 H), 2.91 (t, *J* = 7.2 Hz, 2 H), 2.88 (t, *J* = 7.2 Hz, 2 H), 2.10 (quin, *J* = 7.2 Hz, 2 H), 1.37 (t, *J* = 7.1 Hz, 3 H), 1.32 (t, *J* = 7.1 Hz, 3 H).

4.2.3. General procedure for synthesis of **28**–**39**

A solution of compound **16**–**27** (7.36 mmol) in diphenylether (50 mL) was heated at 250 °C for 10 h. After cooling, precipitated solid was separated by filtration, washed with diethylether. The residue was dried under reduced pressure to give corresponding quinolone **28**–**39**.

4.2.3.1. Ethyl 6-methyl-4-quinolone-3-carboxylate (**28**)

Brown slid (50% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (br s, 1 H), 8.50 (s, 1 H), 7.94 (s, 1 H), 7.52 (s, 2 H), 4.21 (q, *J* = 7.1 Hz, 2 H), 2.42 (s, 3 H), 1.28 (t, *J* = 7.1 Hz, 3 H).

4.2.3.2. Ethyl 7-methyl-4-quinolone-3-carboxylate (**29**)

Brown solid (50% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (br s, 1 H), 8.49 (s, 1 H), 8.04 (d, *J* = 8.2 Hz, 1 H), 7.37 (br s, 1 H), 7.23 (dd, *J* = 8.2, 1.0 Hz, 1 H), 4.20 (q, *J* = 7.1 Hz, 2 H), 2.43 (s, 3 H), 1.27 (t, *J* = 7.1 Hz 3 H).

4.2.3.3. Ethyl 6-methoxy-4-quinolone-3-carboxylate (**30**)

Brown solid (51% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (br s, 1 H), 8.49 (s, 1 H), 7.58 (d, *J* = 8.6 Hz, 1 H), 7.57 (d, *J* = 2.6 Hz, 1 H), 7.34 (dd, *J* = 9.0, 3.0 Hz, 1 H), 4.21(q, *J* = 7.1 Hz, 2 H), 3.85 (s, 3 H), 1.28 (t, *J* = 7.1 Hz, 3 H).

4.2.3.4. Ethyl 7-methoxy-4-quinolone-3-carboxylate (**31**)

Brown solid (40% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.10 (br s, 1 H), 8.48 (s, 1 H), 8.05 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.01 (s, 1 H), 7.00 (dd, *J* = 8.6, 1.5 Hz, 1 H), 4.20 (q, *J* = 7.1 Hz, 2 H), 3.86 (s, 3 H), 1.27 (t, *J* = 7.1 Hz, 3 H).

4.2.3.5. Ethyl 8-methoxy-4-quinolone-3-carboxylate (**32**)

Brown solid (55% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1 H), 8.33 (s, 1 H), 7.69 (dd, *J* = 7.2, 1.6 Hz, 1 H), 7.34 (t, *J* = 8.0 Hz, 1 H), 7.31 (dd, *J* = 8.0, 2.0 Hz, 1 H), 4.19 (q, *J* = 7.2 Hz, 1 H), 3.98 (s, 3 H), 1.26 (t, *J* = 7.2 Hz, 3 H).

4.2.3.6. Ethyl 7-(methylthio)-4-quinolone-3-carboxylate (**33**)

Brown solid (52% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (br s, 1 H), 8.51 (s, 1 H), 8.02 (d, *J* = 8.4 Hz, 1 H), 7.35 (d, *J* = 1.6 Hz, 1 H), 7.26 (dd, *J* = 8.8, 2.0 Hz, 1 H), 4.20 (q, *J* = 6.8 Hz, 2 H), 2.56 (s, 3 H), 1.27 (t, *J* = 6.8 Hz, 3 H).

4.2.3.7. Ethyl 7-(trifluoromethyl)-4-quinolone-3-carboxylate (**34**)

Brown solid (21% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (br s, 1 H), 8.69 (s, 1 H), 8.33 (d, *J* = 8.3 Hz, 1 H), 7.95 (br s, 1 H), 7.64 (d, *J* = 8.2 Hz, 1 H), 4.21 (q, *J* = 7.0 Hz, 2 H), 1.28 (t, *J* = 7.0 Hz, 3 H).

4.2.3.8. Ethyl 7-(trifluoromethyloxy)-4-quinolone-3-carboxylate (**35**)

Brown solid (45% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1 H), 8.24 (d, *J* = 8.9 Hz, 1 H), 7.55 (s, 1 H), 7.33 (d, *J* = 8.7 Hz, 1 H), 4.21 (q, *J* = 6.8 Hz, 2 H), 1.28 (t, *J* = 7.1 Hz, 3 H).

4.2.3.9. Ethyl 6,7-dimethoxy-4-quinolone-3-carboxylate (**36**)

Brown solid (28% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (s, 1 H), 8.44 (s, 1 H), 7.51 (s, 1 H), 7.05 (s, 1 H), 4.42 (q, *J* = 7.2 Hz, 2 H), 3.88 (s, 3 H), 3.84 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3 H).

4.2.3.10. Ethyl 7,8-dimethoxy-4-quinolone-3-carboxylate (**37**)

Brown solid (68% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1 H), 8.33 (s, 1 H), 7.87 (d, *J* = 8.8 Hz, 1 H), 7.23 (d, *J* = 9.2 Hz, 1 H), 4.19 (q, *J* = 7.2 Hz, 2 H), 3.94 (s, 3 H), 3.88 (s, 3 H), 1.26 (t, *J* = 7.2 Hz, 3 H).

4.2.3.11. Ethyl 6,7-(methylenedioxy)-4-quinolone-3-carboxylate (**38**)

Brown solid (62% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1 H), 7.46 (s, 1 H), 7.02 (s, 1 H), 6.12 (s, 2 H), 4.17 (q, *J* = 7.0 Hz, 2 H), 1.26 (t, *J* = 7.1 Hz, 3 H).

4.2.3.12. Ethyl 6,7-(trimethylene)-4-quinolone-3-carboxylate (**39**)

Brown solid (25% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1 H), 7.97 (s, 1 H), 7.42 (s, 1 H), 4.20 (q, *J* = 7.1 Hz, 2 H), 2.97 (t, *J* = 7.6 Hz, 2 H), 2.95 (t, *J* = 7.2 Hz, 2 H), 2.06 (quin, *J* = 7.4 Hz, 2 H), 1.27 (t, *J* = 7.1 Hz, 3 H).

4.2.4. General procedure for synthesis of **40**–**51**

A solution of **28**–**39** (0.17 mmol) in dry DMF (2 mL) was added to a suspension of sodium hydride (60%, 0.34 mmol) in dry DMF (3 mL) at 0 °C. After stirring for 10 min at room temperature, a

solution of corresponding alkyl halide (0.34 mmol) was added and the mixture was heated at 55 °C for 9 h. The solvent was removed in vacuo and the residue was extracted with ethyl acetate and water. The organic layer was washed with brine and dried over sodium sulfate. After evaporation, residue was purified by silica gel column chromatography (ethyl acetate/hexane 3:1) to give corresponding compound **40**–**51**. The obtained products were the mixtures of the ethyl esters and transesterification products with the alkylating reagents. They were used in the next hydrolysis step without further separation.

4.2.4.1. Propyl 6-methyl-1-propyl-4-quinolone-3-carboxylate (**40c**)

Orange oil (72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1 H), 8.36 (d, *J* = 1.0 Hz, 1 H), 7.50 (dd, *J* = 8.7, 2.1 Hz, 1 H), 7.35 (d, *J* = 8.6 Hz, 1 H), 4.30 (t, *J* = 6.8 Hz, 2 H), 4.14 (t, *J* = 7.3 Hz, 2 H), 2.48 (s, 3 H), 1.94 (sext, *J* = 6.6 Hz, 2 H), 1.83 (sext, *J* = 7.2 Hz, 2 H), 1.06 (t, *J* = 7.5 Hz, 3 H), 1.03 (t, *J* = 7.4 Hz, 3 H),.

4.2.4.2. Octyl 6-methyl-1-octyl-4-quinolone-3-carboxylate (**40h**)

Orange oil (54% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1 H), 8.36 (d, *J* = 1.1 Hz, 1 H), 7.50 (dd, *J* = 8.6, 2.0 Hz, 1 H), 7.35 (d, *J* = 8.7 Hz, 1 H), 4.33 (t, *J* = 6.9 Hz, 2 H), 4.16 (t, *J* = 7.5 Hz, 2 H), 2.49 (s, 3 H), 1.89 (quin, *J* = 7.4 Hz, 2 H), 1.81 (quin, *J* = 7.3 Hz, 2 H), 1.48–1.27 (m, 20 H), 0.89 (t, *J* = 6.9 Hz, 6 H).

4.2.4.3. Propyl 7-methyl-1-propyl-4-quinolone-3-carboxylate (**41c**)

Orange oil (90% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (br s, 1 H), 8.41 (d, *J* = 9.0 Hz, 1 H), 7.23 (d, *J* = 8.4 Hz, 1 H), 7.18 (s, 1 H), 4.27 (t, *J* = 6.8 Hz, 2 H), 4.12 (t, *J* = 7.4 Hz, 2 H), 2.50 (s, 3 H), 1.92 (sext, *J* = 6.1 Hz, 2 H), 1.80 (sext, *J* = 7.1 Hz, 2 H),, 1.04–0.99 (m, 6 H).

4.2.4.4. Octyl 7-methyl-1-octyl-4-quinolone-3-carboxylate (**41h**)

Orange oil (64% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (br s, 1 H), 8.44 (d, *J* = 8.4 Hz, 1 H), 7.25 (d, *J* = 8.4 Hz, 1 H), 7.19 (s, 1 H), 4.32 (t, *J* = 7.0 Hz, 2 H), 4.13 (t, *J* = 7.2 Hz, 2 H), 2.52 (s, 3 H), 1.96–1.86 (m, 2 H), 1.80 (quin, *J* = 7.3 Hz, 2 H), 1.48–1.25 (m, 20 H), 0.88 (t, *J* = 5.8 Hz 6 H).

4.2.4.5. Propyl 6-methoxy-1-propyl-4-quinolone-3-carboxylate (**42c**)

Orange oil (81% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (s, 1 H), 7.98 (d, *J* = 3.1 Hz, 1 H), 7.40 (d, *J* = 9.2 Hz, 1 H), 7.29 (dd, *J* = 9.2, 3.1 Hz, 1 H), 4.30 (t, *J* = 6.8 Hz, 2 H), 4.16 (t, *J* = 7.4 Hz, 2 H), 3.92 (s, 3 H), 1.93 (sext, *J* = 7.4 Hz, 2 H), 1.83 (sext, *J* = 7.3 Hz, 2 H), 1.06 (t, *J* = 7.5 Hz, 3 H), 1.02 (t, *J* = 7.4 Hz, 3 H).

4.2.4.6. Octyl 6-methoxy-1-octyl-4-quinolone-3-carboxylate (**42h**)

Orange oil (75 % yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (s, 1 H), 7.97 (d, *J* = 3.0 Hz, 1 H), 7.38 (d, *J* = 9.2 Hz, 1 H), 7.28 (dd, *J* = 9.2, 3.0 Hz, 1 H), 4.32 (t, *J* = 7.0 Hz, 2 H), 4.16 (t, *J* = 7.5 Hz, 2 H), 3.92 (s, 3 H), 1.87 (quin, *J* = 7.5 Hz, 2 H), 1.83 (quin, *J* = 7.3 Hz, 2 H), 1.48–1.19 (m, 20 H), 0.88 (t, *J* = 7.1 Hz, 3 H), 0.87 (t, *J* = 6.9 Hz, 3 H).

4.2.4.7. Ethyl 7-methoxy-1-propyl-4-quinolone-3-carboxylate (**43c**)

Orange oil (77% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 1 H), 8.50 (d, *J* = 9.0 Hz, 1 H), 7.18 (dd, *J* = 9.0, 2.2 Hz, 1 H), 6.93 (d, *J* = 2.2 Hz, 1 H), 4.22 (t, *J* = 7.4 Hz, 2 H), 3.99 (s, 3 H), 3.73 (q, *J* = 7.0 Hz, 2 H), 1.99 (sext, *J* = 7.4 Hz, 2 H), 1.25 (t, *J* = 7.1 Hz, 3 H), 1.06 (t, *J* = 7.4 Hz, 3 H).

4.2.4.8. Octyl 7-methoxy-1-octyl-4-quinolone-3-carboxylate (**43h**)

Orange oil (73% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, *J* = 9.0 Hz, 1 H), 8.36 (s, 1 H), 6.98 (dd, *J* = 9.1, 2.3 Hz, 1 H), 6.76 (d, *J* = 2.2 Hz, 1 H), 4.29 (t, *J* = 7.0 Hz, 2 H), 4.08 (t, *J* = 7.5 Hz, 2 H), 3.91 (s, 3 H), 1.86 (quin, *J* = 7.4 Hz, 2 H), 1.77 (quin, *J* = 7.3 Hz, 2 H), 1.45–1.23 (m, 20 H), 0.86 (t, *J* = 7.1 Hz, 6 H).

4.2.4.9. Ethyl 8-methoxy-1-octyl-4-quinolone-3-carboxylate (**44h**)

Orange oil (45% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1 H), 8.18 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.34 (t, *J* = 8.0 Hz, 1 H), 7.15 (dd, *J* = 8.0, 1.2 Hz, 1 H), 4.46 (t, *J* = 7.2 Hz, 2 H), 4.39 (q, *J* = 7.2 Hz, 2 H), 3.95 (s, 3 H), 1.78 (quin, *J* = 7.2 Hz, 2 H), 1.40 (t, *J* = 7.2 Hz, 3 H), 1.31–1.25 (m, 10 H), 0.87 (t, *J* = 6.8 Hz, 3H).

4.2.4.10. Ethyl 7-(methylthio)-1-octyl-4-quinolone-3-carboxylate (**45h**)

Orange oil (73% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 8.36 (d, *J* = 8.0 Hz, 1 H), 7.21 (dd, *J* = 8.8, 1.6 Hz, 1 H), 7.11 (d, *J* = 1.6 Hz, 1 H), 4.35 (q, *J* = 7.2 Hz, 2 H), 4.10 (t, *J* = 7.2 Hz, 2 H), 2.54 (s, 3 H), 1.84 (quin, *J* = 7.2 Hz, 2 H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.35–1.24 (m, 10 H), 0.85 (t, *J* = 6.8 Hz, 3H).

4.2.4.11. Ethyl 1-octyl-7-(trifluoromethyl)-4-quinolone-3-carboxylate (**46h**)

Orange oil (13% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 8.3 Hz, 1 H), 8.53 (s, 1 H), 7.68 (s, 1 H), 7.65 (d, *J* = 8.5 Hz, 1 H), 4.41 (q, *J* = 7.1 Hz, 2 H), 4.21 (t, *J* = 7.4 Hz, 2 H), 1.90 (quin, *J* = 7.4 Hz, 2 H), 1.42 (t, *J* = 7.1, 3 H), 1.25–1.39 (m, 10 H), 0.88 (t, *J* = 6.7 Hz, 3 H).

4.2.4.12. Ethyl 1-octyl-7-(trifluoromethyloxy)-4-quinolone-3-carboxylate (**47h**)

Orange oil (23% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.8 Hz, 1 H), 8.48 (s, 1 H), 7.27

(d, *J* = 8.9 Hz, 1 H), 7.22 (s, 1 H), 4.40 (q, *J* = 7.1 Hz, 2 H), 4.13 (t, *J* = 7.4 Hz, 2 H), 1.88 (quin, *J* = 7.6 Hz, 2 H), 1.42 (t, *J* = 7.1 Hz, 3 H), 1.40–1.27 (m, 10 H), 0.88 (t, *J* = 6.0 Hz, 3 H).

4.2.4.13. Ethyl 6,7-dimethoxy-1-octyl-4-quinolone-3-carboxylate (**48h**)

Yellow solid (12% yield): ^{1}H NMR (400 MHz, methanol-d_4) δ 8.91 (s, 1 H), 7.50 (s, 1 H), 7.34 (s, 1 H), 4.44 (q, *J* = 7.2 Hz, 2 H), 4.24 (t, *J* = 6.4 Hz, 2 H), 4.02 (s, 3 H), 3.99 (s, 3 H), 1.91 (quin, *J* = 6.4 Hz, 2 H), 1.57 (quin, *J* = 6.8 Hz, 2 H), 1.45 (t, *J* = 7.2 Hz, 3 H), 1.42–1.31 (m, 8 H), 0.90 (t, *J* = 7.2 Hz, 3H).

4.2.4.14. Octyl 7,8-dimethoxy-1-octyl-4-quinolone-3-carboxylate (**49h**)

Orange oil (15% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 8.34 (d, *J* = 8.8 Hz, 1 H), 7.10 (d, *J* = 9.2 Hz, 1 H), 4.38 (t, *J* = 7.2 Hz, 2 H), 4.32 (t, *J* = 6.8 Hz, 2 H), 4.00 (s, 3 H), 3.88 (s, 3 H), 1.79 (quin, *J* = 6.8 Hz, 2 H), 1.78 (quin, *J* = 6.8 Hz, 2 H), 1.48–1.24 (m, 20 H), 0.88 (t, *J* = 6.8 Hz, 3 H), 0.86 (t, *J* = 7.2 Hz, 3 H).

4.2.4.15. Octyl 6,7-(methylenedioxy)-1-octyl-4-quinolone-3-carboxylate (**50h**)

Pale yellow solid (40% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1 H), 7.91 (s, 1 H), 6.82 (s, 1 H), 6.10 (s, 2 H), 4.31 (t, *J* = 7.0 Hz, 2 H), 4.08 (t, *J* = 7.4 Hz, 2 H), 1.86 (quin, *J* = 7.7 Hz, 2 H), 1.79 (quin, *J* = 7.2 Hz, 2 H), 1.47–1.27 (m, 20 H), 0.88 (t, *J* = 7.2 Hz, 3 H), 0.87 (t, *J* = 6.8 Hz, 3 H).

4.2.4.16. Ethyl 1-decyl-6,7-(methylenedioxy)-4-quinolone-3-carboxylate (**50j**)

Pale orange solid (40% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1 H), 7.49 (s, 1 H), 7.41 (s, 1 H), 6.15 (2 H), 4.44 (q, *J* = 7.2 Hz, 2 H), 4.18 (t, *J* = 6.4 Hz, 2 H), 1.89 (quin, *J* = 6.8 Hz, 2 H), 1.44 (t, *J* = 7.2 Hz, 3 H), 1.34–1.27 (m, 10 H), 0.88 (t, *J* = 7.2 Hz, 3H).

4.2.4.17. Ethyl 1-octyl-6,7-(trimethylene)-4-quinolone-3-carboxylate (**51h**)

Pale orange solid (35% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1 H), 8.36 (s, 1 H), 7.25 (s, 1 H), 4.40 (q, *J* = 7.1 Hz, 2 H), 4.14 (t, *J* = 7.4 Hz, 2 H), 3.04 (t, *J* = 7.2 Hz, 2 H), 3.02 (t, *J* = 7.2 Hz, 2 H), 2.17 (quin, *J* = 7.6 Hz, 2 H), 1.88 (quin, *J* = 7.5 Hz, 2 H), 1.41 (t, *J* = 7.1, 3 H), 1.36–1.26 (m, 10 H), 0.87 (t, *J* = 7.2 Hz, 3 H).

4.2.5. General procedure for synthesis of **3**–**15**

2 M NaOH (3 mL) was added to a solution of compound **2** or **40**–**51** (130 mg, 0.431 mmol) in MeOH (5 mL), and the mixture was stirred at room temperature for 8 h. The reaction mixture was quenched with ice /2 M HCl and stirred for 1 h to form a white precipitate. After the mixture was filtrated, the precipitate was washed with H_2O and recrystallized from each suitable solvent to give

desired carboxylic acid **3**–**15**.

4.2.5.1. 1-Methyl-4-quinolone-3-carboxylic acid (**3a**)

Colorless needle (99% yield) recrystallized from EtOH/AcOEt: mp 291–293 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.89 (s, 1 H), 8.78 (s, 1 H), 8.59 (dd, *J* = 8.8, 2.0 Hz, 1 H), 7.64 (br t, *J* = 8.0, 1 H), 7.63 (br d, *J* = 8.8), 4.05 (s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.8, 166.1, 150.1, 140.3, 134.2, 126.4, 125.6, 125.2, 118.2, 107.4, 41.7; HRMS (ESI, m/z) Calcd. for $C_{11}H_{10}NO_3$ $[M+H]^+$: 204.0661. Found 204.0656.

4.2.5.2. 1-Ethyl-4-quinolone-3-carboxylic acid (**3b**)

Colorless needle (97% yield) recrystallized from EtOH/AcOEt: mp 249–250 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.89 (s, 1 H), 9.07 (s, 1 H), 8.40 (dd, *J* = 8.2, 1.5 Hz, 1 H), 8.05 (br d, *J* = 8.7, 1 H), 7.98 (ddd, *J* = 8.7, 7.0, 1.6, 1 H), 7.67 (ddd, *J* = 8.1, 7.2, 0.9, 1 H), 4.62 (q, *J* = 7.2, 2 H), 1.43 (t, *J* = 7.2, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.7, 166.0, 149.1, 139.0, 134.2, 126.2, 125.9, 125.5, 118.0, 107.7, 48.9, 14.5; HRMS (ESI, m/z) Calcd. for $C_{12}H_{12}NO_3$ $[M+H]^+$: 218.0817. Found 218.0812.

4.2.5.3. 1-Propyl-4-quinolone-3-carboxylic acid (**3c**)

Colorless block (91% yield) recrystallized from EtOH/AcOEt: mp 155–157 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.97 (s, 1 H), 8.78 (s, 1 H), 8.58 (dd, J = 8.0, 1.4 Hz, 1 H), 7.86 (ddd, *J* = 8.2, 7.0, 1.5 Hz, 1 H), 7.64–7.59 (m, 2 H), 4.31 (t, *J* = 7.5 Hz, 2 H), 2.00 (sxt, *J* = 7.5 Hz, 2 H), 1.07 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.7, 166.0, 149.5, 139.2, 134.2, 126.3, 125.9, 125.5, 118.2, 107.4, 54.9, 22.0, 10.5; HRMS (ESI, m/z) Calcd. for $C_{13}H_{14}NO_3$ $[M+H]^+$: 232.0974. Found 232.0971.

4.2.5.4. 1-Butyl-4-quinolone-3-carboxylic acid (**3d**)

Colorless plate (90% yield) recrystallized from *n*-Hex/AcOEt: mp 173–176 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (s, 1 H), 8.77 (s, 1 H), 8.56 (d, *J* = 8.1 Hz, 1 H), 7.86 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1 H), 7.63 (d, *J* = 8.6 Hz, 1 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 4.34 (t, *J* = 7.5 Hz, 2 H), 1.92 (quin, *J* = 7.6 Hz, 2 H), 1.47 (sext, *J* = 7.5 Hz, 2 H), 1.02 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.4, 139.3, 134.0, 127.4, 126.6, 126.2, 116.4, 108.7, 54.7, 31.0, 19.9, 13.5; HRMS (ESI, m/z) Calcd. for $C_{14}H_{16}NO_3$ $[M+H]^+$: 246.1130. Found 246.1125.

4.2.5.5. 1-Pentyl-4-quinolone-3-carboxylic acid (**3e**)

Colorless plate (80% yield) recrystallized from *n*-Hex/AcOEt: mp 144–146 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (br s, 1 H), 8.77 (s, 1 H), 8.57 (dd, *J* = 8.1, 1.5, Hz, 1 H), 7.85 (ddd, *J* = 8.7, 7.1, 1.6

Hz, 1 H), 7.62 (d, *J* = 8.5 Hz, 1 H), 7.60 (ddd, *J* = 8.1, 7.2, 0.7 Hz, 2 H), 4.32 (t, *J* = 7.6 Hz, 2 H), 1.95 (quin, *J* = 7.5 Hz, 2 H), 1.43–1.38 (m, 4 H), 0.93 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.4, 139.3, 134.0, 127.4, 126.7, 126.2, 116.4, 108.7, 54.9, 28.8, 28.7, 22.2, 13.8; HRMS (ESI, m/z) Calcd. for $C_{15}H_{18}NO_3$ $[M+H]^+$: 260.1287. Found 260.1282.

4.2.5.6. 1-Hexyl-4-quinolone-3-carboxylic acid (**3f**)

White solid (92% yield) recrystallized from *n*-Hex/AcOEt: mp 153–155 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (br s, 1 H), 8.76 (s, 1 H), 8.58 (dd, *J* = 8.1, 1.4 Hz, 1 H), 7.86 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1 H), 7.62 (d, *J* = 7.9 Hz, 2 H), 7.60 (t, J = 7.1 Hz, 2 H), 4.32 (t, *J* = 7.6 Hz, 2 H), 1.94 (quin, *J* = 7.5 Hz, 2 H), 1.45 (quin, *J* = 7.5 Hz, 2 H), 1.34 (m, 4 H), 0.91 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.2, 148.4, 139.3, 134.0, 127.5, 126.7, 126.2, 116.4, 108.7, 54.9, 31.2, 29.0, 26.3, 22.4, 13.9; HRMS (ESI, m/z) Calcd. for $C_{16}H_{20}NO_3$ $[M+H]^+$: 274.1443. Found 274.1442.

4.2.5.7. 1-Heptyl-4-quinolone-3-carboxylic acid (**3g**)

Colorless plate (99% yield) recrystallized from *n*-Hex/AcOEt: mp 119–121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (br s, 1 H) 8.77 (s, 1 H), 8.58 (dd, *J* = 8.1, 1.4 Hz, 1 H), 7.85 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1 H), 7.62 (d, *J* = 9.1 Hz, 2 H), 7.60 (ddd, *J* = 7.9, 7.2, 0.7 Hz, 2 H), 4.32 (t, *J* = 7.5 Hz, 2 H), 1.94 (quin, *J* = 7.5 Hz, 2 H), 1.46–1.27 (m, 8 H), 0.89 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.4, 139.3, 134.0, 127.5, 126.7, 126.2, 116.4, 108.7, 54.9, 31.5, 29.1, 28.7, 26.6, 22.5, 14.0; HRMS (ESI, m/z) Calcd. for $C_{17}H_{22}NO_3$ $[M+H]^+$: 288.1600. Found. 288.1592.

4.2.5.8. 1-Octyl-4-quinolone-3-carboxylic acid (**3h**)

White solid (88% yield) recrystallized from *n*-Hex/AcOEt: mp 119–122 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (br s, 1 H), 8.77 (s, 1 H), 8.57 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.86 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1 H), 7.62 (d, *J* = 8.6 Hz, 1 H), 7.60 (t, *J* = 7.6 Hz, 1 H), 4.31 (t, *J* = 7.6 Hz, 2 H), 1.94 (quin, *J* = 7.6 Hz, 2 H), 1.45–1.23 (m, 10 H), 0.88 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.4, 139.3, 134.0, 127.4, 126.7, 126.2, 116.4, 108.7, 54.9, 31.6, 29.1, 29.0, 29.0, 26.6, 22.5, 14.0; HRMS (ESI, m/z) Calcd. for $C_{18}H_{24}NO_3$ $[M+H]^+$: 302.1756. Found 302.1749.

4.2.5.9. 1-Nonyl-4-quinolone-3-carboxylic acid (**3i**)

White solid (86% yield) recrystallized from *n*-Hex/AcOEt: mp 103–104 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.97 (s, 1 H), 8.77 (s, 1 H), 8.58 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.86 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1 H), 7.63 (d, *J* = 8.9 Hz, 1 H), 7.59 (t, *J* = 7.2 Hz, 1 H), 4.32 (t, *J* = 7.6 Hz, 2 H), 1.94 (quin, *J* = 7.5 Hz, 2 H), 1.46–1.23 (m, 12 H), 0.88 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.4, 139.3, 134.0, 127.5, 126.7, 126.2, 116.4, 108.7, 54.9, 31.7, 29.3, 29.1, 29.1, 29.1, 26.6, 22.6, 14.1; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_3$ $[M+H]^+$: 316.1913. Found 316.1912.

4.2.5.10. 1-Decyl-4-quinolone-3-carboxylic acid (**3j**)

White solid (82% yield) recrystallized from *n*-Hex/AcOEt: mp 106–107 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (br s, 1 H), 8.77 (s, 1 H), 8.57 (dd, *J* = 8.2, 1.5 Hz, 1 H), 7.86 (ddd, *J* = 8.7, 7.1, 1.7 Hz, 1 H), 7.63 (d, *J* = 9.3 Hz, 1 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 4.32 (t, *J* = 7.6 Hz, 2 H), 1.94 (quin, *J* = 7.5 Hz, 2 H), 1.46–1.22 (m, 14 H), 0.88 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.4, 139.3, 134.0, 127.5, 126.7, 126.2, 116.4, 108.7, 54.9, 31.8, 29.4, 29.3, 29.2, 29.1, 29.1, 26.6, 22.6, 14.1; HRMS (ESI, m/z) Calcd. for $C_{20}H_{28}NO_3$ $[M+H]^+$: 330.2069. Found 330.2060.

4.2.5.11. 1-(Cyclopropylmethyl)-4-quinolone-3-carboxylic acid (**3k**)

White solid (97% yield) recrystallized from *n*-Hex/AcOEt: mp 207–209 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.00 (br s, 1 H), 8.86 (s, 1 H), 8.58 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.86 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1 H), 7.74 (d, *J* = 8.6 Hz, 1 H), 7.61 (ddd, *J* = 8.0, 7.2, 0.8 Hz, 1 H), 4.19 (d, *J* = 7.1 Hz, 2 H), 1.46–1.38 (m, 1 H), 0.81 (m, 2 H), 0.52 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.2, 147.8, 139.7, 134.0, 127.4, 126.5, 126.2, 116.4, 108.8, 58.8, 10.1, 4.78; HRMS (ESI, m/z) Calcd. for $C_{14}H_{14}NO_3$ $[M+H]^+$: 244.0974. Found 244.0967.

4.2.5.12. 1-(Cyclohexylmethyl)-4-quinolone-3-carboxylic acid (**3l**)

White solid (77% yield) recrystallized from *n*-Hex/AcOEt: mp 220–221 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.98 (br s, 1 H), 8.69 (s, 1 H), 8.58 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.85 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1 H), 7.62–7.59 (m, 2 H), 4.13 (d, *J* =7.4 Hz, 2 H), 1.99–1.90 (m, 1 H), 1.80–1.60 (m, 5 H), 1.25–1.06 (m, 5 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 167.1, 148.8, 139.6, 133.9, 127.4, 126.6, 126.2, 116.6, 108.3, 60.9, 37.0, 30.6, 25.9, 25.4; HRMS (ESI, m/z) Calcd. for $C_{17}H_{20}NO_3$ $[M+H]^+$: 286.1443. Found 286.1437.

4.2.5.13. 1-Benzyl-4-quinolone-3-carboxylic acid (**3m**)

Pale pink solid (50% yield) recrystallized from *n*-Hex/AcOEt: mp 235–236 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.91 (s, 1 H), 8.92 (s, 1 H), 8.58 (dd, J = 8.1, 1.5 Hz, 1 H), 7.72 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1 H), 7.55 (ddd, *J* = 8.3, 7.4, 0.7 Hz, 1 H), 7.53 (d, *J* = 8.7 Hz, 1 H), 7.39–7.35 (m, 3 H), 7.18 (d, *J* = 6.5, 2 H), 5.54 (s, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.7, 167.0, 149.2, 139.7, 134.1, 133.3, 129.6, 129.0, 127.3, 126.7, 126.3, 126.1, 117.3, 109.0, 58.2; HRMS (ESI, m/z) Calcd. for $C_{17}H_{14}NO_3$ $[M+H]^+$: 280.0974. Found 280.0970.

4.2.5.14. 6-Methyl-1-propyl-4-quinolone-3-carboxylic acid (**4c**)

White solid (74% yield) recrystallized from *n*-Hex/AcOEt: mp 231–234 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.11 (br s, 1 H) 8.73 (s, 1 H), 8.36 (d, *J* = 0.8 Hz, 1 H), 7.67 (dd, *J* = 8.8, 2.1 Hz, 1 H),

7.53 (d, *J* = 8.7 Hz, 1 H), 4.29 (t, *J* = 7.4 Hz, 2 H), 2.55 (s, 3 H), 1.99 (sext, *J* = 7.4 Hz, 2 H), 1.05 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 167.3, 147.9, 137.4, 136.7, 135.4, 126.8, 126.5, 116.3, 108.4, 56.3, 22.4, 21.0, 11.0; HRMS (ESI, m/z) Calcd. for $C_{14}H_{16}NO_3$ $[M+H]^+$: 246.1130. Found 246.1127.

4.2.5.15. 6-Methyl-1-octyl-4-quinolone-3-carboxylic acid (**4h**)

Colorless block (quant.) recrystallized from *n*-Hex/AcOEt: mp 115–116 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.11 (br s, 1 H), 8.72 (s, 1 H), 8.35 (d, *J* = 0.9 Hz, 1 H), 7.67 (dd, *J* = 8.8, 2.1 Hz, 1 H), 7.52 (d, *J* = 8.8 Hz, 1 H), 4.30 (t, *J* = 7.5 Hz, 2 H), 2.55 (s, 3 H), 1.92 (quin, *J* = 7.5 Hz, 2 H), 1.44–1.21 (m, 10 H), 0.88 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ178.3, 167.3, 147.8, 137.4, 136.6, 135.4, 126.7, 126.5, 116.3, 108.4, 54.9, 31.6, 29.1, 29.0, 29.0, 26.6, 22.5, 21.0, 14.0; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_3$ $[M+H]^+$: 316.1913. Found 316.1906.

4.2.5.16. 7-Methyl-1-propyl-4-quinolone-3-carboxylic acid (**5c**)

White solid (60% yield) recrystallized from *n*-Hex/AcOEt: mp 228–230 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.09 (br s, 1 H), 8.72 (s, 1 H), 8.45 (d, *J* = 8.3 Hz, 1 H), 7.42 (d, *J* = 8.2 Hz, 1 H), 7.37 (br s, 1 H), 4.27 (t, *J* = 7.4 Hz, 2 H), 2.61 (s, 3 H), 1.99 (sext, *J* = 7.4 Hz, 2 H), 1.06 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 167.3, 148.3, 145.4, 139.5, 127.9, 127.2, 124.5, 116.0, 108.4, 56.2, 22.5, 22.3, 11.0; HRMS (ESI, m/z) Calcd. for $C_{14}H_{16}NO_3$ $[M+H]^+$: 246.1130. Found 246.1122.

4.2.5.17. 7-Methyl-1-octyl-4-quinolone-3-carboxylic acid (**5h**)

White solid (70% yield) recrystallized from *n*-Hex/AcOEt: mp 150–152 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.10 (s, 1 H), 8.71 (s, 1 H), 8.44 (d, *J* = 8.3 Hz, 1 H), 7.41 (br d, *J* = 8.2 Hz, 1 H), 7.37 (br s, 1 H), 4.29 (t, *J* = 7.5 Hz, 2 H), 2.61 (s, 3 H), 1.93 (quin, *J* = 7.5 Hz, 2 H), 1.45–1.28 (m, 10 H), 0.89 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 167.3, 148.2, 145.4, 139.5, 127.9, 127.2, 124.5, 116.0, 108.5, 54.8, 31.6, 29.0, 26.6, 22.6, 22.5, 22.5, 14.0; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_3$ $[M+H]^+$: 316.1913. Found 316.1905.

4.2.5.18. 6-Methoxy-1-propyl-4-quinolone-3-carboxylic acid (**6c**)

White solid (60% yield) recrystallized from *n*-Hex/AcOEt: mp 234–236 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.13 (br s, 1 H), 8.70 (s, 1 H), 7.90 (d, *J* = 3.0 Hz, 1 H), 7.57 (d, *J* = 9.4 Hz, 1 H), 7.45 (dd, *J* = 9.3, 3.0 Hz, 1 H), 4.29 (t, *J* = 7.4 Hz, 2 H), 3.97 (s, 3 H), 1.98 (sext, *J* = 7.4 Hz, 2 H), 1.04 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.7, 167.4, 157.9, 146.8, 133.8, 128.1, 124.7, 118.1, 107.9, 106.2, 56.5, 56.0, 22.5, 11.0; HRMS (ESI, m/z) Calcd. for $C_{14}H_{16}NO_4$ $[M+H]^+$: 262.1079. Found 262.1074.

4.2.5.19. 6-Methoxy-1-octyl-4-quinolone-3-carboxylic acid (**6h**)

Colorless needle (82% yield) recrystallized from *n*-Hex/AcOEt: mp 133–134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.13 (s, 1 H), 8.70 (s, 1 H), 7.91 (d, *J* = 2.9 Hz, 1 H), 7.57 (d, *J* = 9.4 Hz, 1 H), 7.45 (dd, *J* = 9.3, 2.7 Hz, 1 H), 4.31 (t, *J* = 7.4 Hz, 2 H), 3.97 (s, 3 H), 1.93 (quin, *J* = 7.3 Hz, 2 H), 1.42–1.27 (m, 10 H), 0.88 (t, *J* = 6.90 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ177.7, 167.4, 157.9, 146.7, 133.8, 128.1, 124.8, 118.1, 108.0, 106.2, 56.0, 55.1, 31.6 29.2, 29.0, 29.0, 26.6, 22.5, 14.0; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1862. Found 332.1855.

4.2.5.20. 7-Methoxy-1-propyl-4-quinolone-3-carboxylic acid (**7c**)

White solid (60% yield) recrystallized from *n*-Hex/AcOEt: mp 221–222 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.15 (br s, 1 H), 8.70 (s, 1 H), 8.50 (d, *J* = 9.0 Hz, 1 H), 7.18 (dd, *J* = 9.0, 1.7 Hz, 1 H), 6.93 (d, *J* = 1.6 Hz, 1 H), 4.23 (t, *J* = 7.1 Hz, 2 H), 3.99 (s, 3 H), 1.99 (sext, *J* = 7.1 Hz, 2 H), 1.06 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ177.9, 167.3, 164.2, 148.6, 141.2, 129.4, 120.5, 114.2, 108.4, 99.9, 56.4, 56.0, 22.1, 11.1; HRMS (ESI, m/z) Calcd. for $C_{14}H_{16}NO_4$ $[M+H]^+$: 262.1079. Found 262.1076.

4.2.5.21. 7-Methoxy-1-octyl-4-quinolone-3-carboxylic acid (**7h**)

Colorless plate (62% yield) recrystallized from *n*-Hex/AcOEt: mp 125–126 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.17 (br s, 1 H), 8.68 (s, 1 H), 8.47 (d, *J* = 9.4 Hz, 1 H), 7.17 (dd, *J* = 9.1, 2.0 Hz, 1 H), 6.93 (d, *J* = 2.1 Hz, 1 H), 4.24 (t, *J* = 7.6 Hz, 2 H), 3.99 (s, 3 H), 1.93 (quin, *J* = 7.4 Hz, 2 H), 1.44–1.27 (m, 10 H), 0.88 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.8, 167.3, 164.2, 148.3, 129.3, 120.4, 114.3, 108.3, 99.7, 55.9, 54.8, 31.6, 29.0, 29.0, 28.7, 26.6, 22.5, 14.0; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1862. Found 332.1861.

4.2.5.22. 8-Methoxy-1-octyl-4-quinolone-3-carboxylic acid (**8h**)

White solid (63% yield) recrystallized from *n*-Hex/AcOEt: mp 139–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.00 (br s, 1 H), 8.62 (s, 1 H), 8.18 (dd, *J* = 8.0, 1.2 Hz, 1 H), 7.50 (t, *J* = 8.0 Hz, 1 H), 7.30 (dd, *J* = 8.8, 1.2 Hz, 1 H), 4.61 (t, *J* = 7.6 Hz, 2 H), 4.02 (s, 3 H), 1.84 (quin, *J* = 7.2 Hz, 2 H), 1.32–1.26 (m, 10 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.09, 167.35, 150.62, 150.48, 130.64, 129.18, 126.85, 119.22, 115.71, 108.39, 60.98, 56.73, 31.87, 31.77, 29.34, 29.28, 26.64, 22.78, 14.24; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1856. Found 332.1849.

4.2.5.23. 7-(Methylthio)-1-octyl-4-quinolone-3-carboxylic acid (**9h**)

White solid (quant.) recrystallized from *n*-Hex/AcOEt: mp 138–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1 H), 8.42 (d, *J* = 8.8 Hz, 1 H), 7.39 (dd, *J* = 8.8, 1.6 Hz, 1 H), 7.28 (d, *J* = 1.2 Hz, 1 H),

4.26 (t, *J* = 7.2 Hz, 2 H), 2.62 (s, 3 H), 1.92 (quin, *J* = 7.6 Hz, 2 H), 1.45–1.22 (m, 10 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.11, 167.26, 148.54, 148.33, 139.92, 127.60, 123.64, 123.58, 111.57, 108.94, 54.96, 31.84, 29.22, 29.20, 29.09, 26.81, 22.75, 15.19, 14.22; HRMS (ESI, m/z) Calcd. for $C_{19}H_{26}NO_3S$ $[M+H]^+$: 348.1628. Found 348.1618.

4.2.5.24. 1-Octyl-7-(trifluoromethyl)-4-quinolone-3-carboxylic acid (**10h**)

White solid (59% yield) recrystallized from *n*-Hex/AcOEt: mp 176–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.5 (s, 1H), 8.82 (s, 1 H), 8.71 (d, *J* = 8.4 Hz, 1 H), 7.85 (s, 1 H), 7.80 (d, *J* = 8.5 Hz, 1 H), 4.35 (t, *J* = 7.5 Hz, 2H), 1.95 (quin, *J* = 7.3 Hz, 2 H), 1.46–1.28 (m, 10 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.0, 162.7, 149.7, 146.2 (q, *J* = 32.0 Hz), 139.3, 131.3 (q, *J* = 272.2 Hz), 129.3, 122.5, 114.2, 113.4, 110.0, 55.3, 31.8, 29.2, 29.2, 29.1, 26.8, 22.8, 14.2; HRMS (ESI, m/z) Calcd. for $C_{19}H_{23}F_3NO_3$ $[M+H]^+$: 370.1630. Found 370.1626.

4.2.5.25. 1-Octyl-7-(trifluoromethyloxy)-4-quinolone-3-carboxylic acid (**11h**)

White solid (91% yield) recrystallized from *n*-Hex/AcOEt: mp 151–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.6 (s, 1H), 8.76 (s, 1 H), 8.62 (d, *J* = 9.0 Hz, 1 H), 7.43 (d, *J* = 9.0 Hz, 1 H), 7.38 (s, 1 H), 4.26 (t, *J* = 7.5 Hz, 2H), 1.92 (quin, *J* = 7.2 Hz, 2 H), 1.44–1.28 (m, 10 H), 0.88 (t, *J* = 7.9 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.9, 166.8, 153.4, 149.6, 140.7, 130.4, 125.1, 120.8 (q, *J* = 260.8 Hz), 118.8, 109.7, 108.2, 55.3, 31.8, 29.2, 29.1, 29.1, 26.8, 22.8, 14.2; HRMS (ESI, m/z) Calcd. for $C_{19}H_{23}F_3NO_4$ $[M+H]^+$: 386.1574. Found 386.1571.

4.2.5.26. 6,7-Dimethoxy-1-octyl-4-quinolone-3-carboxylic acid (**12h**)

White solid (39% yield) recrystallized from *n*-Hex/AcOEt: mp 234–235 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1 H), 8.01 (s, 1 H), 7.52 (s, 1 H), 4.60 (t, *J* = 6.4 Hz, 2 H), 4.10 (s, 3 H), 4.02 (s, 3H), 1.89 (quin, *J* = 6.4 Hz, 2 H), 1.49 (quin, *J* = 6.8 Hz, 2 H), 1.20–1.35 (m, 8 H), 0.84 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.59, 164.22, 155.31, 150.78, 147.63, 118.93, 104.21, 101.19, 57.00, 56.34, 31.94, 30.60, 29.55, 29.45, 26.29, 22.81, 14.26; HRMS (ESI, m/z) Calcd. for $C_{20}H_{28}NO_5$ $[M+H]^+$: 362.1962. Found 362.1951.

4.2.5.27. 7,8-Dimethoxy-1-octyl-4-quinolone-3-carboxylic acid (**13h**)

White solid (80% yield) recrystallized from *n*-Hex/AcOEt: mp 127–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1 H), 8.34 (d, *J* = 8.8 Hz, 1 H), 7.24 (d, *J* = 9.2 Hz, 1 H), 4.49 (t, *J* = 7.6 Hz, 2 H), 4.06 (s, 3 H), 3.93 (s, 3 H), 1.83 (quin, *J* = 7.2 Hz, 2 H), 1.25–1.30 (m, 10 H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.10, 167.39, 157.53, 151.47, 138.49, 134.14, 123.87, 121.97, 111.97, 107.86, 62.08, 59.73, 56.77, 31.88, 31.21, 29.29, 29.29, 26.67, 22.28, 14.24; HRMS (ESI, m/z) Calcd. for $C_{20}H_{28}NO_5$ $[M+H]^+$: 362.1962. Found 362.1957.

4.2.5.28. 6,7-(Methylenedioxy)-1-octyl-4-quinolone-3-carboxylic acid (**14h**)

White solid (quant.) recrystallized from *n*-Hex/AcOEt: mp 154–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.3 (s, 1H), 8.60 (s, 1 H), 7.80 (s, 1 H), 6.96 (s, 1 H), 6.19 (s, 2 H), 4.21 (t, *J* = 7.4 Hz, 2 H), 1.90 (quin, *J* = 7.3 Hz, 2 H), 1.41–1.27 (m, 10 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.1, 167.6, 154.0, 147.5, 146.8, 137.1, 122.9, 108.6, 104.2, 103.2, 95.8, 55.8, 31.9, 29.3, 29.2, 29.2, 26.8, 22.8, 14.3; HRMS (ESI, m/z) Calcd. for $C_{19}H_{24}NO_5$ $[M+H]^+$: 346.1649. Found 346.1648.

4.2.5.29. 1-Decyl-6,7-(methylenedioxy)-4-quinolone-3-carboxylic acid (**14l**)

White solid (quant.) recrystallized from *n*-Hex/AcOEt: mp 77–78 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 9.21 (s, 1 H), 7.60 (s, 1 H), 7.49 (s, 1 H), 6.18 (s, 2 H), 4.42 (t, *J* = 6.5 Hz, 2 H), 1.91 (quin, *J* = 6.5 Hz, 2 H), 1.48 (quin, *J* = 7.5 Hz, 2 H), 1.25–1.37 (m, 12 H), 0.87 (t, *J* = 6.5 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.6, 166.2, 154.6, 150.0, 144.7, 136.0, 126.6, 106.7, 106.1, 103.6, 99.6, 54.7, 32.1, 30.6, 30.5, 29.7, 29.7, 29.5, 26.1, 22.9, 14.3; HRMS (ESI, m/z) Calcd for $C_{21}H_{28}NO_5$ $[M+H]^+$: 374.1962. Found 374.1952.

4.2.5.30. 1-Octyl-6,7-(trimethylene)-4-quinolone-3-carboxylic acid (**15h**)

White solid (36% yield) recrystallized from *n*-Hex/AcOEt: mp 167–169 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.3 (s, 1H), 8.68 (s, 1 H), 8.37 (s, 1 H), 7.42 (s, 1 H), 4.28 (t, *J* = 7.5 Hz, 2 H), 3.12 (t, *J* = 7.4 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 2.21 (quin, *J* = 7.5 Hz, 2 H), 1.92 (quin, *J* = 7.4 Hz, 2 H), 1.41–1.25 (m, 10 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.5, 178.5, 167.7, 152.7, 147.6, 143.9, 138.7, 122.1, 111.7, 108.3, 55.2, 53.6, 33.8, 32.3, 31.8, 29.2, 29.2, 26.8, 25.9, 22.7, 14.2; HRMS (ESI, m/z) Calcd. for $C_{21}H_{28}NO_3$ $[M+H]^+$: 342.2064. Found 342.2062.

4.3. Time-lapse $[Ca^{2+}]_{ER}$ measurements.

Time-lapse $[Ca^{2+}]_{ER}$ measurements were performed as described previously[16]. HEK293 cells stably and inducibly expressing RyRs (RyR1 carrying R2163C mutation, wild-type RyR2, or wild-type RyR3) and R-CEPIA1er were seeded on 96-well, flat, clear-bottom black microplates (#3603; Corning, New York, NY) at a density of 3×10^4 cells/well in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 15 μg/ml blasticidin, 100 μg/ml hygromycin, and 400 μg/ml G418. Expression of RyR1 was induced by addition of doxycycline (2 μg/ml) to the culture medium the next day. After 24-28 hours of induction, the culture medium was replaced with 90 μl of HEPES-buffered Krebs solution (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM glucose, and 5 mM HEPES, pH 7.4), and the microplate was placed in the FlexStation3 fluorometer (Molecular Devices, San Jose, CA) and incubated at 37°C. For RyR3 cells, 1 mM caffeine was added to the solution to appropriately reduce $[Ca^{2+}]_{ER}$ by activating the RyR3

channel. R-CEPIA1er signals (excitation at 560 nm and emission at 610 nm) were captured every 10 seconds for 300 seconds. Sixty microliters of the compounds dissolved in HEPES-Krebs solution was applied to the cells 100 seconds after starting. The fluorescence change induced by the compounds was expressed as F/F_0, in which averaged fluorescence intensity of the last 100 seconds (F) was normalized to that of the initial 100 seconds (F_0).

4.4. [H^3]Ryanodine binding assay.

Microsomes isolated from HEK293 cells stably expressing RyRs (RyR1 carrying R2163C mutation, wild-type RyR2 or wild-type RyR3) were incubated with 5 nM [^{3}H]ryanodine for 2 hours at 37°C (for R2163C RyR1) or 25°C (for RyR2 and RyR3) in a medium containing 0.17 M NaCl, 20 mM 3-morpholino-2-hydroxypropanesulfonic acid (MOPSO) (pH 7.0), 2 mM dithiothreitol, 1 mM β,γ-methyleneadenosine 5'-triphosphate, 1 μM calmodulin, and various concentrations of free Ca^{2+} buffered with 10 mM EGTA. Free Ca^{2+} concentrations were calculated using WEBMAXC STANDARD. Protein-bound [^{3}H]ryanodine was separated by filtration through polyethyleneimine-treated glass filters (Filtermat B, PerkinElmer, Waltham, MA) using a Micro 96 Cell Harvester (Skatron Instruments, Lier, Norway). Nonspecific binding was determined in the presence of 20 μM unlabeled ryanodine.

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Highlights

· Structural development of type-1 ryanodine receptor (RyR1) inhibitors was carried out utilizing the novel assay method based on the measurement of Ca^{2+} in endoplasmic reticulum.

· Among the synthesized quinolone derivatives, compound **14h** exhibited more potent RyR1 channel inhibitory activity than dantrolene, a known RyR1 inhibitor, and exhibited high RyR1 selectivity over RyR2 and RyR3.

· [^{3}H]Ryanodine-binding assay suggested that the synthesized compounds interacted with RyR1 directly to inhibit its channel activity.