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Introduction of amino moiety enhances the inhibitory potency of 1-tetralone chalcone derivatives against LPS-stimulated reactive oxygen species production in RAW 264.7 macrophages

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ABSTRACT

The design and synthesis of a series of thirty-two halogenated 1-tetralone or 6-amino-1-tetralone chalcone derivatives was achieved by the Claisen-Schmidt condensation reaction and were evaluated for their inhibitory effects against ROS production in LPS-stimulated RAW 264.7 macrophages. It was observed that the introduction of amino moiety into 1-tetralone skeleton greatly increased the inhibitory potency compared to corresponding 1-tetralone chalcones. Among the synthesized compounds, compound **18** which consists of 6-amino-1-tetralone skeleton together with *o*-fluorobenzylidene showed the most potent ROS inhibitory effect with IC_{50} value of 0.25 ± 0.13 μM. SAR analysis revealed that amino moiety at the 6th position of 1-tetralone chalcones have an important role for exerting the greater ROS inhibitory potency in LPS-stimulated RAW 264.7 macrophages than those exhibited by 1-tetralone chalcones alone.

1. Introduction

Inflammation is an important part of the body's immune response to any kind of infections, injury, pain, toxin, or stimuli. During the processes of wound healing, these responses are of vital importance for removing infections. However, if they persist longer than necessary, can cause harmful effects, resulting in chronic inflammation. These continuous chronic inflammatory responses that cause detrimental effects to one's body and tissues are thought to be mediated through reactive oxygen species (ROS) [1,2], and has been associated with many diseases such as rheumatoid arthritis [3], atherosclerosis [4], Alzheimer's disease [5], inflammatory bowel disease [6], and cancer [7]. ROS are the products of molecular oxygen, and even while respiring we tend to break down oxygen and generate ROS. They are highly reactive in nature and can oxidize biologically important molecules such as proteins, lipids, including DNA as well. ROS has dual role acting both as a signaling molecule under physiological conditions and mediator of inflammatory processes at high concentrations [8]. Our body is at constant attack from ROS, and in such instances there is a defense mechanism system of antioxidant in our body that keeps it under control. Whenever this balance between the ROS and defense mechanism is disrupted, oxidative stress arises which plays an eminent role in the progression of many pathological disorders [9]. Therefore, controlling the overproduction of ROS and balancing the oxidative stress can be considered as one of the vital strategies in the treatment of inflammation.

Macrophages, as a part of the immune system, play an important role in defending our body against the pathogenic stimuli via engulfing process called phagocytosis. During phagocytosis, many inflammatory mediators like cytokines, chemokines, and nitric oxide (NO) work together with inflammatory response assisting and ensuring a quick resolution and restoration of the normal tissue architecture [10]. Lipopolysaccharide (LPS), acting as a potent toxin, activates macrophages even at very low concentration [11]. Stimulation of the macrophages with LPS induces rapid generation of ROS, which leads to the production of various inflammatory mediators, including tumor necrosis factor-α, interferons and interleukins [12]. Furthermore, the ROS may interact with NO, resulting in the formation of reactive nitrogen species (RNS) and ultimately increasing the oxidative and nitrosative stress responses [13]. Therefore, pharmacological intervention involving the control and balance of ROS production in macrophages would be a promising strategy in the treatment of inflammatory diseases.

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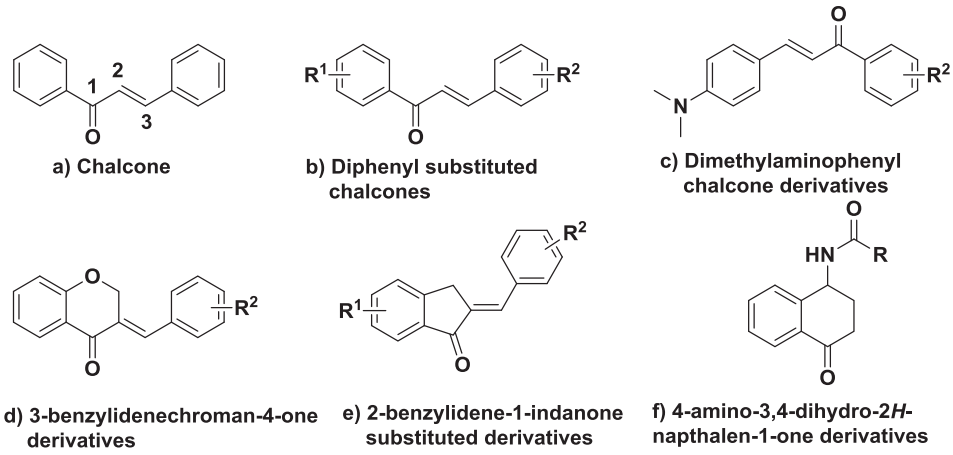


Fig. 1. Structures of (a) chalcone, (b) diphenyl substituted chalcones, (c) dimethylaminophenyl chalcone derivatives (d) 3-benzylidenechroman-4-one derivatives (e) 2-benzylidene-1-indanone substituted derivatives, and (f) 4-amino-3,4-dihydro-2*H*-napthalen-1-one derivatives.

Chalcone and its various derivatives (Fig. 1a and Fig. 1b) are naturally occurring compounds widely found in many plants, fruits, spices, vegetables, etc. possessing valuable properties as anti-inflammatory [14], anti-cancer [15], anti-fungal [16], and anti-oxidant [17]. The use of chalcones as medicines dates back from very ancient times to the present day. These days interest in the synthesis of new chalcone analogues have evolved tremendously owing to their simple structure along with numerous pharmacological activities. The two aryl rings present in the chalcone structure aids flexibility in the structural modifications for designing and in the innovation of various novel pharmaceuticals. Recently, many chalcone-based compounds are available in the market as metochalcone (choleretic drug) and sofalcone (antiulcer and mucoprotective drug) [18]. Many studies illustrating the role of chalcones in inflammation have been reported lately. Compounds with polymethoxychalcones showed potential properties as anti-inflammatory agents, inflammatory cytokine expression inhibitors, and prostaglandin production inhibitors [19]. Dimethylaminophenyl chalcone derivatives (Fig. 1c) have been reported to be potential NO and prostaglandin E_2 generation inhibitors in RAW 264.7 macrophage cell line [20]. Additionally, our research group has continuously explored several modifications in chalcone skeleton and synthesized 3-benzylidenechroman-4-ones (Fig. 1d) and 2-benzylidene-1-indanones (Fig. 1e) as rigid chalcone derivatives with moderate to strong inhibitory activity against ROS production in RAW 264.7 macrophages [21,22].

Halogenation has evolved as a prominent technique in the discovery of new drugs, and about 40% of drugs used in clinical trials are incorporated by addition of halogen functionalities. Similarly, many interesting features of halogen atoms like high electronegativity, its ability to enhance CNS penetration, improvement in metabolic and chemical stability are of great importance in the design and optimization of halogenated drugs [23]. Recent findings reported that insertion of chlorine or nitro groups in the phenyl ring of chalcone resulted in better anti-inflammation properties as well as were selective in the inhibition of NO generation [24]. Studies carried out on chlorinated chalcones suggested that their potent anti-inflammatory and anti-oxidant activity was possible due to the presence of electron withdrawing groups like chlorine [25]. Similarly, the chemical substitution of bromine in methyl hydroquinone revealed the importance of bromination in anti-inflammation [26]. Rojas et al. evaluated a series of fluorinated trimethoxychalcone derivatives, and found that the compounds inhibited the NO production and PGE_2 overproduction in LPS-stimulated RAW 264.7 macrophages [27]. 1-Tetralones, also known as 3,4-dihydro-2*H*-napthalen-1-one, are important class of compounds, and several derivatives of them have shown important biological activities such as anti-tumor [28], neurological disorders [29,30], and antimicrobial activity [31]. Similarly, the novel amine derivatives of 4-amino-3,4-dihydro-2*H*-napthalen-1-one (Fig. 1f) studied by Barlow et al. displayed significant mast cell stabilizing property, and it was also interesting to know that the 1-tetralone nucleus along with aminocyclopentyl group was necessary for exerting the anti-inflammatory effects [32]. Considering these valuable biological properties of 1-tetralone along with the important significance of amino and halogenated functionalities has led us to incorporate these functional groups in this study as shown in Fig. 2.

In this present study, we report the design and synthesis of a series of thirty-two 1-tetralone or 6-amino-1-tetralone along with halogenated functionalities on 2-benzylidene moiety at *ortho, meta*, or *para*-position which were evaluated for their inhibitory effects against the ROS-production in LPS-stimulated 264.7 macrophages. A structure-activity relationship (SAR) study was performed with respect to *ortho, meta*, or *para*- substitution along with different halogenated functionality on the phenyl ring, and with presence or absence of amino group in the 1-tetralone skeleton as well as with the previously synthesized other rigid skeleton of chalcones. Malvidin is a flavonoid present abundantly in blueberries, strawberries, and cranberries which renders them the characteristics color pigments. One of the studies involving malvidin, their mixtures of malvidin-3-glucoside and malvidin-3-galactoside considerably decreased the levels of ROS and oxidative stress in endothelial cells possessing a potent antioxidant activity [33]. Another study on malvidin reported that it inhibits 50% of LPS-induced ROS production in RAW 264.7 macrophages at the concentration of 9.0 ± 0.8 μM and used as the positive control in this study [34].

2. Results and discussion

2.1. Chemistry

At first, halogenated 1-tetralone or 6-amino-1-tetralone derivatives (**1–32**) were synthesized by previously reported [35,36] condensation reaction with 1-tetralone/6-amino-1-tetralone and halogenated aryl aldehyde (R^1 = **a-p**) as summarized in Scheme 1 and Scheme 2. Base-catalyzed *Claisen-Schmidt* condensation reaction was used in which 50% aqueous NaOH or KOH (0.5 mL) was added to a solution of 1-tetralone/6-amino-1-tetralone (1.0 mmol) and halogenated aryl aldehyde (R^1 = **a-p**) (1.2 mmol) in ethanol (4 mL), which resulted in the pure desired chalcone derivatives in 20.0–96.3% yield.

The preparation of compounds **1–13**, **15**, and **17** have been reported in previous studies [37–43] whereas characterization of compounds **8**, **11**, **12**, and **15** were not found so we have included in this study. It was interesting to know that among non-aminolated compounds **1–16**, compounds **14** and **16** were novel and not found to be reported. On the

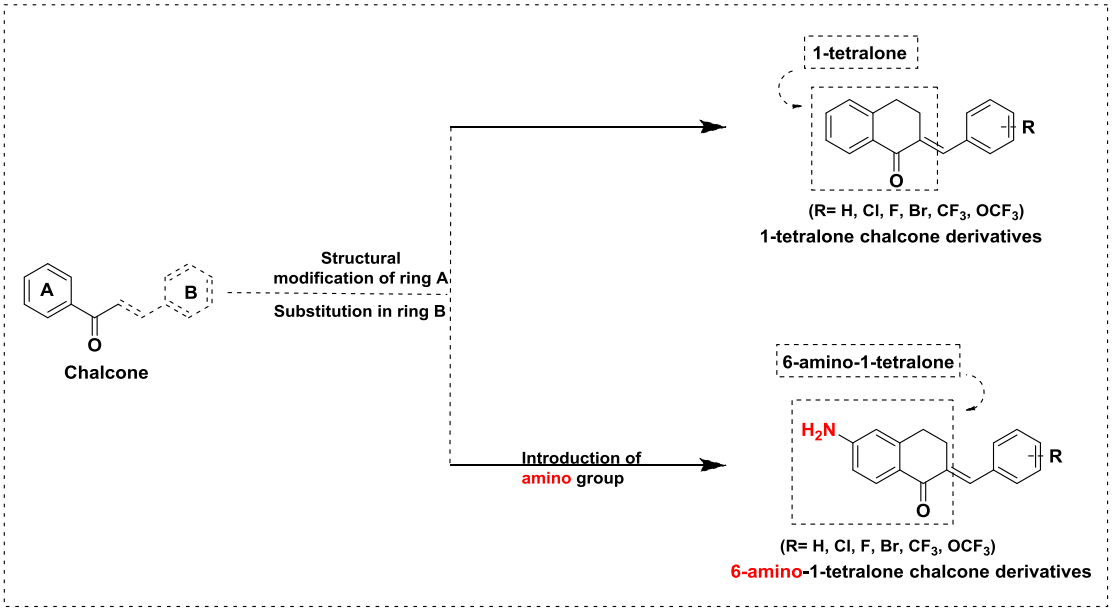
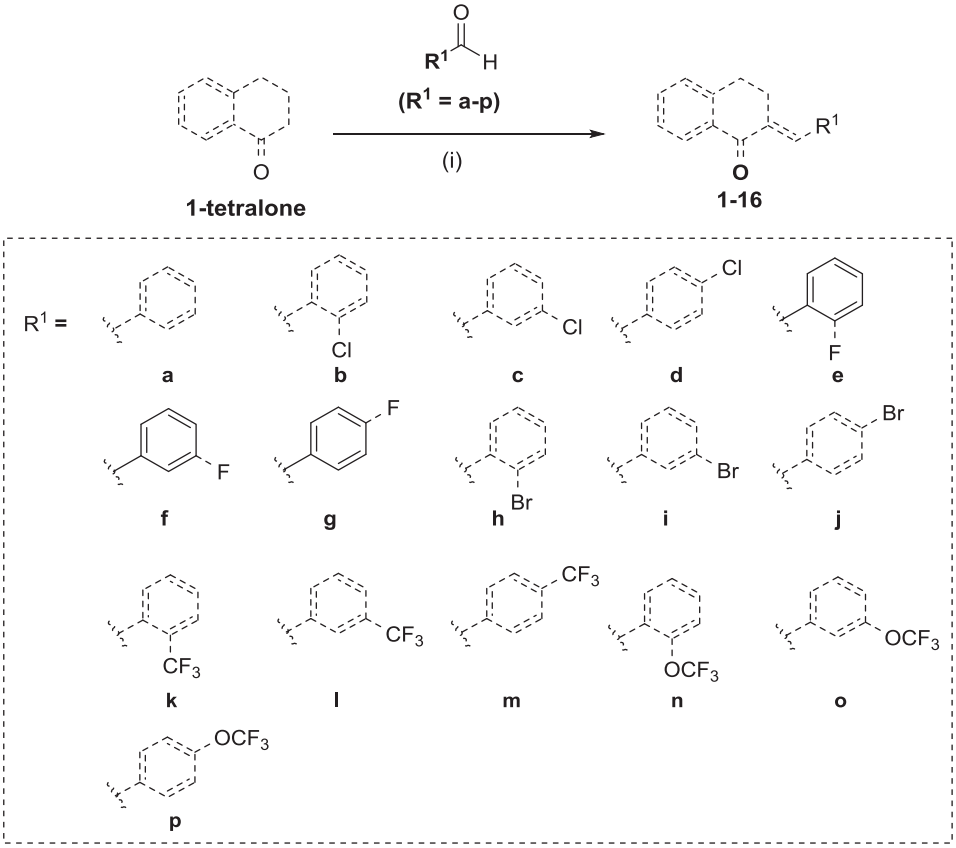


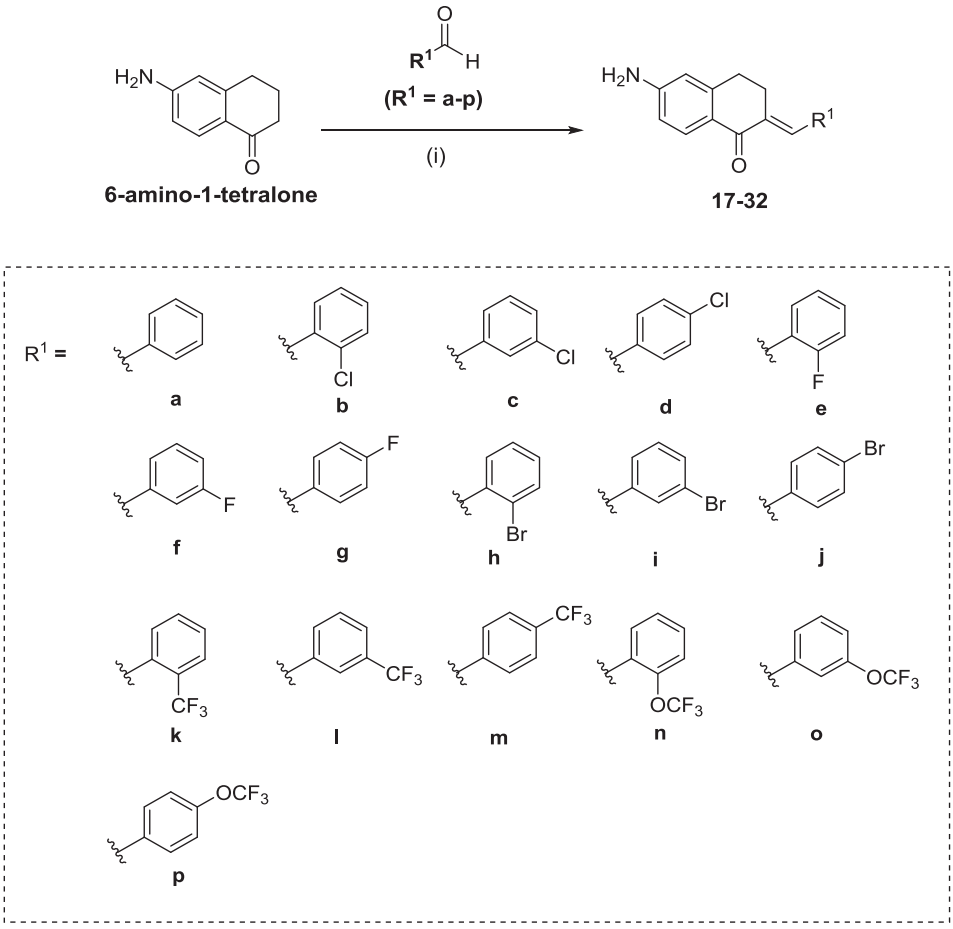
Fig. 2. Strategy design of halogenated 2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one and 6-amino-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one derivatives.

other hand, among aminolated compounds **17–32**, except compound **17**, all of the compounds **18–32** are also novel and are reported for the first time in this study.

The structures of the synthesized target compounds (**1–32**) are illustrated as shown in Fig. 3 and Fig. 4. Amongst, the first sixteen synthesized 1-tetralone chalcones, compound **1** contained 1-tetralone moiety along with phenyl moiety whereas compounds **2–4**, **5–7**, **8–10**, **11–13**, and **14–16** contained 1-tetralone moiety along with *ortho*-, *meta*-, or *para*-, fluorophenyl, chlorophenyl, bromophenyl, trifluoromethylphenyl, and trifluoromethoxyphenyl moiety, respectively (Fig. 3). Similarly, amongst, the next sixteen synthesized 6-amino-1-tetralone chalcones, compound **17** contained 6-amino-1-tetralone moiety along with phenyl moiety whereas compounds **18–20**, **21–23**, **24–26**, **27–29**, and **30–32** contained 6-amino-1-tetralone moiety along



Scheme 1. Schematic representation for the synthesis of halogenated 1-tetralone substituted chalcone derivatives **1–16.** Reagents and conditions: (i) 50% aq. NaOH (4.5 equiv., 0.5 mL), EtOH (4 mL), 3–36 h, 25 °C, 21.5–96.3% yield.



Scheme 2. Schematic representation for the synthesis of halogenated 6-amino-1-tetralone substituted chalcone derivatives **17–32.** Reagents and conditions: (i) 50% aq. KOH (4.5 equiv., 0.5 mL), EtOH (4 mL), 3–24 h, 25 °C, 20.0–52.1% yield.

with *ortho*-, *meta*-, or *para*-, fluorophenyl, chlorophenyl, bromophenyl, trifluoromethylphenyl, and trifluoromethoxyphenyl moiety, respectively (Fig. 4). The SAR was determined according to the different position of fluoro, chloro, bromo, trifluoromethyl, or trifluoromethoxy group, as well as the presence or absence of the amino group of the 1-tetralone or 6-amino-1-tetralone chalcone derivatives.

2.2. Inhibitory activities against the ROS production stimulated by LPS in RAW 264.7 macrophages

The compounds **1–32** have been evaluated for their inhibitory activities against the ROS production stimulated by LPS in RAW 264.7 macrophages, and are illustrated in Table 1. Most of the prepared compounds exhibited potent ROS inhibitory activities, especially 6-amino-1-tetralone substituted chalcone derivatives. Amongst the first sixteen series of synthesized compounds **1–16**, that had the basic skeleton of 1-tetralone together with fluorine, chlorine, bromine, trifluoromethyl, or trifluoromethoxy substituent at the *ortho*-, *meta*-, or *para*position on the 2-benzylidene ring of chalcone, compounds **1**, **5–10**, and **12–16** significantly inhibited LPS-stimulated ROS production in RAW 264.7 macrophages (1.27 to 6.24 μM of IC_{50}). Compared to 1-tetralone with unsubstituted 2-benzylidene moiety of chalcone (compound **1**), compounds **5**, **7**, **10**, **12**, and **16** which employed *o*-chloro, *p*-chloro, *p*-bromo, *m*-trifluoromethyl, and *p*-trifluoromethoxy benzylidene moiety, respectively, greatly increased inhibitory potency, which indicated *o*-chloro, *p*-chloro, *p*-bromo, *m*-trifluoromethyl, and *p*-trifluoromethoxy substitution on 2-benzylidene moiety is important for strong inhibition against LPS-stimulated ROS production in RAW 264.7 macrophages. Likewise, among the next sixteen series of the synthesized compounds **17–32**, that had the basic skeleton of 6-amino-1-tetralone together with fluorine, chlorine, bromine, trifluoromethyl, or trifluoromethoxy substituent at the *ortho*, *meta*, or *para*-position on the 2-benzylidene ring of chalcone, all of the compounds **17–32** showed strong inhibitory activity, and most of the compounds greatly increased inhibitory activity against LPS-stimulated ROS production in RAW 264.7 macrophages (0.25 to 8.77 μM of IC_{50}). Compared to 6-amino-1-tetralone with unsubstituted 2-benzylidene moiety of chalcone (compound **17**), compounds **18**, **20**, and **28** which employed *o*-fluoro, *p*-fluoro, and *m*-trifluoromethyl benzylidene moiety, respectively, greatly increased inhibitory potency, which indicated *o*-fluoro, *p*-fluoro, and *m*-trifluoromethyl substitution on 2-benzylidene moiety, is essential for strong inhibition against LPS-stimulated ROS production in RAW 264.7 macrophages. These findings are quite unexpected results since inhibitory effects depending on substitution of functional groups on 2-benzylidene moiety for 1-tetralone and 6-amino-1-tetralone are not concordance. Among the thirty-two synthesized 1-tetralone/6-amino-1-tetralone substituted chalcone compounds (**1–32**), none of the compounds showed toxicity in RAW 264.7 macrophages from three different experiments performed in triplicate.

Structure-activity relationship study (SAR) of the first sixteen compounds having 1-tetralone as a basic skeleton revealed that the compound possessing *p*-bromophenyl moiety showed the most significant inhibition (1.27 ± 0.29 μM of IC_{50}) whereas compound possessing fluoro and *o*-trifluoromethylphenyl moiety showed the least significant inhibition (> 10 μM of IC_{50}) of LPS-stimulated ROS production in RAW 264.7 macrophages. Comparing inhibitory activities between different halogenated functionalities, it was observed that *p*-bromophenyl (1.27 ± 0.29 μM of IC_{50}) substituent possessed the most

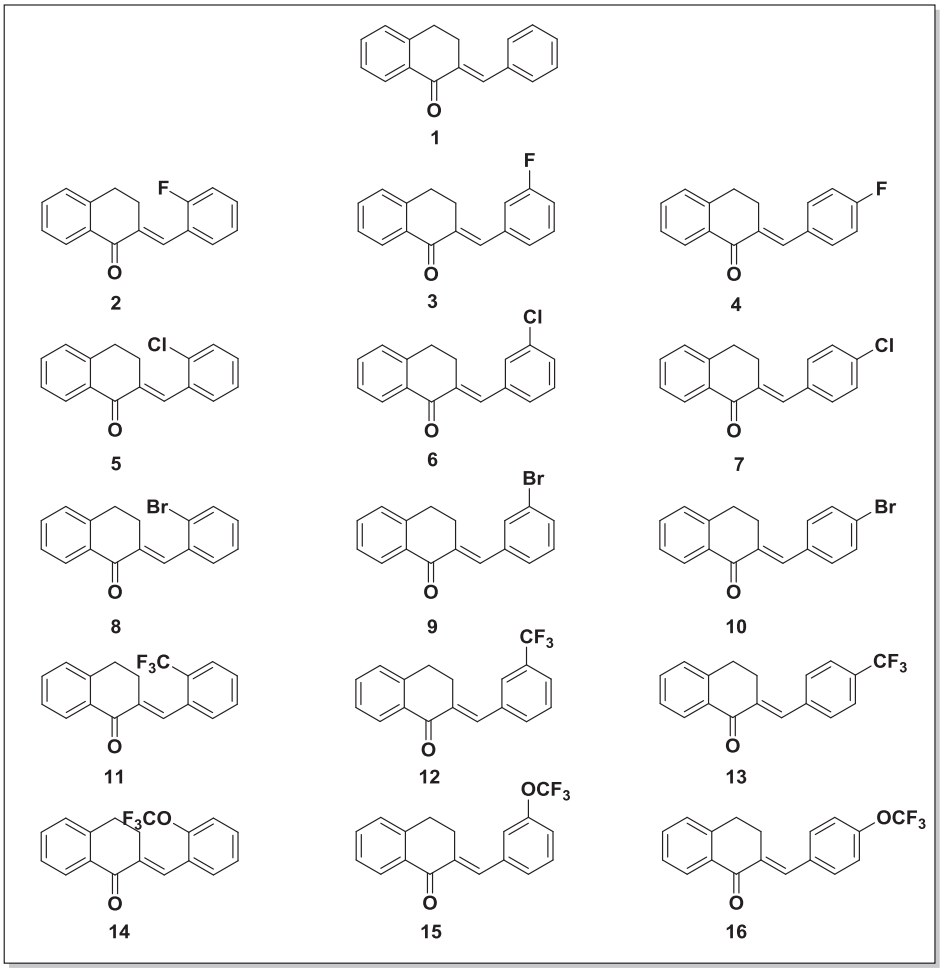


Fig. 3. Synthesized halogenated 1-tetralone substituted chalcone derivatives **1–16**.

significant ROS inhibition followed by *p*-chlorophenyl (1.55 ± 0.64 μM of IC_{50}), *p*-trifluoromethoxyphenyl (1.90 ± 0.23 μM of IC_{50}), *m*-trifluoromethylphenyl (3.02 ± 0.72 μM of IC_{50}), and phenyl (5.43 ± 1.80 μM of IC_{50}) substituent, respectively. SAR between *ortho, meta,* and *para* substituents of halogen functionalities generally indicated that *para* substituent displayed the most significant ROS inhibition followed by *meta*, and then *ortho* substituents.

Inhibitory effects possessing 6-amino-1-tetralone (compounds **17–32**) as a basic skeleton indicated that the compound with *o*-fluorophenyl moiety showed the most significant inhibition (0.25 ± 0.13 μM of IC_{50}) whereas compound possessing *o*-trifluoromethyl moiety showed the least significant inhibition (8.77 ± 1.31 μM of IC_{50}) of LPS-stimulated ROS production in RAW 264.7 macrophages. SAR of compounds **17–32** possessing 6-amino-1-tetralone moiety according to *ortho*-, *meta*-, and *para*substituents of halogen functionalities generally indicated that *meta* substituents showed better inhibitory activities with the exception of fluoro functionalities. Since there is no solid relationship between different functionalities of compounds, it is difficult to determine strict SAR.

However, SAR study revealed that the substitution of amino group at the 6th position of 1-tetralone ring significantly enhanced the ROS inhibition indicating that the 6-amino-1-tetralone moiety has a greater contribution to ROS inhibitory activity than the 1-tetralone moiety as shown in Table 1. Comparing inhibitory activities between 1-tetralone and 6-amino-1-tetralone substituted chalcone derivatives, there was significant increase in the level of ROS inhibition by all the 6-amino-1-tetralone chalcone derivatives **17–32**, except compounds **23** and **29**, than their corresponding 1-tetralone chalcone derivatives **1–16**. Furthermore, among all the halogenated functionalities, fluorine-containing 1- tetralone chalcone derivatives **2–4** did not possess ROS inhibitory activities at all, whereas 6-amino-1-tetralone chalcone derivatives **18–20**, respectively, significantly enhanced the ROS inhibition potency even in sub-micromolar range (0.25–0.76 μM of IC_{50}) as shown in Table 1. Compounds **18** and **20** also exhibited the most potent ROS inhibition compared to all the synthesized 1-tetralone and 6-amino-1-tetralone chalcone derivatives. Further, relative ROS inhibitory potencies of 6-amino-1-tetralone chalcones (**17–32**) and 1-tetralone chalcones (**1–16**) with respect to malvidin showed that 6-amino substituted compounds **17**, **18**, **19**, **20**, **25**, **28**, and **31** were 11.8, 36.0, 11.8, 22.5, 11.3, 22.0, and 10.7 times more potent, respectively, compared to positive control, malvidin, whereas their non-amino substituted compounds **1**, **2**, **3**, **4**, **9**, **12**, and **15** were only 1.7, < 0.9, < 0.9, < 0.9, 2.1, 3.0, and 2.3 times potent, respectively, as shown in Table 1. This relative potency revealed that introduction of amino group at the 6th position of 1-tetraone chalcones significantly enhanced the ROS inhibition in RAW 264.7 macrophages.

Finally, SAR was also analyzed by comparing the various rigid chalcone derivatives reported previously by our research group [21,22] with 1-tetralone and 6-amino-1-tetralone substituted chalcone derivatives. Among various rigid structures, 6-amino-1-tetralone derivatives showed the most potent ROS inhibition whereas 4-chromanone and 1-indanone showed moderate and weak ROS inhibition, respectively, as depicted in Fig. 5.

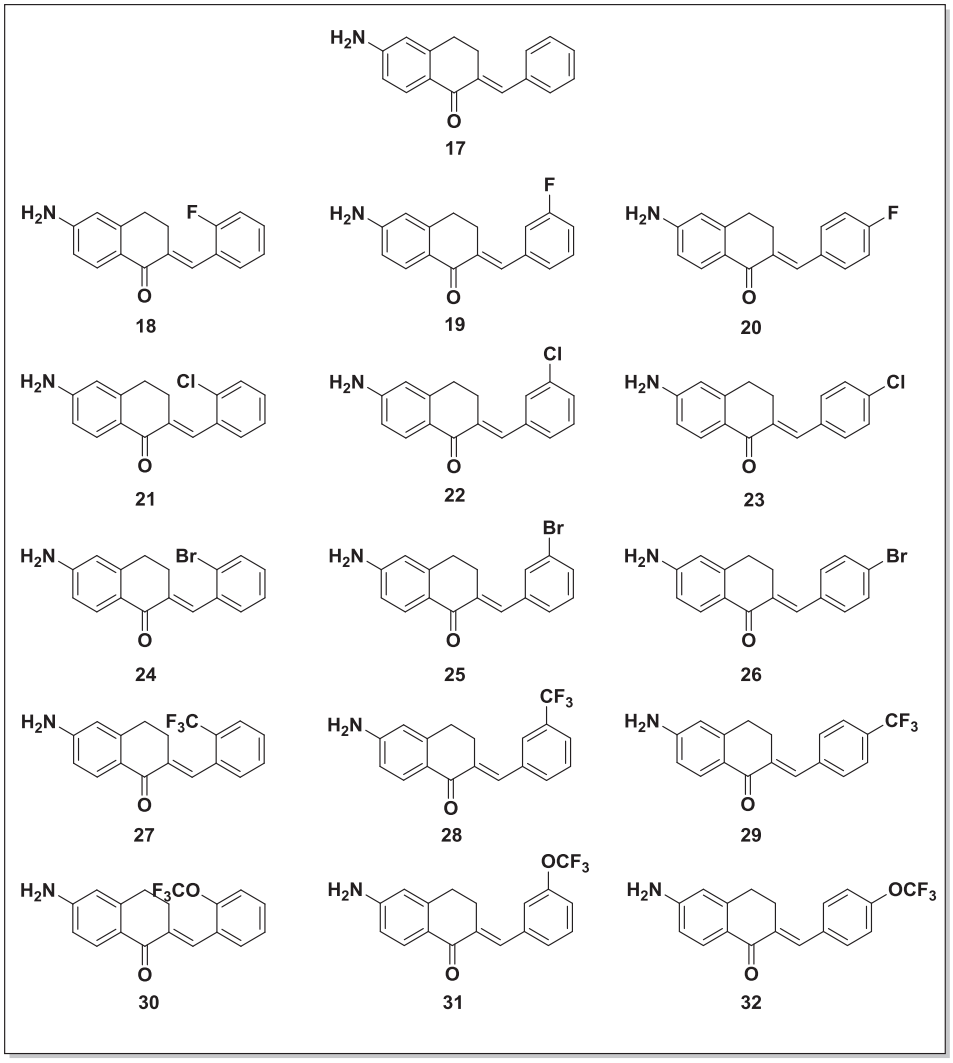


Fig. 4. Synthesized halogenated 6-amino-1-tetralone substituted chalcone derivatives **17–32.**

Table 1
Inhibitory activities of compounds **1–32** on ROS production stimulated by LPS in RAW 264.7 macrophages and relative ROS inhibitory potencies of 1-tetralone chalcones (**1–16**) and 6-amino-1-tetralone chalcones (**17–32**) with respect to positive control, malvidin.

Compounds	ROS Inhibition (IC_{50},[a] μM)	Toxicity	[b] Relative ROS Inhibitory Potency of (**1–16**) with respect to malvidin	Compounds	ROS Inhibition (IC_{50},[a] μM)	Toxicity	[b] Relative ROS Inhibitory Potency of (**17–32**) with respect to malvidin
1	5.43 ± 1.80	NT	1.7	**17**	0.76 ± 0.11	NT	11.8
2	> 10	NT	< 0.9	**18**	0.25 ± 0.13	NT	36.0
3	> 10	NT	< 0.9	**19**	0.76 ± 0.09	NT	11.8
4	> 10	NT	< 0.9	**20**	0.40 ± 0.15	NT	22.5
5	3.11 ± 1.11	NT	2.9	**21**	1.04 ± 0.02	NT	8.7
6	6.24 ± 0.99	NT	1.4	**22**	1.12 ± 0.06	NT	8.0
7	1.55 ± 0.64	NT	5.8	**23**	2.93 ± 0.49	NT	3.1
8	5.35 ± 0.23	NT	1.7	**24**	2.88 ± 0.81	NT	3.1
9	4.31 ± 0.67	NT	2.1	**25**	0.80 ± 0.11	NT	11.3
10	1.27 ± 0.29	NT	7.1	**26**	1.29 ± 0.31	NT	7.0
11	> 10	NT	< 0.9	**27**	8.77 ± 1.31	NT	1.0
12	3.02 ± 0.72	NT	3.0	**28**	0.41 ± 0.04	NT	22.0
13	4.18 ± 1.40	NT	2.2	**29**	4.51 ± 1.39	NT	2.0
14	5.35 ± 0.75	NT	1.7	**30**	3.75 ± 1.38	NT	2.4
15	3.99 ± 0.51	NT	2.3	**31**	0.84 ± 0.12	NT	10.7
16	1.90 ± 0.23	NT	4.7	**32**	1.06 ± 0.29	NT	8.5
				Malvidin [34]	9.0 ± 0.8		

NT: Non-toxic: Cytotoxicity of the compounds was evaluated by cell viability assay (MTS assay). For this, as indicated in the experimental methods, macrophages were treated with the compound for 24 h up to 10 μM. All the compounds used in this study did not affect cell viability in this condition.

[a] Each data represents mean ± S.D. from three different experiments performed in triplicate.

[b] Relative ROS inhibitory potency: IC_{50} (μM) of Malvidin/IC_{50} (μM) of synthesized compounds (**1–32**).

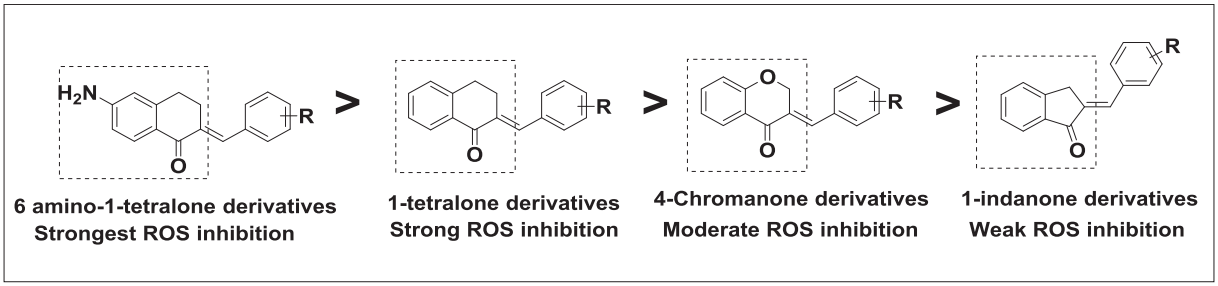


Fig. 5. Comparison of 1-tetralone, 6-amino-1-tetralone chalcone derivatives, and previously reported rigid structure [21,22] for inhibition of ROS production stimulated by LPS in RAW 264.7 macrophages.

3. Conclusion

In conclusion, we have systematically designed and synthesized a series of thirty-two halogenated 1-tetralone and 6-amino-1-tetralone chalcone derivatives using *Claisen-Schmidt* condensation reaction. The synthesized compounds were evaluated for the inhibitory effect on ROS production stimulated by LPS in RAW 264.7 macrophages. Among them, compounds **18** possessing 6-amino-1-tetralone chalcone moiety showed the strongest inhibitory activity of ROS production stimulated by LPS in RAW 264.7 macrophages. SAR study revealed that chalcone derivatives having 1-tetralone skeleton is responsible for exhibiting the potent ROS inhibition than that of previously reported 1-indanone or 4-chromanone skeleton bearing chalcone derivatives. In addition, amino substitution at the 6th position of 1-tetralone skeleton greatly increased inhibitory activity of ROS production stimulated by LPS in RAW 264.7 macrophages. These results would provide new insights to the researchers working to develop novel anti-inflammatory agents.

4. Experimental section

4.1. Chemistry section

All the commercially available starting materials and reagents were purchased from Sigma-Aldrich Chemical Co. (Darmstadt, Germany), TCI Chemicals (Tokyo, Japan), Junsei (Tokyo, Japan) and Alfa-Aesar (Heysham, England) and used without any further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Budrick and Jackson (Shanghai, China). Thin layer chromatography (TLC) was performed with kieselgel 60 F_{254} (Merck, Darmstadt, Germany). Since all the prepared compounds contain aromatic ring, they were visualized and detected on TLC plates with UV light (short wave, long wave or both). NMR spectra were recorded on a Bruker AMX 250 (250 MHz, FT) for ^{1}H NMR and 63 MHz for ^{13}C NMR, and chemical shifts were calibrated according to TMS. Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Melting points were determined in open capillary tubes on electrothermal 1A 9100 digital melting point apparatus and were uncorrected.

The HPLC analysis were performed using an HPLC system consisted of a pump (LC-20AD), an autoinjector (SIL-20-A), a UV–visible detector (SPD-20A), and communications bus module (CBM-20A) from Shimadzu Scientific Instruments (Kyoto, Japan). A Waters COSMOSIL 5C18-MS-II column (5 µm, 4.6 × 250 mm) was used with a gradient solvent system of 95:5 for 20 min with 95% ACN: doubly distilled water, at a flow rate of 0.5 mL/min at 254 nm UV detection. The purity of compound was described as percentage (%), and retention time was given in minutes (min). Mass spectra were measured in positive electrospray ionization (ESI) mode on LCMS-2020 system from Shimadzu Scientific Instruments (Tokyo, Japan).

4.2. General method for preparation of compounds 1–32

The general synthetic method for the preparation of compounds **1–32** has been reported previously [35,36]. The melting points of the reported compounds and those obtained in the present study are also included. The characterization of the compounds are mentioned as follows;

4.2.1. 2-benzylidene-3,4-dihydronaphthalen-1(2H)-one (1) [29,37,38]

mp: 109.8–110.7 °C (ref[29]: 106.0–107.3 °C (ethanol)), HPLC: Retention time: 7.74 min, purity: 98.2%.

4.2.2. 2-(2-fluorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (2) [37,39]

mp: 60.1–61.1 °C (ref[39]: 56.0–58.0 °C), HPLC: Retention time: 8.51 min, purity: 97.2%, ESI LC/MS: m/z calcd for $C_{17}H_{13}FO$ $[MH]^+$ 253.09; found 253.70.

4.2.3. 2-(3-fluorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (3) [37,39]

mp: 87.9–88.7 °C (ref[39]: 89.0–91.0 °C), HPLC: Retention time: 8.41 min, purity: 95.2%, ESI LC/MS: m/z calcd for $C_{17}H_{13}FO$ $[MH]^+$ 253.09; found 253.70.

4.2.4. 2-(4-fluorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (4) [37,39,40]

mp: 113.1–114.1 °C (ref[40]: 112.0–116.0 °C), HPLC: Retention time: 8.50 min, purity: 95.5%, ESI LC/MS: m/z calcd for $C_{17}H_{13}FO$ $[MH]^+$ 253.09; found 253.70.

4.2.5. 2-(2-chlorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (5) [40]

mp: 72.4–73.2 °C (ref[40]: 109.0–112.0 °C), HPLC: Retention time: 9.19 min, purity: 95.2%, ESI LC/MS: m/z calcd for $C_{17}H_{13}ClO$ $[MH]^+$ 269.06; found 269.65.

4.2.6. 2-(3-chlorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (6) [40]

mp: 111.4–112.1 °C (ref[40]: 106.0–108.0 °C), HPLC: Retention time: 9.57 min, purity: 96.2%, ESI LC/MS: m/z calcd for $C_{17}H_{13}ClO$ $[MH]^+$ 269.06; found 269.65.

4.2.7. 2-(4-chlorobenzylidene)-3,4dihydronaphthalen-1(2H)-one (7) [38,40]

mp: 139.0–139.7 °C (ref[40]: 134.0–136.0 °C), HPLC: Retention time: 9.59 min, purity: 95.2%.

4.2.8. 2-(2-bromobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (8) [41]

Yield: 86.6%, white solid; TLC (ethyl acetate/hexanes = 1:27 v/v) R_f: 0.26, mp: 77.9–78.5 °C, (ref[41]: 78.0–80.0 °C), HPLC: Retention time: 9.52 min, purity: 97.2%, ESI LC/MS: m/z calcd for $C_{17}H_{13}BrO$ $[MH]^+$ 313.01; found 313.55.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.15 (d, J = 7.8 Hz, 1H), 7.82 (s, 1H), 7.63 (d, J = 7.9 Hz, 1H), 7.47 (dd, J = 10.5, 4.4 Hz, 1H), 7.32 (dt,

J = 8.3, 6.6 Hz, 3H), 7.25 – 7.15 (m, 2H), 2.93 (s, 4H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 187.57, 143.42, 136.89, 136.34, 135.58, 133.43, 133.27, 132.93, 130.38, 129.67, 128.30, 128.27, 127.05, 126.97, 124.87, 29.01, 27.27.

4.2.9. 2-(3-bromobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (9) [40]

mp: 110.9–111.4 °C (ref[40]: 109.0–110.0 °C), HPLC: Retention time: 9.99 min, purity: 98.7%, ESI LC/MS: m/z calcd for $C_{17}H_{13}BrO$ $[MH]^+$ 313.01; found 313.55.

4.2.10. 2-(4-bromobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (10) [40]

mp: 153.5– 154.2 °C (ref[40]: 139.0–141.0 °C), HPLC: Retention time: 8.84 min, purity: 97.8%, ESI LC/MS: m/z calcd for $C_{17}H_{13}BrO$ $[MH]^+$ 313.01; found 313.55.

4.2.11. 2-(2-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (11) [42]

Yield: 92.0%, white solid; TLC (ethyl acetate/hexanes = 1:27 v/v) R_f: 0.21, mp: 77.4–78.4 °C, HPLC: Retention time: 8.62 min, purity: 98.5%, ESI LC/MS: m/z calcd for $C_{18}H_{13}F_3O$ $[MH]^+$ 303.09; found 303.60.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.15 (dd, J = 7.8, 1.3 Hz, 1H), 7.96 (s, 1H), 7.72 (d, J = 7.7 Hz, 1H), 7.56 (t, J = 7.4 Hz, 1H), 7.52–7.39 (m, 2H), 7.39 – 7.27 (m, 2H), 7.23 (d, J = 7.5 Hz, 1H), 2.92 (dd, J = 9.2, 4.0 Hz, 2H), 2.87 – 2.78 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 187.39, 143.50, 138.05, 134.88 (d, J = 1.9 Hz), 133.50, 133.15, 132.94, 131.47, 130.34, 129.21 (d, J = 30.2 Hz), 128.35, 128.30, 128.04, 127.07, 126.07 (q, J = 5.3 Hz), 121.75, 29.03, 27.36.

4.2.12. 2-(3-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (12)

[42,43] Yield: 71.4%, off-white solid; TLC (ethyl acetate/hexanes = 1:29 v/v) R_f: 0.30, mp: 106.5–107.5 °C, HPLC: Retention time: 8.86 min, purity: 97.6%, ESI LC/MS: m/z calcd for $C_{18}H_{13}F_3O$ $[MH]^+$ 303.09; found 303.60.

^{1}H NMR (250 MHz, DMSO) δ 7.96 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.78 – 7.64 (m, 3H), 7.58 (td, J = 7.4, 1.3 Hz, 1H), 7.39 (dd, J = 13.1, 7.5 Hz, 2H), 3.11 – 3.00 (m, 2H), 3.00 – 2.89 (m, 2H).

^{13}C NMR (63 MHz, DMSO) δ 186.73, 143.65, 137.34, 136.51, 133.96, 133.86, 133.68, 132.83, 129.81, 129.60 (d, J = 31.7 Hz), 128.75, 127.59, 127.21, 126.39 (q, J = 3.9 Hz), 125.25 (q, J = 3.6 Hz), 122.04, 27.99, 26.73.

4.2.13. 2-(4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (13) [38,42,43]

mp: 176.9–177.9 °C, HPLC: Retention time: 9.10 min, purity: 99.5%.

4.2.14. 2-(2-(trifluoromethoxy)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (14)

Yield: 88.9%, light yellowish liquid; TLC (ethyl acetate/hexanes = 1:27 v/v) R_f: 0.28, HPLC: Retention time: 8.47 min, purity: 95.5%, ESI LC/MS: m/z calcd $C_{18}H_{13}F_3O_2$ $[MH]^+$ 319.08; found 319.60

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.14 (dd, J = 7.8, 1.2 Hz, 1H), 7.83 (s, 1H), 7.48 (td, J = 7.4, 1.5 Hz, 1H), 7.43 – 7.27 (m, 5H), 7.23 (d, J = 7.4 Hz, 1H), 2.93 (s, 4H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 187.36, 147.50, 143.43, 138.05, 133.46, 133.19, 130.78, 130.38, 129.76, 129.58, 128.30 (2C), 127.08, 126.54, 121.18 (d, J = 1.3 Hz), 120.49 (d, J = 258.2 Hz), 28.96, 27.55.

4.2.15. 2-(3-(trifluoromethoxy)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (15) [43]

Yield: 91.2%, white solid; TLC (ethyl acetate/hexanes = 1:18 v/v) R_f: 0.27, mp: 150.8–151.2 °C, HPLC: Retention time: 9.06 min, purity: 97.5%, ESI LC/MS: m/z calcd $C_{18}H_{13}F_3O_2$ $[MH]^+$ 319.08; found 319.55.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.12 (d, J = 7.8 Hz, 1H), 7.79 (s, 1H), 7.53 – 7.39 (m, 2H), 7.35 (t, J = 7.9 Hz, 2H), 7.28 – 7.16 (m, 3H), 3.14 – 3.03 (m, 2H), 3.01 – 2.90 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 187.50, 149.26, 143.16, 137.85, 136.85, 134.68, 133.49, 133.26, 129.85, 128.29, 128.24, 128.15, 127.13, 121.98, 120.74, 120.47 (d, J = 257.5 Hz), 28.75, 27.10.

4.2.16. 2-(4-(trifluoromethoxy)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (16)

Yield: 40.6%, white solid; TLC (ethyl acetate/hexanes = 1:27 v/v) R_f: 0.25, mp: 136.8–137.8 °C, HPLC: Retention time: 8.58 min, purity: 98.1%, ESI LC/MS: m/z calcd $C_{18}H_{13}F_3O_2$ $[MH]^+$ 319.08; found 319.55.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.12 (dd, J = 7.7, 1.0 Hz, 1H), 7.81 (s, 1H), 7.52 – 7.40 (m, 3H), 7.35 (t, J = 7.5 Hz, 1H), 7.24 (d, J = 8.1 Hz, 3H), 3.08 (dd, J = 8.9, 4.3 Hz, 2H), 2.94 (t, J = 6.1 Hz, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 187.58, 149.00, 143.12, 136.19, 134.89, 134.47, 133.42, 133.32, 131.27 (2C), 128.27, 128.21, 127.11, 120.81, 120.43 (d, J = 257.6 Hz), 28.77, 27.11.

4.2.17. 6-amino-2-benzylidene-3,4-dihydronaphthalen-1(2H)-one (17) [29]

mp: 175.3–176.2 °C, (ref[29]: 139.0–141.0 °C (ethanol)), HPLC: Retention time: 7.02 min, purity: 98.9%.

4.2.18. 6-amino-2-(2-fluorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (18)

Yield: 34.1%, orange solid, TLC (ethyl acetate/hexanes = 1:6 v/v) R_f: 0.21, mp: 136.4–137.1 °C, HPLC: Retention time: 7.17 min, purity: 98.9%, ESI LC/MS: m/z calcd for $C_{17}H_{14}FNO$ $[MH]^+$ 268.10; found 268.70.

^{1}H NMR (250 MHz, DMSO) δ 7.71 (d, J = 8.6 Hz, 1H), 7.50 (s, 1H), 7.49 – 7.37 (m, 2H), 7.27 (dt, J = 8.4, 6.3 Hz, 2H), 6.51 (dd, J = 8.6, 2.1 Hz, 1H), 6.34 (d, J = 1.9 Hz, 1H), 6.20 (s, 2H), 2.90 – 2.79 (m, 2H), 2.79 – 2.69 (m, 2H).

^{13}C NMR (63 MHz, DMSO) δ 183.88, 162.16, 154.31, 145.99, 139.01, 130.96 (d, J = 3.0 Hz), 130.59 (d, J = 8.5 Hz), 130.40, 125.56 (d, J = 3.2 Hz), 124.54 (d, J = 3.4 Hz), 123.65 (d, J = 14.0 Hz), 121.49, 115.81 (d, J = 21.8 Hz), 112.88, 110.79, 28.64, 27.25.

4.2.19. 6-amino-2-(3-fluorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (19)

Yield: 20.2%, dark yellow solid, TLC (ethyl acetate/hexanes = 1:6 v/v) R_f: 0.21, mp: 150.5–151.5 °C, HPLC: Retention time: 7.37 min, purity: 99.9%, ESI LC/MS: m/z calcd for $C_{17}H_{14}FNO$ $[MH]^+$ 268.10; found 268.70.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.98 (d, J = 8.5 Hz, 1H), 7.71 (s, 1H), 7.34 (dd, J = 14.0, 7.8 Hz, 1H), 7.20 – 6.94 (m, 3H), 6.58 (dd, J = 8.5, 2.0 Hz, 1H), 6.40 (s, 1H), 4.18 (s, 2H), 3.02 (t, J = 5.9 Hz, 2H), 2.81 (t, J = 6.3 Hz, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.99, 151.43, 145.82, 138.43 (d, J = 7.7 Hz), 137.12, 133.67 (d, J = 2.3 Hz), 130.98, 129.84 (d, J = 8.4 Hz), 125.54 (d, J = 2.9 Hz), 124.47, 116.21 (d, J = 21.6 Hz), 114.96 (d, J = 21.3 Hz), 113.54, 112.02, 99.98, 29.07, 27.13.

4.2.20. 6-amino-2-(4-fluorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (20)

Yield: 26.9%, brown solid; TLC (ethyl acetate/hexanes = 1:6 v/v) R_f: 0.20, mp: 215.4–216.3 °C, HPLC: Retention time: 7.1 min, purity: 99.8%, ESI LC/MS: m/z calcd for $C_{17}H_{14}FNO$ $[MH]^+$ 268.10; found 268.70.

^{1}H NMR (250 MHz, DMSO) δ 7.83 – 7.68 (m, J = 8.5 Hz, 3H), 7.61 – 7.48 (m, 3H), 7.26 (t, J = 8.9 Hz, 2H), 6.75 (dd, J = 8.5, 1.9 Hz, 1H),

6.62 (s, 1H), 2.97 (t, J = 6.0 Hz, 2H), 2.78 (t, J = 6.3 Hz, 2H).

^{13}C NMR (63 MHz, DMSO) δ 184.68, 163.97, 145.55, 136.19 (d, J = 1.2 Hz), 133.05, 132.30, 132.24, 132.21, 132.07, 130.06, 124.43, 115.83, 115.49, 115.27, 113.97, 28.42, 26.77.

*4.2.21. 6-amino-2-(2-chlorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one **(21)***

Yield: 22.4%, orange solid; TLC (ethyl acetate/hexanes = 2:5 v/v) R_f: 0.26, mp: 60.7–61.7 °C, HPLC: Retention time: 7.58 min, purity: 95.1%, ESI LC/MS: m/z calcd for $C_{17}H_{14}ClNO$ $[MH]^+$ 284.07; found 284.60.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.00 (d, J = 8.5 Hz, 1H), 7.80 (s, 1H), 7.41 (dd, J = 7.9, 4.0 Hz, 1H), 7.30 – 7.21 (m, 3H), 6.58 (dd, J = 8.5, 2.2 Hz, 1H), 6.39 (d, J = 2.1 Hz, 1H), 4.16 (s, 2H), 2.94 – 2.84 (m, 2H), 2.85 – 2.75 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.98, 151.40, 146.02, 137.78, 134.89, 134.71, 132.02, 130.98, 130.44, 129.65, 129.21, 126.26, 124.52, 113.55, 112.08, 29.30, 27.37.

*4.2.22. 6-amino-2-(3-chlorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one **(22)***

Yield: 24.3%, yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.28, mp: 185.2–186.0 °C, HPLC: Retention time: 7.86 min, purity: 96.9%, ESI LC/MS: m/z calcd for $C_{17}H_{14}ClNO$ $[MH]^+$ 284.07; found 284.65.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.98 (d, J = 8.5 Hz, 1H), 7.69 (s, 1H), 7.36 (s, 1H), 7.33 – 7.22 (m, 3H), 6.58 (dd, J = 8.5, 2.3 Hz, 1H), 6.40 (d, J = 2.1 Hz, 1H), 4.16 (s, 2H), 3.07 – 2.95 (m, 2H), 2.87 – 2.74 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.94, 151.41, 145.82, 138.10, 137.27, 134.25, 133.47, 131.00, 129.61, 129.39, 128.08, 127.88, 124.50, 113.56, 112.04, 29.10, 27.15.

*4.2.23. 6-amino-2-(4-chlorobenzylidene)-3,4-dihydronaphthalen-1(2H)-one **(23)***

Yield: 24.6%, yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.29, mp: 193.4–194.3 °C, HPLC: Retention time: 7.86 min, purity: 99.5%, ESI LC/MS: m/z calcd for $C_{17}H_{14}ClNO$ $[MH]^+$ 284.07; found 284.65.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.97 (d, J = 8.5 Hz, 1H), 7.70 (s, 1H), 7.40 – 7.27 (m, 4H), 6.57 (dd, J = 8.5, 2.3 Hz, 1H), 6.39 (d, J = 2.2 Hz, 1H), 4.14 (s, 2H), 3.06 – 2.93 (m, 2H), 2.87 – 2.73 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.96, 151.40, 145.74, 136.68, 134.77, 134.01, 133.73, 130.98 (3C), 128.60 (2C), 124.64, 113.57, 112.05, 29.12, 27.17.

*4.2.24. 6-amino-2-(2-bromobenzylidene)-3,4-dihydronaphthalen-1(2H)-one **(24)***

Yield: 20.8%, a yellow solid; TLC (ethyl acetate/hexanes = 2:5 v/v) R_f: 0.24, mp: 121.4–122.3 °C, HPLC: Retention time: 8.05 min, purity: 99.7%, ESI LC/MS: m/z calcd for $C_{17}H_{14}BrNO$ $[MH]^+$ 328.02; found 328.50.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.00 (d, J = 8.5 Hz, 1H), 7.73 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.35 – 7.22 (m, 2H), 7.21 – 7.11 (m, 1H), 6.58 (dd, J = 8.5, 2.2 Hz, 1H), 6.39 (d, J = 1.9 Hz, 1H), 4.17 (s, 2H), 2.91 – 2.83 (m, 2H), 2.83 – 2.76 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 186.03, 151.40, 146.05, 137.45, 136.72, 134.22, 132.82, 130.96, 130.46, 129.36, 126.88, 124.84, 124.48, 113.54, 112.08, 29.29, 27.28.

*4.2.25. 6-amino-2-(3-bromobenzylidene)-3,4-dihydronaphthalen-1(2H)-one **(25)***

Yield: 20.1%, yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.24, mp: 177.2–178.2 °C, HPLC: Retention time: 8.10 min, purity: 97.7%, ESI LC/MS: m/z calcd for $C_{17}H_{14}BrNO$ $[MH]^+$ 328.02; found 328.45.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.00 (d, J = 8.5 Hz, 1H), 7.71 (s, 1H), 7.54 (s, 1H), 7.46 (d, J = 7.3 Hz, 1H), 7.36 – 7.22 (m, 2H), 6.60 (dd, J = 8.5, 2.1 Hz, 1H), 6.43 (d, J = 1.7 Hz, 1H), 4.20 (s, 2H), 3.09 – 2.97 (m, J = 5.9 Hz, 2H), 2.90 – 2.78 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.90, 151.42, 145.81, 138.40, 137.31, 133.35, 132.27, 130.99, 130.98, 129.87, 128.31, 124.47, 122.40, 113.56, 112.03, 29.09, 27.12.

*4.2.26. 6-amino-2-(4-bromobenzylidene)-3,4-dihydronaphthalen-1(2H)-one **(26)***

Yield: 28.3%, yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.26, mp: 206.8– 207.7 °C, HPLC: Retention time: 8.20 min, purity: 99.1%, ESI LC/MS: m/z calcd for $C_{17}H_{14}BrNO$ $[MH]^+$ 328.02; found 328.45.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.99 (d, J = 8.5 Hz, 1H), 7.69 (s, 1H), 7.51 (dd, J = 8.3, 1.3 Hz, 2H), 7.30 – 7.24 (m, 2H), 6.58 (dd, J = 8.5, 1.8 Hz, 1H), 6.40 (s, 1H), 4.13 (s, 2H), 3.05 – 2.95 (m, J = 6.6 Hz, 2H), 2.86 – 2.75 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.95, 151.40, 145.76, 136.82, 135.27, 133.76, 131.59 (2C), 131.25 (2C), 131.01, 124.71, 122.24, 113.62, 112.09, 29.14, 27.20.

*4.2.27. 6-amino-2-(2-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one **(27)***

Yield: 20.0%, a yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.22 mp: 144.3–145.2 °C, HPLC: Retention time: 7.43 min, purity: 99.12%, ESI LC/MS: m/z calcd for $C_{18}H_{14}F_3NO$ $[MH]^+$ 318.10; found 318.55.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.00 (d, J = 8.5 Hz, 1H), 7.88 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.53 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 6.57 (dd, J = 8.5, 2.3 Hz, 1H), 6.38 (d, J = 2.2 Hz, 1H), 4.18 (s, 2H), 2.76 (s, 4H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.82, 151.51, 146.12, 138.62, 135.35, 131.51, 131.37, 130.98, 130.48, 129.14 (d, J = 30.1 Hz), 127.73, 125.95 (q, J = 5.4 Hz), 124.35, 123.96 (d, J = 273.9 Hz), 113.55, 112.09, 29.31, 27.37.

*4.2.28. 6-amino-2-(3-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one **(28)***

Yield: 20.7%, yellow solid; TLC (ethyl acetate/hexanes = 1:3 v/v) R_f: 0.27 mp: 161.7–162.7 °C, HPLC: Retention time: 7.53 min, purity: 99.3% ESI LC/MS: m/z calcd for $C_{18}H_{14}F_3NO$ $[MH]^+$ 318.10; found 318.55.

^{1}H NMR (250 MHz, DMSO) δ 7.80 – 7.61 (m, 5H), 7.60 (s, 1H), 6.51 (dd, J = 8.5, 2.2 Hz, 1H), 6.34 (d, J = 1.9 Hz, 1H), 6.20 (s, 2H), 2.95 (t, J = 5.8 Hz, 2H), 2.74 (t, J = 6.2 Hz, 2H).

^{13}C NMR (63 MHz, DMSO) δ 184.01, 154.30, 145.81, 138.43, 137.11, 133.57, 131.61, 130.37, 129.71, 128.83, 126.13 (d, J = 3.9 Hz), 124.72 (dd, J = 7.0, 3.0 Hz), 124.27 (d, J = 272.5 Hz), 121.54, 112.86, 110.73, 28.55, 26.86.

*4.2.29. 6-amino-2-(4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one **(29)***

Yield: 28.0%, yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.22, mp: 196.4–197.4 °C, HPLC: Retention time: 7.58 min, purity: 99.8%, ESI LC/MS: m/z calcd for $C_{18}H_{14}F_3NO$ $[MH]^+$ 318.10; found 318.60.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.99 (d, J = 8.5 Hz, 1H), 7.76 (s, 1H), 7.63 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 8.1 Hz, 2H), 6.58 (dd, J = 8.5, 2.3 Hz, 1H), 6.40 (d, J = 2.1 Hz, 1H), 4.14 (s, 2H), 3.07 – 2.95 (m, 2H), 2.89 – 2.77 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.80, 151.51, 145.82, 139.99, 138.08, 133.24, 131.09, 129.80 (4C), 125.29 (q, J = 3.8 Hz), 124.54, 124.08 (d, J = 272.1 Hz), 113.66, 112.08, 29.15, 27.18.

4.2.30. 6-amino-2-(2-(trifluoromethoxy)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (30)

Yield: 21.0%, yellow solid; TLC (ethyl acetate/hexanes = 1:6 v/v) R_f: 0.22, mp: 155.7–155.6 °C, HPLC: Retention time: 7.47 min, purity: 97.9%, ESI LC/MS: *m/z* calcd for $C_{18}H_{14}F_3NO_2$ $[MH]^+$ 334.09; found 334.55.

^{1}H NMR (250 MHz, DMSO) δ 7.71 (d, J = 8.6 Hz, 1H), 7.57 – 7.41 (m, 5H), 6.52 (dd, J = 8.6, 2.0 Hz, 1H), 6.34 (d, J = 1.8 Hz, 1H), 6.23 (s, 2H), 2.87 – 2.76 (m, 2H), 2.77 – 2.68 (m, 2H).

^{13}C NMR (63 MHz, DMSO) δ 183.82, 154.40, 146.71, 145.98, 139.49, 131.24, 130.42, 130.27, 129.57, 127.67, 126.49, 121.58, 121.39, 120.30 (d, J = 257.0 Hz), 112.92, 110.78, 28.68, 27.13.

4.2.31. 6-amino-2-(3-(trifluoromethoxy)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (31)

Yield: 51.0%, yellow solid; TLC (ethyl acetate/hexanes = 1:4 v/v) R_f: 0.23, mp: 152.5–153.5 °C, HPLC: Retention time: 7.60 min, purity: 99.7%, ESI LC/MS: *m/z* calcd for $C_{18}H_{14}F_3NO_2$ $[MH]^+$ 334.09; found 334.55.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.98 (d, J = 8.5 Hz, 1H), 7.72 (s, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.30 (d, J = 7.7 Hz, 1H), 7.22 (s, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.58 (dd, J = 8.5, 2.3 Hz, 1H), 6.40 (d, J = 2.2 Hz, 1H), 4.17 (s, 2H), 3.07 – 2.96 (m, J = 9.2, 3.9 Hz, 2H), 2.87 – 2.77 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 185.86, 151.45, 149.20, 145.81, 138.29, 137.48, 133.26, 131.02, 129.72, 128.07, 124.46, 121.89, 120.47 (d, J = 257.4 Hz), 120.35, 113.58, 112.03, 29.07, 27.08.

4.2.32. 6-amino-2-(4-(trifluoromethoxy)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (32)

Yield: 20.4%, yellow solid; TLC (ethyl acetate/hexanes = 1:2 v/v) R_f: 0.24, mp: 160.0–161.0 °C, HPLC: Retention time: 7.66 min, purity: 98.8%, ESI LC/MS: *m/z* calcd for $C_{18}H_{14}F_3NO_2$ $[MH]^+$ 334.09; found 334.55.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.99 (d, J = 8.6 Hz, 1H), 7.75 (s, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 6.59 (d, J = 7.4 Hz, 1H), 6.42 (s, 1H), 4.23 (s, 2H), 3.03 (t, J = 6.4 Hz, 2H), 2.88 – 2.77 (m, 2H).

^{13}C NMR (63 MHz, $CDCl_3$) δ 186.00, 151.50, 148.70, 145.78, 136.83, 134.88, 133.44, 131.11 (2C), 130.95, 124.41, 120.71 (2C), 120.42 (d, J = 257.4 Hz), 113.54, 112.00, 29.06, 27.07.

4.3. Pharmacology

4.3.1. Cell culture

RAW 264.7 murine macrophages were purchased from the Korean cell line bank (Seoul, Korea) and routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in an incubator with humidified atmosphere of 95% oxygen and 5% CO_2.

4.3.2. Measurement of cell viability (MTS assay)

Cell viability was assessed using CellTiter 96 Aqueous One Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, RAW 264.7 macrophages were seeded at a density of 5×10^4 cells/well in 96-well plates. After overnight incubation, the cells were treated with the indicated compounds (up to 10 μM) for 24 h followed by incubation with 3-(4,5-dimethylhiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)–2H-tetrazolium (MTS) solution for additional 2 h at 37 °C. Cell viability was determined based on the amount of formazan production, which is produced by the cells metabolically active, by measuring the absorbance at 490 nm using SPECTROstar Nano microplate reader (BMG Labtech Inc., Ortenberg, Germany).

4.3.3. Measurement of reactive oxygen species (ROS) production

ROS production was measured essentially described as previously [44]. Briefly, RAW 264.7 macrophages were initially seeded at a density of 2×10^5/well in 96-well black plates. After overnight culture, the cells were pretreated with the compounds for 1 h and further stimulated with LPS for additional 24 h. Cells were then incubated with 5-chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CMH_2DCFDA) for 30 min in the dark. Excessive dye was removed by washing with HBSS solution and total ROS production was determined by measuring the fluorescence using FLUOstar OPTIMA fluorometer (BMG Labtech, Ortenberg, Germany). Excitation and emission wavelengths were set to 485 and 520 nm, respectively. The results were analyzed by FLUOstar OPTIMA software (BMG Labtech, Ortenberg, Germany).

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