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Short communication

Synthesis of benzamide derivatives of anacardic acid and their cytotoxic activity

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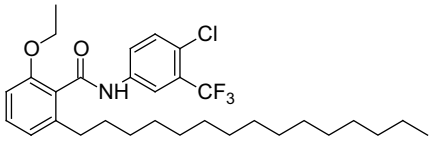
ABSTRACT

Several benzamide derivatives were synthesized from anacardic acid (**1a**) which was the product of hydrogenation of the naturally occurring anacardic acid mixture (**1a–d**), a major constituent of cashew nut shell liquid. Anacardic acid (**1a**) was first alkylated followed by hydrolysis of the ester to obtain synthones namely, 2-ethoxy-6-pentadecylbenzoic acid (**5**) and 2-isopropoxy-6-pentadecylbenzoic acid (**6**). These salicylic acid derivatives were then coupled with a variety of anilines to obtain novel benzamide compounds (**7–39**). Cytotoxic effect of these synthesized compounds was tested on HeLa cell line of wild type with relatively high expression of p300 and on HCT-15, which is p300 negative. Of all the compounds, 2-isopropoxy-6-pentadecyl-N-pyridin-4-ylbenzamide (**27**), 2-ethoxy-N-(3-nitrophenyl)-6-pentadecylbenzamide (**22**) and 2-ethoxy-6-pentadecyl-N-pyridin-4-ylbenzamide (**10**) were found to be more potent with the respective IC_{50} values 11.02 μM, 13.55 μM, 15.29 μM on HeLa cell line. Their activities are comparable with garcinol which is a cell permeable histone acetyltransferase (HAT) inhibitor and 10 fold more active than p300 HAT activators so far reported.

1. Introduction

Cashew nut shell liquid (CNSL) is a by-product of cashew nut industry and anacardic acid mixture (**1a–d**) isolated from CNSL which are salicylic acid derivatives with a nonisoprenoid alk(en)yl side chain [1].

Anacardic acid and its derivatives have been tested for various biological activities viz., antimicrobial activity [2,3] and soybean lipoxygenase-1 inhibitory activity [4,5]. Earlier from our lab we have reported the synthesis of sildenafil analogues [6], dihydropyridine analogues [7] as calcium channel blockers, isonicotinoylhydrazones for antimycobacterial activity [8] starting from anacardic acid. Kubo et al., reported the separation of anacardic acid into monoene (15:1), diene (15:2) and triene (15:3) by preparative HPLC and tested against cancer cells, and found to show moderate cytotoxic activity on BT-20 breast and HeLa epithelioid cervix carcinoma cells [9]. It has been proved that Anacardic acid has a unique function of mediating changes in membrane potential and pH gradient across liposomal membranes [10]. Anacardic acid was found to form highly lipophilic metal derivatives with selectivity towards the first row transition metals such as Fe^{+2}, Cu^{+2} and Zn^{+2} having selective ionophoric properties [11]. An amide derivative of anacardic acid (**2**) has been reported to be the first specific activator of histone acetyltransferase (HAT) activity of p300 [12] while anacardic acid (**1a**) showed to be HAT inhibitor.



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Recently, a few anacardic acid derivatives such as **2** were reported and elucidated their mechanism of HAT activation and suggested that, **2** and its derivatives binds to p300 predominantly to the amide group of α-helix and β-sheets and affect the structure of the enzyme [13]. But all the reported compounds were not cell permeable and showed inhibition at higher concentrations of the compound. More recently, it has been shown that anacardic acid is a specific activator of kinase activity of Aurora Kinase A [14], suppresses expression of nuclear factor-kB regulated gene products leading to potentiation of apoptosis [15] and inhibitor of the HAT activity of recombinant *Plasmodium falciparum* GCN5 [16]. Sbardella et al. recently showed that long chain alkylidenemalonates which are structurally related to anacardic acid as modulators of histone acetyltransferases [17].

In view of potential of anacardic acid and its derivatives as anticancer agents, it was proposed to make and identify potent benzamide derivatives from a natural product anacardic acid isolated from *Anacardium occideltale* L., test them for anticancer activity against HeLa cell line [18].

The aim of the present work is to make use of abundantly and cheaply available anacardic acid and to make synthons for generating novel cell permeable benzamide compounds and check for

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their antiproliferative activity. This was achieved by, (a) Saturation of naturally occurring anacardic acid mixture followed by alkylation of hydroxyl and carboxylic acid groups (b) Hydrolysis of ester to acid (c) Converting acid to acid chloride and then coupling with substituted anilines and (d) testing these compounds for their cytotoxic effect on HeLa and HCT-15 cell lines.

2. Results and discussion

2.1. Chemistry

The anacardic acid mixture (Fig. 1, **1a–d**) was isolated from CNSL by a reported method [19]. Accordingly CNSL was treated with calcium hydroxide, during which anacardic acid present in CNSL becomes calcium anacardate, which was isolated and hydrolyzed with dil. hydrochloric acid to generate anacardic acid ene mixture, which was a mixture of monoene, diene and triene located at (8′), (8′, 11′) and (8′, 11′, 14′) of the C_{15} alkyl chain respectively. O-ethyl anacardic acid (**5**) and O-isopropyl anacardic acid (**6**) (Scheme 1) were prepared by hydrogenation of anacardic acid mixture (**1a–d)** using hydrogen and Pd/C to get saturated anacardic acid (**1a**), which was then reacted with diethyl sulfate and isopropyl bromide respectively in presence of base to obtain diethyl anacardate (**3**) and diisopropyl anacardate (**4**).

The title compounds (**7–39**, Table 1) were synthesized from compounds **5** and **6** (Scheme 2) by treating corresponding 2-alkoxy-6-pentadecylbenzoic acid (**5**/**6**) with thionyl chloride to get acid chloride and then coupled with substituted anilines to get benzamides. Pure compounds were obtained by recrystallization using hexane. All the compounds were fully characterized by using IR, NMR and Mass spectroscopy.

2.2. Antiproliferative activity

Benzamide compounds (**7**–**39**) were tested on HeLa cell line of wild type with relatively more p300 expressed [18] and with p300 negative HCT-15. Inhibition of cell proliferation was measured by MTT assay. Several benzamide molecules exhibited very good cytotoxic activity against HeLa cell line (Table 1). Mantelingu et al. [13] have reported that benzamide molecules derived from anacardic acid, bearing chloro and trifluoromethyl groups on the aniline moiety are poor cell permeable and are specific p300 HAT activators. We have also observed the same effect with our compounds **8** and **12**. In order to find out the influence of other substituents on phenyl ring we have made several compounds having substitution other than halogen and trifluoromethyl groups. We found that, compounds **10**, **22** and **27** showed very good inhibitory activities followed by compounds **16** and **31**.

But all these compounds exhibited poor cytotoxic activity on p300 negative cell line (HCT-15) with inhibitory concentrations of (IC_{50}) >300 μM. Thus, these preliminary results give an indication that, some of benzamide compounds derived from anacardic acid are cell permeable and likely to act as modulators of HAT activity of p300. Hence, of the several compounds synthesized and tested for cytotoxic effect, we found that compound **27** was the most potent ($IC_{50} = 11.02$ μM), which was comparable to a cell permeable HAT inhibitor garcinol ($IC_{50} = 9.13$ μM) and 10 fold more active than p300 HAT activators reported earlier [13].

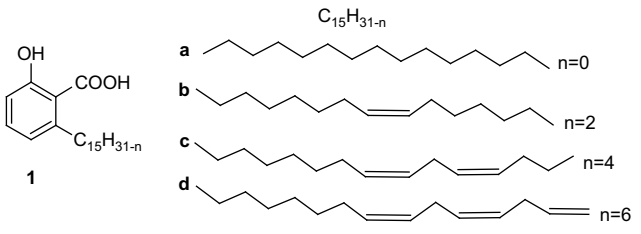


Fig. 1. Anacardic acid mixture.

3. Conclusion

We have synthesized novel benzamide derivatives from anacardic acid synthones. Compounds **27**, **22** and **10** were found to be more potent with IC_{50} values 11.02 μM, 13.55 μM, 15.29 μM respectively on HeLa cell line and their activities are comparable with garcinol, which is a cell permeable HAT inhibitor and 10 fold more active than p300 HAT activators so far reported.

Thus, these novel compounds would be useful as biological switching molecules for probing the role of p300 HAT in cellular physiology.

^{1}H NMR, ^{13}C NMR, mass spectra of compounds **7**–**39** are available free of charge via Internet at http:/www.sciencedirect.org.

4. Experimental

4.1. General methods/instrument

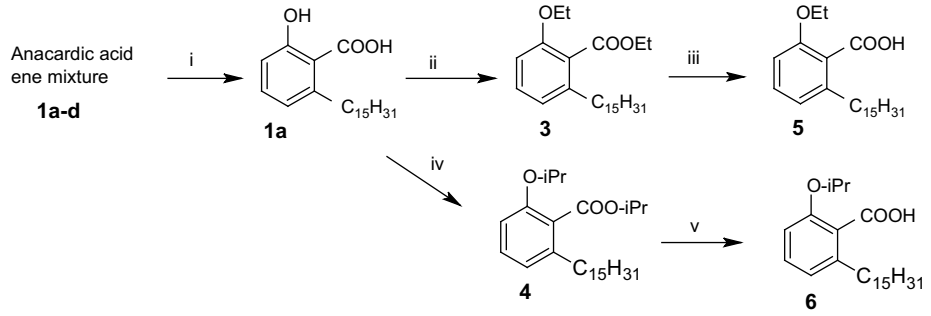
CNSL was obtained from commercial source and all other chemicals and reagents of laboratory grade were obtained from local dealers and were used without further purification. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 at 200 MHz on a Bruker A G Spectrometer. Chemical shifts are reported in δ units downfield from TMS as internal standard. Mass spectra were recorded using GC–MS-QP2010S (Direct probe) and on Q-TOF micro™ AMPS MAX 10/6A system. CHN analysis was recorded using ThermoFinnigan FLASH EA 1112 CHNS analyzer. Melting points were recorded on Acro Steel Pvt. Ltd. melting point apparatus and are uncorrected.

4.2. Solvent extracted CNSL

Solvent extracted CNSL containing anacardic acid mixture 63%, cardanol 10.5% and cardols 22% was obtained from cashew processing industry, Mangalore (Karnataka), India.

4.3. Isolation of anacardic acid mixture from natural CNSL

Commercially available solvent extracted CNSL (100 g) was dissolved in 5% aqueous methanol (600 mL). Activated charcoal (20 g) was added and stirred for 15 min. This solution was filtered over celite to remove insoluble plant materials. The clear filtrate was transferred into a round bottomed flask fitted with a double surface reflux condenser and a mechanical stirrer. Calcium hydroxide (50 g) was added in portions under stirring. After complete addition of calcium hydroxide, the temperature of the reaction mixture was raised to 50 °C and stirred for 3 h. Supernatant solution was monitored by TLC for the absence of anacardic acid. After the completion of the reaction, the precipitated calcium anacardate was filtered and washed thoroughly with methanol (200 mL) and the cake was dried under vacuum at 45–50 °C for 2 h (dry weight 110 g). The calcium anacardate was suspended in distilled water (440 mL) and 11 M HCl (60 mL) was added and stirred for 1 h. The resultant solution was extracted with ethyl acetate (2 × 150 mL). The combined organic layers were washed with distilled water (2 × 100 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield anacardic acid mixture (60 g). The identity of the compound was confirmed by HPLC and in comparison with standard samples [19,21].



Scheme 1. Synthetic pathway of **5** and **6**; Reagents and conditions: (i) MeOH/Pd/C/H_2 2.5 kg/cm^2 at RT for 2 h; (ii) Diethyl sulfate/K_2CO_3/Acetone/Reflux for 3 h; (iii) DMSO/t-BuOK/40 °C for 2 h; (iv) i-PrBr/MIBK/K_2CO_3/Bu_4NHSO_4/Reflux for 24 h; (v) DMSO/t-BuOK/40 °C for 2 h.

4.4. Preparation of intermediates

4.4.1. Preparation of anacardic acid (**1a**)

Anacardic acid mixture (30 g) was dissolved in methanol (120 mL). 5% Pd/C (0.75 g) was added slowly and this solution was transferred into hydrogenation flask. Hydrogenation was carried out at 2.5 kg/cm^2 pressure for 2 h, and the solution was filtered through a celite bed to obtain catalyst-free solution. Organic solvent was evaporated under vacuum to obtain anacardic acid (**1a**), which was recrystallized from petroleum ether (40–60 °C); Yield = 27 g; m.p. 90–91 °C; IR (KBr): 3140, 2917, 1850, 1655, 1604, 1466, 1308, 1248, 1206, 894, 815, 757 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.0 Hz), 1.25(brs, 24H), 1.6(m, 2H), 2.98(t, 2H, J = 8.0 Hz), 6.7–6.8 (dd, 2H, J = 8.0 Hz), 7.36(t, 1H, J = 8.0 Hz).

4.4.2. Preparation of ethyl-2-ethoxy-6-pentadecylbenzoate (**3**)

To a stirred solution of **1a** (12 g, 34.8 mmol) in acetone (60 mL) was added anhydrous powdered potassium carbonate (14.4 g, 104 mmol). Diethyl sulfate (10.7 g, 69.6 mmol) was added in portions for about 10 min at RT. After the addition was complete, the reaction mixture was heated to reflux and stirred for 3 h. The reaction mixture was cooled to RT and organic solvent was concentrated under reduced pressure. Water (100 mL) was added to the reaction mixture and extracted with ethyl acetate (80 mL). The organic layer was separated, washed with water and dried over anhydrous sodium sulfate. The organic layer was evaporated to obtain product **3** as a viscous liquid; Yield: Quantitative; IR (KBr): 2920, 1730, 1260 cm^{-1}; GC–MS (DI, m/z): 404 (M^+).

4.4.3. Preparation of isopropyl-2-isopropoxy-6-pentadecylbenzoate (**4**)

To a stirred solution of **1a** (100 g, 0.29 mol) in methyl isobutyl ketone (700 mL), potassium carbonate (130 g, 0.94 mol) and tetrabutylammonium hydrogen sulfate (2 g), was added isopropyl bromide (90 mL, 0.96 mol). The reaction mass was refluxed for about 24 h, cooled to RT and added water (500 mL) and ethyl acetate (500 mL). Organic layer was separated, washed with water (2 × 100 mL) and brine solution (1 × 100 mL). Organic layer was evaporated to obtain product **4** as a brown colored liquid in quantitative yield; IR (KBr): 2925, 1732, 1264 cm^{-1}; GC–MS (DI, m/z): 432 (M^+).

4.4.4. Preparation of 2-ethoxy-6-pentadecylbenzoic acid (**5**)

To a stirred solution of **3** (10 g, 24.7 mmol) in DMSO (50 mL) was added potassium tert-butoxide (3 g, 24.7 mmol). The reaction mixture was stirred at RT for about 2 h and then heated to 40 °C, stirred for another 2 h. The reaction mass was cooled to RT and quenched in ice-water, adjusted pH to 2 with hydrochloric acid. The resulting precipitate was filtered and dried at RT to obtain compound **5** as a brown solid; (6.5 g, Yield = 70%); m.p. 60–62 °C; IR (KBr): 2916, 2846, 1705, 1585, 1461, 1396, 1265, 1118, 1076 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, J = 6.0 Hz, 3H), 1.25 (brs, 24H), 1.45 (t, J = 7.0 Hz, 3H), 1.59 (m,2H), 2.78 (t, J = 8.0 Hz, 2H), 4.15 (q, J = 7.0 Hz, 2H), 6.80 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 7.8 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H); GC–MS (DI, m/z): 376 (M^+).

4.4.5. Preparation of 2-isopropoxy-6-pentadecylbenzoic acid (**6**)

Using the procedure described for compound **5**, compound **6** was prepared from compound **4** in 70% yield; m.p. 52–54 °C; IR (KBr): 2916, 2850, 1701, 1581, 1465, 1384, 1265, 1118, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, J = 6.2 Hz, 3H), 1.25 (brs, 24H), 1.40 (d, J = 6.0 Hz, 6H), 1.56 (m, 2H), 2.85 (t, J = 8.0 Hz, 2H), 4.58–4.76 (m, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H); GC–MS (m/z): 390 (M^+).

4.5. General procedure for the preparation of final compounds (**7**–**39**)

To a stirred solution of 2-alkoxy-6-pentadecylbenzoic acid (**5** or **6**) (5 mmol) in hexane (10 vol) was added thionyl chloride (1/2 vol) and catalytic amount of DMF (2 drops). The reaction mixture was refluxed for about 2 h. Distilled off excess thionyl chloride along with hexane under reduced pressure to obtain 2-alkoxy-6-pentad ecyl benzoyl chloride. The acid chloride obtained was dissolved in dichloromethane (5 vol) and added slowly to a pre-mixed stirred solution of substituted aniline (6 mmol) and triethylamine (5 mmol) in dichloromethane[1] (5 vol), by keeping the temperature of the reaction mixture at 15–20 °C. After the complete addition of acid chloride, the reaction mixture was allowed to stir at 35–40 °C for about 1 h. Water was added to the reaction mixture. The organic phase was separated and washed with dil. HCl, and water. Finally dried over anhydrous sodium sulfate, filtered and concentrated organic solvent to obtain crude material of title compounds. Pure compounds were obtained by recrystallization in hexane with yields more than 80%.

4.5.1. N-(3-chloro-4-fluorophenyl)-2-ethoxy-6-pentadecylbenzamide (**7**)

Using **5** and 3-chloro-4-fluoroaniline as starting materials, the title compound **7** was obtained as a white solid (2.27 g, Yield = 85%); m.p. 85–89 °C; IR (KBr): 3259, 2920, 1654, 1596, 1527, 1500, 1465, 1392, 1265, 1114, 1080, 813, 763, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.23 (brs, 24H), 1.37 (t, J = 6.9 Hz, 3H), 1.61 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 4.07 (q, J = 6.9 Hz, 2H), 6.78 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 7.11 (t,

[1] DMF was used as solvent for the preparation of compounds **10** and **28**.

Table 1
Cytotoxic effects of compounds **7**–**39** on HeLa cell line[a] (Cervical epithelial adenocarcinoma cell line) and HCT-15 (Colon carcinoma cell line).[b]

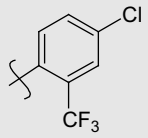
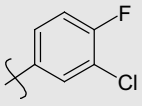
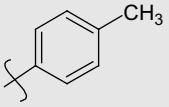
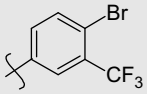
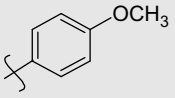
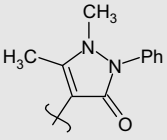
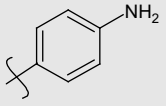
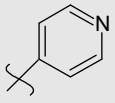
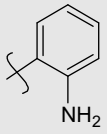
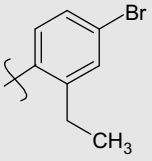
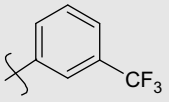
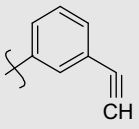
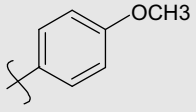
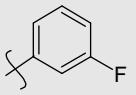
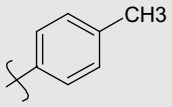
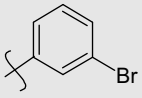
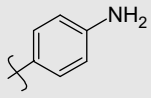
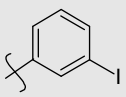
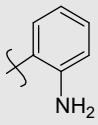
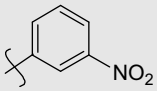
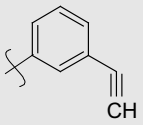
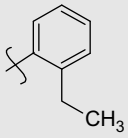
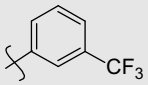
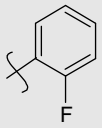
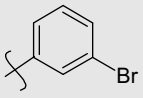
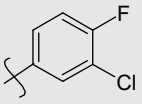
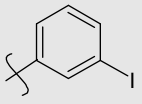
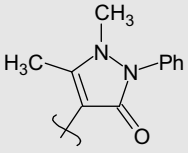
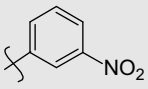
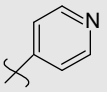
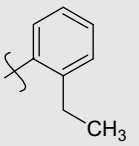
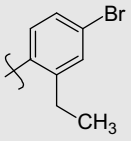
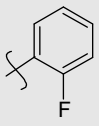
Compound	R	R_1	$[IC_{50}$ (μM) ± SE][c] HeLa	Compound	R	R_1	$[IC_{50}$ (μM) ± SE][c] HeLa
G*			9.13 ± 0.61	**12**	Et		351.01 ± 11.95
7	Et		112.38 ± 6.82	**13**	Et		128.45 ± 6.17
8	Et		335.12 ± 9.59	**14**	Et		140.51 ± 11.83
9	Et		44.25 ± 3.50	**15**	Et		65.41 ± 2.71
10	Et		15.29 ± 0.67	**16**	Et		19.39 ± 0.53
11	Et		342.02 ± 10.10	**17**	Et		100.51 ± 8.68
18	Et		35.60 ± 3.14	**29**	i-Pr		60.44 ± 5.84
19	Et		36.40 ± 2.30	**30**	i-Pr		60.48 ± 6.38
20	Et		50.51 ± 4.73	**31**	i-Pr		32.12 ± 1.81
21	Et		155.34 ± 8.21	**32**	i-Pr		57.97 ± 3.87

Table 1 (*continued*)

Compound	R	R_1	[IC_{50} (μM) ± SE][c] HeLa	Compound	R	R_1	[IC_{50} (μM) ± SE][c] HeLa
22	Et		13.55 ± 0.89	**33**	i-Pr		43.19 ± 2.77
23	Et		79.03 ± 1.70	**34**	i-Pr		275.31 ± 14.61
24	Et		135.25 ± 8.60	**35**	i-Pr		200.20 ± 10.98
25	i-Pr		90.51 ± 6.09	**36**	i-Pr		170.03 ± 7.52
26	i-Pr		220.28 ± 10.77	**37**	i-Pr		92.59 ± 4.54
27	i-Pr		11.02 ± 0.68	**38**	i-Pr		135.81 ± 5.87
28	i-Pr		200.21 ± 17.37	**39**	i-Pr		361.41 ± 9.66

*G = Garcinol, a cell permeable HAT inhibitor was used as control (lit IC_{50} = 7 μM) [20].
[a] Determined by MTT assay.
[b] All compounds showed IC_{50} values >300 μM on HCT-15 which has p300 negative.
[c] Values are average of at least three independent experiments ± standard error, conducted in triplicate samples for each concentration.

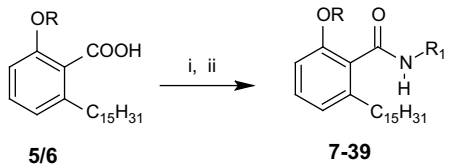
J = 8.6 Hz, 1H), 7.23–7.27 (m, 1H), 7.31–7.36 (m, 1H), 7.52 (s, 1H), 7.80 (dd, *J* = 6.5 and 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.55, 15.28, 23.12, 29.88, 30.10, 31.92, 32.35, 33.72, 64.79, 110.03, 116.84, 117.28, 119.84, 119.97, 122.52, 122.66, 130.93, 135.00, 143.42, 155.88, 157.20, 166.51; GC–MS (DI, *m*/*z*): 503 (M^+).

*4.5.2. N-[4-bromo-3-(trifluoromethyl)phenyl]-2-ethoxy-6-pentadecylbenzamide (**8**)*

Using **5** and 4-bromo-3-(trifluoromethyl)aniline as starting materials, the title compound **8** was obtained as a white solid (3.67 g, Yield = 85%); m.p. 60–63 °C; IR (KBr): 3348, 3271, 3105, 2919, 2850, 1683, 1652, 1582, 1511, 1464, 1407, 1253, 1122, 821, 688 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, *J* = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.37 (t, *J* = 6.9 and 7.0 Hz, 3H), 1.61 (m, 2H), 2.71 (t, *J* = 7.7 Hz, 2H), 4.07 (q, *J* = 6.9 and 7.0 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 8.0 Hz, 1H), 7.65–7.84 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.52, 15.23, 23.10, 29.84, 30.08, 31.89, 32.34, 33.72, 64.85, 110.10, 114.00, 119.23, 119.28, 120.20, 122.79, 124.25, 125.79, 131.11, 135.96, 138.04, 143.62, 155.91, 166.63; GC–MS (DI, *m*/*z*): 597 (M^+).

*4.5.3. N-(4-antipyrinyl)-2-ethoxy-6-pentadecylbenzamide (**9**)*

Using **5** and antipyrine as starting materials, the title compound **9** was obtained as a dark brown solid. (2.53 g, Yield = 85%); m.p.



Scheme 2. Synthetic pathway of **7**–**39**, where R and R_1 are as described in Table 1. Reagents and conditions: (i) $SOCl_2$/Hexane/Reflux for 2 h/Distill off excess $SOCl_2$; (ii) CH_2Cl_2/TEA/Aniline/40 °C for 1 h.

93–96 °C; IR (KBr): 3200, 2920, 2850, 1670, 1625, 1610, 1550, 1465, 1390, 1300, 1261, 1080, 705, 585 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.2$ Hz, 3H), 1.24 (brs, 24H), 1.42 (t, $J = 6.9$ Hz, 3H), 1.62 (m, 2H), 2.40 (s, 3H), 2.71 (t, $J = 7.7$ Hz, 2H), 3.11 (s, 3H), 4.07 (q, $J = 6.9$ Hz, 2H), 6.75 (d, $J = 8.2$ Hz, 1H), 6.84 (d, $J = 7.6$ Hz, 1H), 7.16–7.32 (m, 3H), 7.39–7.50 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.00, 14.55, 15.30, 23.12, 29.75, 30.11, 31.98, 32.35, 33.83, 36.70, 64.60, 109.07, 109.60, 122.15, 124.46, 126.37, 127.08, 129.62, 130.48, 135.28, 142.73, 150.10, 156.04, 162.01, 167.12; GC–MS (DI, *m*/*z*): 561 (M^+).

4.5.4. 2-Ethoxy-6-pentadecyl-N-pyridin-4-ylbenzamide (**10**)

Using **5** and 4-aminopyridine as starting materials, the title compound **10** was obtained as a cream colored solid. (1.92 g, Yield = 80%); m.p. 87–90 °C; IR (KBr): 3232, 3155, 2920, 2850, 1689, 1589, 1527, 1461, 1396, 1307, 1272, 1207, 1114, 995, 829, 763, 594, 540 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.23 (brs, 24H), 1.35 (t, $J = 6.9$ Hz, 3H), 1.59 (m, 2H), 2.70 (t, $J = 7.7$ Hz, 2H), 4.05 (q, $J = 6.9$ Hz, 2H), 6.77 (d, $J = 8.2$ Hz, 1H), 6.89 (d, $J = 7.6$ Hz, 1H), 7.26 (t, $J = 7.6$ Hz, 1H), 7.53 (brs, 2H), 7.95 (s, 1H), 8.48 (brs, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.54, 15.20, 23.10, 29.83, 30.08, 31.85, 32.33, 33.69, 64.69, 109.95, 113.96, 122.61, 125.96, 131.04, 143.30, 145.82, 150.83, 155.87, 167.37; HRMS (*m*/*z*): Calculated for $C_{29}H_{44}N_2O_2$ $(M + H)^+ = 453.2481$, Found = 453.3460.

4.5.5. N-(4-bromo-2-ethylphenyl)-2-ethoxy-6-pentadecylbenzamide (**11**)

Using **5** and 4-bromo-2-ethylaniline as starting materials, the title compound **11** was obtained as a light brown solid (2.61 g, Yield = 88%); m.p. 99–101 °C; IR (KBr): 3232, 2920, 1654, 1593, 1512, 1461, 1392, 1269, 1114, 1076, 810, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.24 (brs, 27H), 1.38 (t, $J = 6.9$ and 7.0 Hz, 3H), 1.64 (m, 2H), 2.67 (m, 4H), 4.08 (q, $J = 6.9$ and 7.0 Hz, 2H), 6.79 (d, $J = 8.2$ Hz, 1H), 6.87 (d, $J = 7.6$ Hz, 1H), 7.23–7.38 (m, 4H), 7.61 (d, $J = 11.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.29, 14.56, 15.27, 23.10, 24.48, 29.80, 30.12, 32.10, 32.36, 33.82, 64.72, 109.91, 119.18, 122.49, 126.10, 127.00, 130.09, 130.67, 131.83, 134.65, 138.35, 143.15, 155.84, 166.95; GC–MS (DI, *m*/*z*): 558 (M^+); Anal. $C_{32}H_{49}NO_2$: C, 80.12; H, 10.30; N, 2.92. Found: C, 79.94; H, 10.25; N, 3.11.

4.5.6. N-[4-chloro-2-(trifluoromethyl)phenyl]-2-ethoxy-6-pentadecylbenzamide (**12**)

Using **5** and 4-chloro-2-(trifluoromethyl)aniline as starting materials, the title compound **12** was obtained as a white solid (2.5 g, Yield = 85%); m.p. 84–86 °C; IR (KBr): 3225, 3082, 2965, 2921, 2850, 1657, 1585, 1506, 1463, 1413, 1264, 1132, 1056, 818, 663 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.25 (brs, 24H), 1.35 (t, $J = 6.9$ Hz, 3H), 1.58 (m, 2H), 2.70 (t, $J = 7.8$ Hz, 2H), 4.08 (q, $J = 6.9$ Hz, 2H), 6.78 (d, $J = 8.3$ Hz, 1H), 6.87 (d, $J = 7.6$ Hz, 1H), 7.28 (t, $J = 8.0$ Hz, 1H), 7.54–7.60 (m, 2H), 7.78 (s, 1H), 8.43 (d, $J = 8.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.52, 14.92, 23.10, 29.83, 30.08, 31.87, 32.34, 33.69, 64.63, 109.83, 121.00, 122.49, 126.06, 126.20, 126.57, 126.65, 130.00, 131.05, 133.29, 134.50, 143.34, 155.90, 167.00; GC–MS (DI, *m*/*z*): 553 (M^+).

4.5.7. 2-Ethoxy-N-(4-methylphenyl)-6-pentadecylbenzamide (**13**)

Using **5** and 4-methylaniline as starting materials, the title compound **13** was obtained as a off-white solid (2.27 g, Yield = 85%); m.p. 78–80 °C; IR (KBr): 3282, 2915, 2846, 1651, 1596, 1523, 1465, 1400, 1257, 1114, 810, 759, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.24 (brs, 24H), 1.36 (t, $J = 6.9$ and 7.0 Hz, 3H), 1.62 (m, 2H), 2.34 (s, 3H), 2.72 (t, $J = 7.8$ Hz, 2H), 4.06 (q, $J = 6.9$ and 7.0 Hz, 2H), 6.77 (d, $J = 8.2$ Hz, 1H), 6.86 (d, $J = 7.6$ Hz, 1H), 7.16 (d, $J = 8.2$ Hz, 2H), 7.21–7.30 (m, 1H), 7.48 (d, $J = 8.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.56, 15.28, 21.33, 23.13, 29.90, 30.11, 31.91, 32.36, 33.71, 64.73, 100.04, 120.52, 122.49, 126.98, 129.96, 130.52, 134.35, 136.01, 143.20, 155.89, 166.36; GC–MS (DI, *m*/*z*): 465 (M^+); Anal. $C_{31}H_{47}NO_2$: C, 79.95; H, 10.17; N, 3.01. Found: C, 79.62; H, 9.92; N, 3.22.

4.5.8. 2-Ethoxy-N-(4-methoxyphenyl)-6-pentadecylbenzamide (**14**)

Using **5** and 4-methoxyaniline as starting materials, the title compound **14** was obtained as a grey solid. (2 g, Yield = 80%); m.p. 84–87 °C; IR (KBr): 3286, 2923, 2850, 1647, 1596, 1515, 1465, 1407, 1249, 1114, 1033, 825, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.29 (brs, 24H), 1.42 (t, $J = 6.9$ Hz, 3H), 1.67 (m, 2H), 2.77 (t, $J = 7.7$ Hz, 2H), 3.85 (s, 3H), 4.10 (q, $J = 6.9$ Hz, 2H), 6.81 (d, $J = 8.2$ Hz, 1H), 6.89 (d, $J = 7.6$ Hz, 1H), 6.94 (d, $J = 8.8$ Hz, 2H), 7.26–7.34 (m, 1H), 7.44 (s, 1H), 7.54 (d, $J = 8.8$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.56, 15.30, 23.12, 29.90, 30.11, 31.92, 32.36, 33.73, 55.94, 64.75, 110.03, 114.65, 122.39, 125.50, 127.20, 130.49, 131.66, 143.17, 155.91, 156.94, 166.37; GC–MS (DI, *m*/*z*): 481 (M^+).

4.5.9. N-(4-aminophenyl)-2-ethoxy-6-pentadecylbenzamide (**15**)

Using **5** and p-phenylenediamine as starting materials, the title compound **15** was obtained as a light brown solid. (2.18 g, Yield = 88%); m.p. 90–93 °C; IR (KBr): 3282, 2920, 2850, 1651, 1593, 1523, 1461, 1423, 1323, 1265, 1114, 1076, 825, 759 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.24 (brs, 24H), 1.37 (t, $J = 7.0$ Hz, 3H), 1.59 (m, 2H), 2.72 (t, $J = 7.8$ Hz, 2H), 3.59 (brs, 2H), 4.05 (q, $J = 7.0$ Hz, 2H), 6.70 (d, $J = 8.6$ Hz, 2H), 6.76 (d, $J = 8.6$ Hz, 1H), 6.85 (d, $J = 7.6$ Hz, 1H), 7.13–7.32 (m, 2H), 7.36 (d, $J = 8.6$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.56, 15.30, 23.12, 29.80, 30.11, 31.92, 32.36, 33.71, 64.73, 110.01, 115.91, 122.40, 122.71, 127.00, 129.50, 130.39, 143.08, 143.82, 155.90, 167.00; HRMS (*m*/*z*): Calculated for $C_{30}H_{46}N_2O_2$ $(M + H)^+ = 467.3637$, Found = 467.3641.

4.5.10. N-(2-aminophenyl)-2-ethoxy-6-pentadecylbenzamide (**16**)

Using **5** and o-phenylenediamine as starting materials, the title compound **16** was obtained as a white solid. (2.1 g, Yield = 85%); m.p. 88–90 °C; IR (KBr): 3267, 2920, 2850, 1647, 1589, 1523, 1461, 1299, 1265, 1114, 1076, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.1$ Hz, 3H), 1.24 (brs, 24H), 1.43 (t, $J = 7.0$ Hz, 3H), 1.60–1.71 (m, 2H), 2.75 (t, $J = 7.8$ Hz, 2H), 4.10 (q, $J = 7.0$ Hz, 2H), 6.76–6.89 (m, 4H), 7.08–7.31 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.54, 15.29, 23.11, 29.78, 30.10, 32.07, 32.34, 33.86, 64.72, 109.68, 117.51, 119.32, 122.40, 123.37, 126.80, 127.08, 128.33, 130.53, 142.60, 142.75, 155.80, 167.21; HRMS (*m*/*z*): Calculated for $C_{30}H_{46}N_2O_2$ $(M + Na)^+ = 489.3457$, Found = 489.3463.

4.5.11. 2-Ethoxy-6-pentadecyl-N-[3-(trifluoromethyl)phenyl]benzamide (**17**)

Using **5** and 3-(trifluoromethyl)aniline as starting materials, the title compound **17** was obtained as a cream colored solid. (2.34 g, Yield = 85%); m.p. 59–61 °C; IR (KBr): 3286, 3066, 2920, 2850, 1658, 1581, 1535, 1469, 1392, 1334, 1172, 1118, 879, 794, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.86 (t, $J = 6.1$ Hz, 3H), 1.23 (brs, 24H), 1.37 (t, $J = 6.9$ Hz, 3H), 1.61 (m, 2H), 2.73 (t, $J = 7.7$ Hz, 2H), 4.08 (q, $J = 6.9$ Hz, 2H), 6.78 (d, $J = 8.2$ Hz, 1H), 6.88 (d, $J = 7.6$ Hz, 1H), 7.28–

7.49 (m, 3H), 7.65 (s, 1H), 7.85 (brs, 2H); GC–MS (DI, *m*/*z*): 519 (M^+).

*4.5.12. 2-Ethoxy-N-(3-ethynylphenyl)-6-pentadecylbenzamide (**18**)*

Using **5** and 3-ethynylaniline as starting materials, the title compound **18** was obtained as a light brown solid (2.27 g, Yield = 90%); m.p. 88–90 °C; IR (KBr): 3281, 3060, 2955, 2920, 2848, 2106, 1654, 1532, 1461, 1401, 1271, 1114, 875, 687 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.36 (t, J = 6.9 Hz, 3H), 1.60 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 3.06 (s, 1H), 4.06 (q, J = 6.9 Hz, 2H), 6.77 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 7.22–7.35 (m, 3H), 7.52 (s, 1H), 7.64 (brd, J = 7.3 Hz, 1H), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.51, 15.25, 23.10, 29.88, 30.09, 31.87, 32.34, 33.70, 64.83, 77.81, 83.69, 110.13, 120.88, 120.61, 123.31, 123.66, 126.57, 128.40, 129.48, 130.74, 138.64, 143.36, 155.90, 166.49; GC–MS (DI, *m*/*z*): 475 (M^+).

*4.5.13. 2-Ethoxy-N-(3-fluorophenyl)-6-pentadecylbenzamide (**19**)*

Using **5** and 3-fluoroaniline as starting materials, the title compound **19** was obtained as a light brown solid (3 g, Yield = 90%); m.p. 65–67 °C; IR (KBr): 3284, 3066, 2919, 2850, 1663, 1599, 1528, 1435, 1257, 1114, 1061, 858, 768, 683 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.36 (t, J = 6.9 and 7.0 Hz, 3H), 1.60 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 4.06 (q, J = 6.9 and 7.0 Hz, 2H), 6.75–6.88 (m, 3H), 7.17–7.34 (m, 3H), 7.58 (d, J = 10.6 Hz, 1H), 7.61 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.51, 15.24, 23.10, 29.85, 30.08, 31.87, 32.34, 33.71, 64.83, 110.13, 111.10, 111.53, 122.65, 126.44, 130.37, 130.55, 130.81, 140.02, 140.24, 143.42, 155.90, 166.50; GC–MS (DI, *m*/*z*): 469 (M^+).

*4.5.14. N-(3-bromophenyl)-2-ethoxy-6-pentadecylbenzamide (**20**)*

Using **5** and 3-bromoaniline as starting materials, the title compound **20** was obtained as a cream color solid (2.59 g, Yield = 92%); m.p. 75–77 °C; IR (KBr): 3271, 2920, 2846, 1654, 1585, 1523, 1396, 1269, 1114, 1076, 860, 759, 682 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6 Hz, 3H), 1.23 (brs, 24H), 1.37 (t, J = 6.9 and 7.0 Hz, 3H), 1.61 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 4.07 (q, J = 6.9 and 7.0 Hz, 2H), 6.77 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 7.17–7.31 (m, 3H), 7.47–7.53 (m, 2H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.17, 14.88, 22.74, 29.50, 29.72, 31.50, 31.97, 33.31, 64.42, 109.69, 118.30, 120.90, 122.24, 122.76, 125.96, 127.24, 130.35, 130.45, 139.50, 143.00, 155.49, 166.11; GC–MS (DI, *m*/*z*): 529 (M^+).

*4.5.15. 2-Ethoxy-N-(3-iodophenyl)-6-pentadecylbenzamide (**21**)*

Using **5** and 3-iodoaniline as starting materials, the title compound **21** was obtained as a cream colored solid (2.76 g, Yield = 90%); m.p. 83–85 °C; IR (KBr): 3263, 2920, 2846, 1654, 1577, 1519, 1461, 1400, 1269, 1114, 1076, 860, 759 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.23 (brs, 24H), 1.37 (t, J = 6.9 and 7.0 Hz, 3H), 1.61 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 4.08 (q, J = 6.9 and 7.0 Hz, 2H), 6.77 (d, J = 8.2 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.27 (t, J = 8.2 and 7.6 Hz, 1H), 7.45–7.58(m, 3H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.12, 14.85, 22.69, 29.46, 29.68, 31.45, 31.93, 33.27, 64.42, 94.24, 109.70, 119.02, 122.21, 126.00, 128.46, 130.41, 130.50, 133.27, 139.36, 142.98, 155.47, 166.01; GC–MS (DI, *m*/*z*): 577 (M^+).

*4.5.16. 2-Ethoxy-N-(3-nitrophenyl)-6-pentadecylbenzamide (**22**)*

Using **5** and 3-nitroaniline as starting materials, the title compound **22** was obtained as a light yellow solid (2.32 g, Yield = 88%); m.p. 94–96 °C; IR (KBr): 3282, 2920, 2846, 1654, 1535, 1465, 1400, 1346, 1257, 1110, 871, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (t, J = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.38 (t, J = 6.9 Hz, 3H), 1.60 (m, 2H), 2.73 (t, J = 7.7 Hz, 2H), 4.09 (q, J = 6.9 Hz, 2H), 6.80 (d, J = 8.2 Hz, 1H), 6.89 (d, J = 7.5 Hz, 1H), 7.30 (t, J = 8.2 Hz, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.79 (s, 1H), 7.99 (d, J = 8.1 Hz, 1H), 8.06 (d, J = 7.8 Hz, 1H), 8.40 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.53, 15.26, 23.11, 29.87, 30.09, 31.90, 32.34, 33.74, 64.82, 110.06, 114.89, 119.20, 122.75, 125.80, 125.94, 130.29, 131.13, 139.72, 143.59, 149.08, 155.93, 166.83; Anal. $C_{30}H_{44}N_2O_4$: C, 72.55; H, 8.93; N, 5.64. Found: C, 72.65; H, 9.05; N, 5.54.

*4.5.17. 2-Ethoxy-N-(2-ethylphenyl)-6-pentadecylbenzamide (**23**)*

Using **5** and 2-ethylaniline as starting materials, the title compound **23** was obtained as a off-white solid (2.42 g, Yield = 95%); m.p. 77–79 °C; IR (KBr): 3244, 2923, 2846, 1647, 1523, 1461, 1392, 1265, 1114, 1076, 798, 744 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.24 (brs, 27H), 1.39 (t, J = 6.9 and 7.0 Hz, 3H), 1.62 (m, 2H), 2.70 (m, 4H), 4.09 (q, J = 6.9 and 7.0 Hz, 2H), 6.79 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 7.13–7.30 (m, 5H), 7.88 (d, J = 7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.10, 14.20, 14.86, 22.70, 24.24, 29.37, 29.63, 31.69, 31.94, 33.40, 64.33, 109.54, 121.99, 124.41, 125.80, 126.70, 128.60, 130.03, 135.12, 136.09, 140.00, 142.67, 155.49, 166.54; GC–MS (DI, *m*/*z*): 479 (M^+).

*4.5.18. 2-Ethoxy-N-(2-fluorophenyl)-6-pentadecylbenzamide (**24**)*

Using **5** and 2-fluoroaniline as starting materials, the title compound **24** was obtained as a cream colored solid (2.37 g, Yield = 95%); m.p. 65–68 °C; IR (KBr): 3244, 2920, 2846, 1658, 1593, 1523, 1461, 1392, 1265, 1110, 1072, 914, 748, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.24 (brs, 24H), 1.36 (t, J = 6.9 and 7.0 Hz, 3H), 1.61 (m, 2H), 2.75 (t, J = 7.7 Hz, 2H), 4.08 (q, J = 6.9 and 7.0 Hz, 2H), 6.78 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 7.07–7.31 (m, 4H), 7.86 (s, 1H), 8.48 (t, J = 7.4 and 7.9 Hz, 1H); HRMS (*m*/*z*): Calculated for $C_{30}H_{44}NO_2F$ $(M+H)^+$ = 470.3434, Found = 470.3419.

*4.5.19. N-(3-chloro-4-fluorophenyl)-2-isopropoxy-6-pentadecylbenzamide (**25**)*

Using **6** and 3-chloro-4-fluoroaniline as starting materials, the title compound **25** was obtained as a brown solid (2.63 g, Yield = 90%); m.p. 77–80 °C; IR (KBr): 3271, 2920, 2846, 1654, 1593, 1500, 1461, 1384, 1269, 1114, 810, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.56 (brs, 2H), 2.72 (t, J = 7.7 Hz, 2H), 4.54 (m, 1H), 6.80 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 7.12 (t, J = 8.6 Hz, 1H), 7.22–7.38 (m, 2H), 7.53 (s, 1H), 7.80 (dd, J = 6.5 and 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.10, 22.18, 22.68, 29.45, 29.67, 31.46, 31.92, 33.34, 71.67, 111.83, 116.43, 116.87, 119.35, 119.48, 122.06, 122.46, 130.41, 134.84, 143.34, 154.53, 157.20, 166.13; GC–MS (DI, *m*/*z*): 517 (M^+).

*4.5.20. N-(4-antipyrinyl)-2-isopropoxy-6-pentadecylbenzamide (**26**)*

Using **6** and antipyrine as starting materials, the title compound **26** was obtained as a brown solid (2.36 g, Yield = 80%); m.p. 105–107 °C; IR (KBr): 3210, 2923, 2850, 1655, 1630, 1593, 1465, 1390, 1295, 1261, 1114, 1022, 702, 590 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): 0.87 (t, J = 6.2 Hz, 3H), 1.24 (brs, 24H), 1.36 (d, J = 6 Hz, 6H), 1.60 (m, 2H), 2.40 (s, 3H), 2.71 (t, J = 7.7 Hz, 2H), 3.11 (s, 3H), 4.58 (s, 1H), 6.77 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 7.7 Hz, 1H), 7.13 (s, 1H), 7.19–7.32 (m, 2H), 7.43–7.50 (m, 4H); GC–MS (DI, *m*/*z*): 575 (M^+); Anal. $C_{36}H_{53}N_3O_3$: C, 75.09; H, 9.28; N, 7.30. Found: C, 74.99; H, 9.43; N, 7.69.

*4.5.21. 2-Isopropoxy-6-pentadecyl-N-pyridin-4-ylbenzamide (**27**)*

Using **6** and 4-aminopyridine as starting materials, the title compound **27** was obtained as a light brown solid (1.91 g, Yield = 80%); m.p. 70–73 °C; IR (KBr): 3280, 3140, 2916, 2850, 1666, 1589, 1525, 1465, 1400, 1269, 1110, 850 cm^{-1}; ^1H NMR ($CDCl_3$,

200 MHz): δ 0.86 (t, J = 6.6 Hz, 3H), 1.24 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.58 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 4.56 (m, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 7.6 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.63 (brs, 2H), 8.12 (s, 1H), 8.51 (brs, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.12, 22.15, 22.69, 29.44, 29.68, 31.48, 31.93, 33.43, 71.72, 111.69, 114.00, 122.69, 126.15, 130.84, 143.54, 146.56, 148.89, 154.66, 167.03; HRMS (*m*/*z*): Calculated for $C_{30}H_{46}N_2O_2$ $(M+H)^+ = 467.3637$, Found = 467.3647.

4.5.22. N-(4-bromo-2-ethylphenyl)-2-isopropoxy-6-pentadecylbenzamide (**28**)

Using **6** and 4-bromo-2-ethylaniline as starting materials, the title compound **28** was obtained as a light grey solid (2.49 g, Yield = 85%); m.p. 77–80 °C; IR (KBr): 3236, 2920, 2846, 1651, 1600, 1510, 1461, 1400, 1265, 1114, 820, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.24 (brs, 27H), 1.31 (d, J = 6.0 Hz, 6H), 1.60 (m, 2H), 2.67 (m, 4H), 4.56 (m, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 7.22–7.38 (m, 4H), 7.84 (d, J = 9.18 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.84, 14.10, 22.13, 22.67, 23.97, 29.34, 29.66, 31.61, 31.90, 33.37, 71.25, 111.31, 118.59, 122.01, 125.41, 127.34, 129.63, 130.09, 131.34, 134.25, 137.68, 142.82, 154.40, 166.56; GC–MS (DI, *m*/*z*): 572 (M^+).

4.5.23. 2-Isopropoxy-N-(4-methoxyphenyl)-6-pentadecylbenzamide (**29**)

Using **6** and 4-methoxyaniline as starting materials, the title compound **29** was obtained as a light grey solid (2.28 g, Yield = 90%); m.p. 76–78 °C; IR (KBr): 3300, 3100, 2920, 2850, 1643, 1508, 1461, 1400, 1242, 1118, 1037, 840, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, J = 6.1 Hz, 3H), 1.23 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.61 (m, 2H), 2.74 (t, J = 7.7 Hz, 2H), 3.81 (s, 3H), 4.54 (m, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 6.90 (d, J = 8.0 Hz, 2H), 7.24 (t, J = 8.0 Hz, 1H), 7.40 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.09, 22.15, 22.66, 29.45, 29.65, 31.43, 31.89, 33.30, 55.47, 71.51, 111.79, 114.20, 121.89, 122.20, 127.61, 129.93, 131.23, 143.01, 154.47, 156.45, 165.97; GC–MS (DI, *m*/*z*): 495 (M^+).

4.5.24. 2-Isopropoxy-N-(4-methylphenyl)-6-pentadecylbenzamide (**30**)

Using **6** and 4-methylaniline as starting materials, the title compound **30** was obtained as a cream colored solid (2.21 g, Yield = 90%); m.p. 88–90 °C; IR (KBr): 3320, 3100, 2920, 2850, 1651, 1596, 1527, 1461, 1400, 1261, 1114, 850, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.92 (t, J = 6.2 Hz, 3H), 1.28 (brs, 24H), 1.34 (d, J = 6.0 Hz, 6H), 1.67 (m, 2H), 2.38 (s, 3H), 2.78 (t, J = 7.7 Hz, 2H), 4.57 (m, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 7.24 (t, J = 8.4 Hz, 1H), 7.27 (d, J = 8.4 Hz, 2H), 7.49 (s, 1H), 7.51 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.09, 20.86, 22.15, 22.67, 29.45, 29.65, 31.42, 31.90, 33.31, 71.60, 111.90, 120.06, 122.27, 127.71, 129.51, 129.96, 133.84, 135.61, 143.08, 154.49, 165.96; GC–MS (DI, *m*/*z*): 479 (M^+).

4.5.25. N-(4-aminophenyl)-2-isopropoxy-6-pentadecylbenzamide (**31**)

Using **6** and p-phenylenediamine as starting materials, the title compound **31** was obtained as a light brown solid (2.1 g, Yield = 88%); m.p. 69–71 °C; IR (KBr): 3294, 2920, 2850, 1651, 1593, 1519, 1465, 1423, 1323, 1265, 1114, 1026, 825 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.24 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.59–1.62 (m, 2H), 2.73 (t, J = 7.8 Hz, 2H), 4.53 (m, 1H), 6.70 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 7.22–7.26 (m, 1H), 7.33 (s, 1H), 7.35 (d, J = 8.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.13, 22.20, 22.70, 29.38, 29.69, 31.46, 31.93, 33.33, 71.53, 111.84, 115.49, 122.10, 122.24, 127.50, 129.49, 129.85, 142.98, 143.38, 154.49, 165.96; GC–MS (DI, *m*/*z*): 480 (M^+); Anal. $C_{31}H_{48}N_2O_2$: C, 77.45; H, 10.06; N, 5.83, Found: C, 77.40; H, 10.35; N, 5.99.

4.5.26. N-(2-aminophenyl)-2-isopropoxy-6-pentadecylbenzamide (**32**)

Using **6** and o-phenylenediamine as starting materials, the title compound **32** was obtained as a white solid (2.0 g, Yield = 85%); m.p. 76–78 °C; IR (KBr): 3247, 2920, 2846, 1647, 1585, 1523, 1461, 1384, 1269, 1114, 1026, 744 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, J = 6.2 Hz, 3H), 1.24 (brs, 24H), 1.36 (d, J = 6.0 Hz, 6H), 1.63 (m, 2H), 2.75 (t, J = 7.9 Hz, 2H), 4.08 (brs, 2H), 4.62 (m, 1H), 6.78–6.88 (m, 4H), 7.07–7.30 (m, 4H); HRMS (*m*/*z*): Calculated for $C_{31}H_{48}N_2O_2$ $(M+Na)^+ = 503.3613$, Found = 503.3630.

4.5.27. N-(3-ethynylphenyl)-2-isopropoxy-6-pentadecylbenzamide (**33**)

Using **6** and 3-ethynylaniline as starting materials, the title compound **33** was obtained as a cream colored solid (2.25 g, Yield = 90%); m.p. 70–72 °C; IR (KBr): 3283, 3061, 2920, 2849, 2107, 1655, 1531, 1464, 1402, 1264, 1115, 873, 688 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.58 (m, 2H), 2.73 (t, J = 7.7 Hz, 2H), 3.07 (s, 1H), 4.54 (m, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 7.22–7.36 (m, 3H), 7.53 (s, 1H), 7.62 (brd, J = 7.3 Hz, 1H), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.17, 22.21, 22.74, 29.51, 29.72, 31.50, 31.97, 33.37, 71.65, 77.45, 83.32, 111.82, 120.43, 122.39, 123.00, 123.21, 127.20, 127.99, 129.12, 130.29, 138.26, 143.27, 154.53, 166.19; GC–MS (DI, *m*/*z*): 489 (M^+).

4.5.28. 2-Isopropoxy-6-pentadecyl-N-[3-(trifluoromethyl)phenyl]benzamide (**34**)

Using **6** and 3-trifluoromethyl aniline as starting materials, the title compound **34** was obtained as a white solid (2.18 g, Yield = 88%); m.p. 54–56 °C; IR (KBr): 3282, 2920, 2846, 1658, 1610, 1535, 1460, 1400, 1334, 1250, 1118, 800, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.2 Hz, 3H), 1.23 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.60 (m, 2H), 2.74 (t, J = 7.7 Hz, 2H), 4.55 (m, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.87 (d, J = 7.5 Hz, 1H), 7.25–7.52 (m, 3H), 7.67 (s, 1H), 7.82 (d, J = 9.8 Hz, 1H), 7.84 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.16, 22.17, 22.73, 29.49, 29.71, 31.51, 31.97, 33.37, 71.68, 111.79, 116.49, 120.81, 122.46, 122.90, 123.00, 126.86, 129.64, 130.46, 131.50, 138.76, 143.39, 154.56, 166.34; GC–MS (DI, *m*/*z*): 533 (M^+).

4.5.29. N-(3-bromophenyl)-2-isopropoxy-6-pentadecylbenzamide (**35**)

Using **6** and 3-bromoaniline as starting materials, the title compound **35** was obtained as a cream colored solid. (2.65 g, Yield = 95%); m.p. 61–64 °C; IR (KBr): 3274, 2920, 2846, 1654, 1585, 1523, 1465, 1410, 1265, 1114, 860, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.1 Hz, 3H), 1.24 (brs, 24H), 1.31 (d, J = 6.0 Hz, 6H), 1.55 (brs, 2H), 2.72 (t, J = 7.7 Hz, 2H), 4.54 (m, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 7.17–7.30 (m, 3H), 7.45–7.61 (m, 2H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.56, 22.59, 23.13, 29.88, 30.11, 31.88, 32.36, 33.75, 72.04, 112.18, 118.61, 121.00, 122.81, 123.10, 127.61, 128.90, 130.70, 130.76, 139.90, 143.72, 154.92, 166.55; GC–MS (DI, *m*/*z*): 544 (M^+).

4.5.30. N-(3-iodophenyl)-2-isopropoxy-6-pentadecylbenzamide (**36**)

Using **6** and 3-iodoaniline as starting materials, the title compound **36** was obtained as a light brown solid (2.88 g, Yield = 95%); m.p. 66–68 °C; IR (KBr): 3271, 2920, 2846, 1654, 1581, 1523, 1469, 1400, 1268, 1140, 890, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, J = 6.2 Hz, 3H), 1.24 (brs, 24H), 1.31 (d,

$J = 6.0$ Hz, 6H), 1.56 (brs, 2H), 2.72 (t, $J = 7.7$ Hz, 2H), 4.53 (m, 1H), 6.80 (d, $J = 8.3$ Hz, 1H), 6.86 (d, $J = 7.6$ Hz, 1H), 7.08 (t, $J = 8.0$ Hz, 1H), 7.26 (t, $J = 7.6$ and 8.3 Hz, 1H), 7.45–7.56 (m, 3H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.12, 22.15, 22.69, 29.45, 29.67, 31.44, 31.92, 33.29, 71.60, 94.50, 111.75, 118.93, 122.36, 127.00, 128.39, 130.31, 130.51, 133.22, 138.50, 143.25, 154.50, 166.50; GC–MS (DI, *m*/*z*): 591 (M^+).

4.5.31. 2-Isopropoxy-N-(3-nitrophenyl)-6-pentadecylbenzamide (**37**)

Using **6** and 3-nitroaniline as starting materials, the title compound **37** was obtained as a light brown solid (2.19 g, Yield = 84%); m.p. 87–90 °C; IR (KBr): 3286, 2920, 2846, 1654, 1593, 1535, 1465, 1410, 1360, 1253, 1118, 870, 732 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.2$ Hz, 3H), 1.23 (brs, 24H), 1.32 (d, $J = 6.0$ Hz, 6H), 1.62 (m, 2H), 2.74 (t, $J = 7.7$ Hz, 2H), 4.55 (m, 1H), 6.82 (d, $J = 8.3$ Hz, 1H), 6.84 (d, $J = 7.7$ Hz, 1H), 7.25–7.57 (m, 1H), 7.53 (t, $J = 8.1$ Hz, 1H), 7.81 (s, 1H), 7.96–8.05 (m, 2H), 8.41 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.11, 22.18, 22.69, 29.46, 29.67, 31.48, 31.93, 33.39, 71.72, 111.79, 114.41, 118.78, 122.56, 125.44, 126.39, 129.90, 130.65, 139.32, 143.54, 148.74, 154.57, 166.43; GC–MS (DI, *m*/*z*): 510 (M^+).

4.5.32. N-(2-ethylphenyl)-2-isopropoxy-6-pentadecylbenzamide (**38**)

Using **6** and 2-ethylaniline as starting materials, the title compound **38** was obtained as a off-white solid (2.27 g, Yield = 90%); m.p. 88–91 °C; IR (KBr): 3283, 3061, 2920, 2849, 2107, 1655, 1531, 1464, 1402, 1264, 1115, 873, 688 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.2$ Hz, 3H), 1.24 (brs, 27H), 1.32 (d, $J = 6.0$ Hz, 6H), 1.64 (m, 2H), 2.70 (m, 4H), 4.58 (m, 1H), 6.80 (d, $J = 8.3$ Hz, 1H), 6.86 (d, $J = 7.6$ Hz, 1H), 7.13–7.30 (m, 5H), 7.89 (d, $J = 7.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.20, 14.23, 22.20, 22.72, 24.21, 29.40, 29.71, 31.68, 31.96, 33.43, 71.25, 111.34, 121.96, 124.25, 125.73, 126.71, 127.85, 128.59, 129.93, 135.17, 135.95, 142.81, 154.51, 166.65; GC–MS (DI, *m*/*z*): 493 (M^+).

4.5.33. N-(2-fluorophenyl)-2-isopropoxy-6-pentadecylbenzamide (**39**)

Using **6** and 2-fluoroaniline as starting materials, the title compound **39** was obtained as a white solid (2.35 g, Yield = 95%); m.p. 54–57 °C; IR (KBr): 3244, 2920, 2846, 1658, 1610, 1520, 1461, 1400, 1265, 1118, 920, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, $J = 6.0$ Hz, 3H), 1.24 (brs, 24H), 1.32 (d, $J = 6.0$ Hz, 6H), 1.59 (m, 2H), 2.75 (t, $J = 7.7$ Hz, 2H), 4.58 (m, 1H), 6.80 (d, $J = 8.2$ Hz, 1H), 6.86 (d, $J = 7.6$ Hz, 1H), 7.05–7.30 (m, 4H), 7.88 (s, 1H), 8.47 (t, $J = 8.0$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.09, 21.98, 22.68, 29.42, 29.65, 31.47, 31.92, 33.43, 111.28, 114.58, 114.96, 121.99, 122.24, 124.00, 124.14, 124.61, 126.77, 130.30, 143.49, 154.55, 166.08; GC–MS (DI, *m*/*z*): 483 (M^+).

4.6. Biological activity

4.6.1. In vitro growth inhibition assay

The cells were maintained in Dulbecco's Modified Eagle's Medium (Sigma–Aldrich Inc., USA) supplemented with 10% fetal bovine serum (Sigma Chemical Co., USA) in a CO_2 incubator. The cytotoxicity of the compounds was measured by MTT assay [22]. The cells were plated in a 96-well plate at the density of 10,000 cells per well (HeLa) and 10,000 cells per well (HCT-15). After 24 h, the cells were treated with different concentrations of the compound (1 μM to 300 μM, final concentration of DMSO < 1%). The cells were later incubated for 72 h. The cytotoxicity was measured by adding 5 mg/mL of MTT (Sigma–Aldrich Inc., USA) to each well and incubated for another 3 h. The purple formazan crystals were dissolved by adding 100 μl of DMSO to each well. The absorbance was read at 570 nm in a spectrophotometer [Spectra Max 340]. The cell death was calculated as follows:

$$\text{Cell death} = 100 - [(\text{test absorbance}/\text{control absorbance}) \times 100]$$

The test result is expressed as the concentration of a test compound which inhibits the cell growth by 50% (IC_{50}).

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.01.033.

References

[1] J.H.P. Tyman, Chem. Soc. Rev. 8 (1979) 499–537.
[2] I. Kubo, H. Muroi, M. Himejima, Y. Yamigiwa, J. Agric. Food Chem. 41 (1993) 1016–1019.
[3] J.L. Gillerman, N.J. Walsh, N.K. Werner, H. Schlenk, Can. J. Microbiol. 15 (1969) 1219–1223.
[4] S.V. Shoba, C.S. Ramadoss, B. Ravindranath, J. Nat. Prod. 57 (1994) 1755–1757.
[5] T.J. Ha, I. Kubo, J. Agric. Food Chem. 53 (2005) 4350–4354.
[6] R. Paramashivappa, P. Phanikumar, P.V. Subba Rao, A. Srinivasa Rao, J. Agric. Food Chem. 50 (2002) 7709–7713.
[7] P. Phanikumar, S.C. Stotz, R. Paramashivappa, M. aBeedle, G.W. Zamponi, A. Srinivasa Rao, Mol. Pharmacol. 61 (2002) 649–658.
[8] B. Narayana Swamy, T.K. Suma, G. Venkateswara Rao, G. Chandrasekara Reddy, Eur. J. Med. Chem. 42 (2007) 422–424.
[9] I. Kubo, M. Ochi, P.C. Viera, S. Komatsu, J. Agric. Food Chem. 41 (1993) 1012–1015.
[10] M. Toyomizu, K. Okamoto, Y. Akiba, T. Nakatsu, T. Konishi, Biochim. Biophys. Acta 1558 (2002) 54–62.
[11] K.S. Nagabhushana, S.V. Shobha, B. Ravindranath, J. Nat. Prod. 58 (1995) 807–810.
[12] K. Balasubramanyam, V. Swaminathan, R. Anupama, T.K. Kundu, J. Biol. Chem. 278 (2003) 19134–19140 and references cited therein.
[13] K. Mantelingu, A.H. Kishore, K. Balasubramanyam, G.V. Kumar, M. Altaf, S.N. Swamy, R. Selvi, C. Das, C. Narayana, K.S. Rangappa, T.K. Kundu, J. Phys. Chem. B 111 (2007) 4527–4534.
[14] A. Hari Kishore, B.M. Vedamurthy, K. Mantelingu, S. Agrawal, B.A. Ashok Reddy, S. Roy, K.S. Rangappa, T.K. Kundu, J. Med. Chem. 51 (2008) 792–797.
[15] B. Sung, M.K. Pandey, K.S. Ahn, T. Yi, M.M. Chaturvedi, M. Liu, B.B. Aggarwal, Blood 111 (2008) 4880–4891.
[16] L. Cui, J. Miao, T. Furuya, Q. Fan, X. Li, P.K. Rathod, X.Z. Su, L. Cui, Eukaryotic Cell 7 (2008) 1200–1210.
[17] G. Sbardella, S. Castellano, C. Vicidomini, D. Rotili, A. Nebbioso, M. Miceli, L. Altucci, A. Mai, Bioorg. Med. Chem. Lett. 18 (2008) 2788–2792.
[18] G. Marzio, M. Tyagi, M.I. Gutierrez, M. Giacca, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 13519–13524.
[19] R. Paramashivappa, P. Phanikumar, P.J. Vithayathil, A. Srinivasa Rao, J. Agric. Food Chem. 49 (2001) 2548–2551.
[20] K. Balasubramanyam, M. Altaf, R.A. Varier, V. Swaminathan, A. Ravindran, P.P. Sadhale, T.K. Kundu, J. Biol. Chem. 279 (2004) 33716–33726.
[21] L.S. Kiong, J.H.P. Tyman, J. Chem. Soc., Perkin Trans. 1 (1981) 1942–1952.
[22] T. Mossman, J. Immunol. Methods 65 (1983) 55–63.