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Research Article

Synthesis, Characterization and Cytotoxic Studies of Benzamide Derivatives of Anacardic Acid using Human Liver Cancer Cells

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ABSTRACT

Naturally occurring anacardic acid based benzamides were reported to show anti-inflammatory and anticancer activities, where we synthesized and characterized by NMR and HRMS analysis and also tested a series of anacardic acid based benzamides and evaluated against the proliferation of human liver cancer cells (HepG2). Among the tested compounds, 6j-m showed good inhibitory activity against HepG2 cells with IC₅₀ values ranging from 78.2-91.9 μ M. In conclusion, we herein reported the newer series of an academic acid benzamides for the first time.

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Introduction

Cancer is a deadly disease in the world with the highest mortal rate [1]. Based on the source and origin of the cancer cells, they are classified into carcinoma, sarcoma, lymphoma or leukemia [2]. Uncontrolled cell proliferation, anti-apoptosis, angiogenesis, metastasis, and genomic instability are the most common features of all types of cancers [3]. There are many ways to treat cancer such as chemotherapy, radiation therapy, surgery, stem cell transplant, immunotherapy, precision medicine etc. Among them, in chemotherapy small molecules are used to treat cancer via different mechanisms.

Basically, anacardic acid is most active against *Streptococcus mutants* and *Cutibacterium acnes* [4]. Besides, it has also showed anticancer activity by inhibition of the histone acetyltransferase in human liver cancer cells, phosphodiesterase-5 inhibition, cytotoxic activity, RANKL-induced osteoclastogenesis inhibitors, fibroblast-like synoriocycle proliferation suppressor and ameliorates collagen-induced

arthritis, induces cell apoptosis of prostate cancer through autophagy by ER stress/DAPK3/Akt signalling pathway, attenuates phenylephrine-induced cardiac hypertrophy, polyketide biosynthetic pathway modulator in endopytic fungus-Anteaglonium Sp. FL0768, antidepressant, regulates Wnt4 expression in chicken, VEGF-induced angiogenesis inhibitor, excellent paclitaxel transporter in breast cancer therapy, and anti-convulsant activities [5-17]. Thus, in continuation of our work on developing new anacardic acid based benzamides, we designed and synthesized new compounds, which contains alkoxy benzamides as additive groups that acted as cytotoxic agents in the previous reports [18-26].

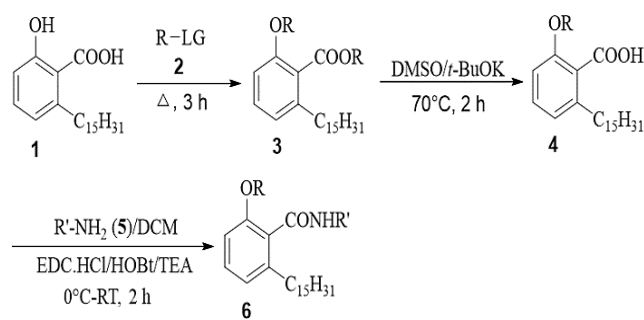
Results and Discussion

I Chemistry

Initially, anacardic acid was isolated according to reported protocol. Further, the liquid was catalytically hydrogenated and purified using column chromatography to obtain pure anacardic acid. Anacardic acid 1

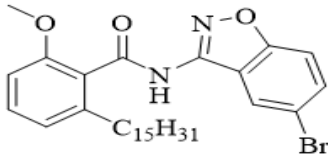
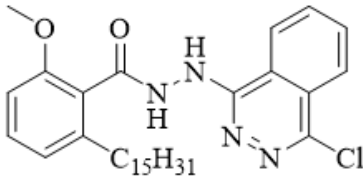
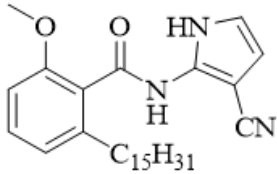
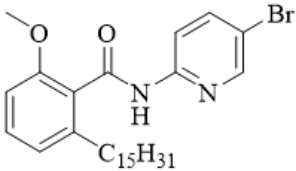
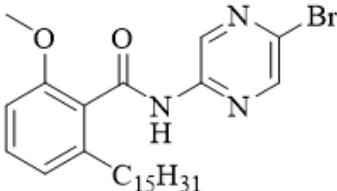
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was converted into its corresponding ester 3 by treatment with dimethyl sulphate (2a), diethyl sulphate (2b) and isopropyl chloride (2c). Meanwhile, phenolic-OH was alkylated by these reagents into respective alkoxy groups according to reported methods [6, 28, 29]. Later, alkylated carboxylic acid group was selectively cleaved into carboxylic acid 4 by heating with potassium tertiary butoxide in DMSO. Coupling of carboxylic acid 4 with various amines 5 in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), hydroxyl benzotriazole (HOBt) and base triethylamine furnished final products: amides of anacardic acid 6. The structures and yields of all title compounds are given in (Table 1). The schematic representation of the synthesis of title compounds were shown in Scheme 1. Further, these compounds were completely characterized by ^1H NMR, ^{13}C NMR and IR spectroscopic techniques.

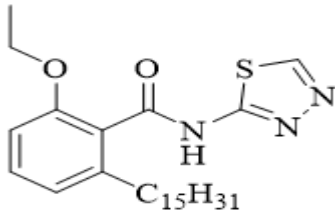
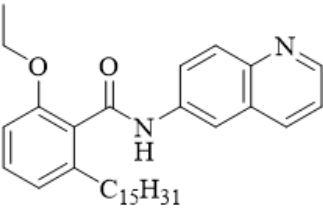
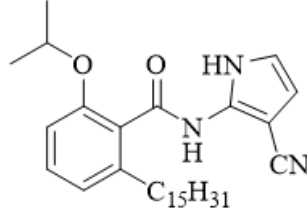


Scheme 1: Synthesis of amides of anacardic acid.

Table 1: Structures of synthesized benzamide derivatives with yields and IC_{50} values.

Entry	Product	Yield (%)	IC_{50} values (μM) on HepG2
1	 6a	76	<100
2	 6b	74	<100
3	 6c	75	<100
4	 6d	74	<100
5		70	<100

6	6e 6f	75	<100
7	6g	75	<100
8	6h	75	<100
9	6i	75	<100
10	6j	70	86.8
11	6k	75	78.2

12	6k 	81.6
13	6l 	91.9
14	6m 	<100

II Biology

Anacardic Acid Derivatives Decreased the Proliferation of Human Liver Cancer Cells (HepG2)

Using a basic MTT colorimetric assay, we carried out the anti-proliferative effect of anacardic acid derivatives on Human liver cancer cells (HepG2) and it was found that the compounds 6j, 6k, 6l and 6m significantly decreased the proliferation of HepG2 cells with an IC_{50} values of 86.8, 78.2, 81.6 and 91.9 μ M respectively (Table 1). The structure activity relationship studies indicated that anacardic acids with methoxy substituents on benzene ring are inactive against HepG2 cells (<100 μ M). While, their analogues with ethoxy substituent are active (6j-m) with aforementioned IC_{50} values. While isobenzofuranone, phenylthiadiazole, 3-bromopyridine and 3-cyano-pyrrole were inactive (<100 μ M). Finally, anacardic acid derivative with isopropoxy group is also inactive (<100 μ M).

Experimental Section

I General Methods/ Materials/Instrumentation

CNSL was purchased from commercial source and all other reagents are of analytical grade purchased from Aldrich. The compounds were analysed and confirmed through 1H and ^{13}C NMR spectra using $CDCl_3$ /DMSO- d_6 as a solvent at 400 & 100MHz respectively on a Bruker A.G Spectrometer. Chemical Shifts were recorded using tetramethyl silane (TMS) as an internal standard. Nicolet avatar 320 FT-IR

spectrometer was used to record IR spectra. Acro Steel Pvt. Ltd. melting point apparatus was used to determine melting points.

II General Procedure for the Preparation of Final Compounds 6

2-alkoxy-6-pentadecylbenzoic acid **4** (0.6 mmol) was dissolved in dichloromethane (5 mL) using a magnetic stirrer and cooled to 0°C. To that EDC.HCl (0.6 mmol), HOBT (0.6 mmol) and amine **5** (0.6 mmol) were added. The completion of the reaction was monitored through TLC and neutralized with dilute HCl (25 mL) extracted with ethyl acetate (25 mL x 3), washed with water and brine (25 mL) and dried over sodium sulphate and concentrated. The obtained crude title compounds were purified by column chromatography using silica gel (60-120 mesh) and 0-5% ethyl acetate in petroleum ether as an effluent to procure white solids of pure title compounds.

III N-(5-Bromobenzo(d) isoxazol-3-yl)-2-methoxy-6pentadecyl benzamide (6a)

White solid. 76%. m.p. 85-87 °C; 1H NMR (DMSO, 400MHz): δ 0.827 (t, J =7.2 Hz, 3H, CH_3), 1.17 (m, 24H, $(CH_2)_{12}$), 1.61 (m, 2H, CH_2), 2.70 (m, 2H, CH_2), 4.00 (s, 3H, OCH_3), 7.0 Hz (d, J =7.6 Hz, 1H, Ar-H), 7.15 (d, J =8.4 Hz, 1H, Ar-H), 7.55 (m, 2H, Ar-H), 7.65 (d, J =8.0 Hz, 1H, Ar-H), 7.7 (t, J =7.6 Hz, 1H, Ar-H), 8.1 (d, J =8.4 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100MHz): δ 14.0, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 56.2, 108.6, 108.7, 120.3, 120.5, 120.8, 122.0, 124.7, 128.5, 128.7, 132.6,

143.6, 143.6, 157.5, 164.3; HRMS (ESI-TOF) *m/z*: [C₃₀H₄₁BrN₂O₃], 557.2379 and 559.2358

IV N-(4-Chlorophthalazin-1-yl)-2-methoxy-6-pentadecyl benzohydrazide (6b)

White solid. 74%. m.p. 84-86 °C; ¹H NMR (DMSO, 400 MHz): δ 0.81 (t, *J*=6.4 Hz, 3H, CH₃), 1.17 (m, 24H, (CH₂)₁₂), 1.61 (m, 2H, CH₂), 2.7 (t, *J*=7.6 Hz, 2H, CH₂), 4.00 (s, 3H, OCH₃), 6.56 (s, 1H, Ar-H) 7.0 Hz (d, *J*=7.6 Hz, 1H, Ar-H), 7.15 (d, *J*=8.4 Hz, 1H, Ar-H), 7.55 (m, 2H, Ar-H, NH), 7.65 (d, *J*=8.0 Hz, 1H, Ar-H), 7.7 (t, *J*=7.6 Hz, 1H, Ar-H), 8.18 (m, 2H, Ar-H, NH); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 56.2, 108.6, 108.7, 120.3, 120.5, 120.8, 122.0, 124.7, 128.5, 128.7, 132.6, 143.6, 143.6, 157.5, 158.0, 164.3; HRMS (ESI-TOF) *m/z*: C₃₁H₄₄ClN₄O₂, 539.3153.

V N-(3-Cyano-1H-pyrrol-2-yl)-2-methoxy-6-pentadecyl benzamide (6c)

White solid. 75%. m.p. 82-85 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.8 Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.70 (m, 2H, CH₂), 2.7 (t, *J*=7.6 Hz, 2H, CH₂), 4.00 (s, 3H, OCH₃) 6.9 (d, *J*=8.4 Hz, 1H, Ar-H), 6.9 (d, *J*=8.0 Hz, 1H, Ar-H), 7.44 (m, 2H, Ar-H), 7.57 (m, 2H, Ar-H, NH), 8.09 (m, 1H, NH); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 56.2, 108.6, 108.7, 120.3, 120.5, 120.8, 122.0, 124.7, 128.5, 128.7, 143.6, 157.5, 158.0, 164.3; HRMS (ESI-TOF) *m/z*: C₂₈H₄₂N₃O₂, 452.3277

VI N-(5-Bromopyridin-2-yl)-2-methoxy-6-pentadecylbenzamide (6d)

White solid. 74%. m.p. 80-82 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.8 Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.68 (m, 2H, CH₂), 2.7 (t, *J*=7.6 Hz, 2H, CH₂), 4.00 (s, 3H, OCH₃), 6.89 (d, *J*=8.4 Hz, 1H, Ar-H), 6.96 (d, *J*=7.6 Hz, 1H, Ar-H), 7.42-7.48 (m, 2H, Ar-H), 7.57-7.58 (m, 2H, Ar-H, NH), 8.09 (d, *J*=8.4 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 56.2, 108.6, 117.4, 120.4, 122.0, 124.7, 128.5, 128.72, 132.6, 143.6, 143.6, 157.5, 164.3; HRMS (ESI-TOF) *m/z*: C₂₈H₄₂BrN₂O₂, 517.2430 and 519.2409

VII N-(5-Bromopyrazin-2-yl)-2-methoxy-6-pentadecylbenzamide (6e)

White solid. 70%. m.p. 80-82 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.8 Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.70 (m, 2H, CH₂), 2.7 (t, *J*=7.6 Hz, 2H, CH₂), 4.00 (s, 3H, OCH₃), 6.90 (d, *J*=8.4 Hz, 1H, Ar-H), 6.96 (d, *J*=8.0 Hz, 1H, Ar-H), 7.44 (m, 2H, Ar-H, NH), 7.58 (d, *J*=3.6 Hz, 1H, Ar-H), 8.09 (d, *J*=8.4 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 56.2, 108.6, 108.7, 117.4, 120.4, 122.0, 124.7, 218.5, 132.6, 143.6, 157.5, 164.3; HRMS (ESI-TOF) *m/z*: C₂₇H₄₁BrN₃O₂, 518.2382 and 520.2362

VIII 2-Ethoxy-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)-6-pentadecylbenzamide (6f)

White solid. 75%. m.p. 86-88 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.4 Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (t, *J*=7.2 Hz, 3H, CH₃), 1.70 (m, 2H, CH₂), 2.78 (t, *J*=8.0 Hz, 2H, CH₂), 4.20 (q, 2H, (OCH₂)₂), 6.87 Hz (d, *J*=8.4 Hz, 1H, Ar-H), 6.94 (d, *J*=7.6 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 7.75 (m, 2H, Ar-H), 7.61 (m, 1H, Ar-H), 8.09 (d, *J*=8.4 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 14.9, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 64.8, 108.71, 109.56, 120.45, 121.78, 124.66, 128.41, 128.78, 132.52, 143.51, 143.55, 156.89, 164.41; HRMS (ESI-TOF) *m/z*: C₃₂H₄₆N₂O₄, 508.3427.

IX 2-Ethoxy-6-pentadecyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-benzamide (6g)

Yellow solid. 75%. m.p. 67-69 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=7.2 Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (t, *J*=7.2 Hz, 3H, CH₃), 1.68 (m, 2H, CH₂), 2.7 (t, *J*=8.0 Hz, 2H, CH₂), 4.20 (m, 2H, OCH₂), 6.87 (d, *J*=8.4 Hz, 1H, Ar-H), 6.94 (d, *J*=7.6 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 7.54 (m, 1H, Ar-H), 7.4 (m, 2H, Ar-H), 8.09 (d, *J*=8.4 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 14.8, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 56.2, 108.7, 109.6, 109.6, 117.7, 120.5, 121.8, 124.6, 128.4, 128.8, 132.5, 132.5, 143.5, 143.6, 156.9, 164.4; HRMS (ESI-TOF) *m/z*: C₃₂H₄₆N₃O₂S, 536.3311.

X N-(3-Bromopyridin-2-yl)-2-ethoxy-6-pentadecyl benzamide (6h)

Yellow solid. 75%. m.p. 82-84 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (m, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (m, 3H, CH₃), 1.70 (m, 2H, CH₂), 2.78 (t, *J*=8.0 Hz, 2H, CH₂), 4.20 (m, 2H, OCH₂), 6.87 (d, *J*=8.4 Hz, 1H, Ar-H), 6.94 (d, *J*=8.0 Hz, 1H, Ar-H), 7.41-7.47 (m, 2H, Ar-H), 7.57-7.63 (m, 2H, Ar-H, NH), 8.10 (d, *J*=8.8 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 14.9, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 64.8, 108.7, 109.6, 117.7, 120.5, 121.8, 124.6, 126.5, 128.4, 132.5, 143.6, 156.9, 164.4; HRMS (ESI-TOF) *m/z*: C₂₉H₄₄BrN₂O₂, 531.2586.

XI N-(3-Cyano-3H-pyrrol-2-yl)-2-ethoxy-6-pentadecyl benzamide (6i)

White solid. 75%. m. p. 90-92 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (m, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (m, 3H, CH₃), 1.68 (m, 2H, CH₂), 2.78 (t, *J*=8.0 Hz, 2H, CH₂), 4.19 (m, 2H, OCH₂), 6.87 (d, *J*=8.0 Hz, 1H, Ar-H), 6.94 (d, *J*=7.6 Hz, 1H, Ar-H), 7.41 (m, 1H, Ar-H), 7.55 (m, 1H, Ar-H), 8.09 (d, *J*=8.0 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 14.9, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 64.8, 109.2, 110.0, 118.0, 120.9, 122.2, 125.2, 128.9, 129.2, 133.0, 143.9, 157.3, 164.9; HRMS (ESI-TOF) *m/z*: C₂₉H₄₄N₃O₂, 466.3434.

XII 2-Ethoxy-N-(3-hydroxy phenyl)-6-pentadecyl benzamide (6j)

White solid. 70%. m.p. 87-89 °C; ¹H NMR (CDCl₃, 400MHz) δ 0.85 (m, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (m, 3H, CH₃), 1.68 (m, 2H, CH₂), 2.78 (t, *J*=8.0 Hz, 2H, CH₂), 4.20 (m, 2H, OCH₂), 6.87 (d, *J*=8.4 Hz, 1H, Ar-H), 6.94 (d, *J*=7.6 Hz, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 7.57 (m, 1H, Ar-H), 7.61 (m, 1H, Ar-H), 8.09 (d, *J*=8.4, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): 14.0, 14.8, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (three peaks), 29.6, 31.3, 31.9, 33.7, 64.8, 108.7, 109.6, 120.4, 120.4, 121.8, 124.6, 128.4, 128.4, 132.5, 143.5, 143.6, 156.9, 164.4; HRMS (ESI-TOF) *m/z*: C₃₀H₄₆NO₃, 468.3478

XIII N-(5-Bromopyrazin-2-yl)-2-ethoxy-6-pentadecyl benzamide (6k)

Yellow solid. 75%. m.p. 82-84 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.8 Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (m, 3H, CH₃), 1.68 (m, 2H, CH₂), 2.77 (t, *J*=8.0 Hz, 2H, CH₂), 4.2 (m, 2H, OCH₂), 6.87 Hz (d, *J*=8.4 Hz, 1H, Ar-H), 6.94 (d, *J*=7.6 Hz, 1H, Ar-H), 7.41 (m, 1H, Ar-H), 7.57 (m, 2H, Ar-H, NH), 8.09 (d, *J*=8.4 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100MHz): δ 14.0, 14.8, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 64.8, 108.7, 109.6, 117.8, 120.5, 121.8, 124.6, 128.4, 132.5, 143.5, 156.9, 164.4; HRMS (ESI-TOF) *m/z*: C₂₈H₁₃BrN₃O₂, 532.2539 and 534.2520

XIV 2-Ethoxy-6-pentadecyl-n-(1,3,4-thiadiazol-2-yl)benzamide (6l)

White solid. 75%. m.p. 85-87°C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.8Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (m, 3H, CH₃), 1.7 (m, 2H, CH₂), 2.77 (t, *J*=8.0 Hz, 2H, CH₂), 4.2 (m, 2H, OCH₂), 6.94 (d, *J*=7.6Hz, 1H, Ar-H), 7.41 (m, 1H, Ar-H), 7.55 (m, 1H, Ar-H), 7.61 (s, 1H, NH), 8.09 (d, *J*=8.4 Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz) δ 14.0, 14.9, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 64.8, 108.7, 109.6, 120.4, 121.8, 124.5, 132.5, 143.5, 156.9, 164.4; HRMS (ESI-TOF) *m/z*: C₂₆H₄₂N₃O₂S, 460.2998.

XV 2-Ethoxy-6-pentadecyl-N-(quinolin-6-yl)benzamide (6m)

Yellow solid. 75%. m.p. 65-67 °C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (t, *J*=6.8Hz, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.53 (m, 3H, CH₃), 1.70 (m, 2H, CH₂), 2.78 (t, *J*=8Hz, 2H, CH₂), 4.2 (m, 2H, OCH₂), 6.87 (d, *J*=8.4 Hz, 1H, Ar-H), 6.94 (d, *J*=7.6Hz, 2H, Ar-H), 7.41 (m, 2H, Ar-H), 7.54 (m, 2H, Ar-H), 7.61 (m, 1H, Ar-H), 8.09 (d, *J*=8.4Hz, 1H, Ar-H); ¹³C NMR (CDCl₃, 50MHz): δ 14.0, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 32.5, 33.7, 64.8, 108.7, 109.6, 117.7, 120.5, 121.8, 124.6, 128.4, 128.8, 132.5, 143.5, 143.6, 155.0, 156.9, 164.4; HRMS (ESI-TOF) *m/z*: C₃₃H₄₇N₂O₂, 503.3638

XVI N-(3-Cyano-1-H-pyrrol-2-yl)-2-isopropoxy-6-pentadecyl benzamide (6n)

White solid. 75%. m.p. 63-65°C; ¹H NMR (CDCl₃, 400 MHz): δ 0.85 (m, 3H, CH₃), 1.23 (m, 24H, (CH₂)₁₂), 1.45 (d, *J*=6.0 Hz, 6H, (CH₃)₂), 1.68 (m, 2H, CH₂), 2.77 (t, *J*=8.0 Hz, 2H, CH₂), 4.72 (m, 1H, CH), 6.89 (d, *J*=8.4 Hz, 1H, Ar-H), 6.92 (d, *J*=7.6Hz, 1H, Ar-H), 7.40 (m, 2H, Ar-H), 7.55 (s, 1H, NH), 7.61 (m, 1H, NH), 8.09 (d, *J*=8.4, 1H, Ar-H); ¹³C NMR (CDCl₃, 100 MHz): δ 14.0, 22.1, 22.6, 29.3 (two peaks), 29.4 (two peaks), 29.5 (two peaks), 29.6 (two peaks), 29.6 (two peaks), 31.3, 31.9, 33.7, 71.7, 108.7, 110.9, 118.9, 120.5, 121.6, 124.6, 128.3, 128.8, 132.3, 143.5, 143.6, 155.9, 164.4; HRMS (ESI-TOF) *m/z*: C₃₀H₄₆N₃O₂, 480.3590

XVII In Vitro Cytotoxicity Assay

The recently synthesized derivatives were assessed for toxicity against hepatocellular carcinoma (HepG2) cell lines as reported earlier [30-32]. Briefly, the cells (1 X 10⁴/ml) were transferred to 96-well microtiter plate in with or without novel compounds of different concentration of 0.15ml and incubated at 37°C for 72h. After incubation, 20 µl of MTT (5 mg/ml in PBS) dye was transferred to the wells and incubated for 2h at 37°C. The incubation was continued for 24h after the addition of 0.1ml lysis buffer (20% SDS, 50%DMF) and their absorbance was measured using a microplate reader at a wavelength of 570nm.

Conclusion

In conclusion, the toxicity of newly synthesized benzamide derivatives of anacardic acid were tested against human liver cancer cell lines (HepG2). Amongst, 6j-m showed good inhibitory activity with IC₅₀ values 78.2-91.9 µM.

Consent for Publication

All the authors given consent for this publication.

Conflicts of Interest

None.

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None.

Supplementary Material

Supplementary material for this article is available at

Abbreviations

RANKL: Receptor Activator of Nuclear factor Kappa-3

ER: Estrogen Receptor

DAPK3: Death Associated Protein Kinase 3

VEGF: Vascular Endothelial Growth Factor

EDC·HCl: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride

HOBt: Hydroxybenzotriazole

NMR: Nuclear Magnetic Resonance

IR: Infra-Red

GCMS: Gas Chromatography Mass Spectra

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