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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and cytotoxicity evaluation of threonine-based galactoceramide with aromatic groups and various fatty-acyl side chains

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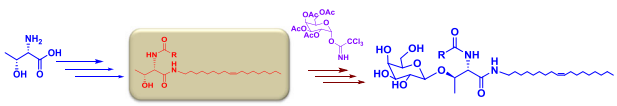
Abstract In galactoceramides, presence of fatty-acyl group on amide moiety or phytosphingosine group is some of the important features that influence the cytotoxicity. Continuous efforts are in progress to modify the fatty-acid moiety and phytosphingosine group present on the galactoceramides to enhance the cytotoxic potential of these compounds. Hence, in the present study, threonine-based β-galactoceramide and its derivatives were prepared by modifying the fatty-acyl group on amide moiety with different fatty-acyl moieties and aromatic acids employing trichloroacetimidate methodology. The structurally related threonine-based ceramide part was synthesized in multi-step process using different reagents. The ceramide part was glycosylated with galactose using trichloroacetimidate as donor. Further, all the synthesized compounds were evaluated for in vitro cytotoxicity against three cancer cell lines and one normal cell line and all the compounds exhibited good to moderate cytotoxicity against all the tested cancer cell lines. In aromatic derivatives, the compound **8i** exhibited promising activity against MCF7, A549 and HeLa cancer cell lines with IC_{50} values of 14.08, 14.78, and 16.70 µM, respectively. In fatty-acid derivatives, two compounds exhibited promising activity, i.e., compound **8m** against HeLa with IC_{50} value 16.34 µM and compound **8n** against MCF7 with IC_{50} value 18.05 µM. Based on structure–activity relationship, aromatic acid derivatives exhibited potential activity as compared to fatty-acid derivatives. Further, the influence of some of the key factors such as spacer chain length between aromatic residue and amide functional group, methoxy substituents on aryl group, terminal unsaturation of fatty acid and branching chain effect on the cytotoxicity are discussed.

Graphical abstract



Keywords Threonine · Galactoceramide · Aromatic acids · Fatty acids · Cytotoxicity

Introduction

Galactoceramide is a bioactive glycolipid and it has been shown to possess antitumor activity (Natori et al. (1993, 1994); Motoki et al. 1995). In galactoceramides, the glycosidic bond exists between sugar moiety and the ceramide part, which consists of a fatty acid and sphingoid chain. In higher organisms, these glycosidic bond has a typically β-configuration. Natori and co-workers from Kirin Brewery, Japan originally isolated these glycolipids from an

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extract of marine sponge, *Agelas mauritianus* (Natori et al. (1993, 1994); Motoki et al. 1995). Since last two decades, galactoceramide and its synthetic analogues have gained a renewed attention in the scientific community due to their high-potential antitumor activity (Natori et al. 1994; Motoki et al. 1995; Nakagawa et al. 1998) in the view of its versatile utility as iNKT activators (Nakagawa et al. 1998; Hayakawa et al. 2004; Kawano et al. 1998) and adjuvants for the treatment of many diseases like malaria (Gonzalez-Aseguinolaza et al. (2000, 2002)) HIV (Huang et al. 2008), tumor immunotherapy (Giaccone et al. 2002), tuberculosis (Sada-Ovalle et al. 2010) and also it inhibited allergic airway inflammation (Matsuda et al. 2005). Moreover, the phase I clinical trials proved that galactoceramide was ineffective in the treatment of solid tumors (Giaccone et al. 2002). Therefore, new galactoceramide analogues are required for the treatment.

The SAR study on α-GalCer showed that the modification on the sphingosine OH group leads to a change in the binding effect without losing total activity of α-GalCer (Zhang et al. 2011) and modification of fatty-acyl group on amide moiety or phytosphingosine group gave better results in enhancing the antitumor promoting activity (Smyth and Godfrey 2000; Yogesh Kumar et al. 2016; Chang et al. 2007). In view of this, Fujio and co-workers reported the design and synthesis of various fatty-acyl chain analogues with replacement of fatty-acyl group on amide moiety with different aromatic acids and evaluated the NKT cell activation by measuring cytokine release profiles. These results demonstrated that the introduction of an aromatic group to the fatty-acyl chain greatly influences the cytokine release and also long chain analogues were more potent than shorter chain aromatic groups (Fujio et al. 2006). In addition, Wu and co-workers reported the presence of an additional hydrogen bond between the phenyl/aryl ring of the fatty-acyl chain and aromatic amino-acid residues present on the wall of the A′ pocket in the CD1d hydrophobic groove (Wu et al. 2006).

In addition, there exist a few other reports where the phytosphingosine group was modified with structurally related amino acids such as serine and evaluated for their different immunoactivities (Hung et al. 2007; Huang et al. 2011; Wallner et al. 2005; Fan et al. 2005). In this regard, it is noteworthy to mention that threonine is structurally related to phytosphingosine group. Moreover, threonine-based vaccines are signaling molecules in the human CD1d cytoplasmic tail (Liu et al. 2010). α-*N*-Acetyl galactosamine-*O*-serine/threonine-based conjugate vaccine works against most of cancers, especially most effectively against prostate cancer (Slovin et al. 2003). Furthermore, glycosphingolipids like *β*-galactosylceramide (**1**) are available in nature with numerous applications (Wallner et al. 2005). Synthesis and evaluation of different activities of synthetic and isolated β-galactosylceramide was reported earlier (Wallner et al. 2005). These glycolipids exhibited different biological activities such as cytotoxicity (Motoki et al. 1995), anti-HIV-1 (Faroux-Corlay et al. 2000), β-glucocerebrosidase activity in keratinocytes (Fukunaga et al. 2003), and also exhibited opposing effects on the production of chemokines and proinflammatory cytokines (Roeske-Nielsen et al. 2004).

Apart from above studies, galactoceramide derivatives with modified phytosphingosine or fatty-acid moiety exhibited different immunoactivities (Smyth and Godfrey 2000; Chang et al. 2007; Hung et al. 2007; Huang et al. 2011; Wallner et al. 2005; Fan et al. 2005) and also in view of the potential antitumor activity of oleic acid derivatives (Garcia-Alvarez et al. 2011; Carrillo et al. 2012) in the present study, synthesis and cytotoxic evaluation of structurally related galactoceramide derivatives of threonine-based β-galactosyl ceramides was carried out. In this study, the fatty-acyl group on amide moiety was replaced with different aromatic acids and various unusual, branched, saturated and unsaturated fatty-acyl moieties. Further, oleyl amine moiety was attached to threonine acid group by mimicking phytosphingosine long chain in ceramide (Fig. 1).

Experimental procedures

Materials and methods

All the chemicals were of analytical grade obtained from different commercial sources and used without any further purification. All the dry reactions were carried out under nitrogen atmosphere using anhydrous freshly distilled solvents and sieved through molecular sieves (4 Å) in flame dried glassware using standard gas-light syringes and septa. Reactions were monitored using micro thin layer chromatograph (TLC) plates (coated with TLC grade silica gel,

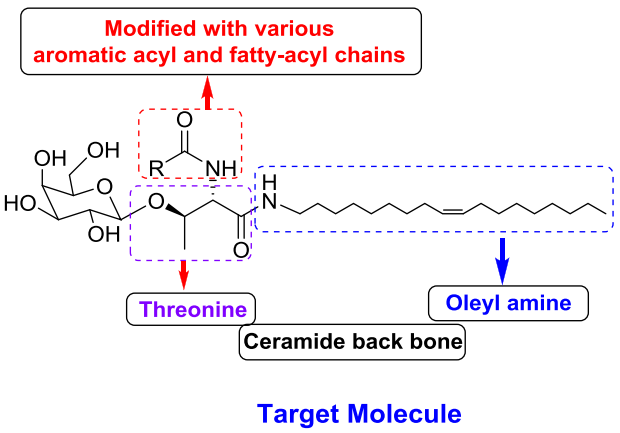


Fig. 1 Structures of target molecule

obtained from Merck) and the spots were detected by iodine vapors. Column chromatography was performed using silica gel (100–200 mesh) procured from Qualigens (India) by eluting freshly distilled solvents. All the ^{1}H-NMR (nuclear magnetic resonance) and ^{13}C-NMR spectra were recorded on a Bruker UXNMR (Operating for ^{1}H-NMR at 300 MHz, 400 MHz, 500 MHz, and for ^{13}C-NMR at 75 MHz, 100 MHz, 125 MHz) spectrometer, using TMS ($\delta = 0$) as an internal standard for chemical shifts (δ) in $CDCl_3$, CD_3OD and DMSO-d_6 at 25 °C. Mass spectra were recorded with HRMS and ESI–MS (electron spray ionization technique). Infrared (IR) spectra were recorded with a Perkin-Elmer Fourier-transform infrared spectroscopy (FT-IR) spectrum BX. The melting points were determined on a Barnstead electro thermal 9200 instrument.

Preparation of L-threonine methyl ester hydrochloride (1)

L-Threonine was dissolved in methanalic HCl solution (2 M) and refluxed the reaction mixture for 1 h. Then the organic solvent was evaporated using rotary evaporator and again same methanalic HCl solution was added to the reaction mixture and refluxed for 1 h. After the reaction time, the organic solvent was evaporated under reduced pressure to give white solid compound. ESI–MS: *m/z* at 134.2 [M^+ +H].

General procedure for synthesis of aromatic acid/fatty acid-based methyl–L-threoninate (2a–2s)

A mixture of 10.25 mmol of L-threonine methyl ester hydrochloride (**1**) and Et_3N (2 mL) was dissolved in ice-cold anhydrous dichloromethane (40 mL) and kept in ice for 20 min with stirring. This was followed by the addition of acid (12.3 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl, 12.3 mmol) and hydroxy benzotriazole (HOBt, 15.38 mmol) successively at 0 °C. The mixture was left at room temperature for 12 h under magnetic stirring. The reaction was monitored using micro TLC (hexane: ethyl acetate, 1:1, v/v). At the end of the reaction, the reaction mixture was dissolved in chloroform (75 mL) and then it was washed by 5% $NaHCO_3$ solution, saturated NaCl solution successively. The chloroform layer was dried with anhydrous Na_2SO_4 and the filtrate was dried under vacuum. The crude mixture was purified by silica-gel chromatography by using hexane, ethyl acetate solvent mixture to get the title compound with 78–84% yield.

*Methyl cinnamoyl-L-threoninate (**2a**)*

Hexane: ethyl acetate (60:40, v/v), yield 81%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.65 (d, $J = 15.71$ Hz, 1H,=CH), 7.49 (m, 2H, Ar–H), 7.33 (t, $J = 3.66$ Hz, 3H, Ar–H), 6.75 (d, $J = 8.85$ Hz, 1H, NH), 6.55 (d, $J = 15.71$ Hz, 1H,=CH), 4.77 (dd, $J = 2.44$, 8.85 Hz, 1H, CH–NH), 4.42 (m, 1H, CH–OH), 3.78 (s, 3H, OCH_3), 1.26 (d, $J = 6.41$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz): 171.7 (O–C=O), 166.7 (HN—C=O), 142.1 (HC=CH), 134.5 (Ar–C), 129.9 (Ar–CH), 128.8 (Ar–CH), 127.9 (Ar–CH), 119.9 (HC=CH), 68.1 (HC-OH), 57.6 (HC–NH), 52.6 (O–CH_3), 20 (HOHC–CH_3); IR (KBr): 3479, 3305, 2964, 1721, 1658, 1543, 1278, 1080, 977, 732, 545; ESI–MS: *m/z* at 286.06 [M + Na].

*Methyl 4—nitro cinnamoyl-L-threoninate (**2b**)*

Hexane: ethyl acetate (50:50, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 8.18–8.24 (m, 2H, Ar–H), 7.61–7.71 (m, 3H, Ar–H,=CH), 6.66–6.72 (m, 2H, NH,=CH), 4.76 (m, 1H, CH–NH), 4.44 (m, 1H, CH–OH), 3.81 (s, 3H, OCH_3), 1.29 (d, $J = 6.25$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 171.5 (O–C=O), 165.4 (HN–C=O), 148.1 (Ar–C–NO_2), 140.7 (HC=CH), 139.1 (Ar–C), 128.4 (Ar–CH), 124.1 (Ar–CH), 124 (HC=CH), 67.9 (HC–OH), 57.6 (HC–NH), 52.7 (O–CH_3), 20 (HOHC–CH_3); IR (KBr): 3478, 3305, 2964, 1716, 1658, 1622, 1543, 1345, 1278, 1086, 977, 732, 545; ESI–MS: *m/z* at 331.04 [M + Na].

*Methyl (2-(1H-indol-3-yl) acetyl)-L-threoninate (**2c**)*

Hexane: ethyl acetate (60:40, v/v), yield 82%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 10.51 (br s, 1H, Ar–NH), 7.57 (d, $J = 7.93$ Hz, 1H, Ar–H), 7.35 (d, $J = 8.08$ Hz, 1H, Ar–H), 7.18 (s, 1H, Ar–H), 7.09 (t, $J = 7.09$ Hz, 1H, Ar–H), 7.02 (t, $J = 7.47$ Hz, 1H, Ar–H), 4.44 (m, 1H, CH–NH), 4.20 (m, 1H, CH–OH), 3.72 (s, 2H, CH_2), 3.64 (s, 3H, OCH_3), 1.06 (d, $J = 6.41$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): 171.8 (O–C=O), 171 (HN–C=O), 136.1 (Ar–C–NH), 126.9 (Ar–C–CH), 123.7 (Ar–CH), 121.1 (Ar–CH), 118.5 (Ar–CH), 118.3 (Ar–CH), 111.2 (Ar–CH), 107.9 (Ar–C), 66.5 (HC–OH), 57.6 (HC–NH), 51.7 (O–CH_3), 32.7 (H_2C–C=O), 19.9 (HOHC–CH_3); IR (KBr): 3409, 3345, 2967, 1738, 1640, 1519, 1457, 1298, 1130, 1015, 747; ESI–MS: *m/z* at 313.1 [M + Na].

*Methyl (3-(1H-indol-3-yl) propanoyl)-L-threoninate (**2d**)*

Hexane: ethyl acetate (60:40, v/v), yield 84%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 8.18 (br s, 1H, Ar–NH), 7.59 (m, 1H, Ar–H), 7.33 (m, 1H, Ar–H), 7.17 (m, 1H, Ar–H), 7.11 (m, 1H, Ar–H), 7.01 (m, 1H, Ar–H), 6.29 (br s, 1H, NH), 4.55 (m, 1H, CH-–NH), 4.21 (m, 1H, CH–OH), 3.70 (s, 3H, OCH_3), 3.12 (m, 2H, CH_2), 2.66 (m, 2H, CH_2), 1.03 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 173.6 (O–C=O), 171.6 (HN–C=O), 136.2 (Ar–C–NH), 127 (Ar–C–CH), 121.8 (Ar–CH), 119.1 (Ar–CH), 118.5 (Ar–CH), 114.4 (Ar–CH), 112.2 (Ar–C), 67.8 (HC–OH), 57.3 (HC–NH),

52.4 (O–$\underline{CH_3}$), 36.8 ($\underline{H_2C}$–C=O), 21.1 ($\underline{H_2C}$–H_2C–C=O), 19.6 (HOHC–$\underline{CH_3}$); IR (KBr): 3412, 3338, 2967, 1742, 1658, 1519, 1462, 1298, 1217, 1130, 1015, 1087, 842, 747, 502; ESI–MS: *m/z* at 327.04 [M + Na].

*Methyl (1H-indazole-3-carbonyl)-L-threoninate (**2e**)*

Hexane: ethyl acetate (50:50, v/v), yield 81%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 8.02 (br s, 1H, Ar–NH), 7.78 (m, 1H, Ar–H), 7.28 (m, 1H, Ar–H), 7.08 (m, 1H, Ar–H), 6.95 (m, 1H, Ar–H), 4.67 (m, 1H, $\underline{CH}$–NH), 4.53 (m, 1H, $\underline{CH}$–OH), 3.48 (s, 3H, OCH_3), 1.03 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 125 MHz): 171.3 (O–$\underline{C}$=O), 163.1 (HN–$\underline{C}$ = O), 141.2 (Ar–$\underline{C}$–NH), 137.5 (O=C–(Ar)$\underline{C}$=NH), 126.3 (Ar–$\underline{CH}$), 122.1 (Ar–$\underline{CH}$), 121.5 (Ar–$\underline{CH}$), 121.4 (Ar-$\underline{CH}$), 110.4 (Ar–$\underline{C}$), 67 ($\underline{HC}$–OH), 57.2 ($\underline{HC}$–NH), 52 (O–$\underline{CH_3}$), 20.1 (HOHC–$\underline{CH_3}$); IR (KBr): 3532, 3358, 3028, 2969, 1742, 1670, 1519, 1458, 1298, 1218, 1130, 1016, 1087, 842, 747; ESI–MS: *m/z* at 300.07 [M + Na].

*Methyl (1H-indole-2-carbonyl)-L-threoninate (**2f**)*

Hexane: ethyl acetate (60:40, v/v), yield 80%. ^{1}H-NMR (DMSO-d_6, 400 MHz): 10.83 (s, 1H, Ar–NH), 7.78 (d, J = 7.33 Hz, 1H, Ar–H), 7.54 (m, 1H, Ar–H), 7.38 (m, 1H, Ar–H), 7.15 (m, 1H, Ar–H), 7.09 (s, 1H, Ar–H), 7.00 (m, 1H, NH), 4.68 (m, 1H, $\underline{CH}$–NH), 4.30 (m, 1H, $\underline{CH}$–OH), 3.67 (s, 3H, OCH_3), 1.18 (d, J = 6.48 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 125 MHz): 171 (O–$\underline{C}$=O), 161.7 (HN–$\underline{C}$=O), 136.4 (Ar–$\underline{C}$–NH), 130.4 (O=C–$\underline{C}$(NH)=CH), 126.8 (Ar–$\underline{C}$), 123.6 (Ar–$\underline{CH}$), 121.3 (Ar–$\underline{CH}$), 119.7 (Ar–$\underline{CH}$), 111.9 (Ar–$\underline{CH}$), 103.7 (Ar–$\underline{CH}$), 67.1 ($\underline{HC}$–OH), 57.8 ($\underline{HC}$–NH), 51.8 (O–$\underline{CH_3}$), 19.9 (HOHC–$\underline{CH_3}$); IR (KBr): 3586, 3362, 2968, 1738, 1645, 1519, 1457, 1298, 1217, 1130, 1015, 1068, 842, 747, 502; ESI–MS: *m/z* at 299.1 [M + Na].

*Methyl (2-phenylacetyl)-L-threoninate (**2g**)*

Hexane: ethyl acetate (65:35, v/v), yield 82%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.28 (m, 5H, Ar–H), 6.68 (d, J = 8.80 Hz, 1H, NH), 4.52 (dd, J = 2.69, 8.92 Hz, 1H, $\underline{CH}$–NH), 4.26 (m, 1H, $\underline{CH}$–OH), 3.67 (s, 3H, OCH_3), 3.58 (s, 2H, CH_2), 1.08 (d, J = 6.35 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 172 (O–$\underline{C}$=O), 171.2 (HN–$\underline{C}$=O), 134.5 (Ar–$\underline{C}$–CH_2), 129 (Ar–$\underline{CH}$), 128.5 (Ar–$\underline{CH}$), 126.9 (Ar–$\underline{CH}$), 67.3 ($\underline{HC}$–OH), 57.4 ($\underline{HC}$–NH), 52.2 (O–$\underline{CH_3}$), 42.9 (Ar–$\underline{CH_2}$–C=O), 19.6 (HOHC–$\underline{CH_3}$); IR (KBr): 3476, 3327, 2955, 1711, 1647, 1513, 1287, 1249, 1083, 1020, 698; ESI–MS: *m/z* at 274.1 [M + Na].

*Methyl (2-(4-methoxyphenyl) acetyl)-L-threoninate (**2h**)*

Hexane: ethyl acetate (60:40, v/v), yield 80%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.21 (d, J = 8.55 Hz, 2H, Ar–H), 6.89 (d, J = 8.55 Hz, 2H, Ar–H), 6.21 (d, J = 8.06 Hz, 1H, NH), 4.58 (dd, J = 2.44, 8.80 Hz, 1H, $\underline{CH}$–NH), 4.31 (m, 1H, $\underline{CH}$–OH), 3.80 (s, 3H, Ar–OCH_3), 3.73 (s, 3H, OCH_3), 3.58 (s, 2H, CH_2), 1.91 (br s, 1H, OH), 1.13 (d, J = 6.48 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 172.1 (O–$\underline{C}$=O), 171.3 (HN–$\underline{C}$=O), 158.8 (Ar–$\underline{C}$–OCH_3), 130.3 (Ar–$\underline{C}$–CH_2), 126.4 (Ar–$\underline{CH}$), 114.3 (Ar–$\underline{CH}$), 67.8 ($\underline{HC}$–OH), 57.2 ($\underline{HC}$–NH), 55.2 (Ar–C–O–$\underline{CH_3}$), 52.5 (O–$\underline{CH_3}$), 42.5 (Ar–$\underline{CH_2}$–C=O), 19.8 (HOHC–$\underline{CH_3}$); IR (KBr): 3478, 3338, 2956, 1728, 1649, 1513, 1287, 1248, 1083, 1018, 698; ESI–MS: *m/z* at 304.1 [M + Na].

*Methyl (4-(4-methoxyphenyl) butanoyl)-L-threoninate (**2i**)*

Hexane: ethyl acetate (60:40, v/v), yield 82%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.09 (d, J = 8.54 Hz, 2H, Ar–H), 6.82 (d, J = 8.69 Hz, 2H, Ar–H), 6.41 (br s, 1H, NH), 4.61 (dd, J = 2.28, 8.85 Hz, 1H, $\underline{CH}$–NH), 4.34 (m, 1H, $\underline{CH}$–OH), 3.78 (s, 3H, Ar–OCH_3), 3.75 (s, 3H, OCH_3), 2.60 (t, J = 7.47 Hz, 2H, CH_2), 2.27 (t, J = 7.32 Hz, 2H, CH_2), 1.95 (m, 2H, CH_2), 1.20 (d, J = 6.41 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 173.2 (O–$\underline{C}$=O), 171.2 (HN–$\underline{C}$=O), 157.4 (Ar–$\underline{C}$–OCH_3), 133.3 (Ar–$\underline{C}$–CH_2), 129 (Ar–$\underline{CH}$), 113.4 (Ar–$\underline{CH}$), 67 ($\underline{HC}$–OH), 57.4 ($\underline{HC}$–NH), 54.8 (Ar–C–O–$\underline{CH_3}$), 51.8 (O–$\underline{CH_3}$), 35.1 (H_2C–$\underline{CH_2}$–C=O), 33.8 (Ar–C–$\underline{CH_2}$), 27.1 ($\underline{CH_2}$), 19.8 (HOHC–$\underline{CH_3}$); IR (KBr): 3458, 3334, 2956, 1711, 1638, 1513, 1287, 1249, 1053, 1020, 698; ESI–MS: *m/z* at 332.1 [M + Na].

*Methyl hexanoyl-L-threoninate (**2j**)*

Hexane: ethyl acetate (70:30, v/v), yield 89%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.46(br s, 1H, NH), 4.58 (m, 1H, $\underline{CH}$–NH), 4.34 (m, 1H, $\underline{CH}$–OH), 3.75 (s, 3H, OCH_3), 2.28 (m, 2H, O=C–$\underline{CH_2}$), 1.65 (m, 2H, CH_2), 1.32 (m, 4H, CH_2), 1.20 (s, 3H, CHOH–$\underline{CH_3}$), 0.89 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 174.1 (O–$\underline{C}$=O), 171.5 (HN–$\underline{C}$=O), 67.6 ($\underline{HC}$–OH), 57.2 ($\underline{HC}$–NH), 52.3 (O–$\underline{CH_3}$), 36.3 (O=C–$\underline{CH_2}$), 31.2 (CH_2), 25.2 (CH_2), 22.2 (CH_2), 19.8 (HOHC–$\underline{CH_3}$), 13.8 (CH_3); IR (KBr): 3353, 2956, 2866, 1723, 1645, 1532, 1287, 1020, 696; ESI–MS: *m/z* at 254.1 [M + Na].

*Methyl octanoyl-L-threoninate (**2k**)*

Hexane: ethyl acetate (70:30, v/v), yield 88%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.44 (d, J = 8.68 Hz, 1H, NH), 4.61 (dd, J = 2.56, 8.92 Hz, 1H, $\underline{CH}$–NH), 4.32–4.37 (m, 1H, $\underline{CH}$–OH), 3.76 (s, 3H, OCH_3), 2.28 (t, J = 7.45 Hz, 2H, O

$=C–CH_2$), 1.62–1.69 (m, 2H, CH_2), 1.28–1.32 (m, 8H, CH_2), 1.21 (d, 3H, CHOH-CH_3), 0.88 (t, $J = 6.72$, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.2 (O–C=O), 171.5 (HN–C=O), 67.6 (HC–OH), 57.2 (HC–NH), 52.3 (O–CH_3), 36.3 (O=C–CH_2), 31.6 (O=C–CH_2–CH_2), 29.1 (CH_2), 28.8 (CH_2), 25.6 (CH_2), 22.4 (CH_2), 19.7 (HOHC–CH_3), 13.9 (CH_3); IR (KBr): 3355, 2968, 2866, 1733, 1645, 1528, 1287, 1020, 696; ESI–MS: *m/z* at 282.2 [M + Na].

*Methyl (2-propylpentanoyl)-L-threoninate (**2l**)*

Hexane: ethyl acetate (70:30, v/v), yield 82%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.43 (br s, 1H, NH), 4.63 (m, 1H, CH–NH), 4.37 (m, 1H, CH–OH), 3.75 (s, 3H, OCH_3), 2.17–2.22 (m, 1H, CH), 1.62 (m, 2H, CH_2), 1.31–1.40 (m, 6H, CH_2), 1.21 (m, 3H, CHOH–CH_3), 0.90–0.92 (m, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz): 177 (O–C=O), 171.6 (HN–C=O), 67.6 (HC–OH), 57 (HC–NH), 52.4 (O–CH_3), 47.5 (O=C–CH), 35.3 (O=C–CH–CH_2), 35.1 (O=C–CH–CH_2), 20.7 (CH_2), 20.6 (CH_2), 19.9 (HOHC–CH_3), 14.1 (CH_3); IR (KBr): 3362, 2958, 2866, 1728, 1647, 1532, 1287, 1018, 696; ESI–MS: *m/z* at 282.1 [M + Na].

*Methyl undec-10-enoyl-L-threoninate (**2m**)*

Hexane: ethyl acetate (70:30, v/v), yield 84%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.45 (d, $J = 8.80$ Hz, 1H, NH), 5.75–5.85 (m, 1H,=CH), 4.96–5.01 (m, 1H,=CH_2), 4.91–4.94 (m, 1H,=CH_2), 4.59 (dd, $J = 2.44$, 8.92 Hz, 1H, CH–NH), 4.31–4.36 (m, 1H, CH–OH), 3.75 (s, 3H, OCH_3), 2.96 (s, 1H, OH), 2.28 (t, $J = 7.45$ Hz, 2H, O=C–CH_2), 2.0–2.06 (m, 2H,=CH–CH_2), 1.61–1.68 (m, 2H, CH_2), 1.28–1.38 (m, 10H, CH_2), 1.20 (d, $J = 6.48$ Hz, 3H, CHOH–CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.04 (O–C=O), 171.5 (HN–C=O), 138.9 (H_2C–HC=CH), 114.0 (H_2C–HC=CH), 67.6 (HC–OH), 57.1 (HC–NH), 52.3 (O–CH_3), 36.3 (O=C–CH_2), 33.6 (H_2C–HC=CH), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 25.5 (CH_2), 19.8 (HOHC–CH_3); IR (KBr): 3495, 3313, 2918, 2850, 1720, 1649, 1545, 1284, 1020, 721; ESI–MS: *m/z* at 322.3 [M + Na].

*Methyl undecanoyl-L-threoninate (**2n**)*

Hexane: ethyl acetate (70:30, v/v), yield 81%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.48 (br s, 1H, NH), 4.59–4.61 (m, 1H, CH–NH), 4.32–4.36 (m, 1H, CH–OH), 3.76 (s, 3H, OCH_3), 2.28 (t, $J = 7.32$ Hz, 2H, O=C–CH_2), 1.65 (m, 2H, CH_2), 1.25 (m, 14H, CH_2), 1.21 (d, $J = 6.4$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.86$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 174 (O–C=O), 171.5 (HN–C=O), 67.7 (HC–OH), 57.1 (HC–NH), 52.4 (O–CH_3), 36.4 (O=C–CH_2), 31.8 (O=C–CH_2–CH_2), 29.5 (CH_2), 29.2 (CH_2), 25.6 (CH_2), 22.5 (CH_2), 19.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3494, 3309, 2918, 2850, 1721, 1646, 1545, 1281, 1019, 716; ESI–MS: *m/z* at 324.2 [M + Na].

*Methyl dodecanoyl-L-threoninate (**2o**)*

Hexane: ethyl acetate (70:30, v/v), yield 80%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.21 (br s, 1H, NH), 4.61 (m, 1H, CH–NH), 4.35 (m, 1H, CH–OH), 3.77 (s, 3H, OCH_3), 2.28 (t, $J = 7.7$ Hz, 2H, O=C–CH_2), 1.66 (m, 2H, CH_2), 1.25 (m, 16H, CH_2), 1.22 (d, $J = 6.35$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.11$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174 (O–C=O), 171.6 (HN–C=O), 67.9 (HC–OH), 57 (HC–NH), 52.4 (O–CH_3), 36.5 (O=C–CH_2), 31.8 (O=C–CH_2-CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29 (CH_2), 25.6 (CH_2), 24.7 (CH_2), 22.6 (CH_2), 19.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3482, 3323, 2918, 2856, 1745, 1658, 1545, 1278, 1019, 718; ESI–MS: *m/z* at 338.1 [M + Na].

*Methyl tetradecanoyl-L-threoninate (**2p**)*

Hexane: ethyl acetate (70:30, v/v), yield 85%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.26 (d, $J = 8.69$ Hz, 1H, NH), 4.63 (dd, $J = 2.59$, 8.85 Hz, 1H, CH–NH), 4.33–4.37 (m, 1H, CH–OH), 3.77 (s, 3H, OCH_3), 2.28 (t, $J = 7.47$ Hz, 2H, O=C–CH_2), 1.61–1.68 (m, 2H, CH_2), 1.25 (m, 20 H, CH_2), 1.22 (d, $J = 6.4$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.86$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 173.9 (O–C=O), 171.6 (HN–C=O), 67.8 (HC–OH), 57 (HC–NH), 52.4 (O–CH_3), 36.5 (O=C–CH_2), 31.8 (O=C–CH_2–CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 19.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3494, 3309, 2918, 2850, 1721, 1646, 1545, 1281, 1019, 716; ESI–MS: *m/z* at 366.2 [M + Na].

*Methyl hexadecanoyl-L-threoninate (**2q**)*

Hexane: ethyl acetate (70:30, v/v), yield 86%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.21 (d, $J = 8.54$ Hz, 1H, NH), 4.62 (dd, $J = 2.44$, 8.85 Hz, 1H, CH–NH), 4.33–4.37 (m, 1H, CH–OH), 3.77 (s, 3H, OCH_3), 2.28 (t, $J = 7.47$ Hz, 2H, O=C–CH_2), 1.63–1.68 (m, 2H, CH_2), 1.25 (m, 24H, CH_2), 1.21 (d, $J = 6.4$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.86$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 173.8 (O–C=O), 171.6 (HN–C=O), 67.9 (HC–OH), 57 (HC–NH), 52.5 (O–CH_3), 36.5 (O=C–CH_2), 31.8 (O=C–CH_2–CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 19.9 (HOHC–CH_3), 14.1 (CH_3); IR (KBr): 3486, 3316, 2918, 2850, 1728, 1642, 1545, 1281, 1014, 716; ESI–MS: *m/z* at 394.1 [M + Na].

*Methyl octadecanoyl-L-threoninate (**2r**)*

Hexane: ethyl acetate (70:30, v/v), yield 82%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.00 (d, $J = 8.85$ Hz, 1H, NH), 4.52 (dd, $J = 2.74$, 9.0 Hz, 1H, CH–NH), 4.26–4.30 (m, 1H, CH–OH), 3.73 (s, 3H, OCH_3), 2.26 (t, $J = 7.62$ Hz, 2H, O=C–CH_2), 1.61–1.67 (m, 2H, CH_2), 1.25 (m, 28H, CH_2), 1.18 (d, $J = 6.41$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.56$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 173.8 (O–C=O), 171.6 (HN–C=O), 67.9 (HC–OH), 57 (HC–NH), 52.5 (O–CH_3), 36.5 (O=C–CH_2), 31.8 (O=C–CH_2–CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 19.8 (HOHC–CH_3), 14.0 (CH_3); IR (KBr): 3478, 3323, 2926, 2836, 1736, 1646, 1547, 1281, 1023, 716; ESI–MS: *m/z* at 422.01 [M + Na].

*Methyl oleoyl-L-threoninate (**2s**)*

Hexane: ethyl acetate (70:30, v/v), yield 80%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.32 (d, $J = 8.39$ Hz, 1H, NH), 5.31–5.37 (m, 2H, HC=CH), 4.62 (dd, $J = 2.44$, 8.85 Hz, 1H, CH–NH), 4.32–4.37 (m, 1H, CH–OH), 3.76 (s, 3H, OCH_3), 2.28 (t, $J = 7.47$ Hz, 2H, O=C–CH_2), 1.99–2.02 (m, 4H, H_2C–CH=), 1.66 (m, 2H, CH_2), 1.26–1.31 (m, 20H, CH_2), 1.22 (d, $J = 6.56$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.86$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 173.9 (O–C=O), 171.6 (HN–C=O), 129.9 (HC=CH), 129.6 (HC=CH), 67.8 (HC–OH), 57.7 (HC–NH), 52.4 (O–CH_3), 36.4 (O=C–CH_2), 31.8 (H_2C–HC=CH), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 19.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3491, 3311, 2920, 2850, 1720, 1647, 1546, 1281, 1021, 726; ESI–MS: *m/z* at 420.2 [M + Na].

General procedure for ester hydrolysis (3a–3s)

Methyl ester (1 mol) was dissolved in tetrahydrofuran, water mixture (7:3) and then lithium hydroxide hydrate (3 mol) was added and the reaction mixture magnetically stirred at room temperature for 16 h. After completion of the reaction, mixture was concentrated under reduced pressure till white precipitate obtained, which was neutralized, extracted successively with 2 M HCl and ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and concentrated to yield a white solid. The crude solid was purified by silica gel column chromatography using chloroform, methanol solvent mixture to obtain 88–95% yield.

*Cinnamoyl-L-threonine (**3a**)*

Chloroform: methanol (95:5 v/v), yield 95%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.57 (d, $J = 15.71$ Hz, 1H,=CH), 7.37 (m, 3H, Ar–H), 7.21 (m, 2H, Ar–H), 6.55 (d, $J = 15.71$ Hz, 1H,=CH), 5.92 (br s, 1H, NH), 4.71 (m, 1H, CH–NH), 4.47 (m, 1H, CH–OH), 1.18 (d, $J = 5.95$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz): 173.7 (O–C=O), 167.6 (HN–C=O), 142.2 (HC=CH), 134.2 (Ar–C), 129.8 (Ar–CH), 128.6 (Ar–CH), 127.9 (Ar–CH), 119.6 (HC=CH), 67.9 (HC–OH), 57.9 (HC–NH), 19.3 (HOHC–CH_3); IR (KBr): 3418, 2924, 2853, 1727, 1661, 1519, 1343, 1216, 760; ESI–MS: *m/z* at 248.1 [M–H].

*4-Nitro cinnamoyl-L-threonine (**3b**)*

Chloroform: methanol (95:5 v/v), yield 94%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 8.23(m, 2H, Ar–H), 7.69 (m, 3H, Ar–H,=CH), 7.63 (m, 1H,=CH), 6.87 (br s, 1H, NH), 4.68 (dd, $J = 2.44$, 8.80 Hz, 1H, CH–NH), 4.43 (m, 1H, CH–OH), 1.26 (d, $J = 6.35$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 172 (O–C=O), 164.8 (HN–C=O), 147.3 (Ar–C–NO_2), 141.2 (HC=CH), 136.8 (Ar–C), 128.1 (Ar–CH), 125.8 (Ar–CH), 123.6 (HC=CH), 66.7 (HC–OH), 57.7 (HC–NH), 20.1 (HOHC–CH_3); IR (KBr): 3426, 2928, 2858, 1736, 1642, 1526, 1343, 1228, 760; ESI–MS: *m/z* at 293.2 [M–H].

*(2-(1H-Indol-3-yl) acetyl)-L-threonine (**3c**)*

Chloroform: methanol (95:5 v/v), yield 92%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 10.1 (br s, 1H, Ar–NH), 7.58 (d, $J = 7.45$ Hz, 1H, Ar–H), 7.37 (m, 1H, Ar–H), 7.19 (s, 1H, Ar–H), 7.12 (m, 1H, Ar–H), 7.04 (m, 1H, Ar–H), 4.46 (dd, $J = 2.56$, 8.80 Hz, 1H, CH–NH), 4.25 (m, 1H, CH–OH), 3.75 (s, 2H, CH_2), 1.08 (d, $J = 6.35$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 171.3 (O–C=O), 171 (HN–C=O), 135.1 (Ar–C–NH), 126 (Ar–C–CH), 122.9 (Ar–CH), 120.1 (Ar–CH), 117.6 (Ar–CH), 117.3 (Ar–CH), 110.3 (Ar–CH), 106.9 (Ar–C), 65.6 (HC–OH), 56.5 (HC–NH), 31.7 (H_2C–C=O), 19 (HOHC–CH_3); IR (KBr): 3424, 3312, 2931, 1742, 1646, 1534, 1498, 1377, 1214, 1107, 1005, 717, 549; ESI–MS: *m/z* at 275.01 [M–H].

*(3-(1H-Indol-3-yl) propanoyl)-L-threonine (**3d**)*

Chloroform: methanol (95:5 v/v), yield 95%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 9.87 (br s, 1H, Ar–NH), 7.55 (m, 1H, Ar–H), 7.35 (d, $J = 8.19$ Hz, 1H, Ar–H), 7.10 (t, $J = 7.58$ Hz, 1H, Ar–H), 7.05 (m, 1H, Ar–H), 7.01 (m, 1H, Ar–H), 4.48 (m, 1H, CH–NH), 4.27 (m, 1H, CH–OH), 3.09 (t, $J = 7.58$ Hz, 2H, CH_2), 2.66 (t, $J = 7.58$ Hz, 2H, CH_2), 1.11 (d, $J = 6.35$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): 172.9 (O–C=O), 172.4 (HN–C=O), 136 (Ar–C–NH), 126.7 (Ar–C–CH), 121.6 (Ar–CH), 120.8 (Ar–CH), 118.1 (Ar–CH), 113.7 (Ar–CH), 110.9 (Ar–C),

67 (HC–OH), 57.2 (HC–NH), 36.5 (H_2C–C=O), 20.7 (H_2C–H_2C–C=O), 19.6 (HOHC–CH_3); IR (KBr): 3456, 3328, 2986, 1758, 1636, 1534, 1498, 1376, 1214, 1126, 1018, 717, 549; ESI–MS: *m/z* at 289.1 [M–H].

*(1H-Indazole-3-carbonyl)-L-threonine (**3e**)*

Chloroform: methanol (95:5 v/v), yield 96%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 8.26 (d, $J = 8.24$ Hz, 1H, Ar–NH), 7.99 (d, $J = 9.15$ Hz, 1H, Ar–H), 7.53 (d, $J = 8.54$ Hz, 1H, Ar–H), 7.33 (t, $J = 7.62$ Hz, 1H, Ar–H), 7.20 (t, $J = 7.47$ Hz, 1H, Ar–H), 4.79 (d, $J = 9.00$ Hz, 1H, CH–NH), 4.51 (m, 1H, CH–OH), 1.29 (d, $J = 6.41$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 172.6 (O–C=O), 162.9 (HN–C=O), 141 (Ar–C–NH), 137.5 (O=C–(Ar)C=NH), 126.1 (Ar–CH), 121.9 (Ar–CH), 121.4 (Ar–CH), 110.1 (Ar–C), 67.2 (HC–OH), 56.8 (HC–NH), 19.8 (HOHC–CH_3); IR (KBr): 3482, 3312, 2931, 1742, 1646, 1534, 1498, 1377, 1214, 1107, 1005, 717, 549; ESI–MS: *m/z* at 263.2 [M–H].

*(1H-Indole-2-carbonyl)-L-threonine (**3f**)*

Chloroform: methanol (95:5 v/v), yield 97%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 10.92 (br s, 1H, Ar–NH), 7.78 (m, 1H, Ar–H), 7.63 (m, 1H, Ar–H), 7.49 (m, 1H, Ar–H), 7.22 (m, 2H, Ar–H), 7.11 (m, 1H, Ar–H), 4.73 (m, 1H, CH–NH), 4.42 (m, 1H, CH–OH), 1.26 (d, $J = 1.25$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 171.5 (O–C=O), 160.9 (HN–C=O), 135.7 (Ar–C–NH), 129.6 (O=C–C(NH)=CH), 126(Ar–C), 122.8 (Ar–CH), 120.5 (Ar–CH), 118.9 (Ar–CH), 111.2 (Ar–CH), 103 (Ar–CH), 66.1 (HC–OH), 56.9 (HC–NH), 19.2 (HOHC–CH_3); IR (KBr): 3424, 3348, 2982, 1758, 1638, 1534, 1498, 1358, 1214, 1107, 1022, 717, 549; ESI–MS: *m/z* at 261.3 [M–H].

*(2-Phenylacetyl)-L-threonine (**3g**)*

Chloroform: methanol (95:5 v/v), yield 96%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 7.27 (m, 5H, Ar–H), 7.13 (s, 1H, NH), 4.47 (dd, $J = 2.59$, 8.85 Hz, 1H, CH–NH), 4.30 (m, 1H, CH–OH), 3.62 (s, 2H, CH_2), 1.12 (d, $J = 5.03$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): 172.2 (O–C=O), 170.9 (HN–C=O), 134.5 (Ar–C–CH_2), 128.8 (Ar–CH), 128.1 (Ar–CH), 126.3 (Ar–CH), 66.9 (HC–OH), 57.3 (HC–NH), 42.7 (Ar–CH_2–C=O), 19.7 (HOHC–CH_3); IR (KBr): 3329, 2932, 1728, 1646, 1513, 1245, 1178, 1034, 673; ESI–MS: *m/z* at 236.02 [M–H].

*(2-(4-Methoxyphenyl) acetyl)-L-threonine (**3h**)*

Chloroform: methanol (95:5 v/v), yield 95%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 7.23 (d, $J = 8.08$ Hz, 2H, Ar–H), 7.08 (br s, 1H, NH), 6.84 (d, $J = 8.39$ Hz, 2H, Ar–H), 4.46 (dd, $J = 1.98$, 8.85 Hz, 1H, CH–NH), 4.29 (m, 1H, CH–OH), 3.77 (s, 3H, Ar–OCH_3), 3.55 (s, 2H, CH_2), 1.12 (d, $J = 4.27$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 172.1 (O–C=O), 171.1 (HN–C=O), 157.8 (Ar–C–OCH_3), 129.8 (Ar–C–CH_2), 126.9 (Ar–CH), 113.4 (Ar–CH), 66.7 (HC–OH), 57.2 (HC–NH), 54.6 (Ar–C–O–CH_3), 41.7 (Ar–CH_2–C=O), 19.8 (HOHC–CH_3); IR (KBr): 3336, 2958, 1768, 1636, 1513, 1236, 1178, 1048, 673; ESI–MS: *m/z* at 266.1 [M–H].

*(4-(4-Methoxyphenyl) butanoyl)-L-threonine (**3i**)*

Chloroform: methanol (95:5 v/v), yield 96%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 7.05 (d, $J = 8.39$ Hz, 2H, Ar–H), 6.90 (br s, 1H, NH), 6.79 (d, $J = 8.39$ Hz, 2H, Ar–H), 4.49 (m, 1H, CH–NH), 4.37 (m, 1H, CH–OH), 3.74 (s, 3H, Ar–OCH_3), 2.54 (t, $J = 7.32$ Hz, 2H, CH_2), 2.26 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 1.16 (br s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 75 MHz): 172.7 (O–C=O), 172.3 (HN–C=O), 157 (Ar–C–OCH_3), 133.1 (Ar–C–CH_2), 128.7 (Ar–CH), 113 (Ar–CH), 66.6 (HC–OH), 56.9 (HC–NH), 54.5 (Ar–C–O–CH_3), 34.9 (H_2C–CH_2–C=O), 33.5 (Ar–C–CH_2), 26.8 (CH_2), 19.5 (HOHC–CH_3); IR (KBr): 3329, 2932, 1728, 1646, 1513, 1245, 1178, 1034, 673; ESI–MS: *m/z* at 294.4 [M–H].

*Hexanoyl-L-threonine (**3j**)*

Chloroform: methanol (95:5 v/v), yield 95%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (br s, 1H, COOH), 7.03 (br s, 1H, NH), 4.51 (m, 1H, CH–NH), 4.40 (m, 1H, CH–OH), 2.29 (t, $J = 7.62$ Hz, 2H, O=C–CH_2), 1.62 (m, 2H, CH_2), 1.31 (m, 4H, CH_2), 1.19 (d, $J = 6.41$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 5.95$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 175.3 (O–C=O), 174.1 (HN–C=O), 67.6 (HC–OH), 57.6 (HC–NH), 36.1 (O=C–CH_2), 31.3 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 19.4 (HOHC–CH_3), 13.8 (CH_3); IR (KBr): 3321, 2927, 2857, 1729, 1643, 1539, 1221, 1113, 758; ESI–MS: *m/z* at 216.02 [M–H].

*Octanoyl-L-threonine (**3k**)*

Chloroform: methanol (92:5 v/v), yield 94%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.05 (br s, 1H, NH), 4.41–4.51 (m, 2H, CH–NH, CH–OH), 2.29 (m, 2H, O=C–CH_2), 1.63 (m, 2H, CH_2), 1.21–1.29 (m, 11H, CHOH–CH_3, CH_2), 0.87 (t, $J = 6.56$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 175.2 (O–C=O), 174.3 (HN–C=O), 67.5 (HC–OH), 57.7 (HC–NH), 36.2 (O=C–CH_2), 31.6((O=C–CH_2–CH_2), 29.2 (CH_2), 28.9 (CH_2), 25.7 (CH_2), 22.5 (CH_2), 19.3 (HOHC–CH_3), 13.9 (CH_3); IR (KBr): 3328, 2938, 2857, 1736, 1628, 1539, 1248, 1113, 758; ESI–MS: *m/z* at 244.1 [M–H].

*(2-Propylpentanoyl)-L-threonine (**3l**)*

Chloroform: methanol (92:8 v/v), yield 92%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.43 (br s, 1H, NH), 4.63 (m, 1H, CH–NH), 4.37 (m, 1H, CH–OH), 3.045 (s, 1H, OH), 2.17–2.22 (m, 1H, CH), 1.62 (m, 2H, CH_2), 1.31–1.40 (m, 6H, CH_2), 1.21 (m, 3H, CHOH–CH_3), 0.90–0.92 (m, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz): 177 (O–C=O), 171.6 (HN–C=O), 67.6 (HC–OH), 57 (HC–NH), 47.5 (O=C–CH), 35.3 (O=C–CH–CH_2), 35.1 (O=C–CH–CH_2), 20.7 (CH_2), 20.6 (CH_2), 19.9 (HOHC–CH_3), 14.1 (CH_3); IR (KBr): 3321, 2923, 2832, 1729, 1623, 1539, 1221, 1113, 758; ESI–MS: *m/z* at 244.2 [M–H].

*Undec-10-enoyl-L-threonine (**3m**)*

Chloroform: methanol (92:8 v/v), yield 95%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.45 (d, $J = 8.80$ Hz, 1H, NH), 5.75–5.85 (m, 1H,=CH), 4.91–5.01 (m, 2H,=CH_2), 4.59 (dd, $J = 2.44$, 8.92 Hz, 1H, CH–NH), 4.31–4.36 (m, 1H, CH–OH), 2.28 (t, $J = 7.45$ Hz, 2H, O=C–CH_2), 2.0–2.06 (m, 2H,=CH–CH_2), 1.61–1.68 (m, 2H, CH_2), 1.28–1.38 (m, 10H, CH_2), 1.20 (d, $J = 6.48$ Hz, 3H, CHOH–CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.04 (O–C=O), 171.5 (HN–C=O), 138.9 (H_2C–HC=CH), 114.0 (H_2C–HC=CH), 67.6 (HC–OH), 57.1 (HC–NH), 36.3 (O=C–CH_2), 33.6 (H_2C–HC=CH), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 25.5 (CH_2), 19.8 (HOHC–CH_3); IR (KBr): 3345, 2923, 2875, 1736, 1656, 1539, 1221, 1113, 758; ESI–MS: *m/z* at 284.1 [M–H].

*Undecanoyl-L-threonine (**3n**)*

Chloroform: methanol (92:8 v/v), yield 96%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.04 (br s, 1H, NH), 4.4–4.5 (m, 2H, CH–NH, CH–OH), 2.29 (m, 2H, O=C–CH_2), 1.62 (m, 2H, CH_2), 1.19–1.25 (m, 17H, CH_2, CHOH–CH_3), 0.87 (t, $J = 6.71$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 175.2 (O–C=O), 174.3 (HN–C=O), 67.5 (HC–OH), 57.7 (HC–NH), 36.4 (O=C–CH_2), 31.8 (O=C–CH_2–CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 19.4 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3321, 2927, 2857, 1736, 1643, 1528, 1221, 1126, 758; ESI–MS: *m/z* at 286.3 [M–H].

*Dodecanoyl-L-threonine (**3o**)*

Chloroform: methanol (92:8 v/v), yield 97%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.92 (br s, 1H, NH), 4.54 (d, $J = 8.39$ Hz, 1H, CH–NH), 4.42 (m, 1H, CH–OH), 2.29 (t, $J = 7.62$ Hz, 2H, O=C–CH_2), 1.63 (m, 2H, CH_2), 1.25 (m, 16H, CH_2), 1.22 (d, $J = 5.03$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.56$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 175.3 (O–C=O), 174.1 (HN–C=O), 67.6 (HC–OH), 57.6 (HC–NH), 36.2 (O=C–CH_2), 31.8 (O=C–CH_2–CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 19.3 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3538, 3315, 2919, 2849, 1711, 1649, 1543, 1281, 1209, 860, 673; ESI–MS: *m/z* at 300.2 [M–H].

*Tetradecanoyl-L-threonine (**3p**)*

Chloroform: methanol (92:8 v/v), yield 96%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.01 (br s, 1H, NH), 4.4–4.5 (m, 2H, CH–NH, CH–OH), 2.29 (m, 2H, O=C–CH_2), 1.62 (m, 2H, CH_2), 1.25 (m, 20H, CH_2), 1.19 (m, 3H, CHOH–CH_3), 0.87 (t, $J = 6.86$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 175.2 (O–C=O), 174.3 (HN–C=O), 67.5 (HC–OH), 57.7 (HC–NH), 36.3 (O=C–CH_2), 31.9 (O=C–CH_2–CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 25.8 (CH_2), 22.6 (CH_2), 19.3 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3536, 3328, 2927, 2857, 1728, 1649, 1523, 1281, 1209, 860, 673; ESI–MS: *m/z* at 328.1 [M–H].

*Hexadecanoyl-L-threonine (**3q**)*

Chloroform: methanol (92:8 v/v), yield 95%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 7.02 (br s, 1H, NH), 4.46 (m, 1H, CH–NH), 4.29 (m, 1H, CH–OH), 2.26 (t, $J = 7.78$ Hz, 2H, O=C–CH_2), 1.63 (m, 2H, CH_2), 1.25 (s, 24H, CH_2), 1.18 (d, $J = 6.4$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.25$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 125 MHz): 173.2 (O–C=O), 172.3 (HN–C=O), 66.8 (HC–OH), 57.2 (HC–NH), 35.8 (O=C–CH_2), 31.3 (O=C–CH_2–CH_2), 29.1 (CH_2), 29 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 25.3 (CH_2), 22.1 (CH_2), 19.9 (HOHC–CH_3), 13.7 (CH_3); IR (KBr): 3538, 3328, 2927, 2848, 1718, 1642, 1543, 1281, 1222, 860, 673; ESI–MS: *m/z* at 356.02 [M–H].

*Octadecanoyl-L-threonine (**3r**)*

Chloroform: methanol (92:8 v/v), yield 96%. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): 7.31 (t, $J = 9.31$ Hz, 1H, NH), 4.36–4.40 (m, 1H, CH–NH), 4.25 (m, 1H, CH–OH), 2.24 (m, 2H, O=C–CH_2), 1.60 (m, 2H, CH_2), 1.25–1.27 (m, 28H, CH_2), 1.13–1.16 (m, 3H, CHOH–CH_3), 0.86–0.91 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6, 125 MHz): 172 (O–C=O), 171.1 (HN–C=O), 65.5 (HC–OH), 56.1 (HC–NH), 34.5 (O=C–CH_2), 30.2 (O=C–CH_2–CH_2), 28 (CH_2), 27.9 (CH_2), 27.7 (CH_2), 27.6 (CH_2), 24.2 (CH_2), 21 (CH_2), 18.9 (HOHC–CH_3), 12.6 (CH_3); IR (KBr): 3538, 3315, 2919, 2849, 1711, 1649, 1543, 1281, 1209, 860, 673; ESI–MS: *m/z* at 384.3 [M–H].

*Oleoyl-L-threonine (**3s**)*

Chloroform: methanol (92:8 v/v), yield 98%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.88 (s, 1H, NH), 5.31–5.37 (m, 2H, HC=CH), 4.48 (m, 1H, CH–NH), 4.42 (m, 1H, CH–OH), 2.29 (t, $J = 7.17$ Hz, 2H, O=C–CH_2), 2.01 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.26–1.31 (m, 20H, CH_2), 1.21 (d, $J = 6.25$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.71$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 175.2 (O–C=O), 174.3 (HN–C=O), 129.9 (HC=CH), 129.6 (HC=CH), 67.5 (HC–OH), 57.7 (HC–NH), 36.3 (O=C–CH_2), 33.8 (H_2C–HC=CH), 31.8 (O=C–CH_2–CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29 (CH_2), 27.2 (CH_2), 25.7 (CH_2), 24.7 (CH_2), 22.6 (CH_2), 19.4 (HOHC–CH_3), 14 (CH_3); IR (Neat): 3316, 2925, 2854, 1724, 1642, 1540, 1461, 1248, 1119, 722; ESI–MS: *m/z* at 382.1 [M–H].

General procedure for synthesis of compounds 4a–4s

Fatty acid or Aromatic acid (**3a–3s**, 10.25 mmol) was dissolved in ice-cold anhydrous dichloromethane (40 mL) and to this oleyl amine (12.3 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC. HCl, 12.3 mmol) and hydroxy benzotriazole (HOBt, 15.38 mmol) were added successively at 0 °C and the mixture was stirred for 12 h at room temperature. The reaction was monitored using micro TLC (hexane: ethyl acetate, 35:65, v/v). At the end of the reaction, the mixture was dissolved in 75 mL of $CHCl_3$, which was then washed by 5% $NaHCO_3$ solution, saturated NaCl solution successively. The chloroform layer was dried with anhydrous Na_2SO_4 and the filtrate was dried under reduced pressure. The crude mixture was purified by silica gel chromatography, the title compounds were obtained with 75–80% yield.

(2S, 3R)-2-Cinnamamido-3-hydroxy-N*-(oleyl) butanamide (**4a**)*

Hexane: ethyl acetate (60:40, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.64 (d, $J = 15.65$ Hz, 1H,=CH), 7.48–7.51 (m, 2H, Ar–H), 7.34–7.35 (m, 3H, Ar–H), 7.20 (d, $J = 7.70$ Hz, 1H, NH), 7.12 (t, $J = 5.50$ Hz, 1H, NH), 6.59 (d, $J = 15.65$ Hz, 1H,=CH), 5.28–5.37 (m, 2H, HC=CH), 4.52 (dd, $J = 2.20$, 7.70 Hz, 1H, CH–NH), 4.39–4.44 (m, 1H, CH–OH), 3.18–3.28 (m, 2H, H_2C–NH), 1.95–2.02 (m, 4H, H_2C–HC=CH), 1.48–1.52 (m, 2H, CH_2), 1.26(s, 22H, CH_2), 1.20 (d, $J = 6.48$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.72$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 171(HN–C=O), 166.9 (HN–C=O), 141.9 (HC=CH), 134.4 (Ar–C), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH, Ar–CH), 128.7 (Ar–CH), 127.8 (Ar–CH), 119.8 (HC=CH), 66.8 (HC–OH), 56.9 (HC–NH), 39.5 (O=C–NH–CH_2), 32.5 (O=C–NH–CH_2–CH_2), 31.8 (H_2C–HC=CH), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 22.6 (CH_2), 18 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3289, 3095, 2925, 2855, 1631, 1552, 1455, 1219, 1103, 978, 758; ESI–MS: *m/z* at 521.03 [M + Na].

(2S, 3R)-2-(4-Nitro-cinnamamido)-3-hydroxy-N*-(oleyl) butanamide (**4b**)*

Hexane: ethyl acetate (60:40, v/v), yield 80%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 8.16–8.24 (m, 2H, Ar–H), 7.63–7.69 (m, 2H, Ar–H), 7.14 (br s, 1H, NH), 6.90 (t, $J = 5.52$ Hz, 1H, NH), 6.71 (d, $J = 15.56$ Hz, 1H,=CH), 6.21 (d, $J = 15.56$ Hz, 1H,=CH), 5.28–5.37 (m, 2H, HC = CH), 4.41–4.46 (m, 1H, CH–NH), 4.31–4.33 (m, 1H, CH-–OH), 3.18–3.30 (m, 2H, H_2C–NH), 1.94–2.01 (m, 4H, H_2C–HC=CH), 1.49–1.51 (m, 2H, CH_2), 1.25 (s, 22H, CH_2), 1.21 (d, $J = 6.56$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.41$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 170.2 (HN–C=O), 165.7 (HN–C=O), 147.9 (Ar–C–NO_2), 140.8 (HC=CH), 138.6 (Ar–C), 129.6 (H_2C–HC=CH), 128.2 (H_2C–HC=CH), 127.6 (Ar–CH), 125.6 (Ar–CH), 124.1 (Ar–CH), 123.8 (Ar–CH), 122.9 (HC=CH), 66.6 (HC–OH), 57.7 (HC–NH), 39.3 (O=C–NH–CH_2), 31.5 (O=C–NH–CH_2–CH_2, H_2C–HC=CH)), 29.4 (CH_2), 29.3 (H_2C), 29.1 (CH_2), 28.9 (CH_2), 26.8 (CH_2), 26.6 (CH_2), 22.3 (CH_2), 18.1 (HOHC–CH_3), 13.6 (CH_3); IR (KBr): 3284, 3081, 2924, 2853, 1626, 1599, 1517, 1346, 973, 721; ESI–MS: *m/z* at 566.1 [M + Na].

(2S, 3R)-2-(2-Indole-3-acetamido)-3-hydroxy-N*-(oleyl) butanamide (**4c**)*

Hexane: ethyl acetate (60:40, v/v), yield 76%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.46 (br s, 1H, Ar–NH), 7.52 (d, $J = 7.94$ Hz, 1H, Ar–H), 7.33 (d, $J = 8.06$ Hz, 1H, Ar–H), 7.18–7.22 (m, 1H, Ar–H), 7.10–7.13 (m, 1H, Ar–H), 7.07–7.08 (s, 1H, Ar–H), 6.71 (t, $J = 6.11$ Hz, 1H, NH), 6.68 (d, $J = 7.70$ Hz, 1H, NH), 5.32–5.38 (m, 2H, HC=CH), 4.29 (m, 1H, CH–NH), 4.27 (m, 1H, CH–OH), 3.77 (s, 2H, CH_2), 3.06–3.17 (m, 2H, H_2C–NH), 1.98–2.03 (m, 4H, H_2C–HC=CH), 1.38 (m, 2H, CH_2), 1.25 (m, 22H, CH_2), 0.94 (d, $J = 6.35$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.72$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 172.8 (HN–C=O), 170.7 (HN–C=O), 136.3 (Ar–C–NH), 129.9 (H_2C–HC=CH), 129.7 (H_2C–HC=CH), 126.7 (Ar–C–CH), 123.6 (Ar–CH), 122.4 (Ar–CH), 119.8 (Ar–CH), 118.2 (Ar–CH), 111.5 (Ar–CH), 108.1 (Ar–C), 66.2 (HC–OH), 56.7 (HC–NH), 39.4 (O=C–NH–CH_2), 33.3 (H_2C–C=O), 31.8 (H_2C–HC=CH, O=C–NH–CH_2–CH_2), 29.7 (CH_2),

29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 22.6 (CH_2), 17.8 (HOHC–$\underline{CH_3}$), 14 (CH_3); IR (KBr): 3395, 3029, 2952, 2832, 1642, 1540, 1243, 1097, 726; ESI–MS: *m/z* at 548.04 [M + Na].

*(2*S*, 3*R*)-2-(3-Indole-3-propanamido)-3-hydroxy*-N*-(oleyl) butanamide (**4d**)*

Hexane: ethyl acetate (60:40, v/v), yield 75%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.0 (br s, 1H, Ar–NH), 7.59 (d, J = 7.78 Hz, 1H, Ar–H), 7.33 (d, J = 7.93 Hz, 1H, Ar–H), 7.18 (t, J = 7.93 Hz, 1H, Ar–H), 7.11 (d, J = 7.62 Hz, 1H, Ar–H), 6.98 (s, 1H, Ar–H), 6.72 (br s, 1H, NH), 6.54 (br s, 1H, NH), 5.31–5.37 (m, 2H, HC=CH), 4.25 (m, 2H, $\underline{CH}$–NH, $\underline{CH}$–OH), 3.89 (br s, 1H, OH), 3.06–3.19 (m (overlap), 4H, $\underline{H_2C}$–NH, CH_2), 2.66 (t, J = 7.32 Hz, 2H, CH_2), 1.95–2.05 (m, 4H, $\underline{H_2C}$–HC=CH), 1.41 (m, 2H, CH_2), 1.25 (m, 22H, CH_2), 0.94 (d, J = 6.41 Hz, 3H, CHOH–$\underline{CH_3}$), 0.87 (t, J = 6.71 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 173.8 (HN–$\underline{C}$=O), 170.7 (HN–$\underline{C}$=O), 136.2 (Ar–$\underline{C}$–NH), 129.9 (H_2C–$\underline{HC}$=CH), 129.7 (H_2C–HC=$\underline{CH}$), 126.9 (Ar–$\underline{C}$–CH), 122 (Ar–$\underline{CH}$), 121.5 (Ar–$\underline{CH}$), 119.3 (Ar–$\underline{CH}$), 118.5 (Ar–$\underline{CH}$), 114.3 (Ar–$\underline{CH}$), 111.1 (Ar–$\underline{C}$), 66.5 ($\underline{HC}$–OH), 56.8 ($\underline{HC}$–NH), 39.4 (O=C–NH–$\underline{CH_2}$), 36.9 ($\underline{H_2C}$–C=O), 32.5 ($\underline{H_2C}$–HC=CH), 31.8 (O=C–NH–CH_2–$\underline{CH_2}$), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 22.6 (CH_2), 21.2 (CH_2), 17.8 (HOHC–$\underline{CH_3}$), 14 (CH_3); IR (KBr): 3295, 3049, 2922, 2852, 1629, 1540, 1213, 1097, 726; ESI–MS: *m/z* at 562.2 [M + Na].

N-*(2*S*, 3*R*)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl)-indazole-3-carboxamide (**4e**)*

Hexane: ethyl acetate (60:40, v/v), yield 76%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 13.05 (br s, 1H, Ar–NH), 9.61 (br s, 1H, NH), 8.30 (d, J = 8.24 Hz, 1H, Ar–H), 8.02 (br s, 1H, NH), 7.57 (d, J = 8.54 Hz, 1H, Ar–H), 7.38 (t, J = 7.32 Hz, 1H, Ar–H), 7.23 (t, J = 7.62 Hz, 1H, Ar–H), 5.29–5.36 (m, 2H, HC=CH), 5.21 (br s, 1H, OH), 4.96 (t, J = 7.78 Hz, 1H, $\underline{CH}$–NH), 4.23 (m, 1H, $\underline{CH}$–OH), 3.37–3.44 (m, 1H, $\underline{H_2C}$–NH), 3.17–3.22 (m, 1H, $\underline{H_2C}$–NH), 1.95–2.01 (m, 4H, $\underline{H_2C}$–HC=CH), 1.35–1.48 (m, 2H, CH_2), 1.15–1.24 (m, 22H, CH_2), 0.99 (d, J = 6.10Hz, 3, CHOH–$\underline{CH_3}$), 0.86 (t, J = 7.02 Hz, 3 H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 171.7 (HN–$\underline{C}$=O), 164.3 (HN–$\underline{C}$=O), 141.2 (Ar–$\underline{C}$–NH), 138.4 (O=C–(Ar)$\underline{C}$=NH), 129.8 (H_2C–$\underline{HC}$=CH), 129.7 (H_2C–HC=$\underline{CH}$), 126.9 (Ar–$\underline{CH}$), 122.3 (Ar–$\underline{CH}$), 121.5 (Ar–$\underline{CH}$), 111 (Ar–$\underline{C}$), 67 ($\underline{HC}$–OH), 60.2 ($\underline{HC}$–NH), 39.9 (O=C–NH–$\underline{CH_2}$), 31.8 ($\underline{H_2C}$–HC=CH, O=C–NH–CH_2–$\underline{CH_2}$), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 27 (CH_2), 22.6 (CH_2), 19.3 (HOHC–$\underline{CH_3}$), 14 (CH_3); IR (KBr): 3403, 3352, 2924, 2853, 1717, 1642, 1515, 1461, 1296, 1158, 745; ESI–MS: *m/z* at 535.01 [M + Na].

N-*(2*S*, 3*R*)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl)-indole-2-carboxamide (**4f**)*

Hexane: ethyl acetate (60:40, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 9.61 (br s, 1H, Ar–NH), 7.59–7.65 (m, 1H, Ar–H), 7.47 (d, J = 7.62 Hz, 1H, Ar–H), 7.40 (d, J = 8.39 Hz, 1H, NH), 7.30 (t, J = 7.17 Hz, 1H, Ar–H), 7.12–7.15 (m, 1H, Ar–H), 7.06 (m, 1H, Ar–H), 6.88 (m, 1H, NH), 5.30–5.37 (m, 2H, HC=CH), 4.57 (m, 1H, $\underline{CH}$–NH), 4.49 (m, 1H, $\underline{CH}$–OH), 4.18 (br s, 1H, OH), 3.19–3.28 (m, 2H, $\underline{H_2C}$–NH), 1.95–2.01 (m, 4H, $\underline{H_2C}$–HC=CH), 1.47 (m, 2H, CH_2), 1.22–1.26 (m, 25H, CH_2, CHOH–$\underline{CH_3}$), 0.87 (t, J = 6.71 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 170.4 (HN–$\underline{C}$=O), 162.2 (HN–$\underline{C}$=O), 136.6 (Ar–$\underline{C}$–NH), 130 (O=C–$\underline{C}$(NH)=CH), 129.7 (H_2C–$\underline{HC}$=CH), 129.5 (H_2C–HC=$\underline{CH}$), 127.2 (Ar–$\underline{C}$), 124.3 (Ar–$\underline{CH}$), 121.8 (Ar–$\underline{CH}$), 120.3 (Ar–$\underline{CH}$), 111.9 (Ar–$\underline{CH}$), 104.4 (Ar–$\underline{CH}$), 66.7 ($\underline{HC}$–OH), 57.5 ($\underline{HC}$–NH), 39.4 (O=C–NH–$\underline{CH_2}$), 31.6 ($\underline{H_2C}$–HC=CH, O=C–NH–CH_2–$\underline{CH_2}$), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 29 (CH_2), 26.9 (CH_2), 26.7 (CH_2), 22.4 (CH_2), 18.4 (HOHC–$\underline{CH_3}$), 13.9 (CH_3); IR (KBr): 3357, 3245, 2922, 2851, 1637, 1509, 1075, 721; ESI–MS: *m/z* at 534.2 [M + Na].

*(2*S*, 3*R*)-3-Hydroxy*-N*-(oleyl)-2-(2-phenylacetamido) butanamide (**4g**)*

Hexane: ethyl acetate (60:40, v/v), yield 77%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.32–7.35 (m, 2H, Ar–H), 7.24–7.29 (m, 3H, Ar–H), 6.82 (br s, 2H, NH), 5.33–5.38 (m, 2H, HC=CH), 4.27–4.30 (m, 2H, $\underline{CH}$–NH, $\underline{CH}$–OH), 3.61 (s, 2H, CH_2), 3.13–3.19 (m, 2H, $\underline{H_2C}$–NH), 1.96–2.03 (m, 4H, $\underline{H_2C}$–HC=CH), 1.39–1.44 (m, 2H, CH_2), 1.25 (m, 22H, CH_2), 1.04 (d, J = 6.48 Hz, 3H, CHOH–$\underline{CH_3}$), 0.88 (t, J = 6.61 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz): 172.3 (HN–$\underline{C}$=O), 170.7 (HN–$\underline{C}$=O), 134.5 (Ar–$\underline{C}$–CH_2), 130.8 (H_2C–$\underline{HC}$=CH), 129.9 (H_2C–HC=$\underline{CH}$), 129.7 (Ar–$\underline{CH}$), 129.1 (Ar–$\underline{CH}$), 128.9 (Ar–$\underline{CH}$), 128.7 (Ar–$\underline{CH}$), 127.3 (Ar–$\underline{CH}$), 66.5 ($\underline{HC}$–OH), 56.8 ($\underline{HC}$–NH), 43.3 (Ar–$\underline{CH_2}$–C=O), 39.5 (O=C–NH–$\underline{CH_2}$), 32.6 (O=C–NH–CH_2–$\underline{CH_2}$), 31.9 ($\underline{H_2C}$–HC=CH), 30.3 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 27.2 (CH_2), 26.8 (CH_2), 23.7 (CH_2), 22.9 (CH_2), 22.6 (CH_2), 17.8 (HOHC–$\underline{CH_3}$), 14.1 (CH_3); IR (KBr): 3354,

3063, 2924, 2853, 1730, 1637, 1513, 1466, 1287, 1074, 719; ESI–MS: *m/z* at 509.1 [M + Na].

*(2S, 3R)-3-Hydroxy-2-(2-(4-methoxyphenyl) acetamido)-N-(oleyl) butanamide (**4h**)*

Hexane: ethyl acetate (60:40, v/v), yield 79%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.16 (d, $J = 8.68$ Hz, 2H, Ar–H), 6.87 (d, $J = 8.68$ Hz, 2H, Ar–H), 6.75 (t, $J = 5.25$ Hz, 1H, NH), 6.56 (d, $J = 7.45$ Hz, 1H, NH), 5.33–5.37 (m, 2H, HC=CH), 4.28–4.33 (m, 1H, CH–NH), 4.24 (dd, $J = 1.83$, 7.58 Hz, 1H, CH–OH), 3.79 (s, 3H, Ar–OCH_3), 3.55 (s, 2H, CH_2), 3.14–3.20 (m, 2H, H_2C–NH), 1.97–2.03 (m, 4H, H_2C–HC=CH), 1.43 (m, 2H, CH_2), 1.25 (m, 22H, CH_2), 1.04 (d, $J = 6.48$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.72$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 172.6 (HN–C=O), 170.7 (HN–C=O), 158.8 (Ar–C–OCH_3), 130.1 (Ar–C–CH_2), 129.9 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 126.2 (Ar–CH), 114.3 (Ar–CH), 66.2 (HC–OH), 56.6 (HC–NH), 55.1 (Ar–C–O-CH_3), 42.5 (Ar–CH_2–C=O), 39.4 (O=C–NH–CH_2), 31.8 (H_2C–HC=CH, O=C–NH–CH_2–CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3292, 3099, 2924, 2853, 1631, 1551, 1512, 1375, 1245, 1040, 753; ESI–MS: *m/z* at 539.06 [M + Na].

*(2S, 3R)-3-Hydroxy-2-(4-(4-methoxyphenyl) butanamide)-N-(oleyl) butanamide (**4i**)*

Hexane: ethyl acetate (60:40, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.07 (d, $J = 8.54$ Hz, 2H, Ar–H), 6.91 (t, $J = 5.18$ Hz, 1H, NH), 6.82 (d, $J = 8.54$ Hz, 2H, Ar–H), 6.67 (d, $J = 7.17$ Hz, 1H, NH), 5.32–5.37 (m, 2H, HC=CH), 4.31–4.32 (m, 2H, CH–NH, CH–OH), 3.78 (s, 3H, Ar–OCH_3), 3.18–3.24 (m, 2H, H_2C–NH), 2.57 (t, $J =$ 7.47 Hz, 2H, CH_2), 2.24–2.28 (m, 2H, CH_2), 1.96–2.02 (m, 4H, H_2C–HC=CH), 1.93 (t, $J = 7.47$ Hz, 2H, CH_2), 1.47 (m, 2H, CH_2), 1.25 (m, 22H, CH_2), 1.13 (d, $J = 6.41$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.86$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 173.9 (HN–C=O), 172.5 (HN–C=O), 157.8 (Ar–C–OCH_3), 133.1 (Ar–C–CH_2), 130.1 (H_2C–HC=CH), 129.8 (H_2C–HC=CH), 129.2 (Ar–CH), 126.2 (Ar–CH), 114.2 (Ar–CH), 113.7 (Ar–CH), 66.2 (HC–OH), 56.7 (HC–NH), 55.1 (Ar–C–O–CH_3), 42.4 (H_2C–CH_2–C=O), 39.4 (O=C–NH–CH_2), 35.4 (Ar–C–CH_2), 31.8 (H_2C–HC=CH, O=C–NH–CH_2–CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.3 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3284, 3081, 2924, 2853, 1626, 1548, 1517, 1346, 1112. 973, 721; ESI–MS: *m/z* at 567.2 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) hexanamide (**4j**)*

Hexane: ethyl acetate (60:40, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.88 (br s, 1H, NH), 6.61 (br s, 1H, NH), 5.29–5.38 (m, 2H, HC=CH), 4.28–4.35(m, 2H, CH–NH, CH–OH), 3.22 (q, $J = 6.48$ Hz, 2H, H_2C–NH), 2.25 (t, $J = 7.58$ Hz, 2H, O=C–CH_2), 1.97–2.01 (m, 4H, H_2C–HC=CH), 1.64 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.25–1.32 (m, 26H, CH_2), 1.14 (d, $J = 6.48$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.72$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.2(HN–C=O), 170.8 (HN–C=O), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.5 (HC–OH), 56.6 (HC–NH), 39.6 (O=C–NH–CH_2), 36.3 (O=C–CH_2), 32.5 (H_2C–HC=CH), 31.8 (O=C–CH_2–CH_2), 31.2 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.3 (CH_2), 22.5 (CH_2), 22.2 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3), 13.8 (CH_3); IR (KBr): 3352, 2933, 2853, 1628, 1568, 1464, 1368, 1290, 1060, 698, 590; ESI–MS: *m/z* at 489.1 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) octanamide (**4k**)*

Hexane: ethyl acetate (60:40, v/v), yield 80%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.86 (br s, 1H, NH), 6.59 (d, $J = 7.45$ Hz, 1H, NH), 5.30–5.38 (m, 2H, HC=CH), 4.32–4.38 (m, 1H, CH–NH), 4.29 (dd, $J = 1.83$, 7.7 Hz, 1H, CH–OH), 3.21 (q, J = 7.09 Hz, 2H, H_2C–NH), 2.25 (t, $J = 7.7$ Hz, 2H, O=C–CH_2), 1.97–2.07 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.27 (m, 30H, CH_2), 1.14 d, $J = 6.6$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.6$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 174.3 (HN–C=O), 171 (HN–C=O), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.3 (HC–OH), 56.4 (HC–NH), 39.4 (O=C–NH–CH_2), 36.4 (O=C–CH_2), 31.8 (H_2C–HC=CH), 31.6 (O=C–CH_2–CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 22.5 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3), 13.9 (CH_3); IR (KBr): 3361, 2923, 2853, 1637, 1558, 1464, 1378, 1290, 1073, 695, 590; ESI–MS: *m/z* at 517.1 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl)-2-propylpentanamide (**4l**)*

Hexane: ethyl acetate (60:40, v/v), yield 76%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.03 (t, $J = 5.64$ Hz, 1H, NH), 6.73 (d, $J = 7.47$ Hz, 1H, NH), 5.30–5.37 (m, 2H, HC=CH), 4.32–4.39 (overlap m, 3H, CH–NH, CH–OH, OH), 3.21 (q, $J = 7.17$ Hz, 2H, H_2C–NH), 2.17–2.23 (m, 1H,

O=C–CH_2), 1.96–2.06 (m, 4H, H_2C–HC=CH), 1.55–1.62 (m, 2H, CH_2), 1.46–1.48 (m, 2H, CH_2), 1.37–1.42 (m, 2H, CH_2), 1.26 (m, 26H, CH_2), 1.14 (d, $J = 6.4$ Hz, 3H, CHOH–CH_3), 0.86–0.91 (m, 9H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz): 177.3 (HN–C=O), 171 (HN–C=O), 129.9 (H_2C–HC=CH), 129.7 (H_2C–HC=CH), 66.3 (HC–OH), 56.2 (HC–NH), 47.1 (O=C–CH), 39.4 (O=C–NH–CH_2), 35.3 (O=C–CH–CH_2), 35.2 (O=C–CH–CH_2), 32.5 (H_2C–HC=CH), 31.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 20.7 (CH_2), 17.7 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3279, 3101, 2923, 2853, 1637, 1551, 1463, 1377, 718; ESI–MS: *m/z* at 517.04 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) undec-10-enamide (**4m**)*

Hexane: ethyl acetate (60:40, v/v), yield 75%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.96 (s, 1H, NH), 6.68 (s, 1H, NH), 5.75–5.83 (m, 1H,=CH), 5.33–5.36 (m, 2H, HC=CH), 4.91–4.99 (m, 2H,=CH_2), 4.32 (m, 2H, CH–NH, CH–OH), 3.19–3.23 (m, 2H, H_2C–NH), 2.25 (t, $J = 6.86$ Hz, 2H, O=C–CH_2), 1.96–2.03 (m, 6H, H_2C–HC = CH, H_2C–HC=CH_2), 1.62 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 1.27 (m, 32H, CH_2), 1.13 (d, $J = 6.41$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 7.02$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 174.3 (HN–C=O), 170.9 (HN–C=O), 138.9 (H_2C–HC=CH), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 114 (H_2C–HC=CH), 66.4 (HC–OH), 56.5 (HC–NH), 39.4 (O=C–NH–CH_2), 36.4 (O=C–CH_2), 33.7 (H_2C–HC=CH), 32.5 (H_2C–HC=CH), 31.8 (O=C–CH_2–CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3358, 2922, 2851, 1635, 1557, 1509, 1291, 1074, 910, 518; ESI–MS: *m/z* at 557.3 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) undecanamide (**4n**)*

Hexane: ethyl acetate (60:40, v/v), yield 76%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.89 (br s, 1H, NH), 6.62 (d, $J = 7.32$ Hz, 1H, NH), 5.30–5.38 (m, 2H, HC=CH), 4.29–4.35 (m, 2H, CH–NH, CH–OH), 4.12 (br s, 1H, OH), 3.21 (q, $J =$ 5.45 Hz, 2H, H_2C–NH), 2.25 (t, $J = 7.62$ Hz, 2H, O=C–CH_2), 1.97–2.02 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.25 (m, 36H, CH_2), 1.14 (d, $J = 6.56$ Hz, 3H, CHOH–CH_3), 0.87 (t, $J = 6.86$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.3 (HN–C=O), 170.9 (HN–C=O), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.4 (HC–OH), 56.5 (HC–NH), 39.4 (O=C–NH–CH_2), 36.3 (O=C–CH_2), 31.8 (H_2C–HC=CH, O=C–CH_2–CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 22.5 (CH_2), 17.8 (HOHC–CH_3), 14(CH_3); IR (KBr): 3371, 2933, 2853, 1632, 1558, 1464, 1368, 1290, 1063, 695, 590; ESI–MS: *m/z* at 559.1 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) dodecanamide (**4o**)*

Hexane: ethyl acetate (60:40, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.85 (t, $J = 5.38$ Hz, 1H, NH), 6.57 (d, $J = 7.58$ Hz, 1H, NH), 5.30–5.38 (m, 2H, HC=CH), 4.33–4.38 (m, 1H, CH–NH), 4.28 (dd, $J = 1.95$, 7.70 Hz, 1H, CH–OH), 3.21 (q, $J = 6.61$ Hz, 2H, H_2C–NH), 2.25 (t, $J = 7.71$ Hz, 2H, O=C–CH_2), 1.97–2.04 (m, 4H, H_2C–HC=CH), 1.59–1.65 (m, 2H, CH_2), 1.46–1.49 (m, 2H, CH_2), 1.25 (m, 38H, CH_2), 1.14 (d, $J = 6.48$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.61$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.4 (HN–C=O), 171.1 (HN–C=O), 129.9 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.3 (HC–OH), 56.4 (HC–NH), 39.4 (O=C–NH–CH_2), 36.4 (O=C–CH_2), 32.5 (H_2C–HC=CH), 31.8 (O=C–CH_2–CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3361, 2923, 2853, 1637, 1558, 1464, 1378, 1290, 1073, 695; ESI–MS: *m/z* at 573.4 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) tetradecanamide (**4p**)*

Hexane: ethyl acetate (60:40, v/v), yield 77%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.99 (br s, 1H, NH), 6.74 (d, $J = 7.21$ Hz, 1H, NH), 5.30–5.38 (m, 2H, HC=CH), 4.34 (m, 2H, CH–NH, CH–OH), 3.18–3.25 (m, 2H, H_2C–NH), 2.26 (t, $J = 7.82$ Hz, 2H, O=C–CH_2), 1.96–2.03 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.25 (m, 42H, CH_2), 1.14 (d, $J = 6.35$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.6$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.3 (HN–C=O), 170.9 (HN–C=O), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.4 (HC–OH), 56.6 (HC–NH), 39.4 (O=C–NH–CH_2), 36.4 (O=C–CH_2), 31.8 (H_2C–HC=CH, O=C–CH_2–CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3429, 3354, 2927, 2851, 1658, 1533, 1475, 1293, 1071, 720; ESI–MS: *m/z* at 601.06 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) hexadecanamide (**4q**)*

Hexane: ethyl acetate (60:40, v/v), yield 79%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.86 (t, $J = 5.49$ Hz, 1H, NH), 6.58

(d, $J = 7.62$ Hz, 1H, NH), 5.31–5.37 (m, 2H, HC=CH), 4.33–4.37 (m, 1H, CH–NH), 4.28 (dd, $J = 1.98$, 7.78 Hz, 1H, CH–OH), 3.21 (q, $J = 5.95$ Hz, 2H, H_2C–NH), 2.25 (t, $J = 7.47$ Hz, 2H, O=C–CH_2), 1.98–2.02 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.25 (m, 46H, CH_2), 1.14 (d, J=6.56 Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.86$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 173.9 (HN–C=O), 170.7 (HN–C=O), 129.6 (H_2C–HC=CH), 129.5 (H_2C–HC=CH), 66.2 (HC–OH), 56.5 (HC–NH), 39.2 (O=C–NH–CH_2), 36.2 (O=C–CH_2), 31.6 (H_2C–HC=CH, O=C–CH_2–CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29 (CH_2), 26.9 (CH_2), 26.6 (CH_2), 25.5 (CH_2), 22.4 (CH_2), 17 (HOHC–CH_3), 13.8 (CH_3); IR (KBr): 3562, 3325, 2932, 2861, 1656, 1543, 1468, 1292, 1081, 720; ESI–MS: at *m/z* 629.1 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) octadecanamide* ***(4r)***

Hexane: ethyl acetate (60:40, v/v), yield 78%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.84 (t, $J = 5.18$ Hz, 1H, NH), 6.58 (d, $J = 7.47$ Hz, 1H, NH), 5.32–5.37 (m, 2H, HC=CH), 4.34–438 (m, 1H, CH–NH), 4.27 (dd, $J = 1.95$, 7.62 Hz, 1H, CH–OH), 3.21 (q, $J = 6.71$ Hz, 2H, H_2C–NH), 2.26 (t, $J = 7.47$ Hz, 2H, O=C–CH_2), 1.97–2.04 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.25 (m, 50H, CH_2), 1.14 (d, $J = 6.56$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.86$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 174.3 (HN–C=O), 171.0 (HN–C=O), 129.8 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.3 (HC–OH), 56.4 (HC–NH), 39.4 (O=C–NH–CH_2), 36.4 (O=C–CH_2), 31.8 (H_2C–HC=CH, O=C–CH_2–CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3409, 3354, 2922, 2851, 1637, 1553, 1465, 1292, 1071, 720; ESI–MS: *m/z* at 657.3 [M + Na].

N-*(2S, 3R)-(3-Hydroxy-1-(oleylamino)-1-oxobutan-2-yl) oleamide* ***(4s)***

Hexane: ethyl acetate (60:40, v/v), yield 77%. ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.86 (s, 1H, NH), 6.57 (s, 1H, NH), 5.30–5.37 (m, 4H, HC=CH), 4.29–4.35 (m, 1H, CH–NH), 4.28 (m, 1H, CH–OH), 4.07 (s, 1H, OH), 3.19–3.23 (m, 2H, H_2C–NH), 2.25 (t, $J = 7.62$ Hz, 2H, O=C–CH_2), 1.98–2.02 (m, 8H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.26–1.30 (m, 42H, CH_2), 1.14 (d, $J = 6.41$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.56$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 174.3 (HN–C=O), 171.1 (HN–C=O), 129.9 (H_2C–HC=CH), 129.6 (H_2C–HC=CH), 66.3 (HC–OH), 56.4 (HC–NH), 39.4 (O=C–NH–CH_2), 36.4 (O=C–CH_2), 32.5 (H_2C–HC=CH), 31.8 (O=C–CH_2–CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 17.8 (HOHC–CH_3), 14 (CH_3); IR (KBr): 3418, 3254, 2934, 2861, 1632, 1543, 1465, 1276, 1071, 720; ESI–MS: *m/z* at 655.2 [M + Na].

1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranoside (5)

Compound was prepared by our previous protocol (Vudhgiri et al. 2017). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.71 (d, $J =$ 8.31 Hz, 1H, Gal-1), 5.43 (m, 1H, Gal-4), 5.33 (t, $J = 8.31$ Hz, 1H, Gal-2), 5.09 (dd, $J = 3.42$, 10.39 Hz, 1H, Gal-3), 4.1–4.19 (m, 2H, Gal-6), 4.04–4.08 (m, 1H, Gal-5), 2.16 (s, 3H, O=C–CH_3), 2.12 (s, 3H, O=C–CH_3), 2.04 (s, 6 H, O=C–CH_3), 1.99 (s, 3H, O=C–CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170(H_3C–C=O), 169.8 (H_3C–C=O), 169.6 (H_3C–C=O), 169.1 (H_3C–C=O), 168.7 (H_3C–C=O), 91.8 (C-1), 71.4 (C-3), 70.5 (C-5), 67.6 (C-2), 66.6 (C-4), 60.8 (C-6), 20.5 (H_3C–C=O), 20.4 (H_3C–C=O), 20.3 (H_3C–C=O); IR ($CHCl_3$) 3027, 2969, 2951, 2907,1756, 1743, 1422, 1371, 1322, 1225, 1067,1048, 912, 756, 704, 641,599. HRMS (ESI) *m/z* [M + Na]-calc. for $C_{16}H_{22}O_{11}Na$ 413.10543 found 413.10449.

2,3,4,6-Tetra-*O*-acetyl-galactopyranose hemiacetal (6)

Compound was prepared by previous protocol (Manzo et al. 2012). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.54 (t, $J =$ 9.9 Hz, 1H, Gal-4), 5.46 (d, $J = 2.8$ Hz, 1H, Gal-2), 5.09 (t, $J = 9.4$ Hz, 1H, Gal-3), 4.8–4.9 (m, 1H, Gal-1), 4.22–4.29 (m, 2H, Gal-6), 4.10–4.16 (m, 1H, Gal-5), 2.10 (s, 3H, O=C–CH_3), 2.08 (s, 3H, O=C–CH_3), 2.04 (s, 3H, O=C–CH_3), 2.02 (s, 3H, O=C–CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.06 (H_3C–C=O), 170.5 (H_3C–C=O), 170.3 (H_3C–C=O), 169.7 (H_3C–C=O), 95.3 (C-1), 89.9 (C-3), 72.9 (C-5), 72.4 (C-5), 71.8 (C-2), 71.1 (C-2), 69.9 (C-4), 68.4 (C-4), 66.9 (C-6), 61.9 (C-6), 30.1 (H_3C–C=O), 29.6 (H_3C–C=O), 20.6 (H_3C–C=O); IR ($CHCl_3$) 3460.6, 3024.4, 1748.8, 1369.5, 1235.7, 1038.6, 756; HRMS (ESI) *m/z* [M + Na]-calc. for $C_{14}H_{20}O_{10}Na$=371.09487 found 371.09459.

2,3,4,6-Tetra-*O*-acetyl-α-galactopyranosyl trichloroacetimidate (7)

2,3,4,6-Tetra-*O*-acetyl-galactopyranose hemiacetal **(2)** (2 g, 5.75 mmol) was treated with trichloroacetonitrile (57.5 mmol) and 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU, 1.15 mmol) in anhydrous dichloromethane (30 mL) and the contents were stirred for 2 h at room temperature. After 2 h, the reaction mixture was concentrated under reduced pressure and purified by silica gel column chromatography. The required product was eluted in solvent mixture (20: 80,

EtOAc: hexane, v/v) with good yield (78%, 2.20 g). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.6 (s, 1H,=NH), 6.61 (d, J = 2.32 Hz, 1H, Gal-1), 5.57 (br s, 1H, Gal-4), 5.34–5.45 (m, 2H, Gal-3, 2), 4.45 (t, J = 6.35 Hz, 1H, Gal-5), 4.06–4.19 (m, 2H, Gal-6), 2.18 (s, 3H, O=C–CH_3), 2.02 (2 s, 9H, O=C–CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): 170.1 (H_3C–C=O), 169.9 (H_3C–C=O), 169.8 (H_3C–C=O), 169.7 (H_3C–C=O), 160.6 (O–C=NH), 93.3 (C–Cl_3), 90.5 (C-1), 68.8 (C-3), 67.3 (C-5), 67.2 (C-2), 66.7 (C-4), 61 (C-6), 20.4 (H_3C–C=O), 20.3 (H_3C–C=O); IR ($CHCl_3$) 3478, 3347, 2135, 1748.8, 1676.44, 1371, 1218, 1073, 756.

General procedure for synthesis of compounds 8a–8s

Imidate (**7**) (1 mmol), compounds **4a–4i** (1.2 mmol) and molecular sieves (4 Å) were taken in freshly distilled dichloromethane (10 mL) at 0 °C and the contents were stirred for 30 min under nitrogen atmosphere. To this reaction mixture, trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.4 mmol) was added dropwise at 0 °C and slowly allowed the reaction mixture to room temperature and magnetically stirred for overnight. After completion of all the starting materials, the reaction mixture was filtered and dissolved in $CHCl_3$ (30 mL). The organic layer was extracted with aq. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. This crude mixture was dissolved in dry methanol that contained a catalytic amount of sodium methoxide. The reaction mixture was allowed to stir at ambient temperature for 30 min under nitrogen. After total consumption of starting material, the reaction mixture was neutralized by the addition of Amberlite IR-120 (H^+) resin. Then, the reaction mixture was filtered and concentrated under reduced pressure to obtain a crude product. This crude product was purified by silica gel chromatography using chloroform: methanol solvent mixture to give title compounds as white solids with 65–74% yields.

(2S, 3R)-2-Cinnamamido-N-*(oleyl)-3-((β-D-galactopyranosyl) oxy) butanamide (**8a**)*

Chloroform: methanol (95: 5, v/v), yield 72%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 300 MHz): 7.61 (d, J = 15.95 Hz, 1H, =CH), 7.55–7.57 (m, 2H, Ar–H), 7.38–7.40 (m, 3H, Ar–H), 6.67 (d, J = 15.95 Hz, 1H,=CH), 5.32–5.39 (m, 2H, HC=CH), 4.70 (d, J = 4.12 Hz, 1H, CH–NH), 4.36–4.39 (m, 1H, CH–OH), 4.26–4.30 (m, 1H, Gal-1), 3.90–3.95 (m, 1H, Gal-4), 3.86 (m, 1H, Gal-6), 3.75–3.78 (m, 1H, Gal-6), 3.57–3.60 (m, 1H, Gal-2), 3.52–3.54 (m (overlap), 2H, Gal-3 & 5), 3.18–3.27 (m, 2H, H_2C–NH), 1.97–2.04 (m, 4H, H_2C–HC=CH), 1.53 (m, 2H, CH_2), 1.22–1.34 (m, (overlap), 25H, CH_2, CHOH–CH_3), 0.88 (t, J = 6.32 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + CD_3OD, 75 MHz): 170.4 (HN–C=O), 167.4 (HN–C=O), 142.2 (HC=CH), 135.2 (Ar–C), 130.3 (H_2C–HC=CH), 130.2 (H_2C–HC=CH, Ar–CH), 129.3 (Ar–CH), 128.3 (Ar–CH), 120.5 (HC=CH), 102.6 (C-1), 76.1 (C-3), 74.9 (C-5), 74 (C-2), 71.7 (C-4), 69.6 (HC–OH), 62.3 (C-6), 58.2 (HC–NH), 40.1 (O=C–NH-CH_2), 32.3 (O=C–NH–CH_2–CH_2), 30.2 (H_2C–HC=CH), 30.1 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 27.6 (CH_2), 27.4 (CH_2), 23.1 (CH_2), 16.3 (HOHC–CH_3), 14.3 (CH_3); IR (KBr): 3289, 3095, 2925, 2855, 1631, 1552, 1455, 1366, 1219, 978, 758; HRMS (ESI) *m/z* [M+H]-calc. for $C_{37}H_{61}O_8N_2$ = 661.44224 found 661.44348.

(2S, 3R)-2-(3-(4-Nitrophenyl) acryl amido)-N-*(oleyl)-3-((β-D-galactopyranosyl) oxy) butanamide (**8b**)*

Chloroform: methanol (95: 5, v/v), yield 70%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 300 MHz): 8.23 (dd, J = 8.49, 21.33 Hz, 2H, Ar–H), 7.73 (dd, J = 8.49, 21.33 Hz, 2H, Ar–H), 7.65 (d, J = 15.86 Hz, 1H,=CH), 6.89 (t (overlap), J = 15.48 Hz, 2H,=CH, NH), 6.28 (d, J = 12.46 Hz, 1H, NH), 5.32–5.37 (m, 2H, HC=CH), 4.68 (d, J = 4.72 Hz, 1H, CH–NH), 4.33–4.39 (m, 1H, CH–OH), 4.24–4.27 (m, 1H, Gal-1), 3.81–3.90 (m, 1H, Gal-4), 3.76–3.77 (m, 1H, Gal-6), 3.72–3.73 (m, 1H, Gal-6), 3.53–3.58 (m, 1H, Gal-2), 3.47–3.51 (m (overlap), 2H, Gal-3 & 5), 3.16–3.28 (m, 2H, H_2C–NH), 1.95–2.02 (m, 4H, H_2C–HC=CH), 1.51 (m, 2H, CH_2), 1.27–1.34 (m (overlap), 25H, CH_2, CHOH–CH_3), 0.88 (t, J = 6.23 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + CD_3OD, 75 MHz): 170.2 (HN–C=O), 166.8 (HN–C=O), 148.7 (Ar–C–NO_2), 142.4 (HC=CH), 139.2 (Ar–C), 130.6 (H_2C–HC=CH), 130.2 (H_2C–HC=CH), 129 (Ar–CH), 126.5 (Ar–CH), 125.1 (Ar–CH), 124.5 (Ar–CH), 123.6 (HC=CH), 102.7 (C-1), 76.1 (C-3), 74.9 (C-5), 74 (C-2), 71 (C-4), 69.7 (HC–OH), 62.4 (C-6), 58.4 (HC–NH), 40.2 (O=C–NH–CH_2), 32.3 (O=C–NH–CH_2–CH_2), 30.1 (H_2C–HC=CH), 29.9 (CH_2), 29.7 (CH_2), 27.6 (CH_2), 27.4 (CH_2), 23.1 (CH_2), 16.4 (HOHC–CH_3), 14.3 (CH_3); IR (KBr): 3284, 3081, 2924, 2853, 1626, 1599, 1517, 1346, 973, 721; HRMS (ESI) *m/z* [M+H]-calc. for $C_{37}H_{60}O_{10}N_3$ = 706.42732 found 706.42840.

(2S, 3R)-2-(2-(1H-Indol-3-yl) acetamido)-N-*(oleyl)-3-((β-D-galactopyranosyl) oxy) butanamide (**8c**)*

Chloroform: methanol (95: 5, v/v), yield 74%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 400 MHz): 7.51–7.57(m, 1H, Ar–H), 7.35–7.39 (m, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 7.08–7.16 (m, 2H, Ar–H), 5.34–5.38 (m, 2H, HC=CH), 4.55 (m, 1H, HC–NH), 4.29 (m, 1H, HC–OH), 4.16 (m, 1H, Gal-1), 4.06 (m, 1H, Gal-4), 3.83 (m, 2H, Gal-6), 3.76 (s, 2H, CH_2), 3.65–3.72 (m, 1H, Gal-2), 3.46 (m (overlap), 2H, Gal-3 & 5), 3.04–3.15 (m, 2H, H_2C–NH), 2.02–2.19 (m, 4H, H_2C–HC=CH), 1.27 (m, 24H, CH_2), 1.09 (m, 3H,

CHOH–$\underline{CH_3}$), 0.89 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + CD_3OD, 75 MHz): 173.8 (HN–$\underline{C}$=O), 170 (HN–$\underline{C}$=O), 137.2 (Ar–$\underline{C}$–NH), 130.3 (H_2C–$\underline{HC}$=CH), 130.3 (H_2C–HC=$\underline{CH}$), 127.5 (Ar–$\underline{C}$–CH), 124.7 (Ar–$\underline{CH}$), 122.3 (Ar–$\underline{CH}$), 120.8 (Ar–$\underline{CH}$), 119.7 (Ar–$\underline{CH}$), 118 (Ar–$\underline{CH}$), 112 (Ar–$\underline{C}$), 102.8 (C-1), 76 (C-3), 74.8 (C-5), 74 (C-2), 71.7 (C-4), 69.5 ($\underline{HC}$–OH), 62.2 (C-6), 58.8 ($\underline{HC}$–NH), 40.1 (O=C–NH–$\underline{CH_2}$), 33.6 ($\underline{H_2C}$–C=O), 32.4 ($\underline{H_2C}$–HC=CH), 30.2 (O=C–NH–CH_2–$\underline{CH_2}$), 30.1 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 27.6 (CH_2), 27.3 (CH_2), 23.1 (CH_2), 16.3 (HOHC–$\underline{CH_3}$), 14.3 (CH_3); IR (KBr): 3295, 3049, 2922, 2852, 1629, 1540, 1213, 1097, 726; HRMS (ESI) *m/z* [M+H]-calc. for $C_{38}H_{62}O_8N_3 = 688.45314$ found 608.45370.

*(2S, 3R)-2-(3-(1H-Indol-3-yl) propanamido)-*N*-(oleyl)-3-((β-D-galactopyranosyl) oxy) butanamide (**8d**)*

Chloroform: methanol (95: 5, v/v), yield 74%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 300 MHz): 7.59 (d, $J = 7.74$ Hz, 1H, Ar–H), 7.35 (d, $J = 7.93$ Hz, 1H, Ar–H), 7.11–7.15 (m, 2H, Ar–H), 7.03–7.05 (m, 1H, Ar–H), 5.33–5.39 (m, 2H, HC=CH), 4.51 (d, $J = 3.96$ Hz, 1H, $\underline{CH}$–NH), 4.26–4.28 (m, 1H, $\underline{CH}$–OH), 4.04–4.08 (m, 1H, Gal-1), 3.81–3.87 (m (overlap), 3H, Gal-4 & 6), 3.67–3.72 (m, 1H, Gal-2), 3.50–3.54 (m, 1H, Gal-3), 3.47–3.49 (m, 1H, Gal-5), 3.03–3.16 (m (overlap), 4H, $\underline{H_2C}$–NH, CH_2), 2.69 (t, $J =$ 6.98 Hz, 2H, CH_2), 1.98–2.02 (m, 4H, $\underline{H_2C}$–HC=CH), 1.26 (m, 24H, CH_2), 1.01 (d, $J = 6.42$ Hz, 3H, CHOH–$\underline{CH_3}$), 0.88 (t, $J = 6.98$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + CD_3OD, 75 MHz): 174.5 (HN–$\underline{C}$=O), 170.1 (HN–$\underline{C}$=O), 136.8 (Ar–$\underline{C}$–NH), 130.2 (H_2C–$\underline{HC}$=CH), 130.1 (H_2C–HC=$\underline{CH}$), 127.4 (Ar–$\underline{C}$–CH), 122.5 (Ar–$\underline{CH}$), 121.8 (Ar–$\underline{CH}$), 119.1 (Ar–$\underline{CH}$), 118.7 (Ar–$\underline{CH}$), 113.9 (Ar–$\underline{CH}$), 111.6 (Ar–$\underline{C}$), 102.2 (C-1), 75.8 (C-3), 74.5 (C-5), 73.8 (C-2), 71.5 (C-4), 69.4 ($\underline{HC}$–OH), 62.1 (C-6), 57.5 ($\underline{HC}$–NH), 39.9 (O=C–NH–$\underline{CH_2}$), 36.9 ($\underline{H_2C}$–C=O), 32.2 ($\underline{H_2C}$–HC=CH), 30 (O=C–NH–CH_2–$\underline{CH_2}$), 29.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 27.5 (CH_2), 27.2 (CH_2), 22.9 (CH_2), 21.5 (CH_2), 15.9 (HOHC–$\underline{CH_3}$), 14.2 (CH_3); IR (KBr): 3395, 3042, 2922, 2862, 1629, 1540, 1458, 1378, 1213, 1097, 924, 726; HRMS (ESI) *m/z* [M+H]-calc. for $C_{39}H_{64}O_8N_3 = 702.46879$ found 702.46902.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl)-1H-indazole-3-carboxamide (**8e**)*

Chloroform: methanol (95: 5, v/v), yield 68%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 400 MHz): 8.24 (d, $J = 7.45$ Hz, 1H, Ar–H), 7.72 (d, $J = 8.68$ Hz, 1H, NH), 7.66 (t, $J = 5.01$ Hz, 1H, NH), 7.55 (d, $J = 8.43$ Hz, 1H, Ar–H), 7.39–7.43 (m, 1H, Ar–H), 7.24–7.28 (m, 1H, Ar–H), 5.29–5.39 (m, 2H, HC=CH), 4.78 (d, $J = 4.64$ Hz, 1H, $\underline{CH}$–NH), 4.48–4.51 (m, 1H, $\underline{CH}$–OH), 4.32–4.35 (m, 1H, Gal-1), 3.97–4.02 (m, 1H, Gal-4), 3.88–3.91 (m, 1H, Gal-6), 3.74–3.78 (m, 1H, Gal-6), 3.63–3.65 (m, 1H, Gal-2), 3.55–3.58 (m (overlap), 2H, Gal-3 & 5), 3.21–3.32 (m, 2H, $\underline{H_2C}$–NH), 2.01 (m, 4H, $\underline{H_2C}$–HC=CH), 1.54 (m, 2H, CH_2), 1.27–1.30 (m (overlap), 25H, CH_2, CHOH–$\underline{CH_3}$), 0.88 (t, $J = 6.96$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + CD_3OD, 75 MHz): 170.4 (HN–$\underline{C}$=O), 163.8 (HN–$\underline{C}$=O), 142 (Ar–$\underline{C}$–NH), 138.2 (O=C–(Ar)$\underline{C}$=NH), 130.3 (H_2C–$\underline{HC}$=CH), 130.2 (H_2C–HC=$\underline{CH}$), 127.4 (Ar–$\underline{CH}$), 123.1 (Ar–$\underline{CH}$), 122.3 (Ar–$\underline{CH}$), 110.9 (Ar–$\underline{C}$), 103.3 (C-1), 76.2 (C-3), 75.7 (C-5), 73.9 (C-2), 71.6 (C-4), 69.7 ($\underline{HC}$–OH), 62.3 (C-6), 57.8 ($\underline{HC}$–NH), 40.2 (O=C–NH–$\underline{CH_2}$), 32.3 ($\underline{H_2C}$–HC=CH), 30.1 (O=C–NH–CH_2–$\underline{CH_2}$), 29.9 (CH_2), 29.7 (CH_2), 27.6 (CH_2), 27.4, 23.1 (CH_2), 16.8 (HOHC–$\underline{CH_3}$), 14.3 (CH_3); IR (KBr): 3403, 3352, 2924, 2853, 1717, 1642, 1515, 1461, 1296, 1158, 745; HRMS (ESI) *m/z* [M+H]-calc. for $C_{36}H_{59}O_8N_4 =$ 675.43274 found 675.43313.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl)-1H-indole-2-carboxamide (**8f**)*

Chloroform: methanol (95: 5, v/v), yield 70%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 300 MHz): 7.66 (d, $J = 7.93$ Hz, 1H, Ar–H), 7.46 (d, $J = 8.49$ Hz, 1H, Ar–H), 7.25–7.29 (m, 1H, Ar–H), 7.17–7.20 (m, 1H, Ar–H), 7.09–7.14 (m, 1H, Ar–H), 5.32–5.38 (m, 2H, HC=CH), 4.75–4.78 (m, 1H, $\underline{CH}$–NH), 4.62 (d, $J = 4.34$ Hz, 1H, $\underline{CH}$–OH), 4.29 (d, $J =$ 6.79 Hz, 1H, Gal-1), 4.16–4.20 (m, 1H, Gal-4), 3.87–3.92 (m, 1H, Gal-6), 3.72–3.81 (m (overlap), 2H, Gal-6 & 2), 3.51–3.61 (m (overlap), 1H, Gal-3 & 5), 3.15–3.28 (m, 2H, $\underline{H_2C}$–NH), 1.98–2.09 (m, 4H, $\underline{H_2C}$–HC=CH), 1.54 (m, 2H, CH_2), 1.26 (m, 25H, CH_2, CHOH–$\underline{CH_3}$), 0.88 (t, $J = 6.42$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$ + CD_3OD, 75 MHz): 170.5 (HN–$\underline{C}$=O), 162.7 (HN–$\underline{C}$=O), 137.7 (Ar–$\underline{C}$–NH), 130.1 (O=C–$\underline{C}$(NH)=CH, H_2C–$\underline{HC}$=CH), 127.8 (Ar–$\underline{C}$), 124.9 (Ar–$\underline{CH}$), 122.3 (Ar–$\underline{CH}$), 120.7 (Ar–$\underline{CH}$), 112.4 (Ar–$\underline{CH}$), 104.4 (Ar–$\underline{CH}$), 99.4 (C-1), 75.6 (C-3), 73.9 (C-5), 73.7 (C-2), 71.4 (C-4), 69.2 ($\underline{HC}$–OH), 62.3 (C-6), 57.8 ($\underline{HC}$–NH), 40.1 (O=C–NH–$\underline{CH_2}$), 32.9 ($\underline{H_2C}$–HC=CH), 32.2 (O=C–NH–CH_2–$\underline{CH_2}$), 30.1 (CH_2), 30 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 27.5 (CH_2), 27.3 (CH_2), 23 (CH_2), 16.8 (HOHC–$\underline{CH_3}$), 14.2 (CH_3); IR (KBr): 3357, 3245, 2922, 2851, 1637, 1509, 1075, 721; HRMS (ESI) *m/z* [M+H]-calc. for $C_{37}H_{60}O_8N_3 = 674.43749$ found 674.43755.

*(2S, 3R)-*N*-(Oleyl)-2-(2-phenylacetamido)-3-((β-D-galactopyranosyl) oxy) butanamide (**8g**)*

Chloroform: methanol (95: 5, v/v), yield 72%. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 300 MHz): 7.26–7.32 (m, 5H, Ar–H), 5.33–5.39 (m, 2H, HC=CH), 4.46 (d, $J = 3.02$ Hz, 1H, $\underline{CH}$–NH), 4.33–4.35 (m, 1H, $\underline{CH}$–OH), 4.16–4.20 (m, 1H, Gal-1), 3.79–3.88 (m (overlap), 2H, Gal-4 & 6), 3.68–3.73

(m, 1H, Gal-6), 3.61 (s, 2H, CH_2), 3.54–3.57 (m, 1H, Gal-2), 3.47–3.51 (m, 2H, Gal-3 & 5), 3.09–3.22 (m, 2H, H_2C–NH), 1.96–2.05 (m, 4H, H_2C–HC=CH), 1.46 (m, 2H, CH_2), 1.27 (m, 22H, CH_2), 1.13 (d, $J = 6.42$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.23$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 171.8 (HN–C=O), 169 (HN–C=O), 134.5 (Ar–C–CH_2), 130 (H_2C–HC=CH), 129.5 (H_2C–HC=CH), 129.4 (Ar–CH), 128.7 (Ar–CH), 128.4 (Ar–CH), 126.7 (Ar–CH), 101.8 (C-1), 75.1 (C-3), 73.9 (C-5), 73.1 (C-2), 70.8 (C-4), 68.7 (HC–OH), 61.4 (C-6), 56.7 (HC–NH), 42.7 (Ar–CH_2–C=O), 39.3 (O=C–NH–CH_2), 32.2 (O=C–NH–CH_2–CH_2), 31.5 (H_2C–HC=CH), 29.3 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 26.8 (CH_2), 26.5 (CH_2), 22.3 (CH_2), 15.2 (HOHC–CH_3), 13.6 (CH_3); IR (KBr): 3354, 3063, 2924, 2853, 1730, 1637, 1513, 1466, 1287, 1074, 719; HRMS (ESI) *m/z* [M+H]-calc. for $C_{36}H_{61}O_8N_2 = 649.44224$ found 649.44309.

(2S, 3R)-2-(2-(4-Methoxyphenyl) acetamido)-N-*(oleyl)-3-((β-D-galactopyranosyl) oxy) butanamide* (***8h***)

Chloroform: methanol (95: 5, v/v), yield 68%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 500 MHz): 7.51 (br s, 1H, NH), 7.22–7.25 (m, 2H, Ar–H), 6.86–6.89 (m, 2H, Ar–H), 5.33–5.38 (m, 2H, HC=CH), 4.52–4.54 (m, 1H, CH–NH), 4.33–436 (m, 1H, CH–OH), 4.17–4.20 (m, 1H, Gal-1), 3.86–3.87 (m, 1H, Gal-4), 3.82–3.84 (m, 2H, Gal-6), 3.81 (s, 3H, Ar–OCH_3), 3.71–3.74 (m, 1H, Gal-2), 3.54–3.57 (m, 1H, Gal-3), 3.50–3.52 (m, 1H, Gal-5), 3.38 (s, 2H, CH_2), 3.11–3.22 (m, 2H, H_2C–NH), 1.97–2.03 (m, 4H, H_2C–HC=CH), 1.48 (m, 2H, CH_2), 1.28–1.29 (m, 22H, CH_2), 1.15 (d, $J = 5.95$ Hz, 3H, CHOH–CH_3), 0.89 (t, $J = 6.56$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 173.3 (HN–C=O), 170.2 (HN–C=O), 159.3 (Ar–C–OCH_3), 130.7 (Ar–C–CH_2), 130.4 (H_2C–HC=CH), 130.2 (H_2C–HC=CH), 127.5 (Ar–CH), 114.6 (Ar–CH), 102.8 (C-1), 76.1 (C-3), 74.9 (C-5), 74 (C-2), 71.7 (C-4), 69.6 (HC–OH), 62.3 (C-6), 57.8 (HC–NH), 55.5 (Ar–C–O–CH_3), 42.6 (Ar–CH_2–C=O), 40.1 (O=C–NH–CH_2), 32.4 (H_2C–HC=CH), 30.2 (O=C–NH–CH_2–CH_2), 30 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 27.6 (CH_2), 27.4 (CH_2), 23.1 (CH_2), 16.1 (HOHC–CH_3), 14.3 (CH_3); IR (KBr): 3292, 3099, 2924, 2853, 1631, 1551, 1512, 1375, 1245, 1040, 753; HRMS (ESI) *m/z* [M+H]-calc. for $C_{37}H_{63}O_9N_2 = 679.45281$ found 679.45298.

(2S, 3R)-2-(4-(4-Methoxyphenyl) butanamido)-N-*(oleyl)-3-((β-D-galactopyranosyl) oxy) butanamide* (***8i***)

Chloroform: methanol (95: 5, v/v), yield 70%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 7.10 (d, $J = 8.52$ Hz, 2H, Ar–H), 6.83 (d, $J = 8.52$ Hz, 2H, Ar–H), 5.32–5.37 (m, 2H, HC=CH), 5.11 (d, $J = 3.57$ Hz, 1H, NH), 4.60 (d, $J = 4.12$ Hz, 1H, NH), 4.47 (d, $J = 3.30$ Hz, 1H, CH–NH), 4.33–4.35 (m, 1H, CH–OH), 4.14–4.17 (m, 1H, Gal-1), 4.07–4.10 (m, 1H, Gal-4), 3.82–3.88 (m, 2H, Gal-6), 3.81 (s, 3H, Ar–OCH_3), 3.67–3.74 (m, 1H, Gal-2), 3.53–3.57 (m, 1H, Gal-3), 3.49–3.51 (m, 1H, Gal-5), 3.13–3.29 (m, 2H, H_2C–NH), 2.59 (t, $J = 7.42$ Hz, 2H, CH_2), 2.28 (t, $J = 7.42$ Hz, 2H, CH_2), 1.87–2.02 (m, 6H, CH_2, H_2C–HC=CH), 1.51 (m, 2H, CH_2), 1.26 (m, 22H), 1.16 (d, $J = 6.60$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.32$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 173.7 (HN–C=O), 169.2 (HN–C=O), 157.5 (Ar–C–OCH_3), 133.3 (Ar–C–CH_2), 129 (H_2C–HC=CH, Ar–CH), 113.5 (Ar–CH), 101.9 (C-1), 75.2 (C-3), 74.2 (C-5), 73.4 (C-2), 71(C-4), 68.8 (HC–OH), 61.6 (C-6), 56.6 (HC–NH), 54.9 (Ar–C–O–CH_3), 39.3 (H_2C–CH_2–C=O), 35.2 (O=C–NH–CH_2), 34 (Ar–C–CH_2), 32.3 (H_2C–HC=CH), 31.6 (O=C–NH–CH_2–CH_2), 29.3 (CH_2), 29.2 (CH_2), 29 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 26.6 (CH_2), 22.3 (CH_2), 15.3 (HOHC–CH_3), 13.7 (CH_3); IR (KBr): 3284, 3081, 2924, 2853, 1626, 1548, 1517, 1346, 1112. 973, 721; HRMS (ESI) *m/z* [M+H]-calc. for $C_{39}H_{67}O_9N_2 = 707.48411$ found 707.48494.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) hexanamide* (***8j***)

Chloroform: methanol (95: 5, v/v), yield 74%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 7.42 (t, $J = 5.77$ Hz, 1H, NH), 7.37 (d, $J = 7.7$ Hz, 1H, NH), 5.29–5.44 (m, 2H, HC=CH), 4.59 (d, $J = 4.4$ Hz, 1H, CH–NH), 4.38 (m, 1H, CH–OH), 4.16–4.20 (m, 1H, Gal-1), 3.88 (m, 1H, Gal-4), 3.84 (d, $J = 7.7$ Hz, 1H, Gal-6), 3.71 (dd, $J = 3.85$, 11.82 Hz, 1H, Gal-6), 3.54–3.57 (m, 1H, Gal-2), 3.51 (m (overlap), 2H, Gal-3 & 5), 3.14–3.29 (m, 2H, H_2C–NH), 2.27 (t, $J = 7.42$ Hz, 2H, O=C–CH_2), 2.02 (m, 4H, H_2C–HC=CH), 1.64 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26–1.30 (m, 26H, CH_2), 1.17 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.89 (t, $J = 6.6$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1 (HN–C=O), 169.3 (HN–C=O), 129.6 (H_2C–HC=CH), 129.4 (H_2C–HC=CH), 101.9 (C-1), 75.1 (C-3), 74.1 (C-5), 73.1 (C-2), 70.9 (C-4), 68.8 (HC–OH), 61.5 (C-6), 56.6 (HC–NH), 39.4 (O=C–NH–CH_2), 35.8 (O=C–CH_2), 32.2 (H_2C–HC=CH), 31.5 (O=C–CH_2–CH_2), 31 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 26.8 (CH_2), 26.6 (CH_2), 25 (CH_2), 22.3 (CH_2), 22 (CH_2), 16 (HOHC–CH_3), 13.6 (CH_3), 13.4 (CH_3); IR (KBr): 3365, 2936, 2845, 1649, 1558, 1461, 1378, 1078, 716; HRMS (ESI) *m/z* [M+H]-calc. for $C_{34}H_{65}O_8N_2 = 629.47354$ found 629.47364.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) octanamide (**8k**)*

Chloroform: methanol (95: 5, v/v), yield 72%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 500 MHz): 7.51 (br s, 1H, NH), 5.30–5.37 (m, 2H, HC=CH), 4.55 (m, 1H, CH–NH), 4.34 (m, 1H, CH–OH), 4.19 (m, 1H, Gal-1), 3.86 (m, 1H, Gal-4), 3.83 (d, $J = 7.32$ Hz, 1H, Gal-6), 3.71 (dd, $J = 4.12$, 11.74 Hz, 1H, Gal-6), 3.55 (m, 1H, Gal-2), 3.5 (m (overlap), 2H, Gal-3 & 5), 3.14–3.27 (m, 2H, H_2C–NH), 2.28 (t, $J = 7.47$ Hz, 2H, O=C–CH_2), 2.02 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.27–1.31 (m, 30H, CH_2), 1.19 (d, $J = 6.25$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.1$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174 (HN–C=O), 169.4 (HN–C=O), 129.1 (H_2C–HC=CH), 129 (H_2C–HC=CH), 101.4 (C-1), 74.9 (C-3), 73.7 (C-5), 72.9 (C-2), 70.5 (C-4), 68.4 (HC–OH), 61.1 (C-6), 56.7 (HC–NH), 38.9 (O=C–NH–CH_2), 35.4 (O=C–CH_2), 31.8 (H_2C–HC=CH), 31.2 (O=C–CH_2–CH_2), 31 (CH_2), 29 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 26.4 (CH_2), 26.2 (CH_2), 25 (CH_2), 21.9 (CH_2), 15.1 (HOHC–CH_3), 13.1 (CH_3); IR (KBr): 3286, 2926, 2855, 1639, 1555, 1377, 1078, 716; HRMS (ESI) *m/z* [M+H]-calc. for $C_{36}H_{69}O_8N_2 = 657.50484$ found 657.50486.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl)-2-propylpentanamide (**8l**)*

Chloroform: methanol (95: 5, v/v), yield 71%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 7.43 (d, $J = 7.42$ Hz, 1H, NH), 7.34 (t, $J = 5.5$ Hz, 1H, NH), 5.29–5.39 (m, 2H, HC=CH), 4.6 (m, 1H, CH–NH), 4.36 (m, 1H, CH–OH), 4.20 (m, 1H, Gal-1), 3.86 (m, 1H, Gal-4), 3.84 (d, $J = 7.42$ Hz, 1H, Gal-6), 3.71 (dd, $J = 4.12$, 11.82 Hz, 1H, Gal-6), 3.56 (m, 1H, Gal-2), 3.50 (m, 2H, Gal-3 5), 3.13–3.29 (m, 2H, H_2C–NH), 2.27 (m, 1H, O=C–CH_2), 2.02 (m, 4H, H_2C–HC=CH), 1.46–1.62 (m, 8H, CH_2), 1.26–1.30 (m, 26H, CH_2), 1.18 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.86–0.93 (m, 9H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 176.8 (HN–C=O), 169.3 (HN–C=O), 129.3 (H_2C–HC=CH), 129.1 (H_2C–HC=CH), 101.6 (C-1), 75 (C-3), 73.7 (C-5), 73 (C-2), 70.6 (C-4), 68.5 (HC–OH), 61.2 (C-6), 56.5 (HC–NH), 46.4 (O=C–CH), 39 (O=C–NH–CH_2), 34.6 (O=C–CH–CH_2), 31.3 (H_2C–HC=CH), 31.9 (CH_2), 29.1 (CH_2), 29 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 26.5 (CH_2), 26.3 (CH_2), 22 (CH_2), 20.1 (CH_2), 15.1 (HOHC–CH_3), 13.2 (CH_3); IR (KBr): 3483, 2925, 2867, 1632, 1556, 1462, 1093, 713; HRMS (ESI) *m/z* [M+H]-calc. for $C_{36}H_{69}O_8N_2 = 657.50484$ found 657.50509.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) undec-10-enamide (**8m**)*

Chloroform: methanol (95: 5, v/v), yield 75%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 5.73–5.87 (m, 1H,=CH), 5.29–5.42 (m, 2H, HC=CH), 4.9–5.01 (m, 2H,=CH_2), 4.59 (d, $J = 4.15$ Hz, 1H, CH–NH), 4.35 (t, $J = 3.58$ Hz, 1H, CH–OH), 4.17–4.20 (m, 1H, Gal-1), 3.86 (m, 1H, Gal-4), 3.83 (d, $J = 7.36$ Hz, 1H, Gal-6), 3.70 (dd, $J = 3.96$, 11.7 Hz, 1H, Gal-6), 3.53–3.57 (m, 1H, Gal-2), 3.50 (m, 2H, Gal-3 & 5), 3.13–3.27 (m, 2H, H_2C–NH), 2.27 (t, $J = 7.36$ Hz, 2H, O=C–CH_2), 2.02 (m, 6H, H_2C–HC=CH, H_2C–HC=CH_2), 1.62 (m, 1H, CH_2), 1.51 (m, 1H, CH_2), 1.26–1.30 (m, 32H, CH_2), 1.17 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.23$ Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1 (HN–C=O), 169.3 (HN–C=O), 138.7 (H_2C–HC=CH), 129.5 (H_2C–HC=CH), 129.4 (H_2C–HC=CH), 113.7 (H_2C–HC=CH), 101.8 (C-1), 75.1 (C-3), 74 (C-5), 73.1 (C-2), 70.8 (C-4), 68.7 (HC–OH), 61.4 (C-6), 56.6 (HC–NH), 39.3 (O=C–NH–CH_2), 35.8 (O=C–CH_2), 33.4 (H_2C–HC=CH), 32.2 (H_2C–HC=CH), 31.5 (O=C–CH_2–CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 26.8 (CH_2), 26.6 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 15.3 (HOHC–CH_3), 13.6 (CH_3); IR (KBr): 3283, 2924, 2853, 1639, 1554, 1377, 1165, 1053, 719; HRMS (ESI) *m/z* [M+H]-calc. for $C_{39}H_{73}O_8N_2 = 697.53614$ found 697.53695.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) undecanamide (**8n**)*

Chloroform: methanol (95: 5, v/v), yield 69%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 5.29–5.39 (m, 2H, HC=CH), 4.59 (d, $J = 3.85$ Hz, 1H, CH–NH), 4.48 (m, 1H, CH–OH), 4.19 (dd, $J = 6.32$, 10.72 Hz, 1H, Gal-1), 3.88 (d, $J = 7.7$ Hz, 1H, Gal-4), 3.84 (m, 1H, Gal-6), 3.70 (dd, $J = 3.85$, 11.82 Hz, 1H, Gal-6), 3.51–3.55 (m, 1H, Gal-2), 3.50 (m, 2H, Gal-3 & 5), 3.16–3.25 (m, 2H, H_2C–NH), 2.27 (t, $J = 7.42$ Hz, 2H, O=C–CH_2), 1.98–2.02 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26–1.29 (m, 36H, CH_2), 1.17 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.05$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1(HN–C=O), 169.4 (HN–C=O), 129.4 (H_2C–HC=CH), 129.2 (H_2C–HC=CH), 101.6 (C-1), 75 (C-3), 73.9 (C-5), 73 (C-2), 70.7 (C-4), 68.6 (HC–OH), 61.3 (C-6), 56.7 (HC–NH), 39.2 (O=C–NH–CH_2), 35.7 (O=C–CH_2), 32.07 (H_2C–HC=CH), 31.4 (O=C–CH_2–CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29 (CH_2), 28.8 (CH_2), 26.7 (CH_2), 26.4 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 15.2 (HOHC–CH_3), 13.4 (CH_3); IR (KBr): 3296, 2924, 2846, 1656, 1526, 1377, 1232, 1165, 1043, 719;

HRMS (ESI) *m/z* [M+H]-calc. for $C_{39}H_{75}O_8N_2 = 699.55179$ found 699.55217.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) dodecanamide (**8o**)*

Chloroform: methanol (95: 5, v/v), yield 67%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 7.4 (t, $J = 5.5$ Hz, 1H, NH), 5.29–5.39 (m, 2H, HC=CH), 4.59 (d, $J = 3.85$ Hz, 1H, CH–NH), 4.36 (m, 1H, CH–OH), 4.19 (dd, $J = 6.32$, 10.72 Hz, 1H, Gal-1), 3.86–3.88 (m, 1H, Gal-4), 3.84 (d, $J = 7.42$ Hz, 1H, Gal-6), 3.70 (dd, $J = 4.12$, 11.82 Hz, 1H, Gal-6), 3.53–3.57 (m, 1H, Gal-2), 3.51 (m, 2H, Gal-3 & 5), 3.16–3.27 (m, 2H, H_2C–NH), 2.27 (t, $J = 7.42$ Hz, 2H, O = C–CH_2), 2.02 (m, 4H, H_2C–HC = CH), 1.63 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26–1.29 (m, 38H, CH_2), 1.17 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.05$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1 (HN–C=O), 169.3 (HN–C=O), 129.3 (H_2C–HC=CH), 129.2 (H_2C–HC=CH), 101.6 (C-1), 75 (C-3), 73.9 (C-5), 73 (C-2), 70.7 (C-4), 68.6 (HC–OH), 61.3 (C-6), 56.6 (HC–NH), 39.1 (O=C–NH–CH_2), 35.7 (O=C–CH_2), 32 (H_2C–HC=CH), 31.4 (O=C–CH_2–CH_2), 29.1 (CH_2), 29 (CH_2), 28.8 (CH_2), 26.6 (CH_2), 26.4 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 15.2 (HOHC–CH_3), 13.3 (CH_3); IR (KBr): 3283, 2924, 2853, 1639, 1554, 1232, 1165, 1053, 719; HRMS (ESI) *m/z* [M+H]-calc. for $C_{40}H_{77}O_8N_2 = 713.56744$ found 713.56740.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) tetradecanamide (**8p**)*

Chloroform: methanol (95: 5, v/v), yield 70%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 5.30–5.38 (m, 2H, HC=CH), 4.60 (d, $J = 4.15$ Hz, 1H, CH–NH), 4.36 (t, $J = 3.58$ Hz, 1H, CH–OH), 4.18 (dd, $J = 6.42$, 10.95 Hz, 1H, Gal-1), 3.87 (m, 1H, Gal-4), 3.83 (d, $J = 7.17$ Hz, 1H, Gal-6), 3.71 (dd, $J = 3.96$, 11.89 Hz, 1H, Gal-6), 3.54–3.58 (m, 1H, Gal-2), 3.51 (m, 2H, Gal-3 & 5), 3.14–3.25 (m, 2H, H_2C–NH), 2.26 (t, $J = 7.36$ Hz, 2H, O=C–CH_2), 2.02 (m, 4H, H_2C–HC=CH), 1.62 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26 (m, 42H, CH_2), 1.16 (d, $J = 6.42$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.23$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1 (HN–C=O), 169.3 (HN–C=O), 129.5 (H_2C–HC=CH), 129.3 (H_2C–HC=CH), 101.7 (C-1), 75.1 (C-3), 74 (C-5), 73.1 (C-2), 70.8 (C-4), 68.7 (HC–OH), 61.4 (C-6), 56.6 (HC–NH), 39.3 (O=C–NH–CH_2), 35.8 (O=C–CH_2), 32.2 (H_2C–HC=CH), 31.5 (O=C–CH_2–CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 26.8 (CH_2), 26.5 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 15.3 (HOHC–CH_3), 13.6 (CH_3); IR (KBr): 3286, 2932, 2854, 1648, 1553, 1368, 1163, 1077, 720; HRMS (ESI) *m/z* [M+H]-calc. for $C_{42}H_{81}O_8N_2 = 741.59874$ found 741.59940.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) hexadecanamide (**8q**)*

Chloroform: methanol (95: 5, v/v), yield 71%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 5.29–5.39 (m, 2H, HC=CH), 4.57 (d, $J = 4.15$, Hz, 1H, CH–NH), 4.34 (t, $J = 3.77$ Hz, 1H, CH–OH), 4.21 (dd, $J = 5.85$, 10.38 Hz, 1H, Gal-1), 3.85 (m, 1H, Gal-4), 3.83 (d, $J = 7.17$ Hz, 1H, Gal-6), 3.71 (dd, $J = 4.15$, 11.7 Hz, 1H, Gal-6), 3.53–3.57 (m, 1H, Gal-2), 3.51 (m, 2H, Gal-3 & 5), 3.16–3.27 (m, 2H, H_2C–NH), 2.28 (t, $J = 7.36$ Hz, 2H, O=C–CH_2), 2.02 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26–1.30 (m, 46H, CH_2), 1.18 (d, $J = 6.42$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.23$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1 (HN–C=O), 169.4 (HN–C=O), 129.4 (H_2C–HC=CH), 129.3 (H_2C–HC=CH), 101.7 (C-1), 75.1 (C-3), 73.9 (C-5), 73.1 (C-2), 70.8 (C-4), 68.6 (HC–OH), 61.3 (C-6), 56.7 (HC–NH), 39.2 (O=C–NH–CH_2), 35.8 (O=C–CH_2), 32.1 (H_2C–HC=CH), 31.4(O=C–CH_2–CH_2), 29.2 (CH_2), 29 (CH_2), 28.8 (CH_2), 26.7 (CH_2), 26.5 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 15.3 (HOHC–CH_3), 13.4 (CH_3); IR (KBr): 3386, 2923, 2852, 1638, 1546, 1379, 1163, 1088, 720; HRMS (ESI) *m/z* [M+H]-calc. for $C_{44}H_{85}O_8N_2 = 769.63004$ found 769.63034.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) octadecanamide (**8r**)*

Chloroform: methanol (95: 5, v/v), yield 72%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 7.46 (t, $J = 6.05$ Hz, 1H, NH), 5.29–5.38 (m, 2H, HC=CH), 4.57 (d, $J = 4.12$ Hz, 1H, CH–NH), 4.34 (t, $J = 3.57$, 1H, CH–OH), 4.21 (dd, $J = 5.77$, 11.82 Hz, 1H, Gal-1), 3.88 (m, 1H, Gal-4), 3.83 (m, 1H, Gal-6), 3.71 (dd, $J = 3.85$, 11.55 Hz, 1H, Gal-6), 3.53–3.57 (m, 1H, Gal-4), 3.51 (m, 2H, Gal-3 & 5), 3.15–3.27 (m, 2H, H_2C–NH), 2.28 (t, $J = 7.15$ Hz, 2H, O=C–CH_2), 2.02 (m, 4H, H_2C–HC=CH), 1.63 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26–1.31 (m, 50H, CH_2), 1.18 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 5.77$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 174.1 (HN–C=O), 169.3 (HN–C=O), 129.5 (H_2C–HC=CH), 129.3 (H_2C–HC=CH), 101.8 (C-1), 75.1 (C-3), 74 (C-5), 73.1 (C-2), 70.9 (C-4), 68.7 (HC–OH), 61.5 (C-6), 56.7 (HC–NH), 39.2 (O=C–NH–CH_2), 35.8 (O=C–CH_2), 31.5 (H_2C–HC=CH), 29.3 (O=C–CH_2–CH_2), 29.1 (CH_2), 28.9 (CH_2), 26.8 (CH_2), 26.5 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 15.3 (HOHC–CH_3), 13.5 (CH_3); IR (KBr): 3286, 2923, 2852, 1638, 1553, 1379, 1163, 1077, 720; HRMS (ESI) *m/z* [M+H]-calc. for $C_{46}H_{89}O_8N_2 = 797.66134$ found 797.66175.

N-*((2S, 3R)-1-(Oleylamino)-1-oxo-3-((β-D-galactopyranosyl) oxy) butan-2-yl) oleamide* (***8s***)

Chloroform: methanol (95: 5, v/v), yield 72%. ^{1}H-NMR ($CDCl_3 + CD_3OD$, 300 MHz): 5.32–539 (m, 4H, HC=CH), 4.56 (d, $J = 4.40$ Hz, 1H, CH–NH), 4.48–4.49 (m, 1H, CH–OH), 4.32–4.35 (m, 1H, Gal-1), 4.18–4.22 (m, 1H, Gal-4), 3.81–3.90 (m, 2H, Gal-6), 3.68–3.74 (m, 1H, Gal-2), 3.53–3.57 (m, 1H, Gal-3), 3.50–3.51 (m, 1H, Gal-5), 3.14–3.28 (m, 2H, H_2C–NH), 2.28 (t, $J = 7.42$ Hz, 2H, O=C–CH_2), 1.90–2.11 (m, 8H, H_2C–HC=CH), 1.64 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.27–1.31 (m, 42H, CH_2), 1.18 (d, $J = 6.32$ Hz, 3H, CHOH–CH_3), 0.88 (t, $J = 6.32$ Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3 + CD_3OD$, 75 MHz): 175 (HN–C=O), 170.4 (HN–C=O), 130.4 (H_2C–HC=CH), 130.1 (H_2C–HC=CH), 102.6 (C-1), 76 (C-3), 74.8 (C-5), 74 (C-2), 71.7 (C-4), 69.6 (HC–OH), 62.2 (C-6), 57.7 (HC–NH), 40.1 (O=C–NH–CH_2), 36.6 (O=C–CH_2), 33.02 (H_2C–HC=CH), 32.3 (H_2C–HC=CH), 30.2 (O=C–CH_2–CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 27.6 (CH_2), 27.4 (CH_2), 26.2 (CH_2), 23.1 (CH_2), 16.1 (HOHC–CH_3), 14.3 (CH_3); IR (Neat): 3286, 2924, 2853, 1638, 1549, 1461, 1146, 1077, 718; HRMS (ESI) *m/z* [M+H]-calc. for $C_{46}H_{87}O_8N_2 = 795.64569$ found 795.64547.

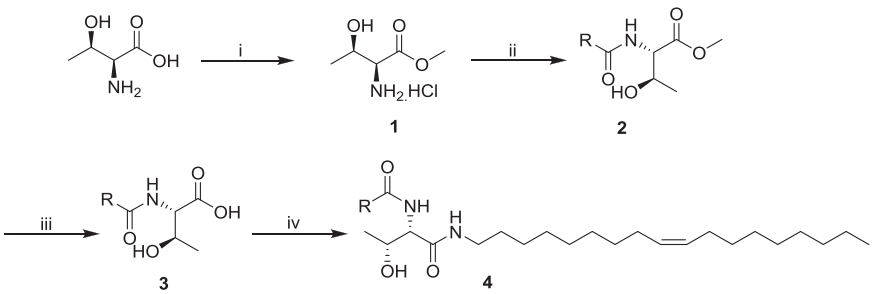
In vitro cytotoxicity evaluation assay

The cytotoxicity of the prepared threonine-based galactoceramide derivatives were evaluated on the basis of measurement of in vitro growth of tumor cell lines in 96-well plates by cell mediated reduction of tetrazolium salt to water insoluble formazan crystals using doxorubicin as a standard control. The cytotoxicity was assessed against a panel of three different tumor cell lines: A549: human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), MCF-7: human breast adenocarcinoma cells (ATCC No. HTB-22), HeLa: human cervical cancer cell line (ATCC No. CCL-2) and HEK 293: normal human embryonic kidney cells (ATCC No. CRL-1573) using the MTT assay (Mosmann 1983). The IC_{50} values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose response curves. IC_{50} values (in μM) are expressed as the average of two independent experiments.

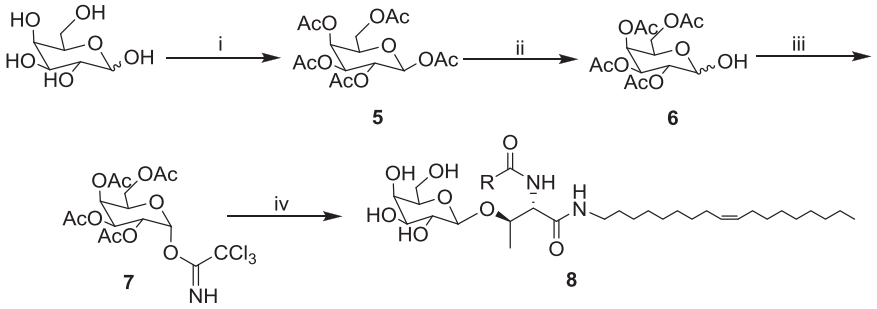
Results and discussion

Synthesis and characterization

New class of threonine-based β-galactosylceramides with replacement of fatty-acyl group on amide moiety with different aromatic acids and various fatty-acyl moieties were synthesized using the starting material L-threonine. As shown in Scheme 1, L-threonine methyl ester hydrochloride (**1**) was prepared from L-threonine using 2 M methanalic HCl solution under reflux conditions. L-threonine methyl ester hydrochloride (**1**) was further derivatized to afford *N*-aromatic/fatty-acyl threonine methyl esters (**2a–2s**) with different aromatic acids/fatty acids using EDC.HCl and HOBt coupling reagents. This amide bond was further confirmed through ^{1}H-NMR, where the N–H proton signals appears as broad doublet/singlet at $\delta = 6.2$–7.0 ppm range. Further to obtain compounds **3a–3s**, *N*-aromatic/fatty-acyl threonine methyl esters **2a–2s** were hydrolyzed with LiOH. H_2O in THF (Tetrahydrofuran)-H_2O solvent mixture and this was followed by acidification of lithium salts with 2 N HCl and these free acid compounds were identified by ^{1}H-NMR, the characteristic methyl group protons disappeared at 3.7 ppm. This free acid compounds **3a–3s** were further derivatized with oleyl amine in presence of EDC.HCl and HOBt coupling reagents to give the desired mixture of di-amide compounds **4a–4s**. Di-amide was confirmed by the



Scheme 1 Reagents and conditions: (i) HCl 2 M, MeOH, reflux, 2 h, 98%; (ii) Acid, EDC.HCl, HOBt, Dichloromethane (DCM), room temperature (rt), Overnight, 78–84%; (iii) LiOH.H_2O, THF, H_2O, rt, 16 h, 88–95%; (iv) Oleyl amine, EDC.HCl, HOBt, DCM, rt, overnight, 75–80%. R = Cinnamoyl (**2a**, **3a**, **4a**), 4-Nitro cinnamoyl (**2b**, **3b**, **4b**), Indole-3-acetanoyl (**2c**, **3c**, **4c**), Indole-3-propanoyl (**2d**, **3d**, **4d**), Indazole-3-cabonyl (**2e**, **3e**, **4e**), Indole-2-cabonyl (**2f**, **3f**, **4f**), Phenyl acetonyl (**2g**, **3g**, **4g**), 4-Methoxy phenylacetonyl (**2h**, **3h**, **4h**), 4-Methoxy phenyl butanoyl (**2i**, **3i**, **4i**), Hexanoyl (**2j**, **3j**, **4j**), Octanoyl (**2k**, **3k**, **4k**), 2-Propylpentanoyl (**2l**, **3l**, **4l**), Undecenoyl (**2m**, **3m**, **4m**), Undecanoyl (**2n**, **3n**, **4n**), Dodecanoyl (**2o**, **3o**, **4o**), Tetradecanoyl (**2p**, **3p**, **4p**), Hexadecanoyl (**2q**, **3q**, **4q**), Octadecanoyl (**2r**, **3r**, **4r**), Oleoyl (**2s**, **3s**, **4s**)



Scheme 2 Reagents and conditions: (i) NaOAc, Ac_2O, reflux, 4 h, 92%; (ii) Benzyl amine, THF, room temperature (rt), 20 h, 84%; (iii) CCl_3CN, DBU, DCM, rt, 2.5 h, 77%; (iv) **4(a–s)**, TMSOTf, DCM, rt, 12 h, NaOMe, MeOH, Amberlite-IR-120, rt, 1 h, 65–74%. R = Cinnamoyl (**8a**), 4-Nitro cinnamoyl (**8b**), Indole-3-acetanoyl (**8c**), Indole-3-propanoyl (**8d**), Indazole-3-cabonyl (**8e**), Indole-2-cabonyl (**8f**), Phenyl acetonyl (**8g**), 4-Methoxy phenylacetonyl (**8h**), 4-Methoxy phenyl butanoyl (**8i**), Hexanoyl (**8j**), Octanoyl (**8k**), 2-Propylpentanoyl (**8l**), Undecenoyl (**8m**), Undecanoyl (**8n**), Dodecanoyl (**8o**), Tetradecanoyl (**8p**), Hexadecanoyl (**8q**), Octadecanoyl (**8r**), Oleoyl (**8s**)

presence of oleyl moiety double bond protons at $\delta =$ 5.35–5.40 ppm as multiplet and one more N–H proton of new amide bond appeared as a singlet at $\delta = 6.8$–7.0 ppm in the ^{1}H-NMR spectrum of the corresponding compounds. All the compounds were fully characterized by ^{1}H-NMR, ^{13}C-NMR, IR, and ESI–MS spectral analysis.

The mixture of di-amide compounds **4a–4s** were glycosylated by employing trichloroacetimidate methodology. As depicted in the Scheme 2, 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**5**) was prepared by our previous protocol (Vudhgiri et al. 2017). Further, the anomeric acetyl group was chemoselectively deprotected by using benzyl amine (Manzo et al. 2012) and the formation of hemiacetal was confirmed by the disappearance of one acetate group protons present at 2 ppm range in proton NMR spectrum.

The glycosyl donor α-trichloroacetimidate (**7**) was prepared from hemiacetal (**6**) by using trichloroacetonitrile in the presence of DBU at room temperature (Manzo et al. 2012). The imidate formation was confirmed through the imine proton which appeared at $\delta = 8.6$ ppm as singlet in proton NMR spectrum. The coupling of imidate (**7**) was carried out with di-amide compounds **4a–4s** in presence of TMSOTf for overnight at room temperature (Schmidt 1986). After completion of all the starting materials, the reaction mixture was filtered and dissolved in chloroform. The organic layer was extracted with aq. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. This crude mixture was subjected to Zemplen deacetylation (Zemplen and Kunz 1923) to produce compounds **8a–8s** in β–configuration. This β-orientation was achieved by NGP of C-2′ acetate group of sugar with C-1′ carbon of sugar as it allowed only equatorial attack of acceptor. Further, β–configuration was confirmed by the presence of anomeric carbon (for compounds **8a–8i** 102 ppm range and for compounds **8j–8s** 101 ppm range) as single peak in ^{13}C-NMR spectrum. These results further corroborated with previously reported spectral data (Sjolin et al. 1996).

In vitro cytotoxicity evaluation

Galactoceramides and its derivatives have gained much attention from a cancer chemoprevention perspective due to their promising inhibitory effect on tumor growth. Fatty-acyl group on amide moiety or phytosphingosine group, and chain length are some of the important features that influence the cytotoxicity. In this regard, all the synthesized compounds were evaluated for cytotoxicity (Mosmann 1983) against three cancer cell lines such as human alveolar adenocarcinoma epithelial cells (A549), human breast adenocarcinoma cells (MCF-7), human cervical cancer cell line (HeLa), and one normal cell line such as normal human embryonic kidney cells (HEK-293) and the results to this regard are tabulated in Table 1.

Based on the results, all the tested compounds **8a–8s** exhibited moderate to good cytotoxicity against all the tested cancer cell lines with IC_{50} values ranging between 14.08 to 64.05 μM. Among all the synthesized compounds, aromatic-acid derivatives were more promising as compared to fatty-acid derivatives because of the absence of additional hydrogen bond on the wall of the A′ pocket in the CD1d hydrophobic groove in fatty-acid derivatives as compared to the aromatic-acid derivatives. These results corroborated with the earlier reports (Fujio et al. 2006; Wu et al. 2006).

In addition, among all the tested aromatic derivatives, compound **8i** exhibited promising activity against MCF7, A549, and HeLa cancer cell lines with IC_{50} values of 14.08, 14.78, and 16.70 μM, respectively. Further, the compounds **8f**, **8g**, **8h**, and **8i** showed promising activity against MCF7 cancer cell line with IC_{50} values of 15.49, 15.70, 15.43, and 14.08 μM, while compound **8e** exhibited good activity against HeLa cancer cell line with IC_{50} value of 16.78 μM. Based on the cytotoxicity results for compounds **8h** and **8i**, the cytotoxicity increases with an increase in the spacer chain length between aromatic residue and amide functional group. These results support an earlier report (Fujio et al. 2006). Based on the results for compounds **8g** and **8h**,

Table 1 In vitro cytotoxicity evaluation of different threonine-based galactoceramide aromatic and fatty-acid derivatives (**8a–8s**)

Test compound	IC_{50} values (μM)			
	A549	MCF 7	HeLa	HEK 293
8a	27.82 ± 0.17	21.62 ± 0.41	26.20 ± 0.32	96.23 ± 0.63
8b	28.35 ± 0.21	26.08 ± 0.91	30.61 ± 0.29	88.63 ± 0.56
8c	64.05 ± 0.12	49.62 ± 0.06	61.07 ± 0.15	91.26 ± 0.36
8d	36.37 ± 0.92	38.85 ± 0.41	39.00 ± 0.08	99.36 ± 0.49
8e	18.35 ± 0.32	19.19 ± 0.21	16.78 ± 0.16	74.26 ± 0.96
8f	17.64 ± 0.02	15.49 ± 0.81	16.04 ± 0.36	86.23 ± 0.65
8g	19.59 ± 0.42	15.70 ± 0.07	18.17 ± 0.78	92.34 ± 0.26
8h	18.71 ± 0.32	15.43 ± 0.91	17.99 ± 0.26	97.56 ± 0.13
8i	14.78 ± 0.32	14.08 ± 0.36	16.70 ± 0.11	88.16 ± 0.26
8j	26.09 ± 0.18	37.25 ± 0.43	27.48 ± 0.76	60.412 ± 0.23
8k	27.58 ± 0.77	34.67 ± 0.06	24.50 ± 0.99	64.69 ± 0.32
8l	22.54 ± 0.17	25.69 ± 0.06	23.78 ± 0.12	61.32 ± 0.36
8m	20.66 ± 0.52	19.78 ± 0.77	16.34 ± 0.23	98.26 ± 0.96
8n	22.28 ± 0.31	18.05 ± 0.42	19.74 ± 0.06	86.92 ± 0.59
8o	22.75 ± 0.65	20.37 ± 0.84	20.11 ± 0.02	73.69 ± 0.63
8p	21.77 ± 0.77	18.83 ± 0.91	19.40 ± 0.21	97.26 ± 0.34
8q	23.02 ± 0.02	21.36 ± 0.81	24.40 ± 0.62	84.26 ± 0.69
8r	22.92 ± 0.96	22.92 ± 0.51	24.60 ± 0.99	95.26 ± 0.54
8s	25.91 ± 0.10	22.18 ± 0.61	22.70 ± 0.87	84.26 ± 0.46
Doxorubicin	2.10 ± 0.09	3.12 ± 0.12	1.78 ± 0.24	78.26 ± 0.89

A549: human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), MCF-7: human breast adenocarcinoma cells (ATCC No. HTB-22), HeLa: human cervical cancer cell line; HEK 293: human embryonic kidney cells 293

methoxy group also influenced the cytotoxicity of the compounds. The indazole derivative (**8e**) exhibited good activity due to the presence of more nitrogen atoms as it may provide additional hydrogen bond stability as discussed in an earlier report (Yogesh Kumar et al. 2016). Furthermore, all the aromatic-acid derivatives showed good to moderate cytotoxicity against all the tested cancer cell lines.

In case of fatty-acid derivatives, compounds **8j–8s** exhibited good cytotoxicity against all the tested cancer cell lines with IC_{50} values ranging between 16.34 to 37.25 μM. The unusual undecenoic acid (**8m**), lauric acid (**8o**) and undecanoic acid (**8n**), myristic acid (**8p**) derivatives exhibited promising activity against HeLa and MCF7 cancer cell lines with IC_{50} values of 16.34, 20.11 and 18.05, 18.33 μM, respectively. All these compounds exhibited good cytotoxicity against the remaining cancer cell lines with ≤ 20 μM. The results for the compounds **8k** and **8l** demonstrated that the branched chain acid derivative (**8l**) exhibited good cytotoxicity against all the tested cancer cell lines as compared to straight chain fatty-acid derivative (**8k**). The results of same chain length terminal unsaturated (**8m**) and saturated (**8n**) fatty-acid derivatives revealed that terminal unsaturated fatty-acid derivative showed considerable effect on cytotoxicity as compared to the saturated fatty-acid derivative. Furthermore, all the fatty-acid derivatives showed good to moderate cytotoxicity against all the tested cancer cell lines.

Conclusions

In the present study, the synthesis of threonine-based β-galactoceramide was carried out and its derivatives were prepared by modifying the fatty-acyl group on amide moiety with different unusual, branched, saturated, and unsaturated fatty-acyl moieties and aromatic acids employing trichloroacetimidate methodology. Further, all the synthesized compounds were evaluated for in vitro cytotoxicity and all these compounds exhibited good to moderate cytotoxicity against all the tested cancer cell lines with IC_{50} values ranging between 14.08 to 64.05 μM. Among all the compounds, the compounds **8i**, **8m**, and **8n** exhibited promising cytotoxicity. Based on structure–activity relationship, aromatic-acid derivatives exhibited promising activity as compared to fatty-acid derivatives. Further, the compounds bearing longer spacer chain length between aromatic residue and amide functional group, methoxy

substituents on aryl group compounds, terminal unsaturation of fatty-acid compounds and branching chain compounds showed good cytotoxicity when compared to their respective counterparts.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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