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Short communication

A facile, one-pot synthesis, characterization and antimicrobial activity of *o*-hydroxy anilide derivatives and 1-substituted-1,3-dicyclohexylurea analogs of long chain carboxylic acids

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ABSTRACT

A series of novel *o*-hydroxy anilide derivatives and 1-substituted-1,3-dicyclohexylurea analogs of long chain carboxylic acids have been synthesized. The structures of the synthesized compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds were tested for their antimicrobial activity by disk diffusion assay with slight modifications against Gram-positive, Gram-negative strains of bacteria as well as fungal strains. After that minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs) of all the synthesized compounds were determined. The investigation of antimicrobial screening data revealed that all the tested compounds showed moderate to good microbial inhibitions. Compounds **3e**, **4e**, **3f** and **4f** were found to be the most potent antimicrobial agents.

1. Introduction

The development of antimicrobial agents to treat infectious diseases has been one of the most notable achievements of the past century. However the natural phenomenon known as “antimicrobial resistance” has posed certain threats to these advancements. The increased use of antimicrobial agents available in the market has resulted in the development of resistance to the commonly used drugs with important implications for morbidity, mortality [1,2] and health care costs. Inspite of a large number of antibiotics and chemotherapeutics available for medical use, the antimicrobial resistance has created a substantial need for design of new class of antimicrobials and this field will always remain an area of immense significance.

Aminophenol is an important intermediate in the preparation of several analgesic and antipyretic drugs such as paracetamol, acetanilide, phenacetin and so forth [3]. Long chain carboxylic acids or their esters are established to be pharmacologically active antimicrobial agents [4] and have also been efficiently used in the treatment of cardiovascular, hepatic and renal disorders [5]. Various anilides have also found wide applicability as bioactive (antimicrobial [6], antioxidant [7], anti-atherosclerotic [8] and anticonvulsant [9]) agents. The 4′-hydroxyacetanilide (acetaminophen) has also been reported to be effective in the treatment of osteoarthritis [10].

The use of 1,3-dicyclohexylcarbodiimide (DCC) as an activating agent of carboxylic acids to form amides is undoubtedly one of the most important reactions. According to the known mechanism, the side reactions during DCC/4-dimethylaminopyridine (DMAP) mediated synthesis of amides is the formation of side products belonging to the group of acylureas [11]. *N*-acylurea and its derivatives exhibit a wide range of biological activities and a number of them possess analgesic and anticonvulsant [12], antioxidant [13], anti-inflammatory and anti-poliferation [14], antifungal [15] and larvicidal [16] properties. Bearing in mind the aforementioned facts relating to the pharmacological importance of various anilides and acylurea derivatives, we herein report the design and synthesis of hitherto unknown *o*-hydroxy anilide derivatives and acylurea analogs of long chain carboxylic acids. The newly synthesized compounds were further screened for their antibacterial and antifungal potencies.

2. Chemistry

A solution of fatty acid (FA) (0.011 mol), DCC (0.011 mol) and 2-aminophenol (0.011 mol) in dichloromethane (50 mL) with 0.001 mol of DMAP were stirred mechanically at room temperature for 28 h. Evaporation of solvent under reduced pressure yielded crude liquid showing two spots on TLC. Silica gel chromatographic

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Table 1
Antibacterial activity of compounds **3a**–**f** and **4a**–**f**.

Compounds	Diameter of zone of inhibition (mm)				
	Gram-positive bacteria		Gram-negative bacteria		
	S. pyogenes	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
3a	10.6 ± 0.2	9.8 ± 0.5	11.5 ± 0.4	10.5 ± 0.3	11.8 ± 0.2
4a	11.2 ± 0.3	10.1 ± 0.6	14.9 ± 0.5	10.7 ± 0.4	12.3 ± 0.2
3b	10.8 ± 0.3	10.5 ± 0.7	11.9 ± 0.2	11.4 ± 0.6	12.4 ± 0.5
4b	11.5 ± 0.5	11.3 ± 0.9	12.2 ± 0.5	12.7 ± 0.7	13.3 ± 0.4
3c	11.1 ± 0.7	10.9 ± 0.3	14.3 ± 0.6	–	12.7 ± 0.4
4c	–	–	12.1 ± 0.5	–	10.6 ± 0.2
3d	11.4 ± 0.3	11.1 ± 0.5	15.2 ± 0.4	10.8 ± 0.2	13.2 ± 0.4
4d	13.5 ± 0.2	13.4 ± 0.2	17.2 ± 0.5	12.8 ± 0.3	15.4 ± 0.3
3e	16.1 ± 0.5	15.6 ± 0.4	15.2 ± 0.5	14.7 ± 0.3	17.7 ± 0.4
4e	19.1 ± 0.3	18.5 ± 0.6	23.2 ± 0.2	17.5 ± 0.2	20.7 ± 0.3
3f	19.2 ± 0.2	18.7 ± 0.6	23.4 ± 0. 4	17.8 ± 0.2	20.9 ± 0.9
4f	20.8 ± 0.3	19.5 ± 0.8	27.1 ± 0.6	20.6 ± 0.2	23.4 ± 0.5
Standard	23.0 ± 0.2	22.0 ± 0.2	32.0 ± 0.3	19.0 ± 0.2	270 ± 0.2
DMSO	–	–	–	–	–

– Indicates bacteria are resistant to the compounds >100 μg/ml Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

separation using mixtures of *n*-hexane–ethylacetate (94:6 v/v) and *n*-hexane–ethylacetate (90:10 v/v) as eluents, yielded two homogenous products: *o*-hydroxy anilide derivatives **3a**–**f** and acylurea analogs, **4a**–**f** respectively. All these novel compounds were characterized from their spectral data.

3. Pharmacology

3.1. Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method [17]. A standard inoculums ($1–2 \times 10^7$ c.f.u./ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Ciprofloxacin (30 μg) was used as positive control. While the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 1.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5×10^5 c.f.u./ml of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bactericidal concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums was killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 2.

3.2. Antifungal studies

Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method [18]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 3.

The nutrient broth, which obtained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6 \times 10^4–6 \times 10^4$ c.f.u./ml. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest

Table 2
MIC and MBC results of compounds **3a**–**f** and **4a**–**f** positive control Ciprofloxacin.

Compounds	Gram-positive bacteria				Gram-negative bacteria					
	S. pyogenes		*S. aureus*		*P. aeruginosa*		*K. pneumoniae*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3a	50	100	50	100	100	100	50	100	100	100
4a	50	100	50	100	25	100	50	100	50	100
3b	50	100	100	100	100	100	50	100	100	100
4b	50	100	50	100	25	100	100	100	50	100
3c	–	–	100	–	–	–	100	100	–	–
4c	–	–	–	–	50	100	–	–	100	100
3d	50	100	100	100	100	100	–	–	100	100
4d	–	–	–	–	100	100	–	–	100	100
3e	50	100	50	100	25	100	–	–	25	100
4e	50	100	50	100	50	100	50	100	50	100
3f	50	100	25	50	25	25	25	50	25	50
4f	25	25	25	50	25	100	25	100	50	100
Standard	12.5	12.5	6.25	12.5	6.5	12.5	12.5	12.5	6.25	12.5

– Indicates bacteria are resistant to the compounds >100 μg/ml; MIC (μg/ml) = minimum inhibitory concentration, i.e., the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bactericidal concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

Table 3
Antifungal activity of compounds **3a**–**f** and **4a**–**f**: Positive control (Greseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Compounds	Diameter of zone of inhibition (mm)			
	CA	AF	TM	PM
3a	13.7 ± 0.2	11.6 ± 0.7	9.8 ± 0.5	9.3 ± 0.4
4a	14.2 ± 0.4	12.5 ± 0.3	10.7 ± 0.8	9.9 ± 0.7
3b	14.7 ± 0.3	12.4 ± 0.9	10.5 ± 0.3	10.2 ± 0.8
4b	15.1 ± 0.7	13.6 ± 0.5	12.2 ± 0.8	10.5 ± 1.2
3c	12.5 ± 0.2	9.2 ± 0.2	8.3 ± 0.4	8.9 ± 0.2
4c	12.1 ± 0.9	–	–	–
3d	15.3 ± 1.4	11.7 ± 0.2	8.9 ± 0.2	9.2 ± 1.2
4d	13.9 ± 0.4	9.9 ± 0.3	–	–
3e	16.2 ± 0.7	12.8 ± 0.3	10.5 ± 0.5	10.1 ± 0.9
4e	18.3 ± 0.2	14.9 ± 1.2	13.1 ± 0.7	–
3f	21.4 ± 0.5	18.3 ± 0.4	15.2 ± 0.3	10.7 ± 0.5
4f	24.2 ± 0.3	21.2 ± 0.2	18.3 ± 1.2	13.8 ± 0.3
Standard	30.0 ± 0.2	27.0 ± 0.2	24.0 ± 0.3	20.0 ± 0.5
DMSO	–	–	–	–

– Indicates bacteria are resistant to the compounds >100 μg/ml; CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*.

concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 4.

4. Results and discussion

The present study is based on the synthesis, characterization and evaluation of antimicrobial potency of several *o*-hydroxy anilide and acylurea derivatives of long chain carboxylic acids. The carboxylic acid reacts with 2-aminophenol in presence of DCC and DMAP in dichloromethane to yield various *o*-hydroxy anilide derivatives **3a**–**f** and 1-substituted-1,3-dicyclohexylurea analogs of carboxylic acids **4a**–**f** (Scheme 1).

During the DCC mediated reaction for the amide synthesis the 1,3-dicyclohexylurea analogs are formed as a side products [11]. However, bearing in mind the pharmacological importance of these 1,3-dicyclohexylurea analogs the development of suitable reaction conditions for optimization of DCC mediated anilide synthesis is very important and there is a serious requisite for selection of appropriate reaction conditions ensuring satisfactory yields of *o*-hydroxy anilide derivatives as well as acylurea analogs.

The reaction of 2-aminophenol with hexadecanoic acid was chosen as the model to optimize the conditions for the preparation of *o*-hydroxy pentadecanilide, **3a** and 1-(hexadecanoyl)-1,3-dicyclohexylurea, **4a**. At first the reaction was carried using equimolar amount of acid, DCC and 2-aminophenol with a catalytic amount of DMAP (0.005 mol). Under such reaction conditions the anilide was formed as the major product. However by lowering the amount of catalyst (DMAP) to 0.001 mol and keeping the rest of conditions constant the anilide and acylurea derivative were formed in comparable yields. The optimum conditions were set up and the generality and scope of the reaction was determined using various long chain alkanyl, alkenyl and hydroxyalkenyl carboxylic acids.

The structure of compounds **3a**–**f** and **4a**–**f** were established from their IR, ^{1}H NMR, ^{13}C NMR and MS spectra.

The newly synthesized compounds **3a**–**f** and **4a**–**f** were also tested for their antimicrobial activities by disk diffusion assay against Gram-positive, Gram-negative strains of bacteria as well as selected fungal strains. The antimicrobial screening data revealed that all the tested compounds showed moderate to good inhibitions. Among the tested bacterial strains, good inhibitory results were obtained against *S. pyogenes*, *S. aureus* and *E. coli* species. The data pertaining to antibacterial screening reveal that the compounds **3e**, **4e**, **3f** and **4f** have exerted a significant inhibitory activity against the selected bacterial strains. The compounds **3f** and **4f** showed antibacterial activity nearly equivalent to that of reference drug Ciprofloxacin. The MBC of a few compounds was found to be same as MIC but in most of the cases it was two or four fold higher than the corresponding MIC results. In another set of experiments all the newly synthesized compounds were also examined for their antifungal activities. Among the screened compounds **3e**, **4e**, **3f** and **4f** showed good inhibition against all the fungal strains. The MFC of few compounds was found to be the same as MIC or four fold higher but in most of the compounds it was two folds higher than the corresponding MIC results. Compound **3f** and **4f** showed good fungicidal activity against *C. albicans*, *A. fumigatus* and *P. marneffei* fungal strains. Thus, the data revealed that the compounds **3a**–**f** and **4a**–**f** have produced the marked enhancement in the potency of these analogs as antibacterial and antifungal agents. However the mode of action of these compounds and their effect on mammalian cell still needs to be evaluated.

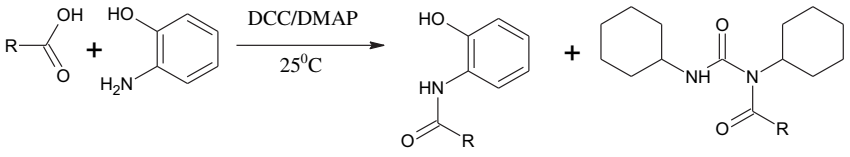
Table 4
MIC and MFC of compounds **3a**–**f** and **4a**–**f**.

Comp.	CA		AF		TM		PM	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
3a	50	100	50	100	25	100	50	100
4a	25	50	50	100	12.5	25	50	100
3b	12.5	25	25	50	50	100	50	100
4b	25	50	50	100	50	100	50	100
3c	50	100	–	–	–	–	–	–
4c	50	100	–	–	–	–	–	–
3d	50	50	25	100	50	100	50	100
4d	50	100	50	100	–	–	–	–
3e	25	50	50	100	50	100	50	100
4e	25	50	25	100	25	100	–	–
3f	12.5	25	25	50	25	50	25	100
4f	12.5	12.5	12.5	25	12.5	25	12.5	25
Standard	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5

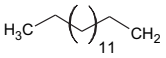
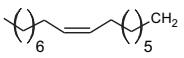
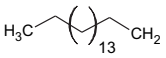
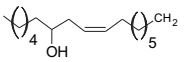
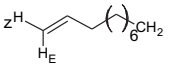
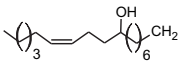
– Indicates bacteria are resistant to the compounds >100 μg/ml; CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. MIC (μg/ml) = minimum inhibitory concentration, i.e., the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (μg/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

5. Conclusion

The present communication reports the synthesis and characterization of hitherto unknown *o*-hydroxy anilide derivatives and acylurea analogs of long chain carboxylic acids. It was found that the molar ratio acid/2-aminophenol/DCC/catalyst(DMAP) = 0.011/0.011/0.011/0.001 is optimal for the preparation of *o*-hydroxy anilide derivative and acylurea analog in comparable yields. The newly synthesized compounds were further screened for their antimicrobial potency. All the compounds showed moderate to good inhibition and hence the possibility of turning the DCC mediated anilide synthesis to the formation of pharmacologically active molecules was found. Further studies regarding the structure–activity relationship, toxicity and evaluation of other biological effects might be helpful in designing new antimicrobials for therapeutic use.



1a-f **2** **3a-f** **4a-f**

Compound	R	Compound	R
1a, 3a, 4a		1d, 3d, 4d	
1b, 3b, 4b		1e, 3e, 4e	
1c, 3c, 4c		1f, 3f, 4f	

Scheme 1. Synthesis of various *o*-hydroxy anilides and *N*-acylurea analogs of long chain carboxylic acids.

6. Experimental protocol

6.1. Physical and spectroscopic measurements

Undec-10-enoic (purity, 98%) and (*Z*)-octadec-9-enoic (97%), stearic and palmitic acids were purchased from Fluka chemicals (Buck: Switzerland). (9*Z*,12*R*)-12-hydroxyoctadec-9-enoic acid (ricinoleic acid, 98%) (**1e**) and (9*R*,12*Z*)-9-hydroxyoctadec-12-enoic acid (isoricinoleic acid 98%) (**1f**) were isolated from *Ricinus communis* and *Wrightia tinctoria* seed oils respectively, following Gunstone's partition procedure [19] and were characterized on the basis of IR and ^{1}H NMR spectral data. DCC, DMAP and 2-aminophenol were purchased from Merck, Mumbai, India. Thin layer chromatography (TLC) was done on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India 0.5 mm thickness). Mixtures of petroleum ether–diethyl ether–acetic acid (70:30:1; v/v) were used as developing solvent. The column chromatography was carried out with silica gel (Merck, Mumbai, India, 60–120 mesh). IR spectra were recorded on Shimadzu 8201 PC spectrophotometer.^{1}H NMR spectra were recorded with a Bruker, DRX 400 spectrometer (400 MHz) in $CDCl_3$, using TMS as internal standard. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are given in Hz. ^{13}C NMR spectra were recorded at Bruker DRX 400 spectrometer in $CDCl_3$ with $CDCl_3$ ($\delta = 77.00$). The mass spectra were recorded on JEOL-SX 102/DA-600 mass spectrometer.

6.1.1. (9Z,12R)-12-Hydroxyoctadec-9-enoic acid (**1e**)

Yellow oily compound, IR (KBr, cm^{-1}): 3400 (O–H), 1678 (C=O). ^{1}H NMR ($CDCl_3$, δ_H): 10.8 (s, 1H, –COO*H*), 5.42 (m, 2H, –C*H*=C*H*–), 3.84 (m, 1H, –C*H*OH), 2.30 (m, 1H, –CHO*H*–), 2.02 (m, 4H, –CH_2–CH=CH–CH_2–), 1.32 (br.s, 18H, chain CH_2), 0.84 (dist.t, 3H, CH_3).

6.1.2. (9R,12Z)-9-Hydroxyoctadec-12-enoic acid (**1f**)

Yellow oily compound, IR (KBr, cm^{-1}): 3406 (O–H), 1675 (C=O). ^{1}H NMR ($CDCl_3$, δ_H): 10.6 (s, 1H, –COO*H*), 5.37 (m, 2H, –C*H*=C*H*–), 3.82 (m, 1H, –C*H*OH), 2.27 (m, 1H, –CHO*H*), 2.09 (m, 4H, –CH_2–CH=CH–CH_2–), 1.30 (br.s, 18H, chain CH_2), 0.86 (dist.t, 3H, CH_3).

6.2. General procedure for the synthesis of o-hydroxy anilide derivatives (**3a–f**) and 1-substituted-1,3-dicyclohexylurea analogs (**4a–f**) of long chain carboxylic acids

A solution of FA (0.011 mol), DCC (0.011 mol) and 2-aminophenol (0.011 mol) in dichloromethane (50 mL) with 0.001 mol of DMAP were stirred mechanically at room temperature for 28 h. The *N*,*N*-dicyclohexylurea was filtered off and the filtrate was washed with water (3 × 50 mL), 5% acetic acid (3 × 50 mL) again with water (3 × 50 mL) and then dried over anhydrous sodium sulfate. Evaporation of solvent under reduced pressure yielded crude liquid showing two spots on TLC. Silica gel column chromatographic separation using mixtures of *n*-hexane–ethylacetate (94:6 v/v) and *n*-hexane–ethylacetate (90:10 v/v) as eluents, yielded two homogenous products: *o*-hydroxy anilide derivatives **3a–f** and acylurea analogs, **4a–f** respectively. All these novel compounds were characterized from their spectral data.

6.2.1. o-Hydroxy-hexadecanilide (**3a**)

Yellow oily liquid, Yield = 48%, IR (KBr, cm^{-1}): 3438 (O–H), 3304 (N–H stretch), 2923 (C–H asym), 2848 (C–H sym), 1706, 1652 (anilide). ^{1}H NMR ($CDCl_3$, δ_H) 9.21 (s, 1H, OH), 8.20 (s, 1H, NH), 7.07–7.05 (m, 4H, *Ph*), 2.22 (t, 2H, $J = 7.4$ Hz, CH_2–CO), 1.63 (m, 2H, CH_2–CH_2–CO), 1.24 (br.s, 24H, chain CH_2), 0.86 (dist.t, 3H, terminal –CH_3). ^{13}C NMR ($CDCl_3$, δ_C) 170.2, 131.7, 127.6, 126.5, 125.4, 122.9, 120.6, 33.7, 33.4, 30.1, 29.6,"two signals are hidden", 29.4, 29.2, 28.5, 27.3, 26.8, 25.9, 25.0, 24.8, 14.0. ESI-MS: found $[M + Na]^+$ 370.45; $C_{22}H_{37}O_2N$ $[M + Na]^+$ requires 370.48.

6.2.2. 1-(Hexadecanoyl)-1,3-dicyclohexylurea (**4a**)

Yellow oily liquid, Yield = 40%, IR (KBr, cm^{-1}): 3321 (N–H stretch), 2925 (C–H asym), 2852 (C–H sym), 1639 (C=O), 1550 (N–H bend), 1225 (C–N). ^{1}H NMR ($CDCl_3$, δ_H) 7.24 (s, 1H, N*H*), 3.83–3.81 (m, 1H, C*H* of C_6H_{11}), 3.65–3.62 (m, 1H, C*H* of C_6H_{11}), 2.31 (t, 2H, $J = 7.6$ Hz, CH_2–CO), 1.65 (m, 8H, 4CH_2), 1.58 (m, 2H, CH_2–CH_2–CO), 1.35 (m, 36H, 18CH_2), 0.83 (dist.t, 3H, terminal –CH_3). ^{13}C NMR ($CDCl_3$, δ_C) 154.9, 135.3, 49.1, 48.7, 33.9, 33.7, 33.6, 33.4, 32.9, 32.5, 31.7, 31.4, 31.3, 31.0, 30.9, 29.7, 29.6, 29.1, "two signals are hidden", 28.8, 28.5, 27.7,

26.3, "one signal is hidden", 25.9, 25.4, 24.9, 14.7. ESI-MS: found $[M + Na]^+$ 485.66; $C_{29}H_{54}O_2N_2$ $[M + Na]^+$ requires 485.68.

*6.2.3. o-Hydroxy-octadecanilide (**3b**)*

Yellow oily liquid, Yield = 48%, IR (KBr, cm^{-1}): 3428 (O–H), 3308 (N–H stretch), 2927 (C–H asym), 2845 (C–H sym), 1715, 1658 (anilide). ^1H NMR ($CDCl_3$, δ_H) 9.25 (s, 1H, OH) 8.18 (s, 1H, NH), 7.07–7.03 (m, 4H, *Ph*), 2.27 (t, 2H, J = 7.4 Hz, CH_2–CO), 1.62 (m, 2H, CH_2–CH_2–CO), 1.27 (br.s, 24H, chain CH_2), 0.88 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 170.3, 131.4, 127.5, 126.7, 125.6, 122.7, 120.6, 33.6, 33.5, 30.3, 29.5, 29.3, 29.2, 28.7, 28.2, 27.5, 27.3, 26.6, 26.3, 25.1, 24.8, 24.3, 25.0, 14.0. ESI-MS: found $[M + Na]^+$ 398.51; $C_{24}H_{41}O_2N$ $[M + Na]^+$ requires 398.53.

*6.2.4. 1-(Octadecanoyl)-1,3-dicyclohexylurea (**4b**)*

Yellow oily liquid, Yield = 39%, IR (KBr, cm^{-1}): 3325 (N–H stretch), 2923 (C–H asym), 2857 (C–H sym), 1635 (C=O), 1525 (N–H bend), 1228 (C–N). ^1H NMR ($CDCl_3$, δ_H) 7.20 (s, 1H, N*H*), 3.88–0.86 (m, 1H, C*H* of C_6H_{11}), 3.68–3.65 (m, 1H, C*H* of C_6H_{11}), 2.33 (t, 2H, J = 7.4 Hz, CH_2–CO), 1.67 (m, 8H, $4CH_2$), 1.55 (m, 2H, CH_2–CH_2–CO), 1.37 (m, 40H, chain $20CH_2$), 0.86 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 154.4, 135.2, 49.2, 48.1, 33.9, 33.5, 32.9, 32.7, 32.1, 31.9, 31.5, 30.6, 30.5, 29.9, 29.7, 29.3, 29.5, 29.1, 28.9, 28.5, 28.2, 27.9, 27.3, 26.3, 26.1, "one signal hidden", 25.7, 25.2, 25.7, 24.2, 14.6. ESI-MS: found $[M + Na]^+$ 513.70; $C_{31}H_{58}O_2N_2$ $[M + Na]^+$ requires 513.73.

*6.2.5. o-Hydroxy-undec-10-anilide (**3c**)*

Yellow oily liquid, Yield = 47%, IR (KBr, cm^{-1}): 3424 (O–H), 3305 (N–H stretch), 2926 (C–H asym), 2855 (C–H sym), 1705, 1669 (anilide). ^1H NMR ($CDCl_3$, δ_H) 9.19 (s, 1H, OH), 8.10 (s, 1H, NH), 7.08–7.05 (m, 4H, *Ph*), 5.74 (tdd, 1H, $J_{H-^8CH_2}$ = 6.6 Hz, J_{H-H_Z} = 10.1 Hz, J_{H-H_E} = 17.0 Hz, CH_2=C*H*–), 4.93 (dd, 1H, J_{H_Z-H} = 10.1 Hz, $J_{H_Z-H_E}$ = 2.6 Hz, H_ZC = CH), 4.91 (dd, 1H, J_{H_E-H} = 17.1 Hz, $J_{H_E-H_Z}$ = 2.8 Hz, H_EC=CH–), 2.28 (t, 2H, J = 7.4 Hz, CH_2–CO), 2.10 (m, 2H, $-CH_2$–CH=CH_2), 1.65 (m, 2H, CH_2–CH_2–CO), 1.27 (br.s, 10H, chain CH_2). ^{13}C NMR ($CDCl_3$, δ_C) 170.2, 131.4, 127.5, 126.6, 125.8, 122.9, 120.7, 129.3, 33.9, 33.5, 29.8, 28.3, 27.5, 24.7, 23.5, 22.9, 14.0. ESI-MS: found $[M + Na]^+$ 298.32; $C_{17}H_{25}O_2N$ $[M + Na]^+$ requires 298.34.

*6.2.6. 1-(Undec-10-enoyl)-1,3-dicyclohexylurea (**4c**)*

Yellow oily liquid, Yield = 41%, IR (KBr, cm^{-1}): 3315 (N–H stretch), 2925 (C–H asym), 2852 (C–H sym), 1639 (C=O), 1520 (N–H bend), 1225 (C–N). ^1H NMR ($CDCl_3$, δ_H) 7.26 (s, 1H, N*H*), 5.80 (tdd, 1H, $J_{H-^8CH_2}$ = 6.7 Hz, J_{H-H_Z} = 10.8 Hz, J_{H-H_E} = 16.9 Hz, CH_2=C*H*–), 4.97 (dd, 1H, J_{H_Z-H} = 10.0 Hz, $J_{H_Z-H_E}$ = 2.3 Hz, H_ZC=CH), 4.94 (dd, 1H, J_{H_E-H} = 17.4 Hz, $J_{H_E-H_Z}$ = 2.0 Hz, H_EC=CH–), 3.88–3.84 (m, 1H, C*H* of C_6H_{11}), 3.70–3.66 (m, 1H, C*H* of C_6H_{11}), 2.40 (t, 2H, J = 7.4 Hz, CH_2–CO), 2.12 (m, 2H, $-CH_2$–CH=CH_2), 1.70 (m, 10H, $5CH_2$), 1.65 (m, 2H, CH_2–CH_2–CO), 1.36 (m, 20H, $10CH_2$). ^{13}C NMR ($CDCl_3$, δ_C) 154.6, 135.9, 129.7, 115.3, 49.3, 48.3, 33.9, 33.7, 33.4, 32.9, 32.3, 32.0, 31.5, 31.1, 30.8, 30.2, 29.3, "one signal hidden", 28.2, 26.3, 26.4, 25.0, 25.3, 25.0. ESI-MS: found $[M + Na]^+$ 413.51; $C_{24}H_{42}O_2N_2$ $[M + Na]^+$ requires 413.54.

*6.2.7. o-Hydroxy-(Z)-octadec-9-anilide (**3d**)*

Yellow oily liquid, Yield = 45%, IR (KBr, cm^{-1}): 3412 (O–H), 3306 (N–H stretch), 2925 (C–H asym), 2852 (C–H sym), 1706, 1643 (anilide). ^1H NMR ($CDCl_3$, δ_H) 9.22 (s, 1H, OH), 8.80 (s, 1H, NH), 7.07–7.05 (m, 4H, *Ph*), 5.28 (m, 2H, $-CH$=C*H*–), 2.26 (t, 2H, J = 7.4 Hz, CH_2–CO), 1.94 (m, 4H, $-CH_2$–CH=CH–CH_2), 1.67 (m, 2H, CH_2–CH_2–CO), 1.27 (br.s, 20H, chain CH_2), 0.80 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 170.6, 131.5, 127.2, 126.2, 125.5, 122.9, 120.6, 129.7, 35.9, 29.7, "two signal hidden", 29.4, 28.7, 27.1, 27.0, 26.8, 26.7, 25.3, 24.8, 23.4, 23.2, 22.9, 22.8, 14.1. ESI-MS: found $[M + Na]^+$ 396.50; $C_{24}H_{39}O_2N$ $[M + Na]^+$ requires 396.52.

*6.2.8. 1-[(Z)-Octadec-9-enoyl]-1,3-dicyclohexylurea (**4d**)*

Yellow oily liquid, Yield = 40%, IR (KBr, cm^{-1}): 3318 (N–H stretch), 2923 (C–H asym), 2859 (C–H sym), 1649, 1597 (C=O), 1457 (N–H bend), 1226 (C–N). ^1H NMR ($CDCl_3$, δ_H) 7.19 (s, 1H, N*H*), 5.28 (m, 2H, $-CH$=C*H*–), 3.81–3.76 (m, 1H, C*H* of C_6H_{11}), 3.62–3.60 (m, 1H, C*H* of C_6H_{11}), 2.54 (t, 2H, J = 7.4 Hz, CH_2–CO), 1.96 (m, 4H, $-CH_2$–CH=CH–CH_2), 1.69 (m, 14H, $7CH_2$), 1.65 (m, 2H, CH_2–CH_2–CO), 1.35 (m, 26H, $13CH_2$), 0.81 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 154.7, 135.5, 129.7, 49.9, 48.7, 35.9, 35.4, 34.9, 34.3, 33.9, 33.7, 32.6, 31.5, 30.3, 29.5, 29.4, 27.9, 27.7, 26.7, 26.5, "one signal hidden", 26.2, 26.0, 25.8, 25.5, 25.4, 24.9, "two signal hidden", 24.3, 23.7, 14.3. ESI-MS: found $[M + Na]^+$ 511.70; $C_{31}H_{56}O_2N_2$ $[M + Na]^+$ requires 511.72.

*6.2.9. o-Hydroxy-(9Z,12R)-12-hydroxyoctadec-9-anilide (**3e**)*

Yellow oily liquid, Yield = 44%, IR (KBr, cm^{-1}): 3446 (O–H), 3302 (N–H stretch), 2928 (C–H asym), 2860 (C–H sym), 1715, 1654 (anilide). ^1H NMR ($CDCl_3$, δ_H) 9.11 (s, 1H, OH), 8.46 (s, 1H, NH), 7.06–7.04 (m, 4H, *Ph*), 5.38 (m, 2H, $-CH$=C*H*–), 3.55 (m, 1H, $-CH$–OH), 2.29 (t, 2H, J = 7.4 Hz, CH_2–CO), 2.27 (s, 1H, CH–O*H*), 1.94 (m, 4H, $-CH_2$–CH=CH–CH_2), 1.65 (m, 2H, CH_2–CH_2–CO), 1.20 (br.s, 18H, chain CH_2), 0.81 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 170.7, 131.7, 127.6, 126.5, 125.3, 122.3, 120.1, 119.3, 76.4, 42.7, 36.6, "two signals hidden", 32.8, 31.5, 31.4, 30.8, "one signal hidden", 29.5, 29.3, 29.1, 27.3, 26.6, 14.0. ESI-MS: found $[M + Na]^+$ 412.51; $C_{24}H_{39}O_3N$ $[M + Na]^+$ requires 412.52.

*6.2.10. 1-[(9Z,12R)-12-Hydroxyoctadec-9-enoyl]-1,3-dicyclohexylurea (**4e**)*

Yellow oily liquid, Yield = 41%, IR (KBr, cm^{-1}): 3316 (N–H stretch), 2927 (C–H asym), 2857 (C–H sym), 1639 (C=O), 1530 (N–H bend), 1231 (C–N). ^1H NMR ($CDCl_3$, δ_H) 7.25 (s, 1H, N*H*), 5.42 (m, 2H, $-CH$=C*H*–), 3.87–3.84 (m, 1H, C*H* of C_6H_{11}), 3.69–3.65 (m, 1H, C*H* of C_6H_{11}), 3.55 (m, 1H, $-CH$–OH), 2.38 (t, 2H, J = 7.4 Hz, CH_2–CO), 2.28 (s, 1H, CH–O*H*), 1.95 (m, 4H, $-CH_2$–CH=CH–CH_2), 1.66 (m, 10H, $5CH_2$), 1.64(m, 2H, CH_2–CH_2–CO), 1.38 (m, 28H, $14CH_2$), 0.81 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 154.9, 135.6, 129.3, 71.7, 49.6, 48.1, 37.5, 36.9, 36.1, 35.2, 34.7, 34.5, 33.9, 33.3, 31.4, 31.2, 30.7, 30.3, 29.6, 29.4, 29.1, 28.9, 28.8, "two signals hidden", 28.5, 27.2, 26.1, "one signal hidden", 25.2, 24.7, 14.4. ESI-MS: found $[M + Na]^+$ 527.70; $C_{31}H_{56}O_3N_2$ $[M + Na]^+$ requires 527.72.

*6.2.11. o-Hydroxy-(9R,12Z)-9-hydroxyoctadec-12-anilide (**3f**)*

Yellow oily liquid, Yield = 46%, IR (KBr, cm^{-1}): 3440 (O–H), 3309 (N–H stretch), 2926 (C–H asym), 2855 (C–H sym), 1705, 1655 (anilide). ^1H NMR ($CDCl_3$, δ_H) 9.28 (s, 1H, OH), 8.50 (s, 1H, NH), 7.04–7.02 (m, 4H, *Ph*), 5.28 (m, 2H, $-CH$=C*H*–), 3.54 (m, 1H, $-CH$–OH), 2.25 (t, 2H, J = 7.1 Hz, CH_2–CO), 2.21 (s, 1H, OH), 1.94 (m, 4H, $-CH_2$–CH=CH–CH_2), 1.64 (m, 2H, CH_2–CH_2–CO), 1.22 (br.s, 18H, chain CH_2), 0.81 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 170.5, 131.3, 127.6, 126.8, 125.8, 122.0, 120.3, 119.5, 76.7, 42.7, 36.9, 35.8, 34.7, 32.7, 31.9, 31.5, 30.9, 29.7, 29.3, 29.1, 28.6, 27.1, 26.3, 14.5. ESI-MS: found $[M + Na]^+$ 412.49; $C_{24}H_{39}O_3N$ $[M + Na]^+$ requires 412.52.

*6.2.12. 1-[(9R,12Z)-9-Hydroxyoctadec-12-enoyl]-1,3-dicyclohexylurea (**4f**)*

Yellow oily liquid, Yield = 40%, IR (KBr, cm^{-1}): 3321 (N–H stretch), 2926 (C–H asym), 2855 (C–H sym), 1629 (C=O), 1574 (N–H bend), 1227 (C–N). ^1H NMR ($CDCl_3$, δ_H) 7.21 (s, 1H, N*H*), 5.30 (m, 2H, $-CH$=C*H*–), 3.86–3.78 (m, 1H, C*H* of C_6H_{11}), 3.65–3.61 (m, 1H, C*H* of C_6H_{11}), 3.56 (m, 1H, $-CH$–OH), 2.32 (t, 2H, J = 7.4 Hz, CH_2–CO), 2.25 (s, 1H, CH–O*H*), 1.97 (m, 4H, $-CH_2$–CH=CH–CH_2), 1.71 (m, 12H, $6CH_2$), 1.64 (m, 2H, CH_2–CH_2–CO), 1.34 (m, 26H, $13CH_2$), 0.81 (dist.t, 3H, terminal $-CH_3$). ^{13}C NMR ($CDCl_3$, δ_C) 154.1, 135.8, 129.1, 71.6, 49.0, 48.3, 37.3, 36.5, 36.0, 35.7, 34.5, 34.3, 33.8,

32.7, 31.9, 31.5, 30.5, "one signal hidden", 29.6, 29.3, 29.1, 29.0, 28.7, 28.5, 27.1, 26.6, 26.3, 25.4, 25.8, 25.1, 24.7, 14.0. ESI-MS: found $[M + Na]^+$ 527.70; $C_{31}H_{56}O_3N_2$ $[M + Na]^+$ requires 527.72.

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References

[1] F. Qadri, A.M. Svennerholm, A.S.G. Faruque, R.B. Sack, Clin. Microbiol. Rev. 18 (2005) 465–483.
[2] R.A. Devasia, T.F. Jones, J. Ward, L. Stafford, H. Hardin, C. Bopp, M. Beatty, E. Mintz, W. Schaffner, Am. J. Med. 119 (2006) 168.e7–168.e10.
[3] S. Mitchell, Kirk-othmer Encyclopedia of Chemical Technology, fourth ed., vol.II, Wiley-Interscience, New York, 1992, 580.
[4] A. Rauf, H. Parveen, Ind. J. Chem. 44B (2005) 1273–1276.
[5] A. Greetings, L.E. Lopez-Huertas, S.T.C. Morales, P.J. Boza, L.J. Jimenez, WO 0382798, Chem. Abstr. 139 (2003) 307634n.
[6] D. Nuta, C. Limban, C. Stecoza, L. Morusciag, C. Iliec, M.T. Caproiu, G.M. Nitulescu, C. Badiceanu, Farmacia LV (2007) 622–631.
[7] F. Karatas, A. Cansiz, H. Kara, M. Karatepe, M. Koparir, Russ. J. Bioorg. Chem. 31 (2005) 499–501.
[8] M. Sato, Y. Kawashima, K. Hatayama, U.S. Patent 5,475,130, (1995).
[9] Z. Akturk, F. Kilic, K. Erol, V. Pabuccuo, II Farmaco 57 (2002) 201–206.
[10] J. Flood, Am. J. Manag. Care 16 (2010) S48–S54.
[11] L.F. Fieser, M. Fieser, Reagents for Organic Synthesis. John Wiley and Sons, Inc, London, 1967, 233.
[12] T. Librowski, M. Kubacka, M. Meusel, S. Scolari, C.E. Muller, M. Gutschow, Eur. J. Pharmacol. 559 (2007) 138–149.
[13] K. Amornraksa, A. Worachartcheewan, V. Prachayasittikul, Eur. J. Sci. Res. 31 (2009) 510–518.
[14] A. Ranise, S. Schenone, O. Bruno, F. Bondavalli, W. Filippelli, G. Falcone, B. Rivaldi, II Farmaco 56 (2001) 647–657.
[15] R.T. Greene, R.C. Bartsch, EP 0896532, (2002).
[16] P. Hofmeister, C. Kunast, A. Lange, Pestic. Sci. 22 (2006) 221–230.
[17] A.H. Collins, Microbiological Methods, second ed. Butterworth, London, 1976.
[18] In vitro and vivo screening techniques for bioactivity screening and evaluation. in: Z.K. Khan (Ed.), Proc. Int. Workshop UNIDO-CDRI (1997), pp. 210–211.
[19] F.D. Gunstone, J. Chem. Soc. (1954) 1611–1616.