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ORIGINAL RESEARCH

Synthesis and biological evaluation of fatty imidazolines

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Abstract A series of eight fatty imidazolines were synthesized under microwave-assisted conditions using different fatty acids namely octyl, decyl, dodecyl, tetradecyl and octadecyl, mixed fatty acids prepared from *Sterculia foetida* (containing cyclopropene-rich fatty acids), coconut (containing medium-chain-rich fatty acids), palm (containing saturated-rich fatty acids) and sunflower (containing unsaturated-rich fatty acids). Coconut, sunflower and non-edible oil *S. foetida*-based imidazolines were synthesized for the first time. The fatty imidazolines were evaluated for anti-fungal activity and found to be good to excellent (MIC, 4.68–18.75 μg ml^{-1}) against the tested *Candida* strains as compared with fluconazole (MIC, 16–64 μg ml^{-1}) as standard. The fatty imidazolines also exhibited excellent to moderate anti-bacterial activity (MIC, 4.68–75 μg ml^{-1}) against *Staphylococcus aureus* MTCC 96, *S. aureus* MLS 16 MTCC 2940 and *Pseudomonas aeruginosa* MTCC 2453 as compared with neomycin (MIC, 18.75 μg ml^{-1}) as standard. The cytotoxic evaluation of the imidazolines against different cancer cell lines such as HeLa (Human Cervical Cancer Cell line), A549 (Human Alveolar Adenocarcinoma Cell line), MDA-MB-231& MCF7 (Human Breast Adenocarcinoma Cell line) and Neuro2a (Mouse neuroblastoma cell line) showed excellent cytotoxicity for dodecyl (**3b**), tetradecyl (**3c**), octadecyl (**3d**) and coconut (**3f**)-based imidazolines. Sunflower-based imidazoline (**3g**) exhibited good anti-cancer activity towards A549, Neuro2a and palm-based imidazoline (**3h**) towards HeLa, A549 and MCF-7 cell lines.

Keywords Fatty acids · Fatty imidazolines · Anti-microbial agents · Cytotoxicity · Anti-cancer agents

Introduction

There has been a remarkable progress in the prevention, control and even eradication of infectious diseases with the development of novel anti-microbial drugs and vaccines. However, there has been a constant threat to the new drugs developed due to increase in the microbial resistance and evolution of newer microbial strains. The impact of the diseases emerging and re-emerging in tropical countries like India was found to be more during the past three decades. Cancer is one such disease, which is increasing day-by-day globally. The control of cancer requires effective implementation of knowledge by carrying out more and more research on bioactive compounds.

In this context, all substituted imidazolines exhibited a vast array of pharmacological properties such as anti-parasitic (Mukherjee *et al.,* 1989; Popa *et al.,* 2001), anti-fungal (Norman *et al.,* 1986) and anti-microbial (Khabnadideh *et al.,* 2003) activities. Their simple structures are known to possess inhibitory effects on microsomal oxidations (Wilkinson and Hetnarski, 1974), cytotoxic (Miller

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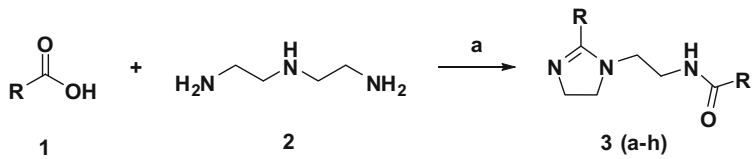
et al., 1983) and anti-fungal (Norman *et al.,* 1986) activities. They also act as anti-inflammatory, anti-diabetic and anti-hypertensive agents (Baltork and Alibeik, 2003).

On the other hand, vegetable oils are good renewable resources for the synthesis of a wide variety of oleochemicals (Karlheinz, 2000). Further, the presence of imidazolines in natural products and pharmacologically active compounds has instituted a diverse array of synthetic approaches to develop biologically active heterocycles (Grammitt *et al.,* 1989). A survey of literature on the synthesis and applications of imidazoline derivatives revealed the following published reports. Ismail *et al.,* 2010 synthesized cationic imidazolinium salts by amidation of lauric, myristic, palmitic, stearic and oleic acids followed by reaction with chloroacetic acid for 8 h. The salts were found to exhibit excellent surface-active and anti-corrosion properties. An efficient method for the synthesis of long-chain 2-alkyl-1-(2-hydroxy ethyl)-2-imidazolines and their amide precursors using fatty acids such as lauric, myristic, palmitic and stearic acids (Palou *et al.,* 2003) and palm fatty acids (Bajpai and Tyagi, 2007) under solvent-free microwave conditions were earlier demonstrated, which resulted in good yields and high purity. The fatty imidazolines also find a wide range of industrial applications, particularly as corrosion inhibitors in oil field applications (Ismail *et al.,* 2010). Imidazole-based surfactants were prepared using ionic liquids for 6–8 h with 70–98 % yield and the compounds with alkyl amido chains containing decyl, dodecyl and hexadecyl chain lengths were found to exhibit good anti-microbial activities (Kanjilal *et al.,* 2009). A microwave irradiation method was adopted by Bajpai (Bajpai and Tyagi, 2008) to prepare long-chain cationic dialkyldiamido imidazolinium salts using diethylenetriamine (DETA) under solvent-free conditions and calcium oxide (CaO) as support, followed by quarternization. The fatty acids used were stearic, palmitic, myristic and lauric acids. However, the imidazoline derivatives synthesized were used for surfactant applications.

Most fatty imidazolines reported were prepared from fatty acids, which were derived from edible oil sources. Also there are no literature reports on the synthesis of imidazolines from non-edible oil-based fatty acids and use of vegetable oil-based fatty imidazolines as anti-microbial and cytotoxic agents. The present study involves the synthesis of a series of fatty imidazolines under microwave-assisted conditions. The fatty acids were selected from different vegetable oil sources, namely coconut oil (containing medium-chain-rich fatty acids), sunflower oil (containing unsaturated-rich fatty acids), palm oil (containing saturated-rich fatty acids) and *Sterculia foetida* seed oil (containing cyclopropene-rich fatty acids). In addition, the products were also prepared by employing selected fatty acids namely octyl (**3a**), dodecyl (**3b**), tetradecyl (**3c**) and octadecyl (**3d**) fatty acids. The synthesized fatty imidazolines were further evaluated for anti-bacterial, anti-*Candida* and cytotoxic activities.

Results and discussion

The fatty imidazolines were synthesized from selected fatty acids namely octyl, dodecyl, tetradecyl, octadecyl and fatty acid mixtures of *S. foetida* (containing sterulic and malvalic fatty acids), coconut (containing medium-chain fatty acids), sunflower (containing unsaturated-rich fatty acids) and palm fatty acids (containing saturated-rich fatty acids). In the present study, coconut, sunflower and *S. foetida* (non-edible oil) fatty acids-based imidazolines **3a–h** were synthesized for the first time under microwave-assisted conditions (Scheme 1). The products that were formed in just 4 min showed an acid value <6 and an amine value <4. The lower acid and amine values indicated the completion of the



R= octyl, 3a
R= dodecyl, 3b
R= tetradecyl, 3c
R= octadecyl, 3d
R= mixture of *sterculia foetida* fatty acyl, 3e
R= mixture of coconut fatty acyl, 3f
R= mixture of sunflower fatty acyl, 3g
R= mixture of palm fatty acyl, 3h

Scheme 1 Microwave-assisted synthesis of fatty imidazolines (**3a–h**). Reagents and conditions: (a) Calcium oxide (CaO) as support, 100 °C, microwave power at 600 W, reaction time, 4 min

Table 1 Anti-bacterial activity of fatty imidazolines **3a–h**

Compounds	Minimum Inhibitory concentration (MIC, μg ml^{-1})		
	S. aureus MTCC 96	*S. aureus* MLS 16 MTCC 2940	*P. aeruginosa* MTCC 2453
3a	–[a]	–	–
3b	18.75	37.5	75
3c	4.68	4.68	18.75
3d	18.75	4.68	75
3e	75	–	75
3f	75	37.5	37.5
3g	4.68	4.68	4.68
3h	4.68	18.75	18.75
Imidazole	–	–	–
Neomycin	18.75	18.75	18.75

[a] No activity

reaction between the fatty acid and the amine, resulting in the formation of fatty imidazoline ring. The synthesized molecules in the present study were further characterized using FTIR, ^{1}H NMR and ESI–MS studies. Although in some earlier studies, fatty acids such as lauric, myristic, palmitic and stearic acids (Palou *et al.,* 2003) and palm fatty acids-based imidazolines (Bajpai and Tyagi, 2007) were synthesized under microwave-assisted conditions. However, the biological evaluation of these fatty acid-based imidazolines was not reported. Hence, in the present study, those compounds having varied chain lengths and nature of the fatty acids were further evaluated for anti-microbial and anti-cancer activities for the first time. Among the different fatty acid-based imidazolines tested, compounds **3c**, **3d**, **3g** and **3h** exhibited excellent anti-bacterial activities (MIC value of 4.68 μg ml^{-1}) towards *Staphylococcus aureus* MTCC 96, *S. aureus* MLS 16 MTCC 2940, *Pseudomonas aeruginosa* MTCC 2453 as compared with the standard, neomycin (MIC value of 18.75 μg ml^{-1}) (Table 1). The different fatty acid-based imidazolines prepared, including dodecyl fatty acid (**3b**), which is a medium-chain fatty acid, coconut-based with medium-chain-rich fatty acid mixture (**3f**) and *Sterculia* (with unusual cyclopropene fatty acid) (**3e**) exhibited poor activity which suggested that the medium-chain fatty acids and cyclopropene fatty acids were poor anti-bacterial agents.

The anti-*Candida* activity of *Sterculia* containing cyclopropene-rich fatty acid-based imidazolines (**3e**) showed promising activity (MIC value of 4.68 μg ml^{-1}) towards *C. albicans* MTCC 183. The activity was found to be eight times more potent as compared with fluconazole (MIC value of 32 μg ml^{-1}). While, the compounds **3b**, **3c**, **3g** and **3h** exhibited good anti-*Candida* activity (MIC value of 18.75 μg ml^{-1}) against the tested *C. albicans* MTCC 3958, *C. albicans* MTCC 7315, *C. parapsilosis* MTCC 1744, *C. albicans* MTCC 4748, *C. glabrata* MTCC 3019 and moderate activity (MIC value of 37.5 μg ml^{-1}) towards *C. albicans* MTCC 7315, *C. albicans* MTCC 1637, *C. albicans* MTCC 227, *C. parapsilosis* MTCC 1744 and *C. glabrata* MTCC 3019 as compared with the standard drug, fluconazole (MIC value of 16–64 μg ml^{-1}; Table 2). These selected vegetable oils containing medium-chain fatty acids and long-chain fatty acids exhibited a twofold increase in activity as compared with the standard drug, fluconazole. However, the short-chain fatty acid, octyl-based imidazoline (**3a**) did not exhibit any activity towards the tested *Candida* strains. Therefore, all these tested fatty acid-based imidazolines can be well-exploited in different anti-*Candida* formulations.

The different fatty acid-based imidazolines were further screened against different cancer cell lines such as HeLa,

Table 2 Anti-*Candida* activity of fatty imidazolines **3a–h**

Compounds	Minimum inhibitory concentration (MIC, μg ml^{-1})								
	Candida albicans MTCC 183	*Candida albicans* MTCC 3018	*Candida albicans* MTCC 3958	*Candida albicans* MTCC 7315	*Candida parapsilosis* MTCC 1744	*Candida albicans* MTCC 4748	*Candida albicans* MTCC 1637	*Candida albicans* MTCC 227	*Candida glabrata* MTCC 3019
3a	–[a]	–	–	–	–	–	–	–	–
3b	–	75	150	37.5	18.75	75	37.5	–	37.5
3c	–	300	75	37.5	18.75	18.75	–	–	37.5
3d	–	–	150	75	–	150	–	–	300
3e	4.68	75	18.75	18.75	18.75	150	37.5	37.5	–
3f	–	75	75	37.5	37.5	150	–	–	150
3g	–	300	150	75	37.5	75	–	–	18.75
3h	–	300	150	37.5	37.5	75	–	–	18.75
Imidazole	–	–	–	–	–	–	–	–	–
Fluconazole	32	32	64	32	16	32	32	32	64

[a] No activity

Table 3 Cytotoxicity of fatty imidazolines **3a–h**

Compounds	IC_{50} values (μM)				
	HeLa	A549	MDA-MB-231	MCF-7	Neuro2a
3a	–[a]	–	–	44.09 ± 0.02	7.87 ± 0.01
3b	10 ± 0.05	12 ± 0.04	15 ± 0.02	8 ± 0.09	7.56 ± 0.03
3c	23.85 ± 0.04	19.62 ± 0.04	6.27 ± 0.03	50.2 ± 0.02	7.70 ± 0.03
3d	15 ± 0.05	74.28 ± 0.05	12.41 ± 0.06	–	8.38 ± 0.07
3e	34 ± 0.04	40 ± 0.03	43 ± 0.02	–	–
3f	15 ± 0.08	17.5 ± 0.05	17 ± 0.04	8.45 ± 0.07	6.61 ± 0.04
3g	–	29.71 ± 0.06	–	–	15.57 ± 0.03
3h	31.98 ± 0.02	8.24 ± 0.05	–	6.79 ± 0.06	–
Imidazole	–	–	–	–	–
Doxorubicin	0.509 ± 0.01	0.459 ± 0.02	0.91 ± 0.02	1.07 ± 0.02	1.04 ± 0.04

HeLa—human cervical cancer cell line, A549—human alveolar adenocarcinoma cell line; MDA-MB-231 and MCF-7—human breast adenocarcinoma cell line, Neuro2a—mouse neuroblastoma cell line

[a] No activity

A549, MDA-MB-231, MCF7 and Neuro2a, and the results to this regard showed that they exhibited promising cytotoxicity for medium- and long-chain-based compounds such as **3b** (IC_{50} values ranging between 7.56 and 15 μM), **3c** (IC_{50} values ranging between 6.27 and 50.2 μM), **3d** (IC_{50} value of 8.38 μM) and **3f** (IC_{50} values ranging between 6.61 and 17.5 μM). However, the long-chain saturated-rich fatty acids-containing palm-based imidazoline (**3h**) showed excellent activity with IC_{50} values ranging between 6.79 and 31.9 μM towards A549 and MCF 7 cell lines, while long-chain unsaturated-rich sunflower fatty acids-containing imidazoline (**3g**) exhibited good activity with IC_{50} values ranging between 15.5 and 29.7 μM towards A549 and Neuro 2a cell lines (Table 3). However, the shorter chain fatty acid, octyl-based imidazoline (**3a**) exhibited promising activity with IC_{50} value of 7.87 μM against the neuroblastoma cell line (Neuro 2a). Based on these results, it was suggested that all the above tested fatty acid-based imidazolines find application in a wide range of anti-cancer formulations.

Conclusions

In conclusion, the fatty imidazolines (**3a–h**) was synthesized under microwave conditions. Long-chain fatty acid-based imidazolines exhibited excellent anti-microbial activity as compared with the standard, neomycin. *Sterculia* containing cyclopropene-rich fatty acids-based imidazolines exhibited promising activity against *Candida albicans* MTCC 183 as compared with flucanozole, while the medium-chain fatty acid-based imidazolines exhibited good activity against the different *Candida* strains. Palm containing-long-chain saturated-rich fatty acids-based imidazolines (**3h**) exhibited excellent cytotoxicity against A549 and MCF 7 cell lines. However, the shorter chain fatty acid, octyl-based imidazoline (**3a**) exhibited promising cytotoxicity against neuroblastoma cell lines (Neuro2a). These results suggest that the above fatty acid-based imidazolines can find possible application in wide range of pharmaceutical formulations.

Experimental section

Materials

All chemicals such as sodium hydroxide (NaOH), diethylenetriamine (DETA), calcium oxide (CaO) and solvents were procured from different commercial suppliers such as S.D. Fine Chemicals Limited, Mumbai, India and Avra Chemicals Pvt. Ltd, Hyderabad, India and were used without further purification. Pure fatty acids namely octanoic, dodecanoic, tetradecanoic and octadecanoic acids were commercially purchased from S.D. Fine Chemicals Limited, Mumbai, India, and mixture of fatty acids were obtained from vegetable oil sources such as coconut, sunflower and palm oil. *S. foetida* oil was obtained from *S. foetida* seeds obtained from Indian Institute of Chemical Technology (IICT) campus. Acid value and amine value of the synthesized imidazolines were determined according to the AOCS standard methods (Firestone, 2010).

Spectral analysis

^{1}H NMR and ^{13}C NMR spectras were recorded on AVANCE 300 and 75 MHz, respectively, in $CDCl_3$.

Chemical shifts relative to TMS as internal standards were given as δ values in ppm. Infrared (IR) spectra were obtained on a 1600 FTIR Perkin-Elmer Spectrometer (Norwalk, CT) with a liquid film between NaCl cells. Mass spectrometry was recorded by electrospray ionization (ESI) on Shimadzu LC/MS instrument.

Determination of fatty acid composition

The composition of the fatty acids was determined using Agilent 6890N series Gas Chromatograph equipped with a flame ionization detector (FID); column, DB-225, 30 m × 0.25 mm × 0.2 μm). The oven temperature was kept at 160 °C for 2 min and programmed from 160 to 180 °C at 6 °C/min, kept for 2 min at 180 °C and finally raised to 230 °C at 4 °C/min which was maintained at 230 °C for 25 min. The injector and detector temperatures were set at 250 °C.

General procedure for the synthesis of fatty imidazolines

Typical procedure for the synthesis of N-[2-(2-Alkyl-4, 5-dihydro-imidazol-1-yl)-ethyl] amide of Sterculia fatty acid

Sterculia foetida fatty acids (2 mol), DETA (1 mol) and CaO were mixed and irradiated under a microwave power of 600 W at 100 °C for 4 min (Bajpai and Tyagi, 2008). The reaction mixture was allowed to cool to room temperature, dissolved in ethyl acetate and heated until boiling and filtered off while hot. The filtrate was concentrated under vacuum to dryness and passed through a silica gel column chromatography to obtain the corresponding N-[2-(2-alkyl-4,5-dihydro-imidazol-1-yl)-ethyl] amide of *Sterculia* fatty acid. The product was characterized for acid and amine values. A similar procedure was followed for the preparation of the fatty imidazolines of octyl (**3a**), dodecyl (**3b**), tetradecyl (**3c**), octadecyl (**3d**), coconut (**3f**), sunflower (**3g**) and palm fatty acids (**3h**). All these compounds were further characterized for FTIR, ^{1}H NMR, ^{13}C NMR and ESI–MS studies.

*N-(2-(2-Heptyl-4,5-dihydro-1H-imidazol-1-yl)ethyl) octanamide (**3a**)* Yield 87 %; $C_{20}H_{39}N_3O$; FTIR (cm^{-1}, neat) 3,397 (N–H str), 2,928 (CH_3, C–H str), 1,711 (–C=O str), 1,663 (C–N str), 715 ((CH_2)$_n$ skeletal, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.89 (t, J = 6.7 Hz, (CH_3)$_2$, 6H), 1.26 (m, –(CH_2)$_8$–, 16H), 1.59 (m, –(CH_2)$_2$–, 4H), 1.96 (m, –CH_2–NH–CO–, 2H), 2.18 (t, –CH_2–CH_2–CO–NH–, 2H), 2.22–2.26 (m, –CH_2 attached to imidazole ring, 4H), 3.35–3.37 (m, imidazole ring protons, 4H), 6.10 (br, –CON*H*–, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 14.1, 21.5, 22.6, 25.8, 28.7, 29.5, 31.8, 36.5, 37.5, 48.1, 49.2, 56.3, 174.1; MS/ESI (*m/z*) Calcd: 337 [M+], found 338 [M +1].

*N-(2-(2-Undecyl-4,5-dihydro-1H-imidazol-1-yl)ethyl) dodecanamide (**3b**)* Yield 89 %; $C_{28}H_{55}N_3O$; FTIR (cm^{-1}, neat): 3,389 (N–H str), 2,927 (CH_3, C–H str), 1,715 (–C=O str), 1,663 (C–N str), 716 ((CH_2)$_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.87 (t, J = 6.7 Hz, (CH_3)$_2$, 6H), 1.27 (m, –(CH_2)$_{16}$–, 32H), 1.58 (m, –(CH_2)$_2$–, 4H), 1.97 (m, –CH_2–NH–CO–, 2H), 2.18 (t, –CH_2–CH_2–CO–NH–, 2H), 2.22–2.26 (m, –CH_2 attached imidazole ring, 4H), 3.33–3.60 (m, imidazole ring protons, 4H), 6.09 (br, –CON*H*–, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 14.1, 21.6, 22.7, 25.9, 25.8, 28.7, 29.3, 29.4, 29.9, 31.8, 36.6, 37.5, 49.3, 56.3, 174.2; MS/ESI (*m/z*) Calcd: 449 [M], found 450 [M + 1], 451 [M + 2].

*N-(2-(2-Tridecyl-4,5-dihydro-1H-imidazol-1-yl)ethyl) tetradecanamide (**3c**)* Yield 90 %; $C_{32}H_{63}N_3O$; FT-IR (cm^{-1}, neat): 3,387 (N–H str), 2,928 (CH_3, C–H str), 1,711 (–C=O str), 1,661 (C–N str), 715 ((CH_2)$_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.88 (t, J = 6.7 Hz, (CH_3)$_2$, 6H), 1.27 (m, –(CH_2)$_{20}$–, 40H), 1.59 (m, –(CH_2)$_2$–, 4H), 1.98 (m, –CH_2–NH–CO–, 2H), 2.19 (t, –CH_2–CH_2–CO–NH–, 2H), 2.22–2.27 (m, –CH_2 attached imidazole ring, 4H), 3.33–3.50 (m, imidazole ring protons, 4H), 6.17 (br, –CON*H*–, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 14.0, 22.6, 25.6, 26.0, 29.3, 29.6, 31.8, 36.5, 37.6, 48.1, 49.1, 56.2, 174.2; MS/ESI (*m/z*) Calcd: 505 [M+], found 506 [M + 1], 528 [M + Na].

*N-(2-(2-Heptadecyl-4,5-dihydro-1H-imidazol-1-yl)ethyl) stearamide (**3d**)* Yield 91 %; $C_{40}H_{79}N_3O$; FT-IR (cm^{-1}, neat): 3,397 (N–H str), 2,928 (CH_3, C–H str), 1,711 (–C=O str), 1,663 (C–N str), 715 ((CH_2)$_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.88 (t, J = 6.7 Hz, (CH_3)$_2$, 6H), 1.27 (m, –(CH_2)$_{28}$–, 56H), 1.59 (m, –(CH_2)$_2$–, 4H), 1.97 (m, –CH_2–NH–CO–, 2H), 2.18 (t, –CH_2–CH_2–CO–NH–, 2H), 2.23–2.27 (m, –CH_2 attached imidazole ring, 4H), 3.35–3.50 (m, imidazole ring protons, 4H), 6.18 (br, –CON*H*–, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 14.1, 21.8, 25.6, 26.8, 29.7, 31.7, 36.7, 37.8, 48.2, 49.3, 56.0, 174.1; MS/ESI (*m/z*) Calcd: 617 [M+], found 618 [M + 1].

*N-[2-(2-Alkyl-4, 5-dihydro-imidazol-1-yl)-ethyl] amide of Sterculia fatty acid (**3e**)* Yield 85 %; FT-IR (cm^{-1}, neat) 3,397 (N–H str), 2,928 (CH_3, C–H str), 1,711 (–C=O str), 1,664 (C–N str), 716 ((CH_2)$_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.78 (s, 2H,

cyclopropene ring protan), 0.88 (t, J = 6.7 Hz, $(CH_3)_2$, 6H), 1.26 (m, $-(CH_2)_n$), 1.62 (m, $-(CH_2)_2-$, 4H), 1.81 (m, $-CH_2-$NH–CO–, 2H), 2.18 (t, $-CH_2-CH_2-$CO–NH–, 2H), 2.74 (m, $-CH_2$ attached imidazole ring, 4H), 3.34 (m, imidazole ring protons, 4H), 5.34 (m, –CH=CH–, 2H), 6.16 (br, –CONH–, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 8.0, 14.1, 21.6, 23.5, 26.2, 28.4, 28.6, 29.6, 29.9, 31.7, 35.8, 37.2, 49.2, 55.8, 57.5, 112.7, 113.8, 174.6; MS/ESI (*m/z*) Calcd: 637 (*sterculic*, [M+]), found 660 (steruculic, [M + Na]).

*N-[2-(2-Alkyl-4, 5-dihydro-imidazol-1-yl)-ethyl] amide of coconut fatty acid (**3f**)* Yield 89 %; FT-IR (cm^{-1}, neat): 3,397 (N–H str), 2,928 (CH_3, C–H str), 1,711 (–C=O str), 1,663 (C–N str), 715 ($(CH_2)_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.87 (t, J = 6.7 Hz, $(CH_3)_2$, 6H), 1.25 (m, $-(CH_2)_n$), 1.61 (m, $-(CH_2)_2-$, 4H), 1.77 (m, $-CH_2-$NH–CO–, 2H), 2.17 (t, $-CH_2-CH_2-$CO–NH–, 2H), 2.67–2.79 (m, $-CH_2$ attached imidazole ring, 4H), 3.20–3.32 (m, imidazole ring protons, 4H), 5.30–5.38 (m, –CH=CH–, 2H), 6.13 (br, –CONH–, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 14.1, 21.7, 23.6, 26.1, 28.5, 29.8, 31.6, 37.2, 49.2, 55.8, 134.5, 135.6, 174.3; MS/ESI (*m/z*) Calcd:449 [M+], found 450 [M + 1].

*N-[2-(2-Alkyl-4, 5-dihydro-imidazol-1-yl)-ethyl] amide of sunflower fatty acid (**3g**)* Yield 86 %; FT-IR (cm^{-1}, neat): 3,395 (N–H str), 2,928 (CH_3, C–H str), 1,711 (–C=O str in amide), 1,663 (C–N str), 715 ($(CH_2)_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.88 (t, J = 6.7 Hz, $(CH_3)_2$, 6H), 1.26 (m, $-(CH_2)_n$), 1.58 (m, $-(CH_2)_2-$, 4H), 1.82 (m, $-CH_2-$NH–CO–, 2H), 2.18 (t, $-CH_2-CH_2-$CO–NH–, 2H), 2.70–2.79 (m, $-CH_2$ attached imidazole ring, 4H), 3.20–3.32 (m, imidazole ring protons, 4H), 5.30–5.35 (m, –CH=CH–, 2H), 6.13 (br, -CONH-, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 14.1, 21.3, 22.8, 23.7, 26.5, 28.6, 29.7, 29.9, 32.0, 37.5, 49.5, 55.3, 134.2, 135.6, 174.1; MS/ESI (*m/z*): Calcd: 613 (18:1, [M+]), 609 (18:2, [M+]), found 636 (18:1, [M + Na]), 632 (18:2, [M + Na]).

*N-[2-(2-Alkyl-4, 5-dihydro-imidazol-1-yl)-ethyl] amide of palm fatty acid (**3h**)* Yield 89 %; FT-IR (cm^{-1}, neat) 3,397 (N–H str), 2,928 (CH_3, C–H str), 1,712 (–C=O str), 1,662 (C–N str), 716 $(CH_2)_n$ skeleton, C–H str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm 0.88 (t, J = 6.9 Hz, $(CH_3)_2$, 6H), 1.25 (m, $-(CH_2)_n$), 1.61 (m, $-(CH_2)_2-$, 4H), 2.00 (m, $-CH_2$–NH–CO–, 2H), 2.30 (t, $-CH_2-CH_2-$CO–NH–, 2H), 2.69–2.77 (m, $-CH_2$ attached imidazole ring, 4H), 3.20–3.32 (m, imidazole ring protons, 4H), 5.31–5.37 (m, –CH=CH–, 2H), 6.13 (br, -CONH-, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 14.1, 18.4, 22.6, 23.8, 25.9, 29.1, 29.6, 31.8, 37.6, 49.5, 55.2, 134.1, 135.6, 174.2; MS/ESI (*m/z*): Calcd 561 (16:0, M+), 589 (16:0 + 18:0) found 562 (16:0, [M + H]), 606 [16:0 + 18:0].

Biological evaluation

The prepared fatty imidazolines were evaluated for antimicrobial activity against different Gram-positive and Gram-negative bacteria such as *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC 2940 and *P. aeruginosa* MTCC 2453 and different *Candida* strains such as *Candida albicans* MTCC 183, *Candida albicans* MTCC 3018, *Candida albicans* MTCC 3958, *Candida albicans* MTCC 7315, *Candida parapsilosis* MTCC 1744, *Candida albicans* MTCC 4748, *Candida albicans* MTCC 1637, *Candida albicans* MTCC 227 and *Candida glabrata* MTCC 3019 using the well-diffusion method (Amsterdam, 1996). The pathogenic bacterial and *Candida* reference strains were seeded on the surface of the media petri plates, containing Muller–Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media petri plates using a cork borer, and the synthesized compounds at a dose range of 300–1.4 μg $well^{-1}$ were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of neomycin and fluconazole at a dose range of 300–1.4 μg $well^{-1}$ were used as positive controls, and the well containing methanol served as negative control. Imidazole was also run in parallel as a reference. The plates were incubated for 24 h at 30 °C, and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicates, and mean values are represented.

Cytotoxicity of the products were assessed against a panel of different cancer cell lines obtained from the American Type Culture Collection (ATCC), Manassas, VA, USA, such as HeLa derived from human cervical cancer cells (ATCC No. CCL-2), A549 derived from human lung adenocarcinoma epithelial cells (ATCC No. CCL-185) and MDA-MB-231 derived from human breast adenocarcinoma cells (ATCC No. HTB-26), MCF-7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22) and Neuro2a derived from mouse neuroblastoma cell line (ATCC No. CCL-131) using the MTT colorimetric assay (Mosmann, 1983), which is based on the measurement of in vitro growth of tumour cell lines in 96-well plates by cell-mediated reduction in tetrazolium salt to water insoluble formazan crystals according to the literature procedures using doxorubicin as a standard and imidazole as a reference. After 48 h incubation, the cells were subjected to MTT colorimetric assay (5 mg ml^{-1} MTT). The effect of the different test compounds on the viability of tumour cell lines was measured at the wavelength of 540 nm on a multimode reader (Infinite® M200, Tecan, Switzerland). Dose–response curves were plotted

for the test compounds and controls after correction by subtracting the background absorbance from that of the blanks. The anti-tumour potency of the compounds indicated by 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 three independent experiments, which was calculated using the statistical tool available in Microsoft Excel program.

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