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SYNTHESIS AND CYTOTOXIC EVALUATION OF FATTY ACID BASED-AMINO ALCOHOLS

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ABSTRACT
In the present investigation, we describe the synthesis of β-amino alcohols from a medium chain fatty acid, 10-undecenoic acid and evaluation of their cytotoxicity. Nucleophilic ring opening reactions of epoxy undecanoates of varying ester chain lengths (C_1-C_8) with aniline in equimolar ratio (1:1) was carried out using Bronsted acidic ionic liquid, 1-methylimidazolium tetrafluoroborate, thus playing a dual role of solvent as well as a reaction medium. The cytotoxic properties of the amino alcohols of 10-undecenoic acid were evaluated. It was observed that the compound 4f showed promising cytotoxicity against HeLa, compound 4a showed good activity against A549 cell line, while compounds 4a, 4c showed good activity against MDA-MB-231 cell line. All other compounds showed moderate activity against all the other tested cell lines.

KEYWORDS: 10-Undecenoic acid, Amino alcohols, Ionic liquid, Cytotoxicity, Anticancer agents.

INTRODUCTION

Amino alcohols are versatile intermediates in the synthesis of a vast range of biologically active natural and synthetic products. β-amino alcohols find diverse applications as chemotherapeutic agents, precursors for the synthesis of heterocycles, chiral auxiliaries, and ligands[1]. Amino alcohol N-acyl derivatives are identified as therapeutic agents against the neurogenic endoneural edema of the peripheral nerve[2]. Spingosine, (2S, 3R, 4E)-2-amino-4-octadecene-l, 3-diol, a long chain amino alcohol is known to play a vital role in the sphingolipid metabolism[3,4]. Some researchers have investigated the structure-activity relationships between the lipid chain length and the bipolar sphingolipid-like head groups and their potential use in antifungal therapy[5]. Various starting materials such as lipidic amino acids, serine, glyceraldehydes, and long chain 1, 2-diols[6] were used to synthesize the sphingosine analogues[7,8] and long chain 2-amino alcohols. In the literature, the reported methodologies for the synthesis of amino alcohols employed catalysts like rare earth metal halides, alkali metal perchlorates, and metal triflates by ring opening of epoxide with amines [9-13]. However, the reported methodologies have certain drawbacks such as potential rearrangement to allylic alcohols, requirement of stoichiometric amounts of catalyst, elevated temperatures, moderate yields *etc*. The synthesis of β-amino alcohols from terminal epoxy fatty acid methyl esters like methyl epoxy stearate and 10-undecenoic acid using zinc (II) per chlorate hexahydrate as catalyst at 80°C using cyclic, aliphatic and aromatic amines except aniline was reported. However, their performance evaluation for biological studies was not reported [14, 15]. Ionic liquids have emerged as environmentally benign and an alternate reaction media to the organic solvents particularly the chlorinated hydrocarbons are widely used in organic synthesis for various chemical and biotransformations [16, 17]. An ionic liquid exhibits unique properties such as tunable polarity, high thermal stability, immiscibility with a number of organic solvents, negligible vapor pressure, and recyclability [18]. The enhanced rates of chemical processes are due to their high polarity and solubilizing ability in both inorganic and organic compounds, we herein report synthesis of six alkyl 11-anilino-10-hydroxyundecanoates from their epoxy derivatives of varying ester chain length (C_1-C_8) using [Hmim] BF_4 as reaction medium for the synthesis. The synthesized β-amino alcohols were characterized by spectral analyses and evaluated for their cytotoxic activities.

MATERIALS AND METHODS

Materials

Aniline, diethyl ether, dichloromethane and meta chloroperbenzoic acid (mCPBA) were purchased from Sigma Aldrich (St. Louis, USA). 2-ethylhexanol, n-butanol, i-butanol, iso-propanol, methanol, hexane, ethyl acetate and ethanol were purchased from Qualigens Fine Chemicals (Mumbai, India). *p*-Toluene sulphonic acid (p-TSA), sulfuric acid (AR grade), Tetraflouro boric acid and methyl imidazole were purchased from M/s S.D. Fine-Chem Ltd., Mumbai. All the chemicals were used directly without further purification.

Spectral analysis

^{1}H NMR spectra were recorded on a Brucker (Wissenbourg, France) AR x 400 Spectrometer (400 MHz) with $CDCl_3$ solvent using TMS as internal standard. Infrared (IR) spectra were recorded on a 1600 FT-IR Perkin-Elmer Spectrophotometer (Norwalk, CT) with a liquid film between NaCl discs. Agilent 6890 Series Gas Chromatograph equipped with FID detector was used in GC analysis, using the capillary column HP1 (30 m x 0.25

mm i.d. x 0.5µ m film thickness). The oven temperature programmed from 150-300°C at 10°C/ min with a holding time of 20 min at final temperature. The injector and detector temperatures were set at 300°C and 325°C respectively. Agilent GC-MS 5973 mass spectrometer (Palo Alto, USA) in the EI mode was used to record GC-MS spectra and data is given in mass units (m/z).

Biological evaluation

In vitro cytotoxicity assay

Cytotoxicity of all the synthesized amino alcohols was assessed on the basis of measurement of *in vitro* growth inhibition 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. The synthesized amino alcohols were tested for cytotoxicity against a panel of four different tumor cell lines: A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), MDA-MB-231 derived from human breast adenocarcinoma cells (ATCC No. HTB-26) and MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22) using the MTT assay[19]. 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 means ± SD of three independent experiments.

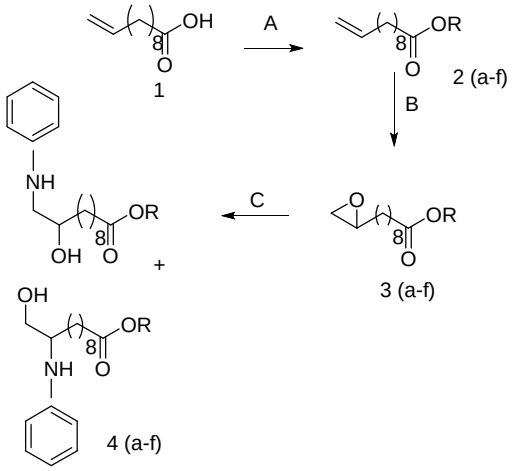
General synthetic protocols

1. Synthesis of 1-methylimidazolium tetrafluoroborate

1-methylimidazole (24 g, 0.01476 mol) was taken in a three-necked flask and cooled to 0°C under magnetic stirring and tetrafluoroboric acid (38mL, 48% in water) was added slowly in order to maintain the reaction mixture at 0-5°C over a period of 30 min. The reaction was further continued for 2 h at room temperature. High vacuum was applied to remove water and a colorless viscous liquid was obtained as 1-methylimidazolium tetrafluoroborate, which solidified on cooling.

Synthesis of alkyl 11-anilino-10-hydroxy undecanoates (4 a-f)

Alkyl-10-epoxy undecanoate (1 m mol) and aniline (1m mol) were taken in 25 ml round bottom flask and ionic liquid [HMIM]BF_4 (0.5 g), was added and stirred at 80ºC. The reaction was monitored by TLC using the solvent system hexane/ ethyl acetate (70:30 v/v). At a reaction time of 3 h, the completion of the reaction was observed. The contents of the flask was extracted with diethyl ether (3 × 10 ml) and washed with water to remove any traces of catalyst, passed through the sodium sulphate and concentrated using rotary evaporator. The product was purified using silica gel column with n-hexane and ethyl acetate (85:15, v/v) to afford the alkyl 11-anilino-10-hydroxy undecanoates (methyl, 2-propyl, 1-butyl, 2-methyl-1-propyl, 2-ethyl hexyl, octyl) [4 (a-f)].



Scheme1. Synthesis of alkyl11-anilino-10-hydroxy undecanoates. Reagents and conditions: (a) Acid catalyst, R-OH; 92%-97% (b) DCM, mCPBA, 38-40°C, 93-96%; (C) aniline, 80 °C, [bmim]BF4. Where R; a;-CH_3, b; -CH-$(CH_3)_2$, c; -$(CH_2)_3$-CH_3, d; -CH_2-CH-$(CH_3)_2$, e; -$(CH_2)_5$-CH-$(CH_3)_2$, f; -$(CH_2)_7$-CH_3.

The spectral data of the final amino alcohols are given below

*Methyl 11-anilino-10-hydroxy undecanoate (*4a): yield 98.5 %; IR (neat, cm^{-1}): 3339 (-OH, -NH); 1599 (N-H bending); 2928 (C-H stretching); ^{1}H NMR: ($CDCl_3$), δ (ppm/TMS): 1.22-1.60 (m, 14 H, -C<u>H</u>$_2$); 2.2-2.3 (t, 2H, -CO-C<u>H</u>$_2$); 2.84-2.94 (m,1H, (-C<u>H</u>-OH); 3.10-3.20 (dd, 2H, -NH-C<u>H</u>$_2$-CH-OH); 3.7-3.78 (s broad, 1H, -N<u>H</u>-, -O<u>H</u>-); 3.60-3.64 (s sharp, 3H, -O-C<u>H</u>$_3$); 6.50-7.I2 (m, 5<u>H</u>, Aryl); EIMS, *m/z* 307 $[M]^{\cdot+}$, $C_6H_5NHCH_2^+$ (*m/z* 106).

Isopropyl 11-anilino-10-hydroxy undecanoate (4b): yield 98%; IR (neat, cm^{-1}): 3393 (-OH, -NH); 1604 (N-H bending); 2927 (C-H stretching); ^{1}H NMR: ($CDCl_3$), δ (ppm/TMS): 1.20-1.36 (q,6H, -CH-(C<u>H</u>$_3$)$_2$); 1.42-1.66 (m, 14 H, -C<u>H</u>$_2$); 2.2-2.3 (t, 2H, -CO-C<u>H</u>$_2$); 3.14-3.18 (d ,2H , O-CH_2); 3.18-3.26 (dd, 2H, -NH-C<u>H</u>$_2$-CH-OH); 3.64-3.65 (s, 1H, -N<u>H</u>-, -O<u>H</u>-); 3.72-3.82 (m,1H, (-C<u>H</u>-OH); 4.05-4.14 (q,1H, -O-CH-$(CH_3)_2$); 6.52-7.14 (m, 5<u>H</u>, Aryl); EIMS, *m/z* 335 $[M]^{\cdot+}$, $C_6H_5NHCH_2^+$ (*m/z* 106).

Butyl11-anilino-10-hydroxy undecanoate (4c): yield 96 %; IR (neat, cm^{-1}): 3403 (-OH, -NH); 1603 (-N-H bending); 2929 (C-H stretching); ^{1}H NMR: ($CDCl_3$), δ (ppm/TMS): 0.9-1.0 (t, 3H, -C<u>H</u>$_3$); 1.2-1.65 (m, 18 H, -C<u>H</u>$_2$); 2.2-2.3 (t, 2H, -CO-C<u>H</u>$_2$); 2.9-3.0 (m,1H, (-C<u>H</u>-OH); 3.15-3.22 (dd, 2H, -NH-C<u>H</u>$_2$-CH-OH); 3.7-3.8 (s, 1H, -N<u>H</u>-, -O<u>H</u>-); 4.0-

4.1 (t,2H, -O-C<u>H</u>$_2$) 6.48-7.2I (m, 5<u>H</u>, Aryl); EIMS, *m/z* 349 $[M]^{\cdot+}$, $C_6H_5NHCH_2^+$ (*m/z* 106).

2-Methyl-1-propyl11-anilino-10-hydroxy undecanoate (4d): yield 97 %; IR (neat, cm^{-1}): 3403 (-OH, -NH); 1603 (-N-H bending); 2929 (C-H stretching); ^{1}H NMR: ($CDCl_3$), δ (ppm/TMS): 0.86-0.94 (m,7H, -C<u>H</u>-(C<u>H</u>$_3$)$_2$); 1.24-1.62 (m, 14 H, -C<u>H</u>$_2$); 2.2-2.3 (t, 2H, -CO-C<u>H</u>$_2$); 2.88-2.96 (m,1H, (-C<u>H</u>-OH) 3.14-3.22 (dd, 2H, -NH-C<u>H</u>$_2$-CH-OH); 3.7-3.8 (s, 1H, -N<u>H</u>-, -O<u>H</u>-); 3.92-3.98 (d ,2H , O-C<u>H</u>$_2$) ; 6.52-7.14 (m, 5<u>H</u>, Aryl); EI-MS, *m/z* 349 $[M]^{\cdot+}$, $C_6H_5NHCH_2^+$ (*m/z* 106)

2-ethyl-1-hexyl 11-anilino-10-hydroxy undecanoate (4e): yield 96.5 %; IR (neat, cm^{-1}): 3397 (-OH, -NH); 1603 (-N-H bending); 2929 (C-H stretching); ^{1}H NMR: ($CDCl_3$), δ (ppm/TMS): 0.82-0.96 (m,6H, -(C<u>H</u>$_3$)$_2$); 1.20-1.60 (m, 21 H, -C<u>H</u>$_2$); 2.2-2.3 (t, 2H, -CO-C<u>H</u>$_2$); 2.90-2.80 (q,2H,-OH-CH-C<u>H</u>$_2$-CH$_2$-); 3.18-2.95 (m,1H, (-C<u>H</u>-OH); 4.56-4.32 (dd, 2H, -NH-C<u>H</u>$_2$-CH-OH); 3.56-3.70 (s,1H, -N<u>H</u>-, -O<u>H</u>-); 3.94-3.90 (d, 2H, O-C<u>H</u>$_2$); 6.70-7.18 (m, 5<u>H</u>, Aryl); EIMS: *m/z* 405 $[M]^{\cdot+}$, $C_6H_5NHCH_2^+$ (*m/z* 106).

Octyl 11-anilino-10-hydroxy undecanoate (4f): yield 95 %; IR (neat, cm^{-1}): 3396 (-OH, -NH); 1603 (-N-H bending); 2929 (C-H stretching); ^{1}H NMR: ($CDCl_3$), δ (ppm/TMS): 0.9-1.0 (t, 3H, -C<u>H</u>$_3$); 1.2-1.65 (m, 26 H, -C<u>H</u>$_2$); 1.85-2.0 (m, -CO-CH$_2$-C<u>H</u>$_2$); 2.25-2.35 (t, 2H, -CO-C<u>H</u>$_2$); 2.95-3.05 (m,1H, (-C<u>H</u>-OH); 3.2-3.3 (dd, 2H, -NH-C<u>H</u>$_2$-CH-OH); 3.8-3.82 (s, 1H, -N<u>H</u>-, -O<u>H</u>-); 3.83-3.85 (t, 2H, -O-C<u>H</u>$_2$) 6.6-7.2 (m, 5<u>H</u>, Aryl); EIMS, *m/z* 405 $[M]^{\cdot+}$, $C_6H_5NHCH_2^+$ (*m/z* 106).

RESULTS & DISCUSSION

10-Undecenoic acid was esterified using different alcohols such as methanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, 2-ethylhexanol and octanol using pTSA (1% of the acid weight) at reflux temperature for 4 h. The yields of the products are as follows (2a) 96%, (2b) 91.5%, (2c) 92.5%, (2d) 96%, (2e) 97% and (2f) 96%. These alkyl 10-undecenoates were epoxidised using m-chloroperbenzoic acid in dichloromethane (45 ml) and stirred at reflux temperature (38-40 ºC) for 3 h. The yields of the products are as follows: (3a) 93%, (3b) 93%, (3c) 96%, (3d) 95%, (3e) 96% and (3f) 97%. In the present study, these epoxidised derivatives were subjected for aminolysis using $[Hmim]BF_4$ and aniline at 80 ºC for 3 h. The final products alkyl 11-anilino-10-hydroxyundecanoates (Scheme 1) were obtained after simple extraction with ether. The ether washing was repeated on the remaining ionic liquid and recycled in subsequent runs without any further purification. The advantages of the present method are lower reaction times, 100% conversion and greener reaction medium and high yields of 95-99%.

In vitro cytotoxicity: The cytotoxicity of all the synthesized amino alcohols 4a-f was tested on different cell lines using the MTT assay [19] and the results obtained are shown in Table 1. It was observed that the compound 4f showed promising cytotoxicity against HeLa cell line. Compound 4a showed good activity against A549 cell line, and compounds 4a and 4c showed good activity against MDA-MB-231 cell line. All the other compounds showed moderate activity against all the tested cell lines. The synthesized products alkyl 11-anilino-10-hydroxy undecanoates having these CH_2-OH and $COOCH_3$ functional groups exhibited promising cytotoxicity which is in agreement with the earlier reported work as in the case of free amino alcohols towards the two cell lines, A2780 and H322 [6].

TABLE 1: *In vitro* cytotoxicity of β amino alcohols on different human cell lines[a]

Test compounds	IC50 values (in µM)			
	A549	MDA-MB-231	MCF-7	HeLa
4a	15.9 ± 0.06	17.5 ± 0.08	-	-
4b	-[b]	21.58 ± 0.07	-	-
4c	-	14.18 ± 0.06	18.09 ± 0.06	-
4d	23.06 ± 0.09	-	-	-
4e	-	28.36 ± 0.09	33.09 ± 0.01	-
4f	-	-	-	6.3 ± 0.07
Doxorubicin	0.451 ± 0.02	0.501 ± 0.03	1.05 ± 0.02	1.21 ± 0.02

[a] IC_{50} values (in µM) are indicated as means ± SD of three independent experiments.
[b]- No activity.

CONCLUSION

10-undecenoic acid based β-amino alcohols were synthesized and evaluated for their anticancer activities. The compound (4f), exhibited promising cytotoxicity against HeLa cell line, while all the other compounds showed good to moderate activity against all the tested cell lines. The carbon chain length effect and the presence of free hydroxyl and amino groups contributed towards this cytotoxicity. Further, studies are in progress to synthesize different β-amino alcohols using various fatty acids and to evaluate their biological activities.

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