

Short communication

# Synthesis, Characterization and Antimicrobial Activity of Long-Chain Hydrazones

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## Abstract

Fatty acids containing hetero atoms are regarded as potential antimicrobial agents. Thus eight different hydrazones **2a–d** and **3a–d** were synthesized from four fatty acid hydrazides namely undecanoic hydrazide (**1a**), octadecanoic hydrazide (**1b**), 12-hydroxyoctadecanoic hydrazide (**1c**) and 9-hydroxyoctadecanoic hydrazide (**1d**) by condensing them with carbonyl group of methyl acetoacetate and acetylacetone. The structural elucidation of these compounds is based on their spectral data (IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS). These compounds were also screened for their microbial activity against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus albus* by cup-plate method at 100 µg/ml of DMF using chloromycetin as a standard drug.

**Keywords:** Fatty acid hydrazones, antimicrobial activity, IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, MS.

## 1. Introduction

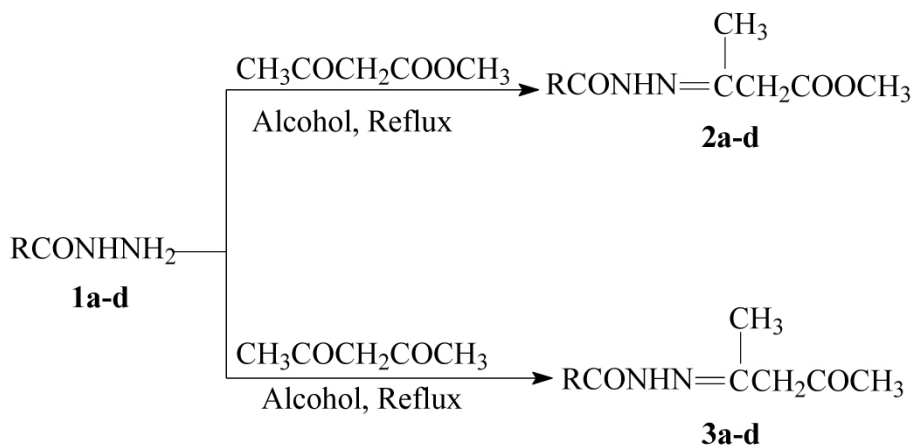
The synthesis, structure and biological activity of some new hydrazones prepared from fatty acid hydrazides has been the focus of research. Different methods have been employed to synthesize different types of hydrazones from different starting materials. Hydrazones have been found to possess many biological activities, e.g. antibacterial,<sup>1,2</sup> anticonvulsant,<sup>3</sup> anti-inflammatory,<sup>4</sup> anti-protzoal,<sup>5</sup> and antitubercular.<sup>6,7</sup> The use of fatty acid substrates as starting materials has become significant because of their own biological activity.<sup>8,9</sup> Thus carbonyl group of methyl acetoacetate and acetylacetone was employed to synthesize the hydrazones **2a–d** and **3a–d** from the aforementioned hydrazides **1a–d**. These hydrazones were also screened for their antimicrobial activity and some of the synthesized compounds showed good antimicrobial activity against *E. coli*, *S. aureus* and *S. albus*.

## 2. Results and Discussion

Hydrazides were prepared by stirring and refluxing the fatty esters with hydrazine hydrate in ethanol in the

presence of air. Absence of peaks for olefinic protons ( $\delta$  5.4–4.6) suggest that hydrogenation of double bond of the fatty acid chain has taken place. This indicates that hydrazine hydrate can hydrogenate double bonds in the presence of air. Such findings are already reported in the literature.<sup>10–12</sup>

The synthetic pathway followed for the synthesis of hydrazones is presented in the Scheme 1. Carbonyl group of methyl acetoacetate and acetylacetone has been employed to build the newly synthesized hydrazones from different fatty acid hydrazides. Reaction of undecanoic hydrazide with carbonyl group of methyl acetoacetate afforded hydrazone **2a**. IR spectra of hydrazone **2a** exhibited intensive bands at 3210, 1743 and 1660 cm<sup>-1</sup> confirming the presence of NH, O=COCH<sub>3</sub> and O=C–NH groups, respectively. <sup>1</sup>H NMR was more informative. In addition to the peak of normal fatty acid chain, other characteristic signals were observed at  $\delta$  10.35 (1H, NH), 3.66 (3H, OCH<sub>3</sub>), 2.30 (2H, t,  $J$  = 7.5 Hz, CH<sub>2</sub>–CONH), 2.01 (2H, s, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>) and 1.56 (3H, s, N=C–CH<sub>3</sub>), confirming the structure of the hydrazone **2a**. In <sup>13</sup>C NMR signals at  $\delta$  174.7 and 174.5 (COOCH<sub>3</sub>, CONH), 159.2 (C=N) and 25.0 (COCH<sub>3</sub>) also supported the structure. Similarly, hydrazones **2b–d** were synthesized from hydrazides **1b–d** by reacting them with methyl acetoace-



Compounds	R
<b>1a, 2a, 3a</b>	$\text{CH}_3(\text{CH}_2)_9$
<b>1b, 2b, 3b</b>	$\text{CH}_3(\text{CH}_2)_{16}$
<b>1c, 2c, 3c</b>	$\text{CH}_3(\text{CH}_2)_5\text{CHOH}(\text{CH}_2)_{10}$
<b>1d, 2d, 3d</b>	$\text{CH}_3(\text{CH}_2)_8\text{CHOH}(\text{CH}_2)_7$

Scheme 1: Synthesis of hydrazones from hydrazides.

tate and were characterized by their spectral data. Reaction of undecanoic hydrazide with carbonyl group of acetylacetone afforded the hydrazone **3a**. Hydrazone **3a** gave intensive IR bands at 3205, 1721 and 1664  $\text{cm}^{-1}$ , confirming the presence of NH, C=O and  $\text{O}=\text{C}-\text{NH}$  groups, respectively. The  $^1\text{H}$  NMR data gave characteristic peaks at  $\delta$  10.33 (1H, NH), 2.30 (2H, t,  $J = 7.5$  Hz,  $\text{CH}_2-\text{CONH}$ ), 2.04 (3H, s,  $\text{CH}_3$ ), 1.94 (2H, s,  $\text{CH}_2-\text{COCH}_3$ ) and 1.56 (3H, s,  $\text{N}=\text{C}-\text{CH}_3$ ), confirming the structure. In  $^{13}\text{C}$  NMR signals at  $\delta$  174.4 and 174.3 ( $\text{COOCH}_3$ , CONH), 161.3 (C=N) and 29.2 ( $\text{COCH}_3$ ) also supported the structure. Similarly, hydrazones **3b-d** were synthesized from hydrazides **1b-d** by reacting them with acetylacetone and their structures were confirmed by the spectral data.

The hydrazones **2** and **3** were screened for their antibacterial activity against *E. coli*, *S. aureus* and *S. albus* by cup-plate method. It was observed that only C-11 hydrazones **2a** and **3a** showed promising results. Whereas hydrazones **2c** and **2d** showed only moderate activity against *E. coli* and *S. albus*. Hydrazone **3d** showed poor activity against *E. coli* and *S. aureus*.

### 3. Experimental

Undecanoic (purity 98%) and (Z)-9-octadecenoic (oleic acid, 97%) acids were purchased from Fluka Chemicals (Buchs, Switzerland). (Z)-12-Hydroxy-9-octadecenoic (ricinolic acid, 98%) and (Z)-9-hydroxy-12-octadecenoic (isoricinolic acid, 98%) were isolated from *Rici-*

*nus communis* and *Wrightia tinctoria* seed oils, respectively, following Gunstone's partition procedure.<sup>13</sup> Methyl acetoacetate, acetylacetone and hydrazine hydrate (80%) were purchased from Sd fine-chem (Mumbai, India). Thin layer chromatography was done on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.5 mm thickness). Mixture of petroleum ether-diethyl ether-acetic acid (80:20:1 v/v) was used as mobile phase. Column chromatography was carried out on silica gel (Merck, Mumbai, India, 60–120 mesh).  $^1\text{H}$  NMR were recorded at 300 MHz and  $^{13}\text{C}$  NMR were recorded at 75 MHz. Melting points were taken in open capillaries and are uncorrected.

**General procedure for the preparation of fatty acid hydrazides 1.** Methyl undecanoate (0.1 mmol) was reacted with hydrazine hydrate (0.25 mmol) while stirring and refluxing in ethanol for 5 h. The resulting solution was cooled and poured into crushed ice. The solid thus obtained was filtered and recrystallized from ethanol to afford the corresponding undecanoic hydrazide **1a**. Similarly, octadecanoic hydrazide **1b**, 12-hydroxyoctadecanoic hydrazide **1c** and 9-hydroxyoctadecanoic hydrazide **1d** were obtained from (Z)-9-octadecenoate, (Z)-12-hydroxy-9-octadecenoate and (Z)-9-hydroxy-12-octadecenoate, respectively.

**Undecanoic hydrazide (1a).** White crystals, yield 80%, m.p. 90–92 °C (lit. m.p. 93–94 °C).<sup>10</sup> IR (KBr,  $\text{cm}^{-1}$ ) 3210–3080 (NH–NH<sub>2</sub>), 1660 ( $\text{O}=\text{C}-\text{NH}$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.74 (1H, s, NH), 3.93 (2H, s, NH<sub>2</sub>), 2.57 (2H, t,  $J = 7.8$  Hz,  $\text{CH}_2-\text{CONH}$ ), 1.55 (2H, m,  $\text{CH}_2\text{CH}_2-\text{CO}$ ), 1.24 (14H, br. s,  $7\times\text{CH}_2$ ), 0.87 (3H, deg. t,  $\text{CH}_3$ ).

**Octadecanoic hydrazide (1b).** White powder, yield 85%, m.p. 110–112 °C (lit. m.p. 112–114 °C).<sup>10,14</sup> IR (KBr,  $\text{cm}^{-1}$ ) 3218–3080 (NH–NH<sub>2</sub>), 1660 ( $\text{O}=\text{C}-\text{NH}$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.85 (1H, s, NH), 3.98 (2H, s, NH<sub>2</sub>), 2.57 (2H, t,  $J = 7.8$  Hz,  $\text{CH}_2-\text{CONH}$ ), 1.57 (2H, m,  $\text{CH}_2\text{CH}_2-\text{CO}$ ), 1.25 (28H, br. s,  $14\times\text{CH}_2$ ), 0.87 (3H, deg. t,  $\text{CH}_3$ ).

**12-Hydroxyoctadecanoic hydrazide (1c).** White crystals, yield 75%, m.p. 112–114 °C. IR (KBr,  $\text{cm}^{-1}$ ) 3327 (OH), 3278–3095 (NH–NH<sub>2</sub>), 1670 ( $O=C-NH$ ). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.70 (1H, s, NH), 4.11 (1H, m, CH–OH), 3.90 (2H, s, NH<sub>2</sub>), 3.66 (1H, br. s, OH), 2.57 (2H, t,  $J = 7.8$  Hz, CH<sub>2</sub>–CONH), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>–CO), 1.27 (26H, br. s, 13×CH<sub>2</sub>), 0.88 (3H, deg. t, CH<sub>3</sub>).

**9-Hydroxyoctadecanoic hydrazide (1d).** Off-white crystals, yield 70%, m.p. 112–114 °C. IR (KBr,  $\text{cm}^{-1}$ ) 3327 (OH), 3270–3095 (NH–NH<sub>2</sub>), 1669 ( $O=C-NH$ ). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.85 (1H, s, NH), 4.11 (1H, m, CH–OH), 3.90 (2H, s, NH<sub>2</sub>), 3.66 (1H, br. s, OH), 2.57 (2H, t,  $J = 7.8$  Hz, CH<sub>2</sub>–CONH), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>–CO), 1.27 (26H, br. s, 13×CH<sub>2</sub>), 0.88 (3H, deg. t, CH<sub>3</sub>).

#### General procedure for the preparation of hydrazones

**2.** Fatty acid hydrazide **1** (0.1 mmol) and methyl acetoacetate (0.1 mmol) were refluxed in absolute ethanol (30 mL) for 3–4 h containing a few drops of HCl. The resulting solution was then concentrated and cooled. The solid thus separated was filtered and crystallized from ethanol, except for the oily compound **2a**, which was chromatographed on silica gel (60–120 mesh) using petrol ether-diethyl ether (98:2, v/v) as eluent.

#### Methyl 3-(2'-undecanoylhydrazono)butanoate (2a).

Oily, yield 86%. IR (neat,  $\text{cm}^{-1}$ ) 3210 (NH), 1743 ( $O=COCH_3$ ), 1660 ( $O=C-NH$ ), 1449 (C=N). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  10.35 (1H, s, NH, exchangeable with D<sub>2</sub>O), 3.66 (3H, s, OCH<sub>3</sub>), 2.30 (2H, t,  $J = 7.5$  Hz, CH<sub>2</sub>–CONH), 2.01 (2H, s, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CO), 1.56 (3H, s, N=C–CH<sub>3</sub>), 1.26 (14H, br. s, 7×CH<sub>2</sub>), 0.87 (3H, deg. t, terminal CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  174.7, 174.5, 159.2, 51.5, 34.2, 31.9, 29.8, 29.7, 29.5, 25.7, 25.0, 22.7, 14.2 (three signals are hidden). MS (FAB)  $m/z$  (%): 298 (M<sup>+</sup>, 3), 267 (50), 154 (100), 127 (25), 99 (20), 95 (45). Anal. Calcd for C<sub>16</sub>H<sub>30</sub>O<sub>3</sub>N<sub>2</sub> (297.97): C, 64.40; H, 10.13; N, 9.39. Found: C, 64.18; H, 9.87; N, 9.08.

#### Methyl 3-(2'-octadecanoylhydrazono)butanoate (2b).

White powder, yield 78%, m.p. 45 °C. IR (KBr,  $\text{cm}^{-1}$ ) 3207 (NH), 1736 ( $O=COCH_3$ ), 1658 ( $O=C-NH$ ), 1455 (C=N). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  10.35 (1H, s, NH, exchangeable with D<sub>2</sub>O), 3.66 (3H, s, OCH<sub>3</sub>), 2.30 (2H, t,  $J = 7.8$  Hz, CH<sub>2</sub>CONH), 1.94 (2H, s, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>–CO), 1.59 (3H, s, N=C–CH<sub>3</sub>), 1.25 (28H, br. s, 14×CH<sub>2</sub>), 0.88 (3H, deg. t, terminal CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  174.8, 174.4, 159.1, 51.6, 34.2, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 25.7, 25.1, 22.7, 14.1 (eight signals are hidden). MS (FAB)  $m/z$  (%): 396 (M<sup>+</sup>, 3), 298 (100), 267 (30), 239 (12), 185 (7), 157 (10), 143 (50), 99 (35). Anal. Calcd for C<sub>23</sub>H<sub>44</sub>O<sub>3</sub>N<sub>2</sub> (395.97): C, 69.65; H, 11.18; N, 7.06. Found: C, 69.59; H, 11.02; N, 6.98.

**Methyl 3-[2'-(12-hydroxyoctadecanoylhydrazono)]butanoate (2c).** Off-white powder, yield 75%, m.p. 47 °C. IR (KBr,  $\text{cm}^{-1}$ ) 3321 (OH), 3212 (NH), 1737 ( $O=COCH_3$ ), 1660 ( $O=C-NH$ ), 1458 (C=N). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  10.34 (1H, s, NH, exchangeable with D<sub>2</sub>O), 3.98 (1H, m, CH–OH), 3.66 (3H, s, OCH<sub>3</sub>), 3.58 (1H, br. s, OH, exchangeable with D<sub>2</sub>O), 2.30 (2H, t,  $J = 7.5$  Hz, CH<sub>2</sub>CONH), 1.91 (2H, s, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>–CO), 1.59 (3H, s, N=C–CH<sub>3</sub>), 1.27 (26H, br. s, 13×CH<sub>2</sub>), 0.88 (3H, deg. t, terminal CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  174.44, 174.40, 159.2, 72.1, 51.5, 37.6, 34.2, 31.9, 29.8, 29.7, 29.57, 29.55, 29.5, 29.2, 25.7, 25.0, 22.8, 14.2 (five signals are hidden). MS (FAB)  $m/z$  (%): 412 (M<sup>+</sup>, 2), 299 (25), 297 (100), 283 (30), 265 (70), 199 (10), 154 (75), 98 (15). Anal. Calcd for C<sub>23</sub>H<sub>44</sub>O<sub>4</sub>N<sub>2</sub> (411.96): C, 66.95; H, 10.75; N, 6.79. Found: C, 66.91; H, 10.55; N, 6.67.

#### Methyl 3-[2'-(9-hydroxyoctadecanoylhydrazono)]butanoate (2d).

White powder, yield 75%, m.p. 43 °C. IR (KBr,  $\text{cm}^{-1}$ ) 3327 (OH), 3214 (NH), 1737 ( $O=COCH_3$ ), 1658 ( $O=C-NH$ ), 1459 (C=N); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  10.35 (1H, s, NH, exchangeable with D<sub>2</sub>O), 4.11 (1H, m, CH–OH), 3.66 (3H, s, OCH<sub>3</sub>), 3.58 (1H, br. s, OH, exchangeable with D<sub>2</sub>O), 2.30 (2H, t,  $J = 7.5$  Hz, CH<sub>2</sub>CONH), 1.91 (2H, s, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CO), 1.55 (3H, s, N=C–CH<sub>3</sub>), 1.25 (26H, br. s, 13×CH<sub>2</sub>), 0.88 (3H, deg. t, terminal CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  174.5, 174.4, 159.2, 72.0, 51.6, 37.6, 37.5, 34.1, 31.9, 29.7, 29.6, 29.53, 29.50, 29.4, 29.2, 25.7, 25.0, 22.7, 14.1 (four signals are hidden). MS (FAB)  $m/z$  (%): 412 (M<sup>+</sup>, 4), 313 (30), 299 (100), 267 (20), 265 (70), 199 (10), 143 (10), 126 (25), 98 (15). Anal. Calcd for C<sub>23</sub>H<sub>44</sub>O<sub>4</sub>N<sub>2</sub> (411.96): C, 66.95; H, 10.75; N, 6.79. Found: C, 66.87; H, 10.52; N, 6.63.

#### General procedure for preparation of hydrazides 3.

Fatty acid hydrazide **1** (0.1 mmol) and acetylacetone (0.1 mmol) were refluxed in absolute ethanol (30 mL) for 4 h containing a few drops of HCl. The resulting solution was then concentrated and cooled at room temperature. The solid thus separated was filtered and crystallized from ethanol, except for the oily compound **3a**, which was chromatographed on silica gel (60–120 mesh) using petrol ether-diethyl ether (96:4, v/v) as eluent.

#### N'-(4-Oxopentane-2-ylidene)undecanohydrazide (3a).

Oily, yield 80%. IR (KBr,  $\text{cm}^{-1}$ ) 3205 (NH), 1721 (C=O), 1664 ( $O=C-NH$ ), 1446 (C=N). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  10.33 (1H, s, NH, exchangeable with D<sub>2</sub>O), 2.30 (2H, t,  $J = 7.8$  Hz, CH<sub>2</sub>CONH), 2.04 (3H, s, CH<sub>3</sub>), 1.94 (2H, s, CH<sub>2</sub>COCH<sub>3</sub>), 1.61 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CO), 1.56 (3H, s, N=C–CH<sub>3</sub>), 1.25 (14H, br. s, 7×CH<sub>2</sub>), 0.87 (3H, deg. t, terminal CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  174.4, 174.3, 161.3, 74.1, 51.4, 32.0, 29.7, 29.6, 29.4, 29.2, 25.1, 22.8, 14.1 (three signals are hidden). MS (FAB)  $m/z$  (%): 282 (M<sup>+</sup>, 3), 281 (65), 267 (40), 239 (15), 169 (10), 141 (10), 137

(100). Anal. Calcd for  $C_{16}H_{30}O_2N_2$  (281.98): C, 68.04; H, 10.71; N, 9.92. Found: C, 67.84; H, 10.37; N, 9.71.

***N'*-(4-Oxopentane-2-ylidene)octadecanohydrazide (3b)**. White crystals, yield 70%, m.p. 43 °C. IR (KBr,  $cm^{-1}$ ) 3207 (NH), 1723 (C=O), 1662 ( $O=C-NH$ ), 1443 (C=N).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  10.33 (1H, s, NH, exchangeable with  $D_2O$ ), 2.30 (2H, t,  $J = 7.8$  Hz,  $CH_2CONH$ ), 2.04 (3H, s,  $CH_3$ ), 1.94 (2H, s,  $CH_2COCH_3$ ), 1.64 (2H, m,  $CH_2CH_2CO$ ), 1.59 (3H, s,  $N=C-CH_3$ ), 1.25 (28H, br s,  $14 \times CH_2$ ), 0.88 (3H, deg. t, terminal  $CH_3$ ).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  174.38, 174.36, 161.3, 51.4, 34.2, 32.1, 29.8, 29.63, 29.58, 29.52, 29.47, 29.41, 29.3, 25.1, 22.7, 14.1 (seven signals are hidden). MS (FAB)  $m/z$  (%): 282 ( $M^+$ , 4), 281 (65), 267 (40), 239 (15), 169 (10), 141 (10), 137 (100). Anal. Calcd for  $C_{23}H_{44}O_2N_2$  (379.98): C, 72.58; H, 11.65; N, 7.36. Found: C, 72.51; H, 11.44; N, 7.23.

**12-Hydroxy-*N'*-(4-oxopentane-2-ylidene)octadecanohydrazide (3c)**. Off-white powder, yield 70%, m.p. 44 °C. IR (KBr,  $cm^{-1}$ ) 3327 (OH), 3205 (NH), 1719 (C=O), 1658 ( $O=C-NH$ ), 1452 (C=N).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  10.35 (1H, s, NH, exchangeable with  $D_2O$ ), 4.13 (1H, m,  $CH-OH$ ), 3.58 (1H, br. s, OH, exchangeable with  $D_2O$ ), 2.32 (2H, t,  $J = 7.5$  Hz,  $CH_2CONH$ ), 2.04 (3H, s,  $CH_3$ ), 1.91 (2H, s,  $CH_2COCH_3$ ), 1.64 (2H, m,  $CH_2CH_2CO$ ), 1.59 (3H, s,  $N=C-CH_3$ ), 1.25 (26H, br. s,  $13 \times CH_2$ ), 0.88 (3H, deg. t, terminal  $CH_3$ ).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  174.36, 174.34, 161.3, 72.1, 51.4, 37.6, 37.5, 34.1, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 25.1, 22.7, 14.2 (six signals are hidden). MS (FAB)  $m/z$  (%): 396 ( $M^+$ , 4), 313 (10), 297 (100), 281 (20), 267 (10), 199 (7), 171 (15), 154 (90), 140 (9), 112 (8), 98 (20). Anal. Calcd for  $C_{23}H_{44}O_3N_2$  (395.97): C, 69.65; H, 11.18; N, 7.06. Found: C, 69.57; H, 10.98; N, 6.96.

**9-Hydroxy-*N'*-(4-oxopentane-2-ylidene)octadecanohydrazide (3d)**. White powder, yield 75%, m.p. 42 °C. IR (KBr,  $cm^{-1}$ ) 3325 (OH), 3208 (NH), 1724 (C=O), 1660 ( $O=C-NH$ ), 1455 (C=N).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  10.35 (1H, s, NH, exchangeable with  $D_2O$ ), 4.11 (1H, m,  $CH-OH$ ), 3.58 (1H, br. s, OH, exchangeable with  $D_2O$ ), 2.30 (2H, t,  $J = 7.5$  Hz,  $CH_2CONH$ ), 2.04 (3H, s,  $CH_3$ ), 1.90 (2H, s,  $CH_2COCH_3$ ), 1.61 (2H, m,  $CH_2CH_2CO$ ), 1.55 (3H, s,  $N=C-CH_3$ ), 1.25 (26H, br. s,  $13 \times CH_2$ ), 0.88 (3H, deg. t, terminal  $CH_3$ ).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  174.37, 174.34, 161.3, 72.0, 51.4, 37.6, 37.5, 34.2, 32.0, 29.7, 29.63, 29.55, 29.50, 29.46, 29.42, 29.3, 25.0, 22.8, 14.2 (four signals are hidden). MS (FAB)  $m/z$  (%): 396 ( $M^+$ , 5), 363 (7), 297 (100), 267 (30), 199 (10), 185 (10), 140 (15), 115 (8), 98 (20). Anal. Calcd for  $C_{23}H_{44}O_3N_2$  (395.97): C, 69.65; H, 11.18; N, 7.06. Found: C, 69.55; H, 10.95; N, 6.92.

### 3. 1. Antibacterial Activity

The *in vitro* antibacterial activity was carried out against *E. coli*, *S. aureus* and *S. albus*. These strains were

streaked on nutrient agar plates separately and grown overnight. Single-well isolated colonies of each type of bacteria were incubated in separate nutrient mediums for 16 h at 37 °C for the experiment. To determine the zone of inhibition cup-plate method was employed.<sup>15</sup> In this technique bacteria liquid culture of each type grown in log phase was added aseptically to the autoclaved LB agar medium maintained at 45 °C, mixed well and poured immediately into sterile Petri dishes separately. After solidification, wells of about 6 mm were cut into agar plates aseptically.

Solution of 100  $\mu g/ml$  of each hydrazone was prepared in DMF. Standard antibiotic chloromycetin was screened under similar conditions. 100  $\mu l$  of these solutions were added to each well and incubated at 37 °C. One of the wells was used as control by adding 100  $\mu g/ml$  of DMF. Zone of inhibition was measured in mm after 24 h and compared with the standard drug. Results of antibacterial screening are reported in Table 1.

**Table 1.** Response of various micro-organism to the new hydrazones 2 and 3 *in vitro* (culture).

Hydrazone	Diameter of zone of inhibition		
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. albus</i>
<b>2a</b>	(+++)	(+++)	(+++)
<b>2b</b>	(-)	(-)	(-)
<b>2c</b>	(++)	(-)	(++)
<b>2d</b>	(++)	(-)	(++)
<b>3a</b>	(+++)	(+++)	(+++)
<b>3b</b>	(-)	(-)	(-)
<b>3c</b>	(-)	(-)	(-)
<b>3d</b>	(+)	(+)	(-)
Chloromycetin	(++++)	(++++)	(++++)

DMF used as the control.

Concentration used = 100  $\mu g/ml$  of DMF.

Symbols: low activity (1–5 mm) (+); moderate activity (6–10 mm) (++) ; high activity (11–15 mm) (+++) ; very high activity (15–20 mm) (++++) ; no activity (-)

## 4. Conclusion

To the best of our knowledge, hydrazones have been for the first time synthesized from fatty acids. Results obtained from the antibacterial activity show that some of the synthesized hydrazones, especially C-11 derivatives, i.e. hydrazones **2a** and **3a**, may be considered promising for development of new antibacterial agents after performing the significant toxicity tests.

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## 6. References

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## Povzetek

Maščobne kisline, ki vsebujejo hetero atome, imajo potencialno vlogo kot antimikrobne učinkovine. S kondenzacijo s karbonilno skupino metil acetoacetata in acetilacetona smo iz štirih različnih hidrazidov maščobnih kislin (in sicer undekanoil hidrazida (**1a**), oktadekanoil hidrazida (**1b**), 12-hidroksioktadekanoil hidrazida (**1c**) and 9-hidroksioktadekanoil hidrazida (**1d**)) sintetizirali osem različnih hidrazonov **2a–d** in **3a–d**. Strukturno produktov smo ugotovili s pomočjo spektroskopskih podatkov (IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR in MS). Raziskali smo tudi njihovo aktivnost proti mikrobom *Escherichia coli*, *Staphylococcus aureus* in *Staphylococcus albus* s pomočjo ploščne metode pri koncentraciji 100 µg/ml v DMF z uporabo kloromicetina kot standarda.