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Short communication

Synthesis, antidepressant and antimicrobial activities of some novel stearic acid analogues

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ABSTRACT

Stearic acid, a saturated fatty acid was isolated from the microalga *Spirulina platensis*. Some novel stearic acid analogues having 1,3,4-oxadiazole, 1,2,4-triazole and 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole are synthesized and characterized by IR, NMR and mass spectral analysis. All the synthesized compounds were screened for antimicrobial activity by using cup plate method. The synthesized compounds have been further screened for their antidepressant activity in swiss albino mice by forced swim test (FST), midbrain dopamine has been estimated and quantified. All the compounds showed good antimicrobial activity and compound 6 showed significant antidepressant activity.

1. Introduction

Spirulina platensis, a blue green microalga, has been used since ancient times as a source of food because of its high protein and nutritional value. Recently, this alga is being widely studied, not only for nutritional reasons but also for its reported medicinal properties. Sseveral studies have shown that Spirulina or its extracts could prevent or inhibit cancer in humans and animals and recent works have indicated that this species has immuno-promoting effects. *S. platensis* was also reported to possess anti-microbial activity [1–4]. Many seed oils, fatty acids (FA) and their derivatives are known for their antimicrobial, antifungal and pesticidal activities [5]. Saturated fatty acids are of interest since they have lower melting points and exhibit good oxidative stability [6]. Fatty acids function as the key ingredients of antimicrobial food additives which inhibit the growth of unwanted microorganisms [7]. Also, fatty acids have been an emerging interest in treating neuropsychological disorders such as depression and schizophrenia [8,9].

During the last two decades, the chemistry of 1,2,4-triazole, 1,3,4-oxadiazoles, 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole and their derivatives have received considerable attention owing to their synthetic and effective biological importance [10–13].

On the basis of this background the present work has been aimed to synthesize some novel stearic acid analogues having 1,3,4-oxadiazole, 1,2,4-triazole and 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole substitution and to evaluate their antidepressant activity and antimicrobial activity.

2. Results and discussion

2.1. Chemistry

We have synthesized three novel stearic acid analogues such as 2-(heptadecyl) − 5 - phenyl −1, 3,4-oxadiazole, 2-(heptadecyl)-5-phenyl-1,2,4-triazole and 6 (-heptadecyl)-3-phenyl-[1,2,4] triazolo [3,4-b]1,3,4-thiadiazole as illustrated in Scheme 1. Structures of all the compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data. Three novel heterocyclic compounds such as 1,3,4- oxadiazole,1,2,4- triazole and 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole substituted stearic acid analogues were synthesized according to the Scheme. Benzoic acid hydrazide **2** was obtained by the reaction of methyl benzoate with

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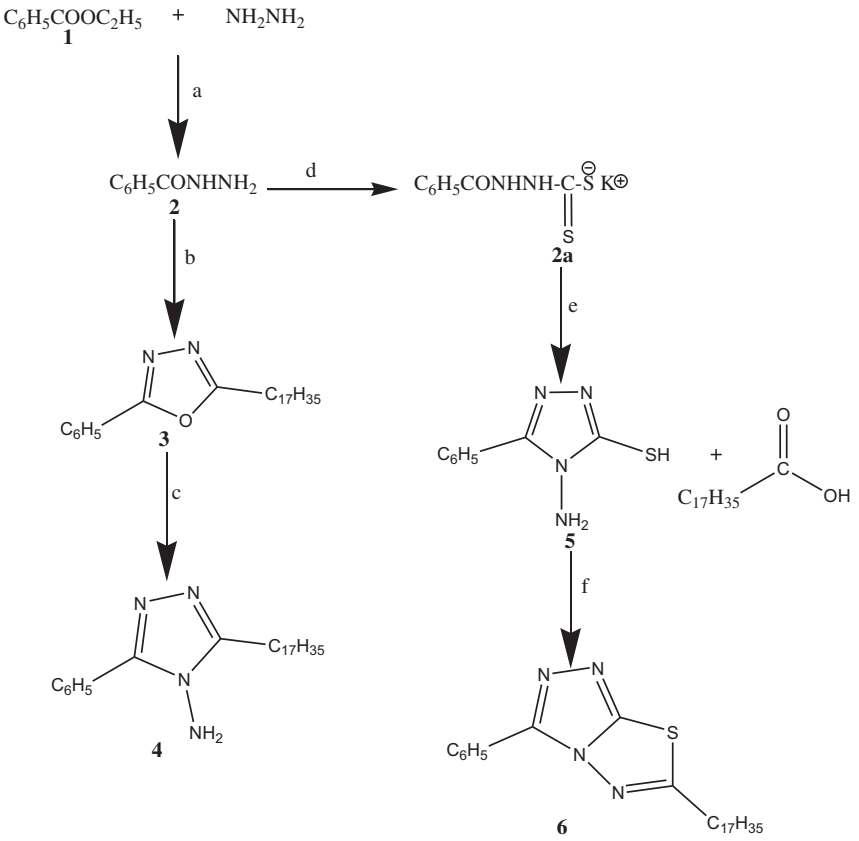

Scheme 1. Synthesis of the compounds. Reagents and conditions. a) NH_2NH_2, reflux 8 h. b) $POCl_3$,$C_{17}H_{35}COOH$, reflux, 8 h, neutralization with NaOH, c) NH_2NH_2, reflux, 8 h, acidification with HCl upto pH3 d) CS_2/Aq.KOH/C_2H_5OH,stirring 10 h e) NH_2NH_2 reflux, 3 h, acidification with HCl. f) $POCl_3$,$C_{17}H_{35}COOH$, reflux, 8 h, neutralization with NaOH.

hydrazine hydrate in the presence of absolute ethanol. Acid hydrazides can be considered as useful intermediates leading to the formation of some heterocyclic rings such as 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole. Condensation of acid hydrazides with various aromatic carboxylic acids in the presence of boiling phosphorous oxychloride yields 1,3,4-oxadiazole. In the present study, in place of aromatic acids a saturated carboxylic acid such as stearic acid was used for the synthesis of our aimed heterocyclic compounds. Compound **2** on treatment with stearic acid in the presence of phosphorous oxychloride afforded 2- (heptadecyl) − 5 - phenyl −1, 3, 4 − oxadiazole **3**. (Scheme 1). The reaction of **3** with excess hydrazine produced 2 - (heptadecyl)−5−phenyl-1,2,4-triazole **4** (Scheme 1). On the other hand potassium dithiocarbazinate [2a] was prepared from compound 2, by using potassium hydroxide, carbon disulphide and ethanol as a solvent. Compound 2a had been undergone ring closure reaction with excess hydrazine resulted in the formation of 4-amino-5-phenyl-4H-1,2,4 triazole-3-thiol **5** Compound **5** was cyclized with stearic acid to get 6(-heptadecyl)-3-phenyl-[1,2,4] triazolo[3,4-b]1,3,4-thiadiazole **6** using $POCl_3$ as a solvent.

2.2. Biological activity

The stearic acid analogues 3,4 and 6 were evaluated for antidepressant activity by FST in rats at a dose of 30 mg/kg and compared with the standard drug (Diazepam, 30 mg/kg). Antidepressant activity was assessed as mean immobility time in seconds and data are presented as Mean ± S.E.M in Table 1. Results of FST revealed that compounds **3**, **4** and **6** exhibited significant ($P < 0.01$) reduction in immobility time compared to control while compound **6** showed the most potent antidepressant activity among the titled compounds. The preliminary structural activity relationship showed that introduction of fused ring system such as [1,2,4] triazolo[3,4-b]1,3,4-thiadiazole substitution exhibited significant antidepressant activity than oxadiazole and triazole substitution. Also the lipophilicity data suggested that compounds **3**, **4** and **6** were most lipophilic in nature (Clog P 9.28, 9.62 and 10.37) and showed highest antidepressant activity (Table 2).

2.3. Effect of stearic acid analogues on dopamine levels

Further to confirm the antidepressant activity we have estimated the dopamine levels of midbrain region. The data showed either increase or maintenance of dopamine levels after treatment with test or standard drugs (Table 3). The retention of dopamine at midbrain region might be the cause to exhibit significant antidepressant activity for the synthesized compounds.

Dopamine concentration in striatal region was measured by HPLC equipped with UV spectrophotometer detector. All animals of the test groups showed significant difference in the dopamine

Table 1
Antidepressant screening by forced swim test.

Compound	Duration of immobility in sec. (mean ± S.E.M)
Control	80.67 ± 0.882
3	70.00 ± 1.065**
4	68.83 ± 1.167**
6	62.67 ± 1.520***
Clomipramine	52.97 ± 0.77***

Data was analysed by Dunnet's test. $n = 6$; (***) equals $p < 0.001$, (**) equals $p < 0.01$ when compared with control.

Table 2
Physicochemical data of compounds **3**, **4** and **6**.

Compound	Yield[a] (%)	M.P.(°C)	R.f.[b]	Clog P[c]
3	65	72–78	0.20	9.28
4	62	76–79	0.23	9.62
6	68	71–74	0.30	10.37

[a] After recrystallization from ethanol.
[b] Ethyl acetate: Chloroform (9:1).
[c] Calculated using Cambridge software 8.0.

levels when compared with control. Compound 6 increased the dopamine concentration while the compounds 3& 4 decreased the dopamine levels.

2.4. Antimicrobial screening

The results of preliminary antimicrobial testing of compounds are shown in Tables 4 and 5. The investigation of antibacterial screening data revealed that all the three tested compounds were found to have moderate to good bacterial inhibition. The antibacterial potency has been calculated as per the procedure given in Indian Pharmacopoeia. The results revealed that compound **3** showed pronounced antibacterial activities against *Escherichia coli* with 93.30% potency and *Staphylococcus aureus* with 94.60% potency. Also it showed moderate antibacterial activities against *Bacillus subtilis* and *P. aeruginosa* with 75.78%, 60.95% respectively. Compound **6** showed pronounced antibacterial activities against *B. subtilis* and *P. aeruginosa* with 93.88%. It showed moderate activities against *E. coli* with 84.08% potency and *S. aureus* with 79.35% potency. The Compound **4** showed pronounced antibacterial activities against *E. coli* and *B. subtils* with 93.30% potency. It showed moderate activities against *S. aureus* with 75.78% potency and *P. aeruginosa* with 59.45%. All the compounds showed good antifungal activity as compared to standard. Compound **6** showed good antifungal activity against *Candida albicans* with 92.57% potency.

3. Conclusion

Owing to the biological importance of fatty acids, three novel fatty acid analogues have been synthesized and confirmed by physical parameters and consistent with their IR, ^{1}H NMR and mass spectra. The synthesized compounds show significant antidepressant activity by forced swim test when compared to the control and moderate to good antimicrobial activities when compared to the standards tetracycline and amphotericin B. In particular compound 6 showed significant antidepressant activity. From the present study, it can be concluded that our synthesized stearic acid analogues have significant antidepressant and antimicrobial activities.

Table 3
Effect of control, stearic acid analogous on concentrations of dopamine level in brain.

Groups	Concentration of dopamine (ng/mg of protein)
Control	0.399 ± 0.151
Clomipramine	0.420 ± 0.181$^{###}$
Comp. **3**	0.250 ± 0.035$^{##}$
Comp. **4**	0.367 ± 0.031$^{##}$
Comp. **6**	1.455 ± 0.030$^{###}$

Data was analysed by Dunnet's test. $n = 6$; ($^{###}$) equals $p < 0.001$, ($^{##}$) equals $p < 0.01$ when compared with control.

4. Experimental protocol

4.1. Materials and methods

The used chemicals were purchased from Fluka chemicals. Their purity was checked by GC. All solvents were purified by distillation and if necessary residual water was removed. The composition of solvents and eluents are given in volume ratios of the components. Plant powder of *S. platensis* was collected from Antenna Research Foundation Pvt. Limited, Madurai, Tamilnadu, India. Stearic acid methyl ester was isolated according to the previously reported method [14]. It was hydrolysed to give free stearic acid. Products were purified by column chromatography and identified using different spectral techniques.

Melting points were taken in glass capillary tubes on a Veego VMP-1 apparatus and are uncorrected. The ^{1}H NMR were recorded on Bruker DRX-300 (300 MHz FT-NMR) using deuterated chloroform as solvent and TMS as internal standard. The IR spectra of compounds were recorded on Shimadzu FT-IR spectrometer by KBr pellet technique and are expressed in cm^{-1}. The mass spectra of compounds were recorded on JEOL GCMATE II GC–MS.

4.2. Synthesis of benzoic acid hydrazide ***2***

Hydrazine hydrate (10 mmol, 1.25 ml) was placed in a round bottom flask fitted with a reflux condenser. To this solution ethyl benzoate (10 mmol, 1.5 ml) was added dropwise and heated gently for 15 min. Then absolute alcohol (5 ml) was added through the condenser to produce clear solution and refluxed for 4–5 h. The ethanol was distilled off and cooled. The crystals were filtered off by the addition of ice water and recrystallised with ethanol.

Yield: 58.82%. MP: 112–116 °C, Rf value: 0.20, TLC: eluent = Ethyl acetate: Chloroform (4:1),

IR (KBr cm^{-1}): 1662 (C=O), 3020 (Ar–CH str), 3300 (N–H str), 1487 (C–N str).

4.3. Synthesis of 2-(heptadecyl-5-phenyl-1, 3, 4-oxadiazole) ***3***

A mixture of benzoic acid hydrazide (10 mmol), stearic acid (10 mmol) and phosphorus oxychloride (10 ml) was refluxed for 8 h, cooled and poured on crushed ice. The solid separated was filtered, and treated with dil. NaOH, washed with water and recrystallised from ethanol.

IR (KBr) cm^{-1}; 3057.27 (Ar C–H), 1602.90 (C=N), 1591 (C=C), 1290.42 (C–O–C), 769.62, 690.54 (Ar–CH).^{1}H NMR (300 MHZ,$CDCl_3$) δ; 7.4–8.2 (m, 5H, Ar–H), 2.55 (t, 2H, CH_2), 1.2 (s, 28H, CH_2), 1.9 (m, 2H, CH_2), 0.96 (t, 3H, CH_3) ppm. FAB-MS: 384 (M^+, 100%): 232 ($C_{17}H_{35}CO^+$),267($C_{17}H_{35}CN^+$)m/z+2103($C_6H_5CN^+$) m/z-2.Anal.Calcd.for.$C_{25}H_{40}N_2O$:C,78.07; H,10.48; N,7.28. Found :C,78.17; H,10.61; N,7.37%.

4.4. Synthesis of 2-(heptadecyl)-5-phenyl-1,2,4-triazole ***4***

A mixture of oxadiazole (10 mmol), hydrazine hydrate (10 mmol) and absolute alcohol (10 ml) was refluxed for 5 h. Then, potassium hydroxide was added to the reaction mixture and the precipitate formed was filtered. The solid obtained was acidified with conc. HCl up to pH-3 and washed with water. The resultant solid was recrystallised from ethanol.

IR (KBr) cm^{-1}; 3057.27 (N–H), 2955.04(Ar–CH), 1602.90 (C=N), 1572.04 (C=C), 767.69, 688.61 (Ar–CH). ^{1}H NMR (300 MHZ,$CDCl_3$) δ; 7.45–7.55 (m, 5H, Ar–H), 7.1 (s, 1H, NH2), 2.8 (m, 2H, CH_2), 1.2 (s, 28H, CH_2), 1.8–1.9 (m, 2H, CH_2), 0.96 (t, 3H, CH_3) ppm. FAB-MS; 398 (M^+,100%)0.103($C_6H_5CN^+$)m/z –2103. Anal.Calcd. for.

Table 4
Antibacterial activity of **3**, **4** & **6**.

Compd.	Ec			Pa			Sa			Bs		
	Conc. (μg/ml)			Conc. (μg/ml)			Conc. (μg/ml)			Conc. (μg/ml)		
	500	1000	% pot	500	1000	% pot	500	1000	% pot	500	1000	% pot
3	1.4	1.8	93.30	1.6	1.7	60.95	1.4	2.1	94.60	1.3	1.6	75.78
4	1.6	1.7	93.30	1.3	1.6	59.45	1.3	1.8	75.78	1.2	1.5	93.30
6	1.6	1.9	84.08	1.5	1.9	93.88	1.6	1.7	79.35	1.3	1.6	93.88
Std	1.8	2.3	–	1.6	2.2	–	1.8	1.9	–	1.3	2.0	–

Sa –*Staphylococcus aureus* Pa – *Pseudomonas aeruginosa*.
Ec – *Escherichia coli* Bs – *Bacillus subtilis*.

$C_{25}H_{42}N_4$:C,75.33; H,10.62; N,14.05. Found :C,75.37; H,10.74; N,14.37%.

4.5. Synthesis of 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol **5**

A mixture of carbon disulphide (15 mmol, 11.2 g), potassium hydroxide (15 mmol, 8.5 g) in absolute ethanol (125 ml), benzoic acid hydrazide (10 mmol, 1.36 g) was stirred for 16 h. To the resulted solution dry ether (250 ml) was added and the precipitate potassium dithiocarbazinate was collected by filtration. The potassium salt was obtained by the above procedure was near to the quantitative yield and used directly without purification. A suspension of the potassium salt (10 mmol, 1.25 g), hydrazine hydrate (3 ml) and water (2 ml) was refluxed for 3–4 h. The colour changes to green, with the evolution of hydrogen sulphide gas. After cooling the solution white precipitate was obtained. It was acidified with conc. hydrochloric acid. The product was collected by filtration and recrystallised with ethanol.

Yield: 88%; m.p. 211–216 °C, Rf value: 0.22, IR (KBr) cm^{-1};943.3 (N–C–S), 1278 (N–N–C), 3365(N–H), 696.33 (C–S), 3082 (Ar–CH).

4.5.1. Synthesis of 6(-heptadecyl-3-phenyl-[1,2,4]triazolo[3,4-b]) 1,3,4-thiadiazole **6**

A mixture of 1,2,4 triazole (10 mmol), stearic acid (10 mmol) and phosphorus oxychloride (10 ml) was refluxed for 8 h, cooled and poured on crushed ice. The solid separated was filtered, and treated with dil. NaOH, washed with water and recrystallised from ethanol.

IR (KBr) cm^{-1};2955.04 (Ar–CH), 1602.90 (C=N), 1573.97 (C=C), 771.55 (Ar–CH). ^{1}H NMR (300 MHZ,$CDCl_3$) δ; 7 (m, 5H, Ar–H), 2.8(t, 2H, CH_2), 1.2 (s, 28H, CH_2), 1.7 (m, 2H, CH_2), 0.9 (t, 3H, CH_3) ppm. FAB-MS; 440 (M^+,100%). 253 (CH_2-$C_{17}H_{35}$),124 (C_3N_4S) 103 (C_6H_5CN *m*/*z*+4), 79(C_6H_5 *m*/*z*+2).Anal.Calcd.for.$C_{26}H_{40}N_4S$:C,70.86; H,9.15; N,12.71.Found:C,70.37; H,9.15; N,12.37%.

4.6. Biology

4.6.1. Animals required

Inbred male albino mice (Swiss strain) weighing between 20 and 30 g and the relative humidity of 50 ± 5%. They received a standard diet and water ad libitum. The animals were transferred to the laboratory 1 h before the start of the experiment. The institutional animal ethics committee approved the study. Experiments were conducted between 9 and 14 h. Each mouse was used only once.

Table 5
Antifungal activity of **3**, **4** & **6**.

Compd.	Ca conc (μg/ml)		
	500	1000	% Pot
3	1.5	2.0	56.71
4	1.5	2.2	65.97
6	1.5	1.9	92.57

Ca – *Candida albicans*.

4.6.2. Anti depressant activity

The activity was screened by forced swim test (FST) [15]. Forced swimming test is a behavioural test used to assess the efficacy of an antidepressant drug. The synthesized compounds were used for the study. They were housed under standard laboratory conditions for a week before the experiments. The housing conditions were maintained at controlled temperature (23 °C) (30 mg/kg) and standard drug clomipramine (30 mg/kg) were suspended in 0.5% carboxymethylcellulose (CMC) and were injected intraperitoneally (i.p), 30 min prior to the test. Control animals received the vehicle only. The mice were forced to swim individually in a glass jar (tank) (25 × 12 × 25 cm^3) containing fresh water of 15 cm height and maintained at 25 ± 3 °C. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The change in immobility duration was studied after administering drugs in separate groups of 6 animals. The percentage change from control [(Test × 100)-100 was calculated for each mouse.

4.6.3. Estimation of dopamine

Dissected striata were immediately frozen on dry ice and stored at −80 °C. Striatal tissue was sonicated in 0.1 M perchloric acid (100 μg/mg tissue). The supernatant fluids were taken for measurement of dopamine levels by HPLC. Briefly, 20 μl supernatant fluid was isocratically eluted through an 4.6 mm C_{18} column with a mobile phase containing 50 mM ammonium phosphate pH 4.6, 25 mM hexane sulfonic acid pH 4.04, 5% acetonitrile and detected by UV detector. The flow rate used was 0.5 ml/min. Paracetamol (100 μg/ml) was used as the internal standard. Concentration of dopamine was expressed as nanogrammes per milligramme of protein [16].

4.7. Antimicrobial activity

Compounds **3**, **4** and **6** were tested for *in vitro* antimicrobial activity against the Gram- positive bacteria *S. aureus* and *B. subtilis*, the Gram negative bacteria *Salmonella typhimuriu*, *E. coli* and fungi *C. albicans*. The primary screen was carried out by agar disc diffusion method [17,18]. Standard inoculum (1–2 × 10^7 c.f.u./Ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, using a sterile glass spreader for even distribution. Disc measuring 6 mm in diameter, sterilized by dry heat at 140 °C for 1 h, were prepared from whatman No.1 filter paper. 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. Tetracycline and amphotericin-B were used as negative

control. Discs with dimethyl sulphoxide alone were used as positive 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 and fungal strain. Inhibition zones were measured and the percentage potency was calculated.

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