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Synthesis and antimicrobial evaluation of fatty chain substituted 2,5-dimethyl pyrrole and 1,3-benzoxazin-4-one derivatives

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Abstract Fatty acids themselves have a number of biological properties and its easy intake by the human body will focus to the synthesis of many heterocyclic moiety substituted with fatty acid residue, to make more gradual intake of heterocycles in the human body. 2,5-Dimethyl pyrrole **2(a–e)** and 1,3-benzoxazin-4-one **4(b–e)** derivatives were synthesized, from cyclization of fatty acid hydrazide **1(a–e)** with acetonyl acetone and from the reaction of fatty esters **3(b–e)** with anthranilic acid in the presence of $POCl_3$, respectively. All these compounds were characterized with the help of IR, ^1H NMR, ^{13}C NMR and mass spectra. The synthesized compounds were screened for antimicrobial evaluation against gram-positive (*Staphylococcus aureus* SA 22, *Bacillus subtilis* MTCC 121), gram-negative (*Escherichia coli* K12, *Klebsiella pneumoniae*) and fungal strains (*Candida albicans* IOA-109) and were found to be good antimicrobial agents.
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1. Introduction

The pyrrole ring is a part of many biological compounds such as the enzyme catalase, the bile pigment bilirubin and the mould pigment prodigiosin; it is also a significant part of macrocyclic porphyrin ring system of chlorophyll and hemin [1,2]. Apart from these properties pyrrole and its derivative possess a number of biological activities such as antiallergic, antitumor [3], antibacterial, antifungal [4], antiinflammatory, analgesic [5], anticonvulsant [6], antimycobacterial [7] antitubercular, anticancer [8] and anti HIV [9]. Substituted dimethyl pyrroles can be synthesized from the widely used Knorr pyrrole synthesis [10]. Other methods are also known for the synthesis of 2,5-dimethyl pyrrole derivatives [11,12]. Sometimes for the synthesis of substituted pyrroles, photochemical reactions are also used, which involves the use of other pyrrole precursor including the migration of group from one nitrogen atom to the ring carbon atom [13]. Despite the biological use of substituted dimethyl pyrroles they have been synthetically
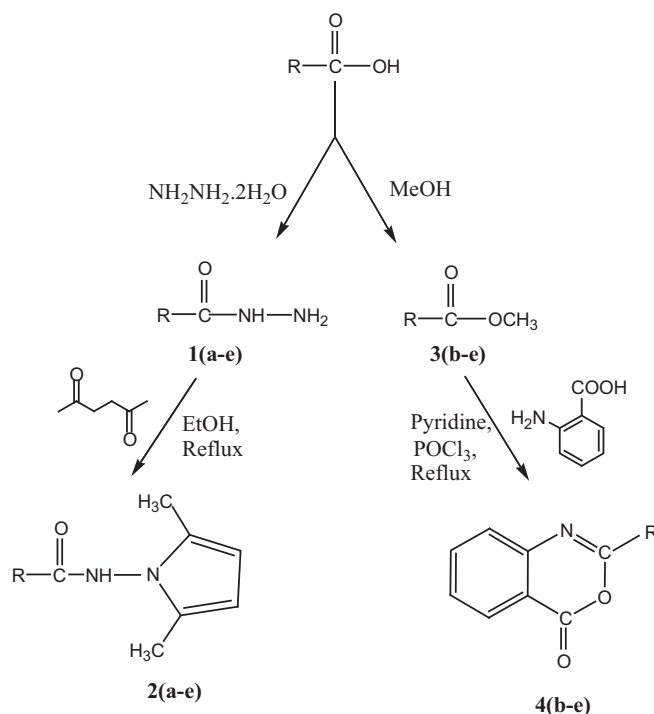
Compound	R
1a, 2a	CH_3-$(CH_2)_{14}$-
1b, 2b, 3b, 4b	$\overset{H_E}{\underset{H_Z}{>}}C=CH$-$(CH_2)_8$-
1c, 2c, 3c, 4c	CH_3-$(CH_2)_7$-$CH=CH$-$(CH_2)_7$-
1d, 2d, 3d, 4d	CH_3-$(CH_2)_5$-$CH(OH)$-CH_2-$CH=CH$-$(CH_2)_7$-
1e, 2e, 3e, 4e	CH_3-$(CH_2)_4$-$CH=CH$-CH_2-CH_2-$CH(OH)$-$(CH_2)_7$-

Scheme 1 Synthetic pathway for the synthesis of a series of N-fatty acid derivative substituted 2,5-dimethyl pyrrole and 2-alkenyl/hydroxyalkenyl chain substituted-benz-1,3-oxazin-4-one.

very useful compounds. In the solid phase synthesis 2,5-dimethyl pyrroles can be used as a good protecting group for protecting the terminal amines. Solid supported 2,5-dimethyl pyrrole was obtained in pure form and quantitative yield by microwave irradiation using dimethyl pyridine (DMP) as solvent. The microwave irradiation speeds up the reaction rate and solid supported pyrrole derivative was obtained only in five minutes [14]. These 2,5-dimethyl pyrroles, undergo reductive condensation (did not undergo self condensation) with acetonyl acetone to form 1,3-diphenyl-4,7-dimethyl isoindoline [1,2]. Various methods are known for the synthesis of dimethyl pyrrole derivatives using different reagents and substrates, these derivatives were found very useful in the field of chemistry as well as in biology [15–20]. Preliminary data on the synthesis of substituted pyrrole derivative of saturated fatty acid (stearic acid) have been already reported [21], but the unsaturated fatty acid amide substitution on 2,5-disubstituted pyrrole has not been reported yet.

On the other hand, 1,3-benzoxazin-4-one derivatives are biologically very useful compounds [22]. These are used either

directly or indirectly in many fields such as industries [23], research field and in clinical work [24]. Clinically used 4-quinazolone derivatives were synthesized from benzoxazinone starting material [25]. Vinyl and phosphate group substituted benzoxazinone derivatives were used as hypnotic drug [26], antiphlogistic drug [27] and possessed antimuscular contraction properties [28]. These derivatives are very useful in natural product chemistry, because they were found in the form of acetal glycosides in plant of different taxonomic positions [29]. Nitrogen atom of these derivatives is a part of the aglyconic cyclo hemiacetal unit, therefore it can act as a plant's own resistance factor towards pests, insects, fungi and other microbial diseases. A conjugate base benzoxazine kanamycin can be used as a suitable fluorescent nucleus (probe) for detecting the mycoplasmas in cell culture [30]. These derivatives also proved to be neuropeptide Y Y5 receptor antagonists through quantitative structure activity relationship (QSAR) studies [31] and also as factor Xa (FXa) inhibitor (it is a trypsin like protease, helps in blood coagulation) [32]. The 1,3-benzoxazin-4-one undergoes nucleophilic ring opening reaction with Ruppert's

reagent (TMS-CF$_3$) to form trifluoro methylated compound (o-amino-2,2,2-trifluoroacetophenones) that is a useful compound in modern drug discovery and material sciences [33]. Benzoxazinones were synthesized from the different methods which include condensation reaction of anthranilic acid amide with cyanuric chloride, elimination of hydrochloride (HCl) and benzoxazinone [25]. Another method of synthesis of benzoxazinone derivatives was refluxing of mixture of benzoic acid derivatives with acetic anhydride [34]. Besides this, the drugs containing fatty acid chain residues played a vital role in modern drug discovery. Some of the newly synthesized bacterial fatty acids were purchased by the number of research groups to develop or produce new antibacterial agents (anti staphylococcal agents) [35]. But we have synthesized these derivatives through the reaction of fatty acid methyl esters and anthranilic acid in dry benzene solvent with catalytic amount of POCl$_3$ and pyridine. Anthranilic acid is used here, because of its amino acidic nature and may be used as a useful precursor for pharmaceutics [36]. Therefore, in view of the above mentioned findings and properties, the aim of the present work was to design, synthesize, analyse and evaluate antimicrobial activity of some novel long chain hydroxy and non hydroxy fatty acid derivatives of 2,5-dimethyl pyrroles and 1,3-benzoxazin-4-ones.

2. Results and discussion

2.1. Chemistry

Long chain alkenyl/hydroxy alkenyl acids (fatty acids) were used as a starting material for the synthesis of fatty acid hydrazides **1(a–e)** and fatty acid methyl esters **3(b–e)**, depicted in Scheme 1. Long chain saturated and unsaturated fatty acid hydrazides **1(a–e)** reacted with the 2,5-hexadione to form the corresponding compounds **2(a–e)** in ethanol presented in Scheme 1. These newly synthesized fatty acid substituted dimethyl pyrrole derivatives **2(a–e)** were found to be good antimicrobial agents because it has been suggested that the fatty acid may increase antibacterial and antifungal activity of number of organic heterocyclic moieties [37–40], graphically depicted in Fig. 1, and the antimicrobial screening data were given in Table 2. Compounds **2b**, **2d**, **2e** were found to be good antibacterial as well as good antifungal agent. Apart from this, the other novel series of heterocyclic derivatives (benzoxazinone derivatives) of fatty acids **4(b–e)** were synthesized from refluxing the mixture of fatty acid methyl esters **3(b–e)** and anthranilic acid in dry benzene solvent, in the presence of catalytic amount of pyridine and POCl$_3$, presented in Scheme 1. The physico-chemical properties of all newly synthesized compounds are given in Table 1. These compounds were also screened for the antimicrobial activity and showed moderate to good results. Compound **4b** and **4c** were found to be potent antibacterial agents against gram-negative bacterial species as compared to the gram-positive bacteria. All the synthesized compounds were characterized with the help of IR, ^{1}H NMR, ^{13}C NMR and mass spectra. Detailed spectral description of the compound **2b** is given below.

This compound showed characteristic peaks in the IR spectrum for the functional groups. The peaks obtained at 3259 cm^{-1} for NH stretching, at 1672 and 1082 cm^{-1} for C=O and C–N stretching, respectively. ^{1}H NMR spectra were

very useful in assigning the structure of the synthesized compound. This newly synthesized compound showed a diagnostic
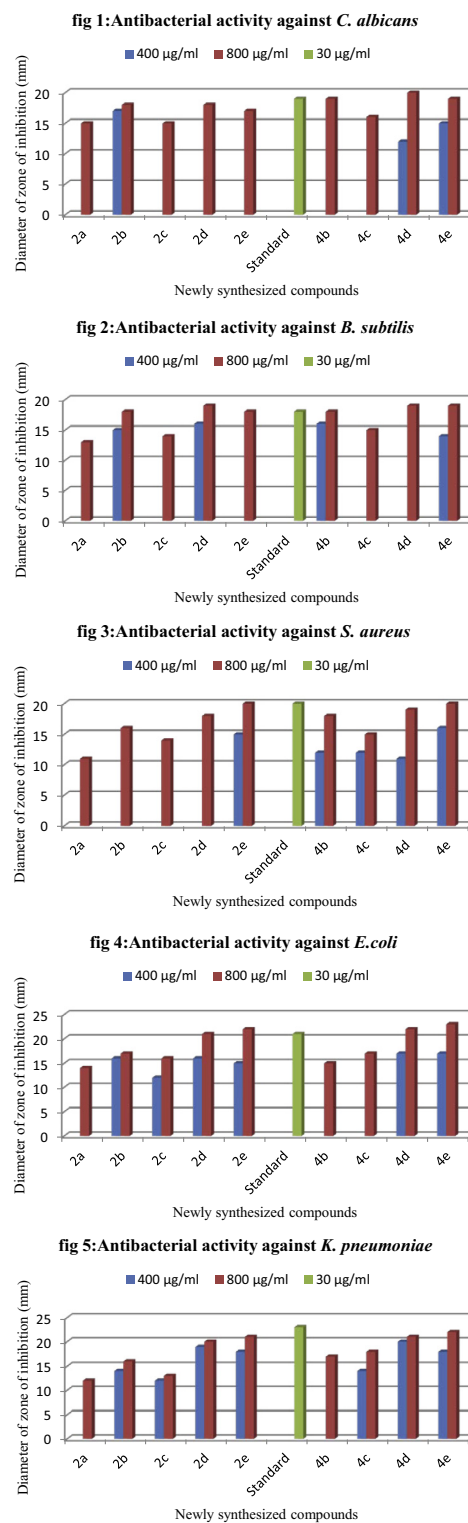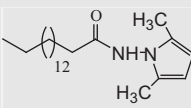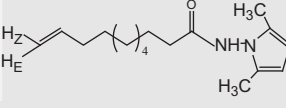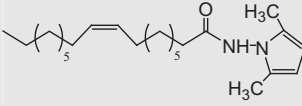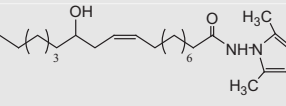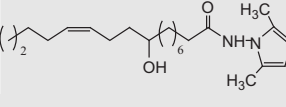

Figure 1 Showing the antimicrobial activity against various microbes. Fig. (1) shows the antifungal activity against *Candida albicans*, standard used was Nystatin. Figs. (2–5) show the antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, respectively, standard used was Ciprofloxacin.

Table 1 Physico-chemical properties of newly synthesized compounds **2(a–e)** and **4(b–e)**.

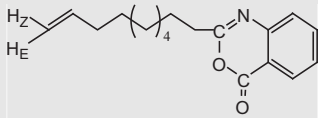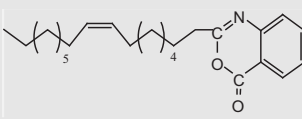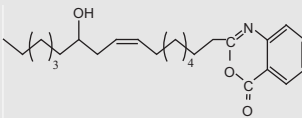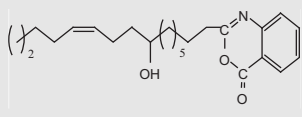| Comp. code	Compound	Mole. weight	Colour and Physical State	M.P. (°C)	% Yield
2(a)		348.49	White powder	120–122	82
2(b)		276.36	White powder	96–98	71
2(c)		374.52	Pale yellow powder	116–118	76
2(d)		390.52	Yellow powder	111–113	73
2(e)		390.52	Dark yellow powder	116–117	69
4(b)		285.34	Pale yellow Oily Liquid	-	69
4(c)		383.50	Light yellow Oily liquid	-	74
4(d)		399.50	Yellow liquid	-	73
4(e)		399.50	Yellow Liquid	-	68

singlet at δ 7.98 for NH proton, a doublet at δ 5.72 for ring protons. In case of unsubstituted pyrrole, ring protons showed the chemical shift value at or above the 6.1 ppm but in case of dimethyl substituted pyrrole derivatives the position of protons attached to the C_3 and C_4 carbons of the pyrrole ring, shifted to lower (δ 5.70–5.85) delta value (upfield side) this is because of the shielding effect of the electron releasing methyl groups (attached at 2 and 5 position of the pyrrole ring) on the neighbouring protons (C_3 and C_4 ring protons). The methine proton of C_{10} showed triplet of doublet of doublet (tdd) at δ 5.76. The C_{11} methylene protons H_Z and H_E showed two distinct doublets of doublet (dd) at δ 4.91 and at δ 4.85, respectively. A singlet was observed at δ 2.00 for six methyl protons. ^{13}C NMR spectrum also showed the peaks of ring

Table 2 *In vitro* antibacterial and antifungal activity data (resulting zones of inhibition were measured in millimetres) against various microbes at 2 different concentrations of compounds **2(a–e)** and **4(b–e)**.

Compound	Concentration (μg/ml)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Candida albicans*
2a	400	–	–	–	–	–
	800	13	11	14	12	15
2b	400	15	–	16	14	17
	800	18	16	17	16	18
2c	400	–	–	12	12	–
	800	14	14	16	13	15
2d	400	16	–	16	19	–
	800	19	18	21	20	18
2e	400	–	15	15	18	–
	800	18	20	22	21	17
4b	400	16	12	–	–	–
	800	18	18	15	17	19
4c	400	–	12	–	14	–
	800	15	15	17	18	16
4d	400	–	11	17	18	16
	800	19	19	22	21	20
4e	400	14	16	17	18	15
	800	19	20	23	22	19
Ciprofloxacin	30 (μg/disc)	18	20	21	23	–
Nystatin	30 (μg/disc)	–	–	–	–	19

carbon atoms at δ 126.7, 125.3, 104.2 and 103.1 as well as for the chain carbon atoms. Mass spectra of the synthesized compound satisfied the assigned molecular formula.

Spectral description of the compound **4b** is discussed here. IR spectra of the compound showed the peaks at 3075 cm^{-1} for the aromatic CH stretching, at 1659 cm^{-1} for C=O stretching, at 1523 cm^{-1} for C=N stretching and at 1090 cm^{-1} for C–N stretching. ^{1}H NMR spectra of the compound showed the following peaks. Two multiplets were observed at δ 8.35, 7.33 for four aromatic protons. The C$_9$ methine proton of the fatty ester substitution showed the tdd at δ 5.80 and two methylene protons H$_Z$ and H$_E$ of C$_{10}$ showed two distinct dd at δ 4.99 and 4.92 respectively. ^{13}C NMR spectra of the compound showed the diagnostic peaks at δ 174.4 (C=O carbon), 171.2 (C=N carbon), 142.2, 134.3, 131.6, 130.8, 129.9, 128.2, 124.5 and 122.6 for benzene ring and unsaturated carbons, respectively. The molecular ion peak was also consistent with the calculated molecular weight of the synthesized compound.

2.2. Biology

All the synthesized compounds were tested for *in vitro* antimicrobial activity against gram- positive, gram-negative bacteria and fungi. Antibacterial activity was tested against two gram-positive species *Staphylococcus aureus* SA 22, *Bacillus subtilis* MTCC 121 and two gram- negative species *Escherichia coli* K12, *Klebsiella pneumoniae*. *In vitro* antifungal activity was tested against the species *Candida albicans* IOA-109. These newly synthesized compounds were tested at 400 and 800 μg/ ml diameter of the zone of inhibition in mm. The minimum inhibitory concentration (MIC) of the synthesized compounds was 400 and 800 μg/ml respectively at two different diameters of zone of inhibition. The standard used for antibacterial activity, was ciprofloxacin (30 μg/disc) and for antifungal activity, was nystatin (30 μg/disc).

2.3. Structure activity relationship

Biological activity of any organic compound is directly related to its structure. The activity of the compound is greatly affected by the presence and absence of different functional groups and substituents. All the synthesized compounds **2(a–e)** and **4(b–e)** were tested against the various bacterial (gram-negative species viz *E. coli* K12, *K. pneumoniae* and gram-positive species *S. aureus* SA 22, *B. subtilis* MTCC 121) and fungal strains (*C. albicans* IOA-109). All the compounds showed the distinct effect of biological activity against these microbes depending upon their structures. Among the two series of compounds, substituted 2,5-dimethyl pyrrole derivatives **2(a–e)** and substituted 1,3-benzoxazine-4-one derivatives **4(b–e)**, compounds **4(b–e)** were found to be more active than the compounds **2(a–e)**, mainly because of more number of hetero atoms in their structures. Among all the synthesized compounds, compound **2d**, **2e**, **4d** and **4e** were found to be good antimicrobial agents. The enhancement of the activity of these compounds is due to the presence of the hydroxyl group in their structures. The hydroxyl group increases the reactivity of the compounds against the various biological reactions. Compounds **2b** and **4b**, showed the good antimicrobial results than the compounds **2c** and **4c**. The presence of terminal double bond in compounds **2b** and **4b**, increases their reactivity than the compounds **2c** and **4c**, that contain internal double bond. Compound **2a**, showed least antimicrobial activity which may be attributed to the presence of long saturated carbon chain (C$_{17}$) substituent. The compounds **2d**, **2e**, **4d** and **4e**, were found to be the most active against *E. coli* and *K. pneumoniae* species. Maximum inhibition values were observed for 1,3-benzoxazin-4-one derivatives with hydroxy substituents that is **4d** and **4e**, against *E. coli* with zone diameter of 17, 17 mm (at 400 μg/ml concentration) and 22, 23 mm (at 800 μg/ml concentration), respectively. From the above discussion it is clear that the structure of the compound has a marked effect on their biological activities.

3. Experimental

3.1. Physical apparatus

Undec-10-enoic acid (purity 98%) and octadec-9Z-enoic acid (purity 97%) were purchased commercially from the Fluka Chemicals (Switzerland). (9Z, 12R)-12-Hydroxyoctadec-9-enoic (Ricinolic) and (9R, 12Z)-9-hydroxyoctadec-12-enoic acid (Isoricinolic) were extracted from the naturally occurring ring seeds; *Ricinus communis* and *Wrightia tinctoria*, through Gunstone's partitioning method [41,42]. The fatty acids obtained by this method were further purified by the column chromatography. Laboratory grade chemicals were used for the purification. Acetonyl acetone (2,5-hexadione), anthranilic acid (amino benzoic acid), phosphorus oxychloride (POCl$_3$) and pyridine were purchased commercially from the Sd-fine Chemicals, Mumbai, India. Melting points were taken in glass capillaries and are uncorrected. Formation of the product was determined by TLC (thin layer chromatography) plates, coated with silica gel-G and spots were visualized in the iodine chamber. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectra.

IR spectra were taken on the Shimadzu 8201 FT-IR instrument and wave lengths were recorded in cm^{-1}. ^{1}H NMR spectra were obtained from Brucker Avance II instrument, spectra were recorded at 400 MHz in CDCl$_3$. ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts for both ^{1}H NMR and ^{13}C NMR, were quoted in ppm and were measured relative to the standard TMS (tetra methyl silane). Coupling constant (J) are expressed in Hertz. Mass spectra of the test compounds were obtained on a Jeol SX-102 spectrometer. The purification of the synthesized compounds was carried out by column chromatography.

3.2. Synthesis

3.2.1. General procedure of the synthesis of fatty acid hydrazides 1(a–e)

The hydrazides of fatty acid were synthesized according to the previously synthesized reported method [43].

3.2.2. General procedure for the synthesis of compounds 2(a–e)

Take 0.01 mol of fatty acid hydrazide and 0.02 mol acetonyl acetone (2,5-hexadione) in a round bottom flask. Dissolve the reaction mixture in about 30 ml of ethanol (EtOH). Reflux this mixture on water bath at temperature of 80 °C. Progress of the reaction was monitored by the TLC, after completion concentrate the reaction mixture, up to half of its original volume by opening the corks of a round bottom flask. Now pour the reaction mixture into crushed ice, solid was obtained. Precipitate was filtered and further purification was done through crystallization from ethanol solvent. Spectra of the synthesized compounds are given below.

3.2.2.1. N-(2′,5′-dimethyl pyrrol-1′-yl)-hexadecamide 2(a).
IR (KBr, cm^{-1}): 3273 (NH stretch), 2919 (CH asymmetric), 2848 (CH symmetric), 1672 (amide C=O), 1096 (C–N stretch); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.15 (s, 1H, CON*H*), 5.79 (d, 2H, ring protons), 2.34 (t, 2H, J = 7.52 Hz, C*H*$_2$CO), 2.08 (s, 6H, methyl substituents), 1.74 (m, 2H, C*H*$_2$CH$_2$CO), 1.25 (broad singlet, 24H, (C*H*$_2$)$_{12}$), 0.88 (distorted triplet, 3H, C*H*$_3$CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 168.1, 127.2, 126.8, 104.5, 103.2, 33.9, 33.7, 32.5, 31.8, 31.1, 30.9, 30.4, 29.9, 29.7, 28.7, 28.4, 27.6, 26.1, 25.2, 14.8, 12.0, 11.7; MS (m/z): 371.3 [M + Na]$^+$, Calculated for C$_{22}$H$_{40}$N$_2$O = 371.4.

3.2.2.2. N-(2′,5′-dimethyl pyrrol-1′-yl)-10-enyl-undecamide 2(b).
IR (KBr, cm^{-1}): 3259, 3228 (NH stretch), 2923 (CH asymmetric), 2856 (CH symmetric), 1673 (amide C=O), 1082 (C–N stretch); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 7.98 (s, 1H, CON*H*), 5.76 (tdd, 1H, $J_{H-{}^9CH_2}$ = 5.9 Hz, J_{H-Hz} = 10.2 Hz, J_{H-H_E} = 16.4 Hz, CH$_2$=C*H*), 5.72 (d, 2H, J = 10.3 Hz, ring protons), 4.91 (dd, 1H, J_{Hz-H} = 10.2 Hz, J_{H_E-Hz} = 1.8 Hz, $_z$*H*C=CH), 4.85 (dd, 1H, J_{H_E-H} = 16.4 Hz, J_{H_E-Hz} = 1.8 Hz, $_E$*H*C=CH), 2.72 (t, 2H, J = 7.6 Hz, C*H*$_2$CO), 2.00 (s, 6H, methyl substituents), 1.66 (m, 2H, =CH–C*H*$_2$), 1.54 (m, 2H, C*H*$_2$CH$_2$CO), 1.26 (broad singlet, 10H, (C*H*$_2$)$_5$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 165.2, 134.5, 133.6, 126.7, 125.3, 104.2, 103.1, 29.8, 29.5, 28.7, 26.5, 26.1, 25.2, 24.3, 22.8, 11.9, 11.6; MS (m/z): 299.2 [M + Na]$^+$, Calculated for C$_{17}$H$_{28}$N$_2$O = 299.3.

3.2.2.3. N-(2′,5′-dimethyl pyrrol-1′-yl)-9-enyl-octadecamide 2(c).
IR (KBr, cm^{-1}): 3233, 3224 (NH stretch), 2923 (CH asymmetric), 2855 (CH symmetric), 1676 (amide C=O), 1080 (C–N stretch); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.02 (s, 1H, CON*H*), 5.82 (d, 2H, J = 11.5 Hz, ring protons), 5.36 (m, 2H, C*H*=C*H*), 2.26 (t, 2H, J = 7.5 Hz, C*H*$_2$CO), 2.09 (s, 6H, methyl substituents), 1.75 (m, 2H, C*H*$_2$CH$_2$CO), 1.73 (m, 4H, C*H*$_2$–CH=CH–C*H*$_2$), 1.27 (broad singlet, 18H, (C*H*$_2$)$_9$), 1.05 (m, 2H, C*H*$_2$CH$_3$), 0.89 (distorted triplet, 3H, C*H*$_3$CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 167.8, 135.5, 134.6, 128.7, 126.9, 105.1, 104.7, 40.8, 39.7, 38.7, 37.6, 34.2, 33.1, 31.5, 28.9, 27.0, 25.1, 24.4, 23.7, 22.8, 22.0, 14.2, 11.9, 11.0; MS (m/z): 397.5 [M + Na]$^+$, Calculated for C$_{24}$H$_{42}$N$_2$O = 397.5.

3.2.2.4. N-(2′,5′-dimethyl pyrrol-1′-yl)-(9Z, 12R)-9-enyl-12-hydroxyoctadecamide 2(d).
IR (KBr, cm^{-1}): 3407 (OH stretch), 3249, 3237 (NH stretch), 2925 (CH asymmetric), 2851 (CH symmetric), 1675 (amide C=O), 1076 (C–N stretch); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.07 (s, 1H, CON*H*), 5.72 (d, 2H, J = 11.1 Hz, ring protons), 5.40 (m, 2H, C*H*=C*H*), 3.54 (m, 1H, CH$_2$C*H*(OH)–), 2.27 (t, 2H, J = 7.6 Hz, C*H*$_2$CO), 2.13 (m, 4H, C*H*$_2$-CH=CH–C*H*$_2$), 2.05 (s, 6H, methyl substituents), 1.90 (m, 2H, C*H*$_2$CH$_2$CO), 1.79 (m, 2H, C*H*$_2$CH(OH)–), 1.67 (m, 1H, CH$_2$CH(O*H*)–), 1.38 (m, 2H, C*H*$_2$CH$_3$), 1.21 (broad singlet, 16H, (C*H*$_2$)$_8$), 0.81 (distorted triplet, 3H, C*H*$_3$CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 168.2, 138.7, 137.6, 127.7, 126.9, 104.2, 103.2, 68.6, 39.8, 38.7, 37.5, 36.6, 35.3, 35.0, 34.8, 31.8, 29.3, 26.5, 23.1, 14.5, 10.9, 10.7; MS (m/z): 413.4 [M + Na]$^+$, Calculated for C$_{24}$H$_{42}$N$_2$O$_2$ = 413.5.

3.2.2.5. N-(2′,5′-dimethyl pyrrol-1′-yl)-(9Z, 12R)-9-enyl-12-hydroxyoctadecamide 2(e).
IR (KBr, cm^{-1}): 3410 (OH stretch), 3240, 3234 (NH stretch), 2928 (CH asymmetric), 2854 (CH symmetric), 1671 (amide C=O), 1081 (C–N stretch); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.10 (s, 1H, CON*H*), 5.73 (d, 2H, J = 10.9 Hz, ring protons), 5.46 (m, 2H,

C*H*==C*H*), 3.52 (m, 1H, CH$_2$C*H*(OH)–), 2.26 (t, 2H, J = 7.4 Hz, C*H$_2$*CO), 2.14 (m, 4H, C*H$_2$*–CH==CH==C*H$_2$*), 2.05 (s, 6H, methyl substituents), 1.88 (m, 2H, C*H$_2$*CH$_2$CO), 1.81 (m, 2H, C*H$_2$*CH(OH)-), 1.69 (m, 1H, CH$_2$CH(O*H*)–), 1.37 (m, 2H, C*H$_2$* CH$_3$), 1.19 (broad singlet, 16H, (C*H$_2$*)$_8$), 0.79 (distorted triplet, 3H, C*H$_3$*CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 169.0, 139.8, 138.8, 129.3, 127.4, 106.0, 105.1, 68.4, 38.9, 38.6, 37.1, 36.0, 34.9, 34.7, 33.9, 32.7, 30.1, 28.7, 26.5, 14.9, 11.9, 10.9; MS (*m/z*): 413.3 [M + Na]$^+$, Calculated for C$_{24}$H$_{42}$N$_2$O$_2$ = 413.5.

3.2.3. General procedure for the synthesis of fatty acid esters 3(b–e)

Methyl esters of fatty acids were synthesized by refluxing the fatty acids in methanol in the presence of catalytic amount of concentrated H$_2$SO$_4$ [44].

3.2.4. General procedure for the synthesis of compounds 4(b–e)

A mixture of 0.01 mol of fatty acid methyl ester and 0.01 mol of anthranilic acid were dissolved in 35 ml dry benzene. In the presence of catalytic amount of pyridine, reflux the reaction mixture on water bath for 6–8 h or till complete conversion of ester into the product. After cooling, the reaction mixture was gradually poured into the diluted HCl, then worked up with dichloromethane–water. An oily liquid was obtained; purify this liquid by column chromatography. Spectra of the synthesized compounds are given below.

3.2.4.1. 2-(Dec-9′-enyl)-1,3-benzoxazin-4-one 4(b).
IR (KBr, cm^{-1}): 3075 (CH aromatic), 2924 (CH asymmetric), 2852 (CH symmetric), 1659 (amide C==O), 1523 (C==N), 1090 (C–N stretch), 1158–786 (C–C); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.35–7.33 (m, 4H, aromatic protons), 5.80 (tdd, 1H, $J_{H-^9CH_2}$ = 5.8 Hz, J_{H-Hz} = 10.1 Hz, J_{H-H_E} = 16.2 Hz, CH$_2$==C*H*), 4.99 (dd, 1H, J_{Hz-H} = 10.1 Hz, J_{H_E-Hz} = 3.0 Hz, $_z$HC==CH), 4.92 (dd, 1H, J_{H_E-H} = 16.2 Hz, J_{H_E-Hz} = 3.0 Hz, $_E$HC==CH), 2.30 (t, 2H, J = 7.4 Hz, C*H$_2$*C–O), 1.60 (m, 2H, ==CH–C*H$_2$*), 1.36 (m, 2H, C*H$_2$*CH$_2$C–O), 1.25 (broad singlet, 10H, (C*H$_2$*)$_5$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 174.4, 171.2, 142.2, 134.3, 131.6, 130.8, 129.9, 128.2, 124.5, 122.6, 37.9, 35.1, 29.6, 27.1, 25.5, 22.5, 22.3; MS (*m/z*): 308.4 [M + Na]$^+$, Calculated for C$_{18}$H$_{23}$O$_2$N = 308.3.

3.2.4.2. 2-(Octadec-8′-enyl)-1,3-benzoxazin-4-one 4(c).
IR (KBr, cm^{-1}): 3064 (CH aromatic), 2925 (CH asymmetric), 2858 (CH symmetric), 1631 (amide C==O), 1539 (C==N), 1002 (C–N stretch), 1167–750 (C–C); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.68–6.99 (m, 4H, aromatic protons), 5.28 (m, 2H, C*H*==C*H*), 2.36 (t, 2H, J = 7.6 Hz, C*H$_2$*C–O), 1.95 (m, 4H, C*H$_2$*CH==CHC*H$_2$*), 1.67 (m, 2H, C*H$_2$*CH$_2$C–O), 1.53 (m, 2H, C*H$_2$* CH$_3$), 1.26 (broad singlet, 18H, (C*H$_2$*)$_9$), 0.80 (distorted triplet, 3H, C*H$_3$*CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 174.5, 172.7, 142.0, 135.1, 130.1, 130.0, 128.0, 127.9, 123.8, 122.4, 38.6, 34.1, 31.9, 31.5, 30.2, 29.7, 29.5, 28.1, 27.2, 26.4, 25.6, 24.9, 22.6, 14.1; MS (*m/z*): 406.3 [M + Na]$^+$, Calculated for C$_{25}$H$_{37}$O$_2$N = 406.4.

3.2.4.3. 2-[(8′R,11′Z)-8′-Hydroxyoctadec-11′-enyl]-1,3-benzoxazin-4-one 4(d).
IR (KBr, cm^{-1}): 3394 (OH stretch), 3014 (CH aromatic), 2927 (CH asymmetric), 2856 (CH symmetric), 1637 (amide C==O), 1534 (C==N), 1004 (C–N stretch), 1173–

751 (C–C); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.74–7.08 (m, 4H, aromatic protons), 5.45 (m, 2H, C*H*==C*H*), 3.50 (m, 1H, CH$_2$C*H*(OH)–), 2.44 (t, 2H, J = 7.1 Hz, C*H$_2$*C–O), 2.27 (m, 4H, C*H$_2$*CH==CHC*H$_2$*), 1.75 (m, 1H, CH-O*H*), 1.63 (m, 2H, C*H$_2$*CH$_2$C–O), 1.50 (m, 2H, C*H$_2$*CHOH), 1.24 (broad singlet, 16H, (C*H$_2$*)$_8$), 0.89 (distorted triplet, 3H, C*H$_3$*CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 179.3, 172.3, 141.8, 135.0, 132.5, 131.6, 125.1, 124.8, 122.4, 120.3, 65.9, 38.5, 36.4, 35.2, 34.0, 31.8, 29.3, 28.8, 27.3, 25.6, 25.3, 24.6, 22.5, 15.2, 14.3; MS (*m/z*): 422.2 [M + Na]$^+$, Calculated for C$_{25}$H$_{37}$O$_3$N = 422.4.

3.2.4.4. 2-[(8′Z,11′R)-11′-Hydroxyoctadec-8′-enyl]-1,3-benzoxazin-4-one 4(e).
IR (KBr, cm^{-1}): 3401 (OH stretch), 3034 (CH aromatic), 2928 (CH asymmetric), 2857 (CH symmetric), 1648 (amide C==O), 1537 (C==N), 1028 (C–N stretch), 1159–772 (C–C); ^{1}H NMR (400 MHz, δ, ppm, CDCl$_3$): 8.72–7.10 (m, 4H, aromatic protons), 5.44 (m, 2H, C*H*==C*H*), 3.53 (m, 1H, CH$_2$C*H*OH), 2.45 (t, 2H, J = 7.2 Hz, C*H$_2$*C–O), 2.27 (m, 4H, C*H$_2$*CH==CHC*H$_2$*), 1.73 (m, 1H, CH–O*H*), 1.62 (m, 2H, C*H$_2$*CH$_2$C–O), 1.51 (m, 2H, C*H$_2$*CHOH), 1.29 (broad singlet, 16H, (C*H$_2$*)$_8$), 0.87 (distorted triplet, 3H, C*H$_3$*CH$_2$); ^{13}C NMR (100 MHz, δ, ppm, CDCl$_3$): 178.7, 171.5, 140.7, 133.3, 132.7, 131.8, 125.6, 124.3, 121.9, 120.8, 66.1, 37.6, 36.5, 35.8, 34.6, 31.9, 29.7, 29.1, 27.4, 25.8, 25.4, 24.9, 23.0, 22.1, 20.7, 14.6; MS (*m/z*): 422.5 [M + Na]$^+$, Calculated for C$_{25}$H$_{37}$O$_3$N = 422.4.

3.3. Biological activity

3.3.1. In vitro antibacterial studies

All the newly synthesized novel compounds were dissolved in DMSO to prepare a stock solution of 1 mg/ml. The antibacterial activity of test compounds and standard ciprofloxacin was carried out by the filter paper disc method [45]. Media with DMSO were set up as control. All cultures were consistently maintained on NA (nutrient agar) and incubated overnight at 37 °C. The culture was centrifuged at 1000 rpm and pellets were resuspended then diluted in sterile NSS (normal saline solution) to obtain viable count 10^5 cfu/ml. About, 0.1 ml of diluted bacterial culture suspension was spread accordingly with the help of spreader on NA plates. Sterile discs of thickness 8 mm (Hi-Media Pvt. Ltd.) were impregnated with the test compounds. Antibiotic disc, ciprofloxacin (30 µg/disc Hi-Media) was used as control. The disc was placed on the nutrient agar plate. Each plate had one control disc impregnated with the solvent. The plates were then incubated for 24 h at 37 °C, and the resulting zones of inhibition were measured in mm.

3.3.2. In vitro antifungal studies

The synthesized compounds were dissolved in DMSO and media with DMSO were set up as control. All cultures were consistently maintained on SDA (sabouraud's dextrose agar) and incubated at 28 °C. Spore formation of filamentous fungi was prepared from 7 day old culture in sterile normal solution (8% NaCl) and approximately diluted to obtain 10^5 cfu/ml. The inoculums of non-sporing fungi, *C. albicans* were performed by growing the culture in SD (sabouraud's) broth at 37 °C for overnight. The culture was centrifuged at 1000 rpm and pellets were resuspended and diluted in sterile NSS (nor-

mal saline solution) to obtain viable count 10^5 cfu/ml. About, 0.1 ml of diluted fungal culture suspension was spread uniformly with the help of spreader on SDA plates. Sterile discs (Hi-Media Pvt. Ltd.) were impregnated with the test compounds. Antibiotic disc, nystatin (30 μg/disc Hi-Media) was used as control. The disc was placed on the SDA plate. Each plate had one control disc impregnated with the solvent. The plates were incubated at 28 °C for filamentous fungi for 72 h or more, while for *C. albicans* plates were incubated at 37 °C for 18–48 h. Antifungal activity was determined by measuring the diameters of the inhibition zone (in mm).

4. Conclusions

Here we report the synthesis of two different types of series of heterocyclic derivatives of fatty acids synthesized from the two different substrates. The method reported here for the synthesis of substituted-2,5-dimethyl pyrrole and 1,3-benoxazin-4-one derivatives, was easy and convenient one, which includes the use of safe and cheaper chemicals. The compounds obtained from the synthesis were in appreciable yield. All these compounds were found to be good antimicrobial agents. Compounds **2d**, **2e**, **4d** and **4e** showed excellent antibacterial activity against gram-negative bacteria and good activity against gram-positive bacteria. These compounds were also found to be good antifungal agent against *C. albicans* species. On the basis of the above mentioned antimicrobial results, these compounds showed promising results in the field of pharmaceutical chemistry for future development.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2014.04.008.

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