ORIGINAL ARTICLE

Synthesis, biological screening of novel long chain derivatives of 1,3-disubstituted-1H-pyrazol-5(4H)-one and 2-substituted-3H-1,4-phthalazin-1,4-dione: Structure-activity relationship studies

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Abstract The main purpose of this study is to synthesize novel heterocyclic derivatives of fatty acids which are also biologically important. The simple, efficient and one-pot synthesis of two novel series of 1-long chain alkanoyl/alkenoyl/hydroxyalkenoyl-3-methyl-1H-pyrazol-5(4H)-ones **2(a–e)** and 2-long chain alkenoyl/hydroxyalkenoyl-3H-phthalazin-1,4-diones **3(b–e)** is achieved by the reaction of ethylacetoacetate/phthalic anhydride and long chain alkyl/alkenyl/hydroxyalkenyl hydrazides **1(a–e)**. Although some methods are available for the synthesis of phthalazindiones and pyrazolones, the development of a new synthetic method for the efficacious build up of hetero-cycles (phthalazindiones and pyrazolones) substituted with long alkanoyl/alkenoyl/hydroxyalke-noyl chain is an interesting challenge in the field of synthesis of novel compounds of fatty acids that includes heterocyclization and derivatization of fatty acids. Compounds **2(a–e)** were synthesized by the cyclization reaction between ethylacetoacetate and long alkyl/alkenyl/hydroxyalkenyl chain hydrazides **1(a–e)**. Compounds **3(b–e)** were synthesized by the reaction of phthalic anhydride and long alkenyl/hydroxyalkenyl chain hydrazides **1(b–e)** in absolute ethanol/glacial AcOH. Structures of all the newly synthesized compounds have been elucidated by means of IR, ^{1}H NMR, ^{13}C NMR and MS. Newly synthesized compounds were evaluated for *in vitro* antibacterial and anti-fungal activities and their structure–activity relationship studies have been carried out.

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1. Introduction

Nowadays, human beings are totally dependent on the medicines derived from heterocyclic rings. In many pharmaceutically active compounds heterocyclic rings are found, because both heterocycles and medicines are interrelated. The phthalazine nucleus also has very marked pharmacological and biological applications due to its antitumour (Loh et al., 2005), antihypertensive (Demirayak et al., 2004), antithrombotic (Johnsen et al., 2003), antidiabetic (Lenz et al., 2002) and anti-inflammatory (Dogruer et al., 2004) activities. Pyrazole ring represents an interesting template for medicinal and combinatorial chemistry (Pevarello et al., 2006) because they play a key role in biologically active compounds. Indeed, several pyrazole derivatives have been reported to possess antimicrobial (Vijesha et al., 2011), antiviral (Narule, 2011), antiandrogenic (Amr et al., 2006), analgesic (Parashar et al., 2010), antiinflammatory (Dohutia et al., 2013), antidiabetic (Das et al., 2008) and antioxidant (Mohan and Ananthan, 2011) properties. Pyrazole and pyrazolone derivatives were also reported to possess antiinflammatory, postmenopausal, osteoporosis, antagonists,

anticoagulants and angiotensin activities (Lee et al., 2003). In drug molecules such as celecoxib (a) (Singh et al., 2004), pyrazofurine (b) (Dömling and Ugi, 2000) and many others, the pyrazole ring is the key moiety. Literature survey also reveals that (Z)-4-(4-hydroxy-3-methoxy benzylidene)-1-(3-nitrophenyl)-3-phenyl-1H-pyrazol-5(4H)-one (c) (a pyrazolone derivative) was found to be a non-steroidal Farnesoid X Receptor (FXR) selective antagonist (Huang et al., 2012; Ma et al., 2010). Azelastine (d) is a potent, second-generation, selective histamine antagonist (histamine-H$_1$-receptor antagonist) and it is a member of the phthalazinone family, it shows relevant bronchodilatory activity used for treatment of asthma (McTavish and Sorkin, 1989) and has also been reported to induce vasorelaxation in vitro assays (Lee et al., 1990). Commercially available drug, hydralazine (e), is one of the first antihypertensive drugs developed in 1950s and due to its vasodilator action it is considered as lead for introducing new drugs. Hydralazine belongs to phthalazine drug family and directly acts as a smooth muscle relaxant used to treat hypertension by acting as a vasodilator in arteries and arterioles. A slight change in the structure of hydralazine led to the discovery of some phthalazine derivatives which were
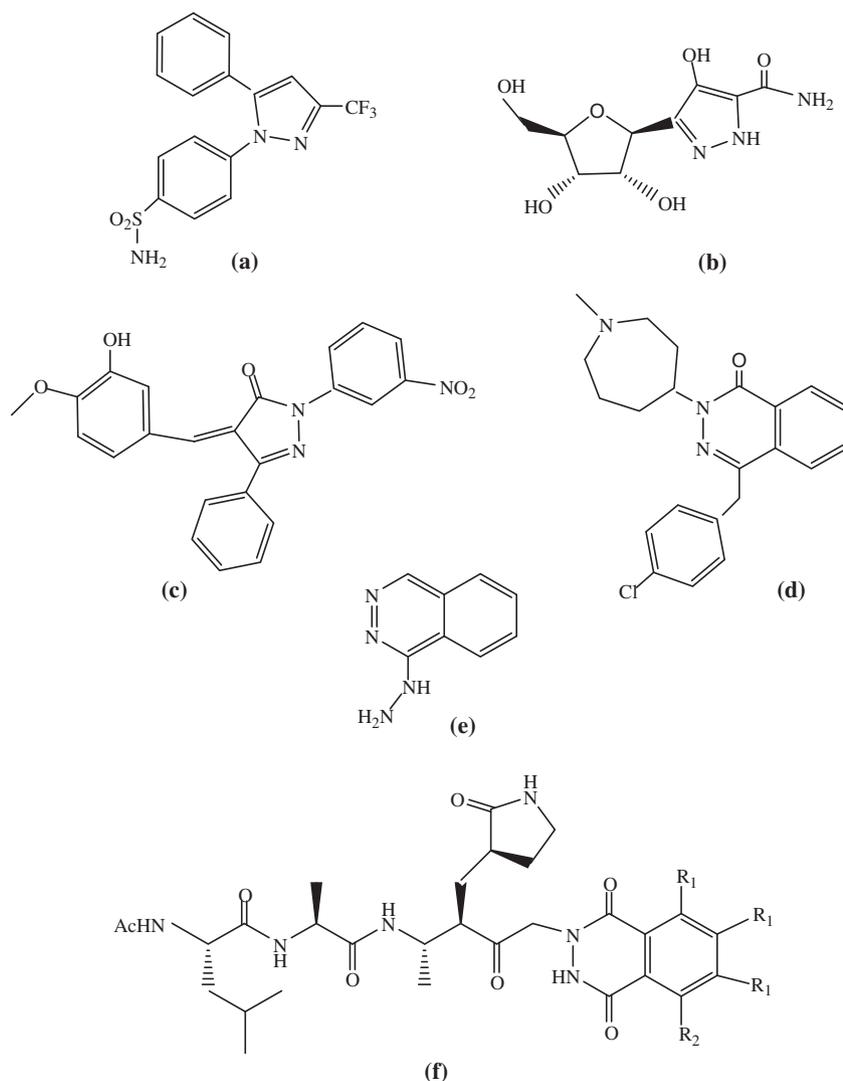

Figure 1 Structurally related drugs to the target compounds.

found to possess antiparasitic, antifungal, antimicrobial, anti-psychotic, antineoplastic activities (Olmo et al., 2006). The well known HAV 3C inhibitor (f) is also a phthalazindione derivative which has been found to possess inhibitory properties against hepatitis A virus 3C proteinase (Jain and Vederas, 2004). Pyrazolo[1,2-b]phthalazine-dione derivatives were reported as antiinflammatory, analgesic, antihypoxic and antipyretic agents (Al'-Assar et al., 2002). Our investigations show that the structure of some drugs which are mainly based on pyrazole, pyrazolone, phthalazine and phthalazinone, phthalazindione nuclei (Fig. 1) is closely related to the structure of the target compounds i.e., 1,3-disubstituted-1H-pyrazol-5(4H)-one and 2-substituted-3H-1,4-phthalazindione fatty acid analogues.

Carboxylic acids having long alkyl/alkenyl/hydroxyalkenyl chains are found to be pharmacologically active antimicrobial agents (Rauf and Parveen, 2005) and these are also useful in the treatment of renal, cardiovascular and hepatic disorders (Greetings et al., 2003). Also, some fatty acid derivatives have been found to possess antitumor activity and antidepressant activity (Khan et al., 2011; Jubie et al., 2012). Vital utility range of pyrazolones and phthalazindiones, fascinating properties of fatty acids and in continuation of our work in derivatization and heterocyclization of selected fatty acids (Farshori et al., 2010, 2011a,b; Varshney et al., 2013) enthused us to design a simple, efficacious and one-pot synthetic route for the synthesis of two novel series of 1,3-disubstituted-1H-pyrazol-5(4H)-one and 2-substituted-3H-1,4-phthalazindione

fatty acid analogues using selected long alkyl/alkenyl/hydroxyalkenyl chain hydrazides as starting materials. Furthermore, this synthetic methodology involves the use of cheap and readily available solvents and commercially available reagents, giving the desired products in good to excellent yields. The ambidexterity of this synthetic route makes it applicable for library synthesis in drug discovery effects.
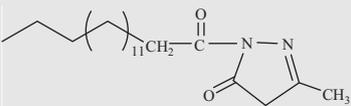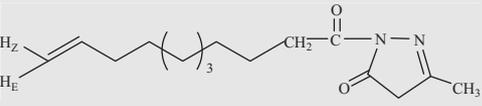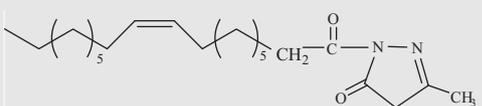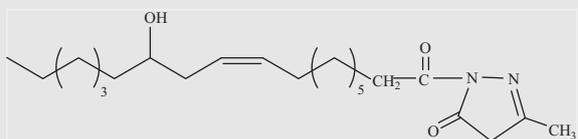
2. Materials and methods

2.1. Physical and spectroscopic measurements

(9Z, 12R)-12-Hydroxyoctadec-9-enoic and (9R, 12Z)-9-hydroxyoctadec-12-enoic acids were isolated from *Ricinus communis* and *Wrightia tinctoria* seed oils respectively following Gunstone's partition (Gunstone, 1954). Undec-10-enoic acid (Purity 98%), (Z)-octadec-9-enoic acid (97%) and palmatic acid were purchased from Fluka Chemicals (Buck Switzerland). Ethylacetoacetate and phthalic anhydride were purchased from Merck, Mumbai, India. Thin layer chromatography (TLC) was done on glass plated with a layer of silica gel G (Merck, Mumbai, India 0.5 mm thickness). Column chromatography was carried out on silica gel (Merck, Mumbai, India, 60–120 mesh)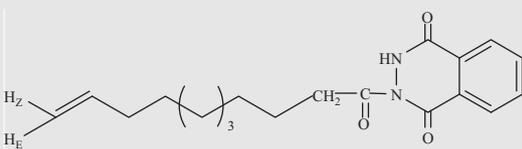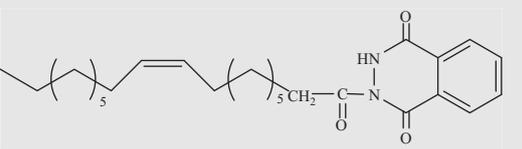. IR spectra were recorded on Shimadzu 8201 PC spectrometer and absorption given in cm^{-1}. ^1H NMR and ^{13}C NMR were recorded in $CDCl_3$ on a Bruker DRX-400 instrument. The chemical shifts (δ) were measured

Compounds	R
1a, 2a	
1b, 2b, 3b	
1c, 2c, 3c	
1d, 2d, 3d	
1e, 2e, 3e	
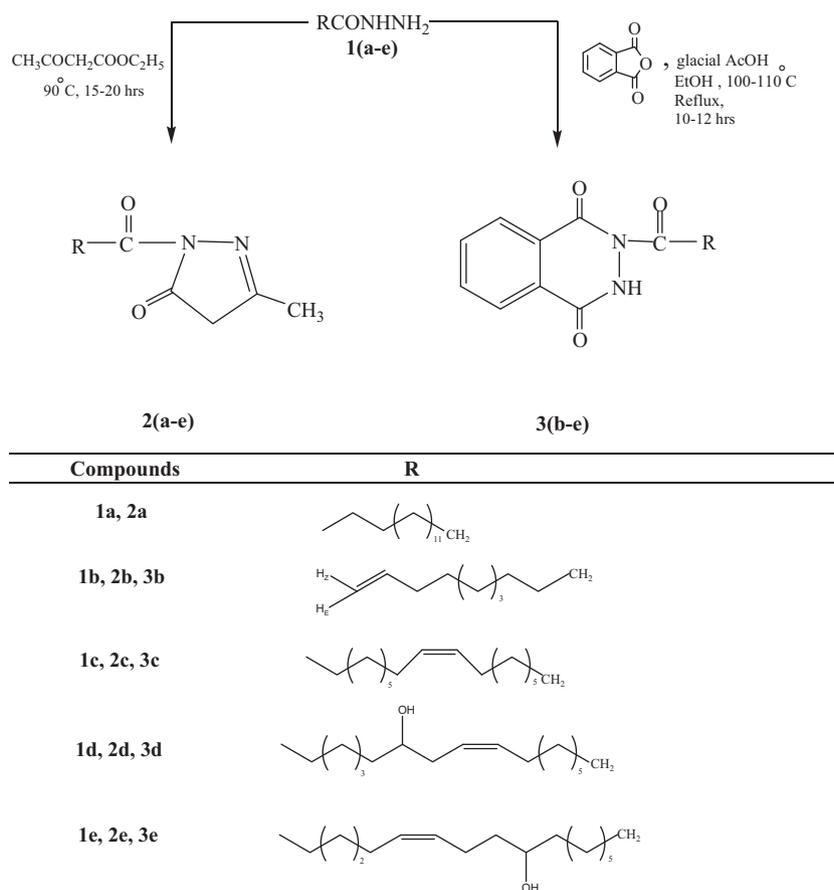
Scheme 1   Synthesis of 1,3-disubstituted-1H-pyrazol-5(4H)-ones and 2-substituted-3H-1,4-phthalazindiones.

Table 1 Structure and physicochemical parameters of all newly synthesized compounds.

Code	Compound	Molecular formula	Molecular weight	Physical state	M.P.	% Yield
2a		$C_{20}H_{36}O_2N_2$	336.44	Colorless oily	–	75
2b		$C_{15}H_{24}O_2N_2$	264.32	Colorless oily	–	74
2c		$C_{22}H_{38}O_2N_2$	362.48	Reddish brown oily	–	70
2d		$C_{22}H_{38}O_3N_2$	378.48	Reddish brown oily	–	71
2e		$C_{22}H_{38}O_3N_2$	378.48	Reddish brown oily	–	73
3b		$C_{19}H_{24}O_3N_2$	328.36	White powder	85–87	85
3c		$C_{26}H_{38}O_3N_2$	426.52	White powder	91–92	82
3d		$C_{26}H_{38}O_4N_2$	442.52	White powder	70–72	70
3e		$C_{26}H_{38}O_4N_2$	442.52	White powder	74–76	74
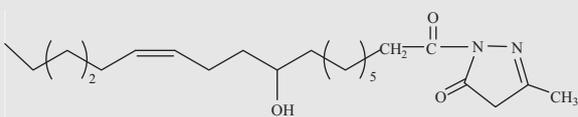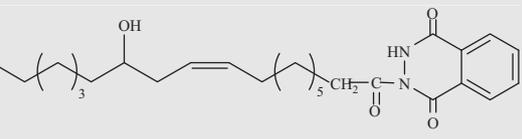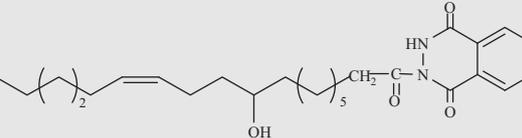
*M.P.: Melting point.

relative to TMS as an internal standard and quoted in ppm. Coupling constants (J) are expressed in Hertz (Hz). The mass spectra were recorded on JEOL-SX 102/DA-600 mass spectrometer.

2.1.1. General procedure for the preparation of 1,3-disubstituted-1H-pyrazol-5(4H)-ones 2(a-e)

Fatty acid hydrazides **1(a–e)** previously synthesized in our laboratory (Rauf et al., 2007) were used as the starting material. A mixture of 0.01 mol of fatty acid hydrazides **1(a–e)** and 0.1 mol (13 mL) of ethylacetoacetate was heated on paraffin bath. After sometime the reaction mixture turned into a reddish oily syrup. The reaction was continued till all the reactants were consumed. After completion of the reaction, the reaction mixture was cooled and worked up with dichloromethane and water. The product was purified by column chromatography. All the products were oily in nature and were elucidated on the basis of their spectral data.

2.1.2. General procedure for the preparation of 2-substituted-3H-1,4-phthalazinediones 3(b-e)

0.1 mole of fatty acid hydrazides **1(b–e)** and 0.1 mol of phthalic anhydride in ethanol (5 mL) were taken in a round bottom flask and 0.005 mol of glacial acetic acid was added. Reaction mixture was refluxed for 10–12 h, and then poured into crushed ice, a solid product was precipitated. All the products were in the form of white powder and were elucidated on the basis of their spectral data. The reaction sequences leading to the formation of these two novel series of compounds are outlined in Scheme 1 and physiochemical parameters of all the newly synthesized compounds are tabulated in the Table 1.

2.2. Biological activity

2.2.1. Antibacterial studies

A stock solution of 1 mg/mL was prepared by dissolving all the newly synthesized compounds in DMSO. Filter paper disc method (FPDM) (Bauer et al., 1966) was used for testing the antibacterial activity of all the target compounds and standard doxycycline Media with DMSO was set up as control. On nutrient agar all cultures were systematically maintained and incubated overnight at 37 °C. At 1000 rpm the culture was centrifuged, pellets were resuspended and then diluted in sterile normal saline solution (NSS) to obtain viable 10^5 cfu/mL. On nutrient agar plates approximately 0.1 mL of diluted bacterial culture suspension was spread uniformly with the help of spreader. Sterile 8 mm discs (Hi-media Pvt. Ltd) were impregnated with the test compounds. Antibiotic disc, doxycycline (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 (mm).

2.2.2. Antifungal studies

The synthesized compounds were dissolved in DMSO. Media with DMSO was 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 saline solution (8% NaCl) and approximately diluted to obtain 10^5 cfu/mL. The inoculums of non-sporing fungi, *Candida albicans* were performed by growing the culture in SD (sabouraud's) broth at 37 °C for overnight. At 1000 rpm the culture was centrifuged, pellets were resuspended and then diluted in sterile normal saline solution (NSS) to obtain viable 10^5 cfu/mL. On SDA plates approximately 0.1 mL of diluted fungal culture suspension was spread uniformly with the help of spreader. Sterile 8 mm 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 onto 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 (mm).

3. Results and discussion

3.1. Chemistry

The present study is based on the synthesis and characterization of two novel series of pyrazolone and phthalazindione derivatives of long chain fatty acids. The fatty acid hydrazides **1(a–e)** were used as the starting material and they were prepared from the fatty acids following previously reported method (Rauf et al., 2007). The derivatives of 1,3-disubstituted-1H-pyrazol-5(4H)-ones **2(a–e)** were synthesized by the condensation of long chain alkyl, alkenyl (internal and terminal) and hydroxyalkenyl carboxylic acid hydrazides **1(a–e)** with ethylacetoacetate, followed by cyclization. The synthesized compounds were identified on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS. The structure of compound **2b** was confirmed by the appearance of an absorption band at 1603 cm^{-1} due to C=N stretch. Another absorption band for C=O was observed at 1698 cm^{-1}. The absorption band at 2932 cm^{-1} was observed for aliphatic stretch. The ^{1}H NMR spectrum was more informative in assigning the structure. In addition to peaks of fatty acid chain, the singlet at δ4.18 for two protons of pyrazolone ring CH$_2$ was observed along with a singlet at δ1.57 for CH$_3$ protons which is directly attached to the ring. In ^{13}C NMR, peaks at δ178.6, δ130.2, δ50.8 were observed. Further evidence was obtained by recording mass spectrum. The mass spectrum showed characteristic molecular ion peak which was in accordance with the molecular formula. Similar type of spectral data were obtained for compounds **2a**, **2c**, **2d** and **2e**.

Similarly, the other novel series of 2-substituted-3H-1,4-phthalazindione derivatives **3(b–e)** were synthesized by the condensation of long chain alkenyl (internal and terminal) and hydroxyalkenyl carboxylic acid hydrazides **1(b–e)** with phthalic anhydride in ethanol and catalytic amount of glacial acetic acid was used. Products were in the form of white powder. All the newly synthesized compounds were characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS. Compound **3b** showed IR absorption bands at 3228 cm^{-1} for N-H stretch, at 2925 cm^{-1} for C-H stretch and at 1599 cm^{-1} for C=O

stretch. The ^{1}H NMR showed a singlet at δ8.01 corresponding to the N–H proton. Also a multiplet at δ7.74–7.89 was observed for four aromatic protons. In ^{13}C NMR, peaks at δ171.8, δ165.3, δ139.2, δ134.7, δ130.9, δ129.1, δ128.4, and δ127.1 were observed. The mass spectrum showed a characteristic molecular ion peak which was in accordance with the molecular formula. Similar type of spectral data were obtained for compounds **3c**, **3d** and **3e**.

3.1.1. The spectroscopic and analytical data for the synthesized compounds 2(a–e) and 3(b–e) are presented below

3.1.1.1.   1-(Hexadecanoyl)-3-methyl-1H-pyrazol-5(4H)-one (2a). IR (KBr, cm^{-1}): 2920 (C–H stretching), 1680 (C=O stretching), 1592 (C=N stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 4.12 (2H, s, CH$_2$ ring), 2.25 (2H, t, J = 7.59 Hz, CH$_2$CO), 1.95 (2H, m, CH$_2$CH$_2$CO), 1.56 (3H, s, CH$_3$ ring), 1.22 (24H, br.s, (CH$_2$)$_{12}$ chain), 0.85 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 176.9, 165.4, 127.9, 45.8, 35.6, 33.8, 33.7, 32.9, 31.8, 31.2, 30.0, 29.9, 29.8, 29.7, 27.4, 27.0, 25.4, 23.8, 22.8, 14.0. MS (ESI): m/z = 359.500 found [M + Na]$^+$, calculated [M + Na]$^+$ = 401.470.

3.1.1.2.   1-(Undec-10′-enoyl)-3-methyl-1H-pyrazol-5(4H)-one (2b). IR (KBr, cm^{-1}): 2932 (C-H stretching), 1698 (C=O stretching), 1603 (C=N stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 5.82 (1H, tdd, $J_{H-^9CH_2}$ = 6.50H_Z, J_{H-H_Z} = 10.30H_Z, J_{H-H_E} = 17.10 H_Z, CH$_2$ = CH), 5.01 (1H, dd, J_{H_Z-H} = 10.30H_Z, $J_{H_Z-H_E}$ = 2.00H_Z, H_ZC = CH), 4.91 (1H, dd, J_{H_E-H} = 16.90H_Z, $J_{H_E-H_Z}$ = 2.00H_Z, H_EC = CH), 4.18 (2H, s, CH$_2$ ring), 2.51 (2H, t, J = 7.38 Hz, CH$_2$CO), 2.06 (2H, m, CH$_2$ = CH-CH$_2$), 1.71 (2H, m, CH$_2$CH$_2$CO), 1.57 (3H, s, CH$_3$), 1.25 (10H, br.s, CH$_2$(CH$_2$)$_5$). ^{13}C NMR (CDCl$_3$, δ$_C$): 178.6, 174.0, 130.2, 130.0, 129.9, 50.8, 34.4, 33.9, 31.9, 30.8, 30.1, 29.7, 27.1, 24.2, 18.0. MS (ESI): m/z = 287.121 found [M + Na]$^+$, calculated [M + Na]$^+$ = 287.310.

3.1.1.3.   1-[(9′Z)   (Octadec-9′-enoyl)]-3-methyl-1H-pyrazol-5(4H)-one (2c). IR (KBr, cm^{-1}): 2924 (C-H stretching), 1700 stretching, 1598 (C = N stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 5.35 (2H, m, CH = CH), 4.20 (2H, s, CH$_2$ ring), 2.36 (2H, t, J = 7.52 Hz, CH$_2$CO), 2.24 (4H, m, CH$_2$CH = CHCH$_2$), 2.00 (2H, m, CH$_2$CH$_2$CO), 1.61 (3H, s, CH$_3$), 1.25 (20H, br.s, (CH$_2$)$_{10}$), 0.88 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 180.1, 165.8, 127.2, 123.1, 122.9, 48.1, 35.0, 33.1, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 27.1, 25.3, 25.0, 24.7, 15.4, 14.1. MS (ESI) m/z = 385.381 found [M + Na]$^+$, calculated [M + Na]$^+$ = 385.471.

3.1.1.4.   1-[(9′Z,   12′R)-12′-Hydroxy-octadec-9′-enoyl]-3-methyl-1H-pyrazol-5(4H)-one (2d). IR (KBr, cm^{-1}): 3400 (O-H stretching), 2929 (C–H stretching), 1685 (C = O stretching), 1579 (C = N stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 5.34 (2H, m, CH = CH), 4.87 (1H, m, CHOH), 4.11 (2H, s, CH$_2$ ring), 2.27 (2H, t, J = 7.47 Hz, CH$_2$CO), 2.21 (1H, m, CHOH), 1.96 (4H, m, CH$_2$CH = CHCH$_2$), 1.85 (2H, m, CH$_2$CH$_2$CO), 1.55 (3H, s, CH$_3$ ring), 1.21 (18H, br.s, (CH$_2$)$_9$), 0.80 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 182.9, 173.9, 133.2, 132.8, 123.8, 72.0, 50.1, 37.4, 36.7, 34.3, 31.9, 31.7, 30.1, 29.6, 29.5, 29.1, 28.9, 27.3, 25.6, 24.9, 15.9, 14.1. MS (ESI) m/z = 401.299 found [M + Na]$^+$, calculated [M + Na]$^+$ = 401.470.

3.1.1.5.   1-[(9′R,   12′Z)-9′-Hydroxy-octadec-12′enoyl]-3-methyl-1H-pyrazol-5(4H)-one (2e). IR (KBr, cm^{-1}): 3391 (O-H stretching), 2933 (C–H stretching), 1680 (C = O stretching), 1570 (C = N stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 5.35 (2H, m, CH = CH), 4.86 (1H, m, CHOH), 4.13 (2H, s, CH$_2$ ring), 2.25 (2H, t, J = 7.50 H$_Z$, CH$_2$CO), 2.19 (1H, m, CHOH), 1.95 (4H, m, CH$_2$CH = CHCH$_2$), 1.86 (2H, m, CH$_2$CH$_2$CO), 1.53 (3H, s, CH$_3$ ring), 1.23 (18H, br.s, (CH$_2$)$_9$), 0.82 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 181.9, 176.9, 133.1, 132.5, 123.0, 72.9, 50.4, 38.4, 37.7, 33.3, 31.8, 31.7, 30.2, 29.5, 29.2, 29.0, 28.6, 27.1, 25.7, 24.6, 15.9, 14.3. MS (ESI) m/z = 401.302 found [M + Na]$^+$, calculated [M + Na]$^+$ = 401.470.

3.1.1.6.   2-(Undec-10′-enoyl)-3H-1,4-phthalazindione (3b). IR (KBr, cm^{-1}): 3228 (N-H stretching), 2925 (C–H stretching), 1599 (C = O stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 8.01 (1H, s, NH), 7.74–7.89 (4H, m, ArH), 5.80 (1H, tdd, $J_{H-^9CH_2}$ = 6.71 H$_Z$, J_{H-H_Z} = 10.90 H$_Z$, J_{H-H_E} = 16.82 H$_Z$, CH$_2$ = CH), 5.01 (1H, dd, J_{H_Z-H} = 10.11 H$_Z$, $J_{H_Z-H_E}$ = 2.20 H$_Z$, H_ZC = CH), 4.93 (1H, dd, J_{H_E-H} = 17.45 H$_Z$, $J_{H_E-H_Z}$ = 2.20 H$_Z$, H_EC = CH), 2.39 (2H, t, J = 7.50 Hz, CH$_2$CO), 2.03 (2H, m, CH$_2$ = CH–CH$_2$), 1.70 (2H, m, CH$_2$CH$_2$CO), 1.28 (10H, br.s, CH$_2$(CH$_2$)$_5$). ^{13}C NMR (CDCl$_3$, δ$_C$): 175.1, 171.8, 165.3, 139.2, 134.7, 130.9, 129.1, 128.4, 127.1, 124.0, 114.1, 33.9, 33.8, 29.3, 29.2, 29.0, 28.9, "one signal hidden", 25.1. MS (ESI): m/z = 351.333 found [M + Na]$^+$, calculated [M + Na]$^+$ = 351.353.

3.1.1.7.   2-[(Z)-Octadec-9′-enoyl]-3H-1,4-phthalazindione (3c). IR (KBr, cm^{-1}): 3232 (N-H stretching), 2922 (C–H stretching), 1594 (C = O stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 8.36 (1H, s, NH), 7.77–7.92 (4H, m, ArH), 5.34 (2H, m, CH = CH), 2.41 (2H, t, J = 7.40 H$_Z$, CH$_2$CO), 2.22 (4H, m, CH$_2$CH = CHCH$_2$), 1.70 (2H, m, CH$_2$CH$_2$CO), 1.29 (20H, br.s, (CH$_2$)$_{10}$), 0.87 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 174.8, 170.1, 167.2, 138.1, 134.9, 133.5, 131.9, 128.1, 127.7, 118.4, 115.6, 36.6, 34.2, 31.5, 31.2, 30.5, "two signals hidden", 29.5, 29.2, 29.0, 28.7, 27.5, 26.2, 24.1, 14.1. MS (ESI) m/z = 449.500 found [M + Na]$^+$, calculated [M + Na]$^+$ = 449.510.

3.1.1.8.   2-[(9′Z, 12′R)-12′-Hydroxy octadec-9′-enoyl]-3H-1,4-phthlazindione (3d). 3392 (O-H stretching), 3227 (N-H stretching), 2921 (C–H stretching), 1585 (C = O stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 8.41 (1H, s, NH), 7.34–7.67 (4H, m, ArH), 5.50 (2H, m, CH = CH), 4.21 (1H, m, CHOH), 2.54 (2H, t, J = 7.79 H$_Z$, CH$_2$CO), 2.19 (1H, m, CHOH), 1.80 (4H, m, CH$_2$CH = CHCH$_2$), 1.65 (2H, m, CH$_2$CH$_2$CO), 1.27 (18H, br.s, (CH$_2$)$_9$), 0.88 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 173.1, 168.8, 163.9, 134.9, 133.4, 132.7, 130.1, 126.7, 123.4, 118.8, 114.7, 72.0, 38.1, 38.0, 37.2, 36.7, 35.1, 33.9, "one signal hidden", 32.1, 29.4, 28.5, 27.6, 24.8, 23.1, 13.9. MS (ESI) m/z = 465.490 found [M + Na]$^+$, calculated [M + Na]$^+$ = 465.513.

3.1.1.9.   2-[(9′R, 12′Z)-9′-Hydroxy octadec-12′-enoyl]-3H-1,4-phthalazindione (3e). 3372 (O–H stretching), 3217 (N–H stretching), 2918 (C–H stretching), 1591 (C = O stretching). ^{1}H NMR (CDCl$_3$, δ$_H$): 8.42 (1H, s, NH), 7.29–7.57 (4H, m,

ArH), 5.480 (2H, m, CH = CH), 4.29 (1H, m, CHOH), 2.34 (2H, t, J = 7.59 H$_Z$, CH$_2$CO), 2.20 (1H, m, CHOH), 1.81 (4H, m, CH$_2$CH = CHCH$_2$), 1.71 (2H, m, CH$_2$CH$_2$CO), 1.25 (18H, br.s, (CH$_2$)$_9$), 0.81 (3H, dist.t, CH$_3$). ^{13}C NMR (CDCl$_3$, δ$_C$): 174.5, 169.1, 162.6, 134.0, 133.9, 132.5, 131.5, 125.7, 124.4, 119.0, 112.9, 71.0, 38.9, 38.1, 37.6, 36.7, 35.8, 33.0, "one signal hidden", 32.1, 30.0, 29.5, 28.4, 26.4, 24.9, 14.4. MS (ESI) m/z = 465.600 found [M + Na]$^+$, calculated [M + Na]$^+$ = 465.513.

3.2. Biology

All the newly synthesized compounds were assessed for *in vitro* antibacterial activity against an assortment of two Gram-positive bacteria, *Staphylococcus aureus* SA 22, *Bacillus subtilis* MTCC 121, and two Gram-negative bacteria, *Escherichia coli* K12, *Pseudomonas aeruginosa*. Doxycycline was used as the standard drug for the comparison of the antibacterial activity results. The *in vitro* antimicrobial screening results are given in Table 2. Graphic representation of the biological screening results is depicted in Fig. 2(A–E) in terms of diameter of zone inhibition (in mm). The newly synthesized compounds **2(a–e)** and **3(b–e)** have exerted significant inhibitory activity against the growth of the tested bacterial strains. The antibacterial screening results showed that among the tested bacterial strains, good inhibitory results were obtained against *E. coli* and *P. aeruginosa* as depicted in Fig. 2(C) and (D). Among the tested compounds, **3d** and **3e** showed more potent inhibitory activity against both types of bacteria. However, depending on the nature of the heterocyclic moiety (phthalazindione/pyrazolone) and substituents (long alkanoyl/alkenoyl/hydroxy alkenoyl chains) attached to it exerted varying inhibitory actions. In another set of experiments, all the newly synthesized compounds **2(a–e)** and **3(b–e)** were also screened for *in vitro* antifungal activity against *C. albicans* IOA 109. Nystatin was
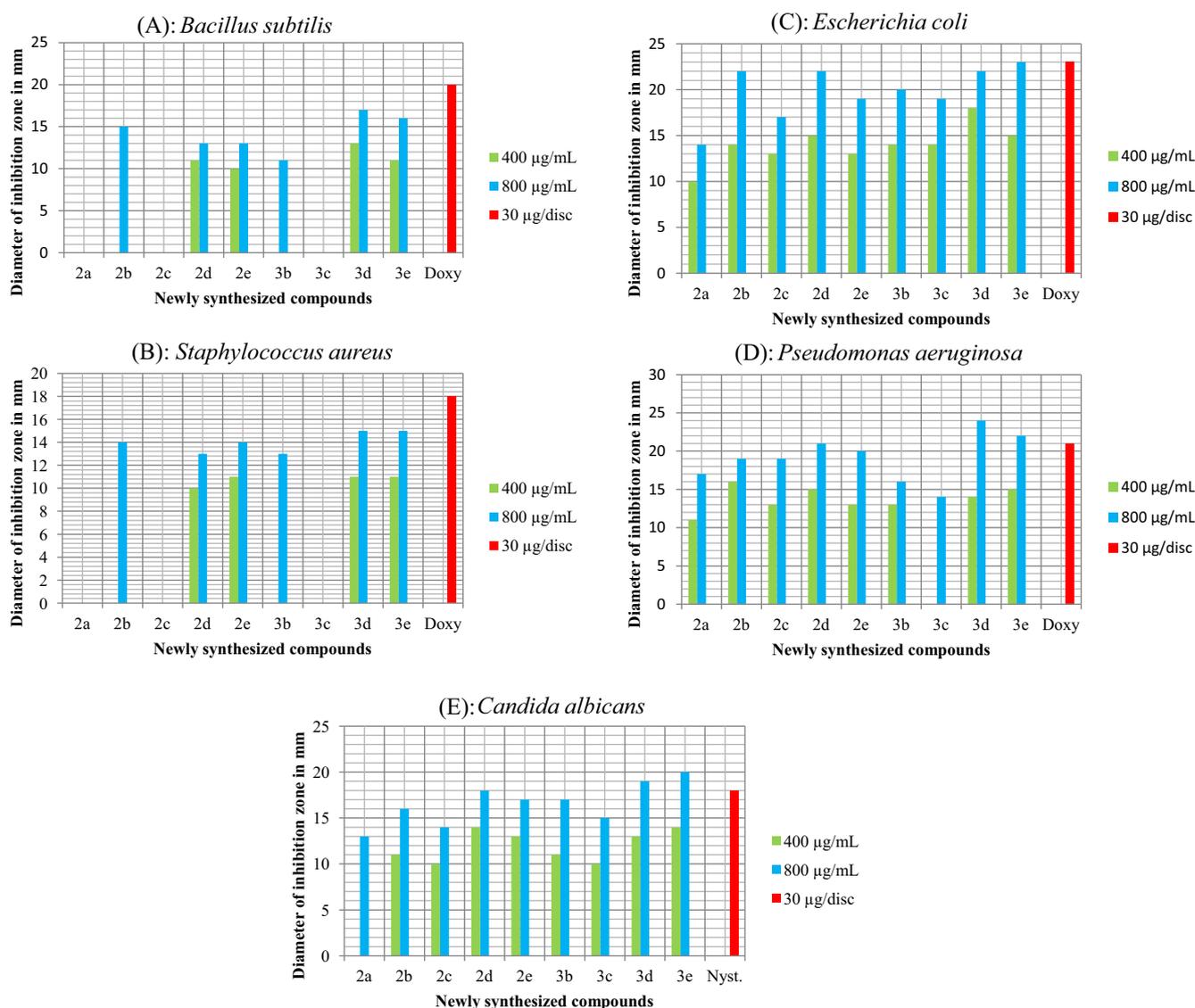
Figure 2 (A–E): Graphic representation of the antimicrobial screening results in terms of diameter of inhibition zone (in mm) against various microbial strains. Fig. 2(A–D) shows the antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively, standard used was Doxycycline. Fig. 2(E) shows the antifungal activity against *Candida albicans*, standard used was Nystatin.

Table 2 *In vitro* antimicrobial screening data of compounds **2(a–e)** and **3(b–e)**.

Test sample	Concentration (μg/mL)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
2a	400	–	–	10	11	–
	800	–	–	14	17	13
2b	400	–	–	14	16	11
	800	15	14	22	19	16
2c	400	–	–	13	13	10
	800	–	–	17	19	14
2d	400	11	10	15	15	14
	800	13	13	22	21	18
2e	400	10	11	13	13	13
	800	13	14	19	20	17
3b	400	–	–	14	13	11
	800	11	13	20	16	17
3c	400	–	–	14	–	10
	800	–	–	19	14	15
3d	400	13	11	18	14	13
	800	17	15	22	24	19
3e	400	11	11	15	15	14
	800	16	15	23	22	20
NA	Doxycycline 30 (μg/disc)	20	18	23	21	
Nystatin	30 (μg/disc)	NA	NA	NA	NA	18

*NA: Not applicable.

used as the standard drug for the comparison of the antifungal results. The synthesized compounds showed moderate to excellent inhibitory results for *C. albicans* as seen in Fig. 2(E).

3.2.1. Structure–activity relationship (SAR) studies

The structure–activity studies showed that depending on the nature of the heterocyclic skeleton and its substituent, the newly synthesized compounds **2(a–e)** and **3(b–e)** exhibit varying degree of microbial inhibition. From the antimicrobial screening results, it has been found that the phthalazindione derivatives **3(b–e)** have shown better activity as compared to their corresponding pyrazolone derivatives **2(a–e)**. Within the same heterocyclic series that is either in phthalazindione derivatives or in pyrazolone derivatives, the inhibitory activity depends on the nature of substituents attached to them. The *in vitro* biological screening indicated that the presence of the hydroxy substituents attached to the heterocyclic moiety as in compounds (**2d, 2e, 3d** and **3e**) increased their inhibitory activities compared to other compounds. Structure activity relationship (SAR) studies cleared that, compounds with terminal alkenoyl substituent (**2b** and **3b**) and smaller carbon chain (C_{10}) have better inhibitory activity compared with compounds (**2c** and **3c**) which have internal alkenoyl substituent and longer carbon chain (C_{17}). Further, SAR studies show that heterocyclic moiety having alkanoyl substituent (**2a**) which is analogue of saturated fatty acid and having long carbon atom chain (C_{15}) shows very small inhibitory results. The values of diameter of zone of inhibition in mm for all the newly synthesized compounds are pictorially represented in graphical form in Fig. 2(A–E) against *B. subtilis*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *C. albicans,* respectively. The maximum inhibition was observed in phthalazindione derivatives substituted with hydroxy substituent i.e., **3d** and **3e** against *P. aeruginosa* with zone diameter of 14 and 15 mm, respectively (at 400 μg/mL concentration) and also with zone diameter of 24 and 22 mm, respectively (at 800 μg/mL concentration) as seen in Fig. 2(D). The compounds **3d** and **3e** also show good inhibitory activity against *E. coli* with zone diameter of 18 and 15 mm, respectively (at 400 μg/mL concentration) and with zone diameter of 22 and 23 mm, respectively (at 800 μg/mL concentartion) as depicted in Fig. 2(C). Hence, we summarized that higher activity of compounds **2d, 2e, 3d** and **3e** may be attributed to the presence of hydroxy substituents. Also, the synthesized compounds with phthalazindione rings substituted with hydroxy group (**3d** and **3e**) show excellent activity as compared to pyrazolone rings substituted with hydroxy group (**2d** and **2e**). Thus, the nature of substituents and the heterocyclic skeleton of molecules have a strong influence on the extent of antibacterial and antifungal activities.

4. Conclusion

To the best of our knowledge these alkanoyl/alkenoyl/hydroxyalkenoyl pyrazolone and phthalazindione derivatives of some selected fatty acids have been synthesized for the first time. The salient features of these procedures includes mild reaction conditions, use of inexpensive reagents and starting material, products were obtained in good to excellent yields. The structure–activity studies showed that depending on the nature of heterocyclic skeleton and its substituent, the newly synthesized compounds **2(a–e)** and **3(b–e)** exhibit varying degree of microbial inhibition. The *in vitro* biological screening results show that compounds having hydroxy substituent attached to the heterocyclic moiety (**2d, 2e, 3d** and **3e**) were the most promis-

ing antimicrobial agents. The maximum inhibition was observed in phthalazindione derivatives substituted with hydroxy substituent i.e., **3d** and **3e**. The synthesis will be a valuable addition to the synthetic methodology available for the synthesis of heterocyclic derivatives of fatty acids which are useful biologically as well as industrially. From these studies it is understandable that further derivatization and heterocyclization of these hetero-analogues of fatty acids can be served as new templates for antimicrobial drug discovery and could probably lead to more potent agents in this field.

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