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Synthesis, characterization, and in vitro antimicrobial activities of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles and thiadiazoles

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ABSTRACT

The long-chain alkenoic acid hydrazides (**1a–d**) on reaction with phenylisocyanate and phenylthiocyanate gave their corresponding semicarbazides (**2a–d**) and thiosemicarbazides (**4a–d**), which on further refluxing with $POCl_3$ and Ac_2O yielded corresponding 1,3,4-oxadiazoles (**3a–d**) and thiadiazoles (**5a–d**), respectively.

The structure elucidation of synthesized compounds is based on the elemental analysis and spectral data (IR, ^1H NMR, ^{13}C NMR and MS). The synthesized oxadiazoles and thiadiazoles have been screened for antibacterial and antifungal activities. The investigation of antimicrobial screening revealed that compounds **3c**, **3d**, **5c**, **5d** and compounds **3b**, **5b**, showed good antibacterial and antifungal activities, respectively.

A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules. Among them the derivatives of oxadiazoles and thiadiazoles have played an important role in the medicinal chemistry. These heterocycles have been found to possess broad-spectrum antimicrobial activity and many other uses.[1,2] The therapeutic effects of compounds containing 1,3,4-thiadiazole rings have been studied for a number of pathological conditions including inflammation[3,4], pain,[5–7] and hypertension.[8] Furthermore, the synthesis of thiadiazoles and oxadiazoles has attracted wide attention due to the diversity of their applications as antibacterial[9], antimycobacterial[10,11], antifungal,[12–14] and antidepressant agents.[15]

Various biological applications such as antimicrobial[16,17], antifungal[18], pesticidal,[19] and anticancer activities[20] have also been reported for seed oils, long-chain alkenoic acids and their derivatives.

The wide range of therapeutic values of alkenoic acids and oxadiazoles/thiadiazole ring systems prompted us to synthesize the title compounds and screen them for various antimicrobial activities. The basic idea was to append the long-chain alkenyl/hydroxyalkenyl moiety of the long-chain alkenoic acid to the oxadiazole/thiadiazole nucleus so as to combine the beneficial effects in a single structure with expected biological activities.

In this communication we are reporting the synthesis of 1,3,4-oxadiazoles/thiadiazoles bearing a long alkenyl/hydroxyalkenyl chain substituent at C-5. To the best of our knowledge the synthesis and antimicrobial activity of these newly synthesized compounds has not been reported so far.

The long-chain alkenoic acid hydrazides (**1a–d**), used as the starting material were prepared from corresponding long-chain alkenoic acids by esterification and further treatment with hydrazine hydrate.[21] Reacting **1a–d** with phenyl isocyanate in dry benzene under reflux and removing excess of solvent under reduced pressure gave semicarbazides (**2a–d**). The treatment of **2a–d** with $POCl_3$ yielded 2,5-disubstituted- 1,3,4-oxadiazoles (**3a–d**) in good yields. The reaction of **1a–d** with phenyl isothiocyanate gave corresponding thiosemicarbazides (**4a–d**) which on dehydrative cyclization by Ac_2O produced 2,5-disubstituted- 1,3,4-thiadiazoles (**5a–d**) in excellent yields. The reaction sequences are outlined in Schemes 1 and 2.

The semicarbazide **2a** showed IR bands at 3236 cm^{-1} (NH, NH–NH) and 1667 cm^{-1} (C=O). ^1H NMR was more informative, characteristic peaks were observed at δ 13.04 (1H, s, CO–*NH*-Ar), 9.16 (2H, br s, CO–*NHNH*–CO), 8.17 (2H, d, J = 7.2 Hz, Ar-H-2″/6″), 7.60 (1H, t, J = 7.4 Hz, Ar-H-4″) and 7.51 (2H, t, J = 7.4 Hz, Ar-H-3″/5″). In ^{13}C NMR peaks at δ 168.2 and 165.4 were observed.

The build up of **3a–d** is evident from their spectral data. Compound **3a**, 5-(Dec-9′-enyl)-2-phenylamine-1,3,4-oxadiazole, showed IR absorption bands at 3228 cm^{-1}, 1504 cm^{-1}, 1258 cm^{-1} due to stretching vibrations of NH, C=N and C–O–C functions. The ^1H NMR was more informative in assigning the structure. Diagonastic peaks at δ 9.07 (1H, s, NH), 7.27 (2H, d, J = 7.6 Hz, Ar-H-2″/6″), 7.18 (1H, t, J = 7.5 Hz, Ar-H-4″) and 6.98 (2H, t, J = 7.3 Hz, Ar-H-3″/5″)

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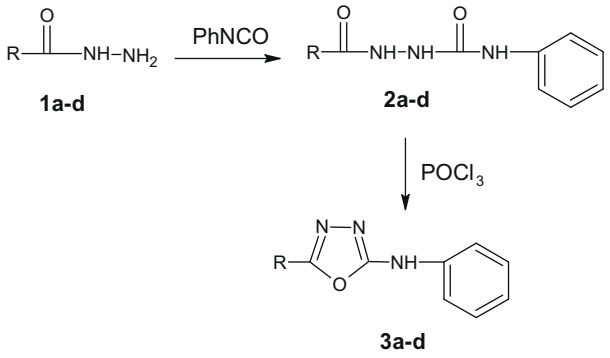
were observed. A triplet at δ 2.24 was observed for CH_2 protons α to oxadiazole ring. In ^{13}C NMR peaks at δ 172.9 and 155.6 were observed for ring carbon atoms. The mass spectra showed characteristic molecular ion peak which were in accordance with the molecular formula.

5-(Dec-9′-enyl)-2-phenylamine-1,3,4-thiadiazole **5a** gave significant IR bands at 3221 cm^{-1} (NH), 1488 cm^{-1} (C=N), and 707 cm^{-1} (C–S–C). ^1H NMR peak at δ 12.20 (1H, s, NH), 8.16 (2H, d, J = 8.5 Hz, Ar-H-2″/6″), 7.55 (1H, t, J = 7.3 Hz, Ar-H-4″), and 7.44 (2H, t, J = 7.8 Hz, Ar-H-3″/5″) were observed in addition to peaks of normal fatty acid chain. In ^{13}C NMR peaks at δ 165.2 and 153.0 were observed for ring carbon atoms. The mass spectra showed characteristic molecular ion peak which were in accordance with the molecular formula. The newly synthesized compounds have been confirmed by their spectral data[28].

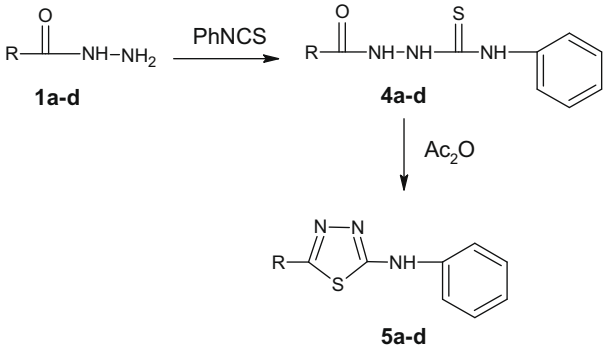
The characterization data of semicarbazides (**2a–d**), oxadiazoles (**3a–d**), and thiadiazoles (**5a–d**) is given in Table 1.

Undec-10-enoic (purity, 98%) and (*Z*)-octadec-9-enoic (97%) acids were purchased from Fluka chemicals (Buck: Switzerland). (9*Z*,12*R*)-12-hydroxyoctadec-9-enoic acid (ricinoleic acid, 98%) and (9*R*,12*Z*)-9-hydroxyoctadec-12-enoic acid (isoricinoleic acid, 98%) were isolated from *Ricinus communis* and *Wrightia tinctoria* seed oils, respectively, following Gunstone's partition procedure.[26] Phenyl thiocyanate, phenyl isocyanate, phosphorous trichloride, and acetic anhydride were purchased from Merck, Mumbai, India. Thin layer chromatography (TLC) was done on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India 0.5 mm thickness). Mixtures of petroleum ether/diethyl ether/acetic acid (70:30:1; v/v) were used as developing solvent. The column chromatography was carried out with silica gel (Merck, Mumbai, India, 60–120 mesh).IR spectra were recorded on Shimadzu 8201 PC spectrophotometer. ^1H NMR spectra were recorded with a Bruker, DRX 400 spectrometer (400 MHz) in $CDCl_3$, using TMS as internal standard. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are given in Hz. ^{13}C NMR spectra were recorded at Bruker DRX 400 spectrometer in $CDCl_3$ with $CDCl_3$ (δ = 77.00).

Antibacterial studies. The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes*, and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method.[22,23] A standard inoculums (1–2 × 10^7 c.f.u./ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar



Scheme 1. Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles **3a–d**.



Scheme 2. Synthesis of 2,5-disubstituted-1,3,4-thiadiazoles **5a–d**.

medium. Solvent and growth controls were kept. Chloramphenicol (30 μg) was used as positive control and the disk poured in DMSO was used as negative 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. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 2.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5 × 10^5 c.f.u./ml of actively dividing bacterial cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 3. The PBE (percentual bacteriostatic efficiency, %) was obtained as,

PBE = 100/MIC

The results have been reported in Table 6.

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds **3a, 3b, 3c, 3d, 5c, 5d** showed good inhibition against all Gram-positive and Gram-negative bacterial strains at 6.25 μg/ml concentrations. The compounds **3c, 3d, 5c** and **5d** were found to be almost equally potent as the reference drug, chloramphenicol, in case of *K. pneumoniae*. The MBC of few compounds was found to be the same as MIC but in most of the compounds it was two or three folds higher than the corresponding MIC results.

Antifungal studies. Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans, Aspergillus fumigatus, Penicillium marneffei,* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method.[24,25] Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty millilitres of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days.

Table 1
Characterization data of synthesized compounds **2a–d**, **3a–d**, and **5a–d**

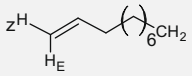
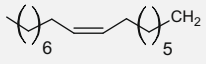
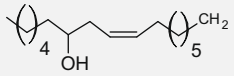
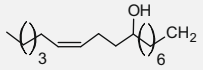
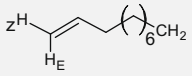
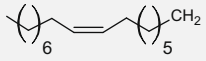
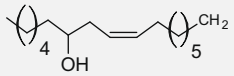
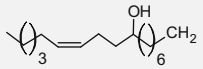
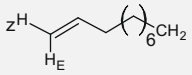
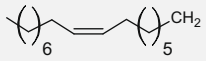
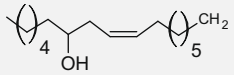
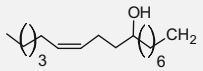
Compounds	R	Mol. formula	Mp (°C)	Yield (%)	Analysis (%) found (calculated)		
					C	H	N
2a		$C_{18}H_{27}O_2N_3$	103–104	80	67.67(68.11)	8.44(8.56)	13.09(13.23)
2b		$C_{25}H_{41}O_2N_3$	101–102	79	71.95(72.25)	9.86(9.93)	10.02(10.11)
2c		$C_{25}H_{41}O_3N_3$	111–113	77	69.23(69.57)	9.50(9.56)	09.65(9.73)
2d		$C_{25}H_{41}O_3N_3$	113–114	72	69.33(69.57)	9.20(9.56)	09.65(9.73)
3[a]		$C_{18}H_{25}ON_3$	133–134	92	72.06(72.21)	8.11(8.40)	13.90(14.03)
3b		$C_{25}H_{39}ON_3$	144–146	90	75.23(75.52)	9.74(9.87)	10.44(10.56)
3c		$C_{25}H_{39}O_2N_3$	133–136	87	72.35(72.60)	9.41(9.49)	10.00(10.15)
3d		$C_{25}H_{39}O_2N_3$	135–137	87	72.28(72.60)	9.36(9.49)	09.97(10.15)
5[a]		$C_{18}H_{25}SN_3$	134–135	95	68.30(68.53)	7.78(7.98)	13.07(13.31)
5b		$C_{25}H_{39}SN_3$	138–140	91	72.23(72.59)	9.44(9.49)	10.10(10.15)
5c		$C_{25}H_{39}OSN_3$	136–138	85	69.61(69.89)	9.04(9.14)	09.78(9.78)
5d		$C_{25}H_{39}OSN_3$	139–141	83	69.54(69.89)	9.02(9.14)	09.72(9.78)

Table 2
Antibacterial activity of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles **3a–d** and thiadiazoles **5a–d**

Compounds	Diameter of zone of inhibition (mm)				
	Gram-positive bacteria			Gram-negative bacteria	
	S. Pyogenes	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
3a	18.0 ± 0.5	17.7 ± 0.6	17.3 ± 0.8	16.8 ± 0.8	19.8 ± 0.6
3b	15.6 ± 0.2	15.3 ± 0.2	19.3 ± 0.5	14.9 ± 0.3	17.5 ± 0.3
3c	21.2 ± 0.3	20.2 ± 0.4	25.6 ± 0.2	19.7 ± 0.2	22.8 ± 0.3
3d	20.1 ± 0.2	21.1 ± 0.3	19.4 ± 0.2	20.2 ± 0.1	21.6 ± 0.2
5a	13.5 ± 0.3	13.1 ± 0.5	17.2 ± 0.4	12.8 ± 0.2	15.2 ± 0.4
5b	13.1 ± 0.7	12.9 ± 0.3	16.3 ± 0.6	12.1 ± 0.5	14.8 ± 0.4
5c	22.9 ± 0.8	21.6 ± 0.3	29.2 ± 0.2	22.7 ± 0.6	25.5 ± 0.5
5d	21.1 ± 0.2	20.8 ± 0.4	25.9 ± 0.6	19.9 ± 0.9	22.9 ± 0.2
Standard	23.0 ± 0.2	22.0 ± 0.2	32.0 ± 0.3	19.0 ± 0.2	27.0 ± 0.2
DMSO	–	–	–	–	–

Positive control (standard); chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

The fungal activity of each compound was compared with greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 4. The nutrient broth, which obtained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4–6×10^4 c.f.u./ml. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC).

To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 5. The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC $\geqslant 4$) or fungicidal (MFC/MIC $\leqslant 4$) activity and the results have been summarized in Table 6.

Table 3
MIC and MBC results of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles **3a–d** and thiadiazoles **5a–d**: positive control chloramphenicol

Compounds	Gram-positive bacteria				Gram-negative bacteria					
	S. Pyogenes		*S. aureus*		*P. aeruginosa*		*K. Pneumoniae*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3a	12.5	50.0	6.25	25.0	12.5	50.0	12.5	25.0	12.5	50
3b	25.0	25.0	12.5	50.0	12.5	50.0	12.5	50.0	12.5	50
3c	6.25	12.5	6.25	12.5	6.25	50.0	6.25	6.25	6.25	25
3d	6.25	25.0	12.5	12.5	6.25	50.0	6.25	25.0	6.25	25
5a	25.0	50.0	12.5	25.0	12.5	25.0	25.0	50.0	25.0	100
5b	12.5	100	12.5	25.0	12.5	25.0	25.0	50.0	25.0	50
5c	6.25	12.5	6.25	12.5	6.25	12.5	6.25	6.25	6.50	12.5
5d	6.25	25.0	6.25	12.5	6.25	25.0	6.25	6.25	6.25	12.5
Standard	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5

MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bacterial concentration, that is, the lowest concentration of the compound for killing the bacteria completely.

Table 4
Antifungal activity of 5-Alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles **3a–d** and thiadiazoles **5a–d**: positive control (greseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm)

Compounds	Diameter of zone of inhibition (mm)			
	CA	AF	TM	PM
3a	24.2 ± 0.4	20.8 ± 0.3	16.9 ± 0.9	15.2 ± 1.2
3b	26.1 ± 0.3	23.1 ± 0.2	20.2 ± 1.2	15.9 ± 0.3
3c	23.3 ± 0.9	20.7 ± 0.4	17.5 ± 0.2	13.9 ± 0.3
3d	23.2 ± 0.2	21.6 ± 0.4	18.7 ± 0.2	16.3 ± 0.2
5a	19.5 ± 0.5	16.5 ± 0.4	14.1 ± 0.3	10.2 ± 0.5
5b	27.2 ± 0.2	23.8 ± 1.2	20.3 ± 0.7	16.1 ± 0.2
5c	25.1 ± 0.7	22.4 ± 0.3	18.9 ± 0.5	15.1 ± 0.9
5d	24.9 ± 1.4	21.8 ± 0.2	19.0 ± 0.2	15.2 ± 1.2
Standard	30.0 ± 0.2	27.0 ± 0.2	24.0 ± 0.3	20.0 ± 0.5
DMSO	—	—	—	—

CA = *Candida albicans*, AF = *Aspergillus fumigatus*, TM = *Trichophyton mentagrophytes*, PM = *Penicillium marneffei*.

Table 6
PBE and fungicidal/fungistatic activity (MFC/MIC) of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4- oxadiazoles **3a–d** and thiadiazoles **5a–d**

Compounds	PBE = 100/MIC					MFC/MIC			
	Bacteria tested					Fungi tested			
	SP	SA	PA	KP	EC	CA	AF	TM	PM
3a	8	16	8	8	8	2	4	4	2
3b	4	8	8	8	8	4	2	2	4
3c	16	16	16	16	16	4	4	4	8
3d	16	8	16	16	16	2	2	2	4
5a	4	8	8	4	4	2	4	2	4
5b	8	8	8	4	4	2	4	2	2
5c	16	16	16	16	15.4	2	4	4	2
5d	16	16	16	16	16	2	2	4	4
Chloramphenicol	16	16	16	16	16	—	—	—	—
Greseofulvin	—	—	—	—	—	2	2	2	2

SP = *S. pyogenes*, SA = *S. aureus*, PA = *P. aeruginosa*, KP = *K. Pneumonia*, EC = *E. Coli*, CA = *C. albicans*, AF = *A. fumigatus*, TM = *T. mentagrophytes*, and PM = *P. Marneffei*.

The antifungal screening data showed moderate to good activity. Among the screened compounds **3b** and **5b** were found to be most active against all test fungal strains. The compound **5b** showed maximum activity against *C. albicans*, *A. fumigatus*, and *T. metagrophyte* strains. The compound **3b** was active against *P. marneffei*. The MFC of most of the compounds was two or three folds higher than the corresponding MIC results. Most of the synthesized compounds showed good fungistatic activity against the fungal strain *C. albicans*.

Table 5
MIC and MFC of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles **3a–d** and thiadiazoles **5a–d**, positive control greseofulvin

Compounds	CA		AF		TM		PM	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
3a	25.0	50.0	25.0	100	12.5	50.0	12.5	25.0
3b	6.25	25.0	6.25	12.5	12.5	25.0	6.25	25.0
3c	25.0	100	12.5	50.0	12.5	50.0	12.5	100
3d	12.5	25.0	25.0	50.0	25.0	50.0	25.0	100
5a	25.5	50.0	25.0	100	25.0	50.0	25.0	100
5b	6.25	12.5	6.25	25.0	6.25	12.5	6.25	12.5
5c	12.5	25.0	12.5	50.0	12.5	25.0	25.0	50.0
5d	25.0	50.0	25.0	50.0	25.0	100	25.0	100
Std	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5

CA = *Candida albicans*, AF = *Aspergillus fumigatus*, TM = *Trichophyton mentagrophytes*, PM = *Penicillium marneffei*; **Std** = standard. MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (μg/ml) = minimum fungicidal.

The present stratagem describes, the synthesis of new 4,5-disubstituted-1,3,4-oxadiazoles and thiadiazoles using long-chain alkenoic acids as the starting material. The described compounds have been variously characterized and identified by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. The potential antimicrobial effects of the synthesized compounds were investigated against *S. pyogenes, S. aureus, P. aeruginosa, K. pneumoniae* and *E. coli* bacterial strains and *C. albicans, A. fumigatus, T. mentagrophytes* and *P. marneffei* fungal strains. Among the synthesized oxadiazoles/thiadiazoles, the compounds with a hydroxyalkenyl chain substituent at fifth position of oxa/thiadiazoles were found to increase the antibacterial activity in compounds **3c**, **3d**, **5c** and **5d**. However the position of the hydroxyl group had no significant effect on the magnitude of the antibacterial activity. Further, the compounds showed parallel activity against Gram-positive and Gram-negative bacterial strains. The compounds with an internal double bond in the long alkenyl substituent of synthesized oxa/thiadiazoles were found to be potent antifungal agents. Contrary to the antibacterial studies, the presence of the hydroxyl on the alkenyl side chain turns out to be detrimental for the antifungal activity perhaps due to pharmacokinetic reasons.

In conclusion the present study showed that the synthesized compounds can be used as template for future development through modification and derivatization to design more potent and selective antimicrobial agents.

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28. *General procedure for the synthesis of long-chain alkenoic acid hydrazide* (**1a–d**): the hydrazides of long-chain alkenoic acids (**1a–d**) which are used as the starting material were prepared by the previously reported methods.[21]
Synthesis of 1-(alkenoyl/hydroxyalkenoyl)-5-phenylsemicarbazide (**2a–d**): hydrazide (**1a–d**) (1.0 mmole) was dissolved in abs. ethanol by heating to make a clear solution. An equal molar amount of phenyl isocyanate was added to it and the solution was refluxed for 5 h, cooled to get a precipitate. The precipitate was filtered, washed with abs ethanol and dried to give analytically pure compounds **2a–d**. The characterization data of compounds **2a–d** is given below.
1-(Undec-10-enoyl)-5-phenylsemicarbazides (**2a**): white powder; yield 80%; mp 103–104 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3236 (NH, NH–NH), 1667 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 13.04 (1H, s, CO–*NH*-Ar), 9.16 (2H, br s, CO–*NHNH*–CO), 8.17 (2H, d, J = 7.2 Hz, Ar-H-2″/6″), 7.60 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.51 (2H, t, J = 7.4 Hz, Ar-H-3″/5″), 5.82 (1H, tdd, $J_{H-^9CH_2}$ = 6.8 Hz, J_{H-H_Z} = 10.0 Hz, J_{H-H_E} = 17.8 Hz, CH_2=C*H*–), 5.01 (1H, dd, J_{H_Z-H} = 10.0 Hz, $J_{H_Z-H_E}$ = 2.8 Hz, H_ZC=CH), 4.94 (1H, dd, J_{H_E-H} = 17.8 Hz, $J_{H_E-H_Z}$ = 2.8 Hz, H_EC=CH–), 2.45 (2H, t, J = 7.4 Hz, CH_2–CO), 2.01 (2H, m, CH_2=CH–*CH*$_2$), 1.82 (2H, m, *CH*$_2$CH$_2$–CO), 1.45–1.25 (10H, br s, $(CH_2)_5$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 168.2, 165.4, 139.2, 133.2, 131.3, 128.9, 128.6, 114.2, 33.8, 29.9, 29.6, 29.3, 29.2, 29.1, 29.0, 28.9.
1-[(9Z)-Octadec-9-enoyl]-5-phenylsemicarbazides (**2b**): white powder; yield 79%; mp 101–102 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3222 (NH, NH–NH), 1665(C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 13.18 (1H, s, NH), 9.18 (2H, br s, NHNH), 8.08 (2H, d, J = 7.2 Hz, Ar-H-2″/6″), 7.64 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.51 (2H, t, J = 7.2 Hz, Ar-H-3″/5″), 5.31 (2H, m, CH_2–*CH*=*CH*–CH_2), 2.54 (2H, t, J = 7.8 Hz, CH_2–CO), 2.36 (4H, m, *CH*$_2$–CH$_2$=CH–*CH*$_2$), 1.82 (2H, m, *CH*$_2$–CH$_2$–CO), 1.37–1.25 (20H, br s, $(CH_2)_{10}$), 0.89 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz $CDCl_3$, δ, ppm): 167.4, 164.7, 139.2, 137.3, 133.2, 131.5, 128.8, 129.0, 31.9, 30.7, 30.4, 30.1, 29.9, 29.7, 29.6, 29.4, 29.2 'two signals are hidden', 29.1, 26.6, 22.7, 14.2.
1-[(9Z,12R)-12-Hydroxy-octadec-9-enoyl]-5-phenylsemicarbazides (**2c**): off-white powder; yield 77%; mp 111–113 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3343 (OH), 3233 (NH, NH–NH) 1661 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 13.23 (1H, s, NH), 9.17 (2H, br s, NHNH), 8.12 (2H, d, J = 7.4 Hz, Ar-H-2″/6″), 7.65 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.51 (2H, t, J = 7.2 Hz, Ar-H-3″/5″), 5.37 (2H, m, CH_2–*CH*=*CH*–CH_2), 4.87 (1H, m, *CH*–OH), 2.55 (2H, t, J = 7.6 Hz, CH_2–CO), 2.36 (4H, m, *CH*$_2$–CH$_2$=CH–*CH*$_2$), 1.83 (2H, m, *CH*$_2$–CH$_2$-CO), 1.76 (1H, m, CH–*OH*), 1.44–1.28 (18H, br s, $(CH_2)_9$), 0.88 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 166.2, 163.3, 138.7, 136.3, 132.2, 128.1, 128.0, 127.8, 70.7, 40.1, 39.9, 39.7, 39.4, 39.2, 31.3, 30.4, 29.4, 29.2, 29.0, 28.8, 26.1, 22.0, 14.1.
1-[(9R,12Z)-9-Hydroxy-octadec-12-enoyl]-5-phenylsemicarbazide (**2d**): off-white powder; yield 72%; mp 113–114 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3353 (OH), 3229 (NH, NH–NH) 1668 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 13.03 (1H, s, NH), 9.09 (2H, br s, NHNH), 8.16 (2H, d, J = 7.2 Hz, Ar-H-2″/6″), 7.61 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.52 (2H, t, J = 7.2 Hz, Ar-H-3″/5″), 5.38 (2H, m, CH_2–*CH*=*CH*–CH_2), 4.89 (1H, m, *CH*–OH), 2.55 (2H, t, J = 7.6 Hz, CH_2–CO), 2.26 (4H, m, *CH*$_2$–CH$_2$=CH–*CH*$_2$), 1.86 (2H, m, *CH*$_2$–CH$_2$–CO), 1.68 (1H, m, CH–*OH*), 1.40–1.25 (18H, br s, $(CH_2)_9$), 0.87 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 167.6, 163.2, 138.4, 137.6, 131.2, 128.7, 128.3, 125.5, 70.56, 40.1, 39.9, 36.3, 24.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.5, 28.4, 25.1, 22.3, 14.01.
Synthesis of 5-(alkenyl/hydroxyalkenyl)-2-phenylamine 1,3,4-oxadiazoles (**3a–d**): 1-alkenoyl-5-phenyl semicarbazides (**2a–d**) (1.0 mmole) in $POCl_3$ (6.0 ml) were refluxed for 4 h. The resulting mixture was then poured into NaOH ice water solution, resulting in deposition that was filtered, washed, dried and recrystallized from aqueous ethanol and acetone (1:4 ml v/v) to give compounds **3a–d**. The characterization data of compounds **3a–d** is given below.
5-(Dec-9′-enyl)-2-phenylamine-1, 3, 4-oxadiazole (**3a**): white powder; yield 92%; mp 133–134 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3228 (NH), 1504 (C=N), 1258(C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 9.07 (1H, s, NH), 7.27 (2H, d, J = 7.6 Hz, Ar-H-2″/6″), 7.18 (1H, t, J = 7.5 Hz, Ar-H-4″), 6.98 (2H, t, J = 7.3 Hz, Ar-H-3″/5″), 5.79 (1H, tdd, $J_{H-^9CH_2}$ = 6.6 Hz, J_{H-H_Z} = 10.1 Hz, J_{H-H_E} = 16.9 Hz, CH_2=C*H*–), 5.00 (1H, dd, J_{H_Z-H} = 10.1 Hz, $J_{H_Z-H_E}$ = 2.2 Hz, H_ZC=CH), 4.94 (1H, dd, J_{H_E-H} = 16.9 Hz, $J_{H_E-H_Z}$ = 2.2 Hz, H_EC=CH–), 2.24 (2H, t, J = 7.5 Hz, CH_2 α to ring), 2.00 (2H, m, CH_2=CH–*CH*$_2$), 1.58 (2H, m, CH_2 β to ring), 1.21 (10H, br s, $(CH_2)_5$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 172.9, 155.6, 139.4, 139.0, 128.7, 122.2, 118.5, 114.2, 40.6, 40.2, 39.8, 39.4, 33.9, 29.2, 28.9, 25.4. MS (ESI): m/z = 322.3 [M+Na]$^+$, calcd = 322.4.
5-[(8′Z)-Heptatadecenyl]-2-phenylamine-1,3,4-oxadiazole (**3b**): white crystals; yield 90%; mp 144–146 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3218 (NH), 1505 (C=N), 1242 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 9.39 (1H, s, NH), 7.26 (2H, d, J = 7.7 Hz, Ar-H-2″/6″), 7.17 (1H, t, J = 7.5 Hz, Ar-H-4″), 6.98 (2H, t, J = 7.3 Hz, Ar-H-3″/5″), 5.33 (2H, m, CH_2–*CH*=*CH*–CH_2), 2.23 (2H, t, J = 7.5 Hz, CH_2 α to ring), 2.01 (4H, m, *CH*$_2$–CH=CH–*CH*$_2$), 1.56 (2H, m, CH_2 β to ring), 1.29 (20H, br s, $(CH_2)_{10}$), 0.87 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 172.9, 152.2, 133.5, 130.4, 129.3, 125.6, 123.2, 114.2, 40.6, 40.2, 39.8, 39.4, 38.6, 38.4, 33.9, 31.8, 31.4, 29.2, 28.4, 28.2, 27.9, 22.6, 14.0. MS (ESI): m/z = 420.3 [M+Na]$^+$, calcd = 420.5.
(8′Z,11′R)-5-(11′-Hydroxy-octadec-8′-enyl)-2-phenylamine-1,3,4-oxadiazole (**3c**): off-white powder; yield 87%; mp 133–136 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3358 (OH), 3224 (NH), 1518 (C=N), 1220 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 9.31 (1H, s, NH), 7.62 (2H, d, J = 7.2 Hz, Ar-H-2″/6″), 7.53 (1H, t, J = 7.0 Hz, Ar-H-4″), 7.41 (2H, t, J = 7.2 Hz, Ar-H-3″/5″), 5.37 (2H, m, CH_2–*CH*=*CH*–CH_2), 4.69 (1H, m, *CH*–OH), 2.38 (2H, t, J = 7.5 Hz, CH_2 α to ring), 1.98 (4H, m, *CH*$_2$–CH = CH–*CH*$_2$), 1.78 (1H, m, CH–*OH*), 1.67 (2H, m, CH_2 β to ring), 1.28 (18H, br s, $(CH_2)_9$), 0.88 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 172.1, 163.2, 138.7 'one signal hidden″, 132.2, 128.1, 128.0, 127.0, 70.7, 40.1, 39.9, 39.7, 39.5, 39.3, 31.3, 30.4, 29.1, 29.0, 28.8, 28.6, 25.1, 22.0, 13.6.167.2, 139.8, 139.0, 133.5, 130.0, 129.8, 127.9, 77.1, 38.6, 38.4, 33.5, 31.7, 30.2, 29.9, 29.6, 29.3, 28.7, 28.5, 28.4, 28.3, 22.4, 14.4. MS (ESI): m/z = 436.5 [M+Na]$^+$, calcd = 436.59.
(8′R,11′Z)-5-(8′-Hydroxy-octadec-11′-enyl)-2-phenylamine-1,3,4-oxadiazole (**3d**): off-white powder; yield 87%; mp 135–137 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3353 (OH), 3217 (NH), 1494 (C=N), 1225 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 9.88 (1H, s, NH), 8.20 (2H, d, J = 7.3 Hz, Ar-H-2″/6″), 7.63 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.54 (2H, t, J = 7.9 Hz, Ar-H-3″/5″), 5.34 (2H, m, CH_2–*CH*=*CH*–CH_2), 4.87 (1H, m, *CH*–OH), 2.50 (2H, t, J = 7.5 Hz, CH_2 α to ring), 1.98 (4H, m, *CH*$_2$–CH=CH–*CH*$_2$), 1.77 (1H, m, CH–*OH*), 1.67 (2H, m, CH_2 β to ring), 1.28 (18H, br s, $(CH_2)_9$), 0.89 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 172.1, 165.7, 139.1, 139.0, 133.0, 130.7, 129.4, 127.6, 77.3, 38.6, 36.4, 30.4, 31.8, 29.8, 29.6, 29.4, 29.2, 29.0, 28.9, 28.6, 25.9, 22.2, 14.3. MS (ESI): m/z = 436.4 [M+Na]$^+$, calcd = 436.59.
Synthesis of 1-(alkenoyl/hydroxyalkenoyl)-5-phenylthiosemicarbazide (**4a–d**): 1-alkenoyl-5-phenylthiosemicarbazides (**4a–d**) were prepared by the reported literature method.[27]
Synthesis of 5-(alkenyl/hydroxyalkenyl)-2-phenylamine-1,3,4-thiadiazoles (**5a–d**): 1.0 mmole of compounds (**4a–d**) in acetic anhydride (Ac_2O) (6.0 ml) was refluxed for 5 hrs. The resulting mixture was poured into crushed ice (100gm) with stirring. The product thus obtained was filtered, washed with cold water, dried and recrystallized from aqueous ethanol and acetone (1:4 ml v/v) to give analytically pure compounds **5a–d**. The characterization data of compounds **5a–d** prepared according to the above procedure is given below.
5-(dec-9′-enyl)-2-Phenylamine-1, 3, 4-thiadiazole (**5a**): white powder; Yield 95%; mp 134–135 °C. IR ($\nu_{max,}$ cm^{-1}, KBr): 3221 (NH), 1488 (C=N), 707 (C–S–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.20 (1H, s, NH), 8.16 (2H, d, J = 8.5 Hz, Ar-H-2″/6″), 7.55 (1H, t, J = 7.3 Hz, Ar-H-4″), 7.44 (2H, t, J = 7.8 Hz, Ar-H-3″/5″), 5.73 (1H, tdd, $J_{H-^9CH_2}$ = 6.7 Hz, J_{H-H_Z} = 10.1 Hz, J_{H-H_E} = 16.9 Hz, CH_2=C*H*–), 4.98 (1H, dd, J_{H_Z-H} = 10.1 Hz, $J_{H_Z-H_E}$ = 2.1 Hz, H_ZC=CH), 4.85 (1H, dd, J_{H_E-H} = 16.9 Hz, $J_{H_E-H_Z}$ = 2.1 Hz, H_EC=CH–), 2.98 (2H, t, J = 7.5 Hz, CH_2 α to ring), 1.96 (2H, m,

CH_2=CH–CH_2), 1.76 (2H, m, CH_2 β to ring), 1.25 (10H, br s, $(CH_2)_5$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.2, 153.0, 139.1, 133.4, 131.3, 129.8, 127.9, 114.2, 33.7, 29.1, 28.9, 28.8, 28.7, 28.4, 26.0, 25.8. MS (ESI): m/z = 338.2 $[M+Na]^+$, calcd = 338.47.

5-(8′Z)(heptadec-8′-enyl)-2-Phenylamine-1,3,4-thiadiazole (**5b**): white powder; Yield 91%; mp 138–140 °C. IR (ν_{max}, cm^{-1}, KBr): 3219 (NH), 1468 (C=N), 701 (C–S–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.10 (1H, s, NH), 8.14 (2H, d, J = 8.5 Hz, Ar-H-2″/6″), 7.55 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.45 (2H, t, J = 7.8 Hz, Ar-H-3″/5″), 5.30 (2H, m, CH_2–*CH*=*CH*–CH_2), 2.98 (2H, t, J = 7.7 Hz, CH_2 α to ring), 2.36 (4H, m, *CH_2*–CH_2=CH–*CH_2*), 1.76 (2H, m, CH_2 β to ring), 1.28 (20H, br s, $(CH_2)_{10}$), 0.80 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.4, 158.0 'one signal hidden', 139.1, 137.2, 133.2, 131.3, 128.8, 128.6, 31.9, 29.9, 29.7, 29.5 'two signals are hidden', 29.4 'two signals are hidden', 29.3, 29.1, 28.7, 26.4, 22.7, 14.2. MS (ESI): m/z = 436.3 $[M+Na]^+$, calcd = 436.6.

5-[(8′Z,11′R)-11′-hydroxy-heptadec-8′-enyl]-2-Phenylamine-1,3,4-thiadiazole (**5c**): white powder; Yield 85%; mp 136–138 °C. IR (ν_{max}, cm^{-1}, KBr): 3310 (OH), 3219 (NH), 1467 (C=N), 695 (C–S–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.37 (1H, s, NH), 8.23 (2H, d, J = 8.5 Hz, Ar-H-2″/6″), 7.62 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.52 (2H, t, J = 7.8 Hz, Ar-H-3″/5″), 5.37 (2H, m, CH_2–*CH*=*CH*–CH_2), 3.59 (1H, s, *CH*–OH), 3.00 (2H, t, J = 7.5 Hz, CH_2 α to ring), 2.38 (4H, m, *CH_2*–CH_2=CH–*CH_2*), 1.83 (2H, m, CH_2 β to ring), 1.71 (1H, m, CH–*OH*), 1.26 (18H, br s, $(CH_2)_9$), 0.87 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 170.9, 165.0, 133.3, 131.1, 130.7, 130.2, 128.8, 128.5, 73.9, 34.1, 31.5, 29.8, 29.7, 29.5, 29.3, 29.1, 28.9, 27.1, 25.2, 23.1, 22.5, 21.3, 14.6. MS (ESI): m/z = 452.4 $[M+Na]^+$, calcd = 452.6.

5-[(8′R,11′Z)-8′-hydroxy-heptadec-11′-enyl]-2-Phenylamine-1,3,4-thiadiazole (**5d**): off-white powder; Yield 83%; mp 139–141 °C. IR (ν_{max}, cm^{-1}, KBr): 3322 (OH), 3236 (NH), 1461 (C=N), 699 (C–S–C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.17 (1H, s, NH), 8.31 (2H, d, J = 8.4 Hz, Ar-H-2″/6″), 7.43 (1H, t, J = 7.4 Hz, Ar-H-4″), 7.25 (2H, t, J = 7.4 Hz, Ar-H-3″/5″), 5.36 (2H, m, CH_2–*CH*=*CH*–CH_2), 3.57 (1H, s, *CH*–OH), 2.81 (2H, t, J = 7.5 Hz, CH_2 α to ring), 2.23 (4H, m, *CH_2*–CH_2=CH–*CH_2*), 2.04 (2H, m, CH_2 β to ring), 1.71 (1H, m, CH–*OH*), 1.33 (18H, br s, $(CH_2)_9$), 0.90 (3H, dist. t, terminus CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 168.9, 163.1, 137.6, 133.2, 131.2, 128.7, 128.3, 125.5, 70.56, 40.1, 39.9, 36.3, 24.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.5, 28.4, 25.1, 22.3, 14.01. MS (ESI): m/z = 452.5 $[M+Na]^+$, calcd = 452.6.