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# Synthesis, antimicrobial and cytotoxicity studies of novel undecenoic acid-based triazolothiadiazole derivatives

Vijayendar Venepally[a,d], K Sirisha[b,d], C Ganesh Kumar[b,d], E Vamshi Krishna[c,d], Sunil Mishra[c,d] & Ram Chandra Reddy Jala*[a,d]

[a] Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 007, India
[b] Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 007, India
[c] Pharmacology and Toxicology Division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 007, India
[d] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201 002, India

E-mail: jrcreddy10@gmail.com; ramchandra@iict.res.in

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In the present study, synthesis, antimicrobial and cytotoxic activity studies of 3,6-disubstituted-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole series compounds have been carried out. Compounds **6d**, **6i**, **6k**, **6p**, **6q**, **6r**, **6s** and **6t** exhibit promising activity with MIC value of 3.9 µg/mL against some of the tested bacterial strains. Compounds **6r** and **6s** consistently show promising minimum bactericidal concentration activity with MBC value of 7.8 µg/mL against *Staphylococcus aureus* MTCC 96 strain. Cytotoxic evaluation study claims that most of the compounds exhibit significant cytotoxicity against all the studied cancer cell lines. Particularly, compounds **6c**, **6k**, **6l**, **6n** and **6t** against HeLa cell line and compounds **6c** and **6h** against B16-F10 cell line exhibit promising activities with $IC_{50}$ values ranging from 6.34 to 9.99 µM. Further, most of the compounds are non-toxic against Chinese hamster ovary cell (CHO-K1) normal cell.

**Keywords**: Undecenoic acid, triazolothiadiazole, antimicrobial, cytotoxic, promising activities

Microbial infections continue to exist as serious threat to human life and are responsible for public health problems due to its emerging resistance to presently using antibiotic drugs. As a result, there is a compulsion to discover novel antimicrobial agents with promising activity against drug-resistant microorganisms. Besides, cancer is a major debilitating disease which ranks 2nd in the world and can affects almost every tissue in the human body and poses a great challenge to medical science. Hence, great efforts are in progress to discover new compounds with enhanced selectivity and activity by chemical modifications. The development of new antimicrobial and anticancer therapeutics is one of the basic goals in medicinal chemistry. In this regard, continual efforts are needed to develop novel compounds exhibiting anticancer and antimicrobial activities to address these global health issues.

Nitrogen, oxygen and sulfur containing compounds are of significant importance and particular interest both in pharmaceutical as well as agrochemical industries. Among these heterocyclic systems, especially those containing 1,2,4-triazoles are associated with diverse pharmacological properties such as antibacterial, antifungal[1], antitubercular[2], anticancer[3], anticonvulsant[4], antiinflammatory[5], analgesic[6] and molluscicidal properties[7-10]. Various studies reported that amino and mercapto groups of 1,2,4-triazoles are readily available nucleophilic centres for the synthesis of fused heterocyclic compounds. Due to their significance in medicinal chemistry lately fused heterocyclic derivatives have been receiving a lot of attention. Among the fused heterocyclic compounds, triazolothiadiazoles and triazolothiadiazines are a class of heterocyclic compounds have gained renewed interest among medicinal chemists exhibiting wide range of pharmacological activities such as antifungal[11,12], antibacterial[13,14], antiviral[15], anthelmentic[16-17], antitumour[18], analgesic[19] and antiinflammatory[20,21].

It is a well known fact that the fatty acids and their derivatives exhibit biological activities such as antimicrobial[22,23], antifungal[24]and pesticidal[25] activities. These fatty acid analogs were reported to show diverse biological activities such as antiinflammatory[26], antioxidant[27], antifeedant[28], antiparasitic[29], antimicrobial[30] and neuroprotective[31] activities. Some studies revealed that a variety of modified fatty acids are important molecules in the treatment of cancers[30,32-33]. Undecenoic acid derivatives also exhibited promising biological activities, namely, antifungal, antibacterial, antiviral and anticancer activities[30]. Based on the above

facts, in the present study it was decided to explore undecenoic acid and triazolothiadiazole-based pharmacophores in the synthesis of various 3,6-disubstituted 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives and evaluate them for various bioactivities.

## Experimental Procedures

### Materials and Methods

Chemicals used in these synthetic schemes were of analytical grade and they were procured from different commercial suppliers and used without any further purification. All the reactions were monitored on micro TLC plates (coated with TLC grade silica gel, procured from Merck). Column chromatography was carried out by using silica gel (100-200 mesh) procured from Qualigens (India) using freshly distilled solvents. All the Proton NMR and $^{13}$C NMR spectra were recorded with a Bruker Avance (for $^{1}$H NMR at 300 MHz, 400 MHz, 500 MHz and for $^{13}$C NMR at 75 MHz, 100 MHz, 125 MHz) spectrometer, using TMS δ = 0 ppm & δ 77.00 ppm as internal standard for chemical shifts (δ) in $CDCl_3$ solvent at 25°C. In NMR studies, the chemical shift values are furnished in ppm (parts per million) units. Mass spectral studies were performed with HRMS. IR spectral studies were conducted in chloroform on a Perkin-Elmer FT-IR spectrum BX.

### Synthesis of methyl undec-10-enoate, 2

Undec-10-enoic acid (73.45 mmol) in methanol (100 mL) was kept under stirring and a few drops of concentrated $H_2SO_4$ were added and the contents were refluxed for about 10 h. The progress of reaction was monitored by micro TLC. As soon as completion of the reaction was confirmed by TLC, methanol was removed under reduced pressure using rotary evaporator and subsequently, water was added and the title compound was extracted employing ethyl acetate solvent and the organic layer was then dried over anhydrous sodium sulphate and the dried solution was concentrated under vacuum to get the desired product.

$^{1}$H NMR (300 MHz, $CDCl_3$): δ 5.75-5.85 (m, -CH=$CH_2$-, 1H), 4.91-5.01 (m, -CH=$CH_2$-, 2H), 3.66 (s, -$OCH_3$, 2H), 2.28-2.31 (t, -$CH_2$-, *J* = 7.4 Hz, 2H), 2.01-2.06 (m, -$CH_2$-, 2H), 1.59-1.65 (m, -$CH_2$-, 2H), 1.26-1.39 (m, -$(CH_2)_5$-, 10H); ESI-MS: $[M+H]^+$ *m/z* = 199.

### Synthesis of undec-10-enehydrazide, 3

Methyl undec-10-enoate (2) (59.93 mmol) was taken in ethanol (90 mL) under stirring and hydrazine hydrate (269.68 mmol) was added to it and mixture was refluxed for 10 h. The reaction progress was periodically monitored by micro TLC. The solvent was removed after completion of reaction using rotary evaporator under reduced pressure, subsequently, chilled water (50 mL) was poured into mixture and was stirred for about 15 min. The solid got after stirring was filtered and dried under vacuum to produce undec-10-enehydrazide as a white solid.

$^{1}$H NMR (300 MHz, $CDCl_3$): δ 5.75-5.85 (m, -CH=$CH_2$-, 1H), 4.91-5.01 (m, -CH=$CH_2$-, 2H), 2.67-3.01 (broad-s, -$NH_2$, 2H), 2.12-2.16 (t, -$CH_2$-, *J* = 7.3 Hz, 2H), 2.00-2.06 (m, -$CH_2$-, 2H), 1.59-1.66 (m, -$CH_2$-, 2H), 1.25-1.38 (m, -$(CH_2)_5$-, 10H); ESI-MS: $[M+H]^+$ *m/z* = 199.

### Synthesis of potassium 2-(undec-10-enoyl) hydrazine-1-carbodithioate, 4

Pellets of potassium hydroxide (106.54 mmol) were dissolved in ethanol (40 mL) and to this solution, undec-10-enehydrazide (53.27 mmol) and carbon disulfide (117.19 mmol) were added successively and subsequently, the contents were stirred at RT for 8 h. The formation of product from reactants was monitored using micro TLC technique. After completion of starting materials, diethyl ether (100 mL) was poured into the reaction mixture and stirred further for about 10 min. The filtration was carried out to obtain off-white solid, potassium 2-(undec-10-enoyl) hydrazine-1-carbodithioate.

### Synthesis of 4-amino-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol, 5

Hydrazine hydrate (45.38 mmol) was added to the product obtained in previous step, potassium 2-(undec-10-enoyl) hydrazine-1-carbodithioate (45.38 mmol) and subsequently, the contents were refluxed for 5 h. The progress in formation of product was periodically monitored by micro TLC. After reactants were consumed, concentrated hydrochloric acid was used to acidify the reaction mixture. The precipitate obtained so was filtered and dried under reduced pressure to obtain the crude product which was further subjected to silica gel column chromatography and the required product, an off white solid, was eluted in hexane, ethyl acetate solvent mixture (85: 15, v/v). ESI-MS: $[M+H]^+$ *m/z* = 255.

### General procedure for the synthesis of bridged compounds 6a-j

4-amino-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (0.1 mol), aromatic and/or heterocyclic acids (0.1 mol) and phosphorus oxychloride (10 L) mixture was

refluxed for about 6 h. The product formation from reactants was monitored by micro TLC. After consuming the reactants during reaction, the mixture was cooled to RT and it was discharged onto small pieces of ice and the title compound was extracted using ethyl acetate solvent and this organic layer was dried over anhydrous sodium sulphate to remove the moisture traces from the product and ethylacetate was evaporated under reduced pressure The crude product was subjected to silica gel column chromatography to get the desired product.

**3-(Dec-9-en-1-yl)-6-(2-nitrobenzyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6a**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as a light yellow semi solid with 65% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.19Hz, 1H) 7.67-7.71 (t, 1H) 7.52-7.59 (m, 2H) 5.75-5.86 (m, 1H) 4.91-5.01 (m, 2H) 4.63 (s, 2H) 3.00-3.04 (t, 2H) 2.00-2.06 (m, 2H) 1.80-1.87 (m, 2H) 1.23-1.42 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 166.6, 153.4, 148.4, 148.2, 139.1, 134.1, 132.8, 129.6, 129.3, 125.7, 114.1, 36.1, 33.7, 31.8, 29.6, 29.0, 28.8, 26.6, 24.9, 22.6, 14.0; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3394, 2923, 2852, 1588, 1479, 1415, 1334, 1129, 1002, 754, 654; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{20}H_{26}O_2N_5S$ is *m/z* 400.18017. Found: *m/z* 400.18036 ($C_{20}H_{26}O_2N_5S$).

**6-(4-Chlorophenyl)-3-(dec-9-en-1-yl)-[1,2,4] triazolo [3,4-b][1,3,4]thiadiazole, 6b**

The crude compound was subjected to silica gel column chromatography and the targeted product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off-white semi-solid with 73% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.84 (d, *J* = 8.69 Hz, 2H), 7.53 (d, *J* = 8.69 Hz, 2H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.10-3.13 (t, 2 H), 2.01-2.05 (m, 2H), 1.88-1.95 (m, 2H), 1.25-1.48 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 164.8, 152.6, 148.6, 139.0, 138.8, 129.7, 128.2, 127.9, 114.1, 33.7, 31.8, 29.2, 29.0, 28.8, 26.6, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3421, 3075, 2924, 2852, 1586, 1468, 1397, 1215, 756; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}ClN_4S$ is *m/z* 375.14047. Found: *m/z* 375.14077 ($C_{19}H_{24}ClN_4S$).

**3-(Dec-9-en-1-yl)-6-(4-iodophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6c**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off-white semi-solid with 69% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.90 (d, *J* = 8.39Hz, 2H), 7.61 (d, *J* = 8.39Hz, 2H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.10-3.13 (t, 2 H), 2.01-2.05 (m, 2H), 1.88-1.94 (m, 2H), 1.25-1.46 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 165.2, 152.5, 148.6, 139.0, 138.6, 128.9, 128.2, 114.1, 99.4, 33.7, 29.6, 29.2, 29.0, 28.8, 26.6, 25.0, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3422, 3074, 2922, 2851, 1958, 1586, 1468, 1274, 1216, 956, 758, 667; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}IN_4S$ is *m/z* 467.07609. Found: *m/z* 467.07603 ($C_{19}H_{24}IN_4S$).

**3-(Dec-9-en-1-yl)-6-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6d**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as a light brown semi-solid with 61% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.19 (d, *J* = 3.35 Hz, 1H), 6.65 (m, 1H), 5.77-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.08-3.11 (t, 2 H), 2.01-2.05 (m, 2H), 1.86-1.93 (m, 2H), 1.28-1.43 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 156.0, 152.4, 148.5, 146.0, 143.7, 131.4, 124.5, 113.4, 112.8, 32.4, 31.8, 29.6, 29.4, 29.0, 26.6, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3412, 3076, 2925, 2854, 2557, 1679, 1589, 1470, 1216, 1069, 770; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{17}H_{23}ON_4S$ is *m/z* 331.15871. Found: *m/z* 331.15832 ($C_{17}H_{23}ON_4S$).

**3-(Dec-9-en-1-yl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6e**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 80: 20, v/v) as an off-white semi-solid with 66% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.90 (m, 2H), 7.52-7.59 (m, 3H), 5.75-5.85 (m, 1H), 4.91-5.01 (m, 2H), 3.10-3.14 (t, 2 H), 2.00-2.05 (m, 2H), 1.88-1.96 (m, 2H), 1.25-1.41 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.2, 152.9, 148.6, 139.1, 132.5, 129.5, 129.3, 127.0, 114.1, 33.7, 31.8, 29.6, 29.0, 28.8, 26.7, 25.0, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3531, 3394, 3021, 2924, 2852, 1588, 1466, 1334, 1129, 1002, 753; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{25}N_4S$ is *m/z* 341.17944. Found: *m/z* 341.17915 ($C_{19}H_{25}N_4S$).

**6-(4-Methoxybenzyl)-3-(dec-9-enyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6f**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30,

v/v) as a light brown semi-solid with 59% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.23 (d, *J* = 8.85 Hz, 2H), 6.92 (d, *J* = 8.85 Hz, 2H), 5.77-5.85 (m, 1H), 4.92-5.00 (m, 2H), 4.21 (s, 2H), 3.90 (s, 3H), 3.04-3.07 (t, 2H), 2.00-2.05 (m, 2H), 1.84-1.90 (m, 2H), 1.25-1.44 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 169.7, 159.4, 153.5, 148.0, 131.4, 130.1, 126.1, 124.5, 114.6, 55.2, 37.6, 32.5, 31.8, 29.6, 29.0, 26.7, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3531, 3394, 3021, 2924, 2852, 1588, 1466, 1334, 1129, 1002, 753; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{21}H_{29}ON_4S$ is *m/z* 385.20566. Found: *m/z* 385.20586 ($C_{21}H_{29}ON_4S$).

**3-(Dec-9-en-1-yl)-6-(p-tolyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6g**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off white semi-solid with 77% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.24 Hz, 1H), 7.34 (d, *J* = 7.93 Hz, 1H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.10-3.13 (t, 2H), 2.45 (s, 3H), 2.00-2.05 (m, 2H), 1.89-1.95 (m, 2H), 1.25-1.48 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.2, 152.7, 148.4, 143.3, 139.0, 130.0, 126.9, 126.7, 114.0, 33.6, 31.8, 29.6, 29.2, 29.0, 28.9, 28.8, 26.6, 24.9, 21.5; IR ($CHCl_3$,$\nu_{max}$ $cm^{-1}$): 3531, 3393, 3021, 2924, 2852, 1588, 1466, 1334, 1129, 1002, 753; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{20}H_{27}N_4S$ is *m/z* 355.19509. Found: *m/z* 355.19463 ($C_{20}H_{27}N_4S$).

**3-(Dec-9-en-1-yl)-6-(2-iodophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6h**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off-white semi-solid with 76% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, *J* = 8.06 Hz, 1H), 7.63 (d, *J* = 9.41Hz, 1H), 7.52-7.54 (t, 1H), 7.25-7.29 (m, 1H), 5.74-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.11-3.15(t, 2H), 1.99-2.05 (m, 2H), 1.89-1.96 (m, 2H), 1.25- 1.48 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.1, 153.7, 148.6, 141.0, 139.0, 133.8, 132.7, 131.3, 128.6, 114.1, 96.0, 33.7, 29.6, 29.2, 29.0, 28.8, 26.7, 25.0, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3423, 3074, 2920, 2850, 1958, 1586, 1468, 1274, 1218, 957, 771, 665.HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}N_4IS$ is *m/z* 467.07609. Found: *m/z* 467.07568 ($C_{19}H_{24}N_4IS$).

**3-(Dec-9-en-1-yl)-6-(3-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6i**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as a light yellow semi-solid with 77% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.46 (d, *J* = 10.22 Hz, 1H), 8.21 (d, *J* = 7.78 Hz, 1H), 7.76-7.80 (t, 1H), 5.76-5.84 (m, 1H), 4.91-4.99 (m, 2H), 3.14-3.17 (t, 2H), 2.01-2.05 (m, 2H), 1.90-1.96 (m, 2H), 1.25-1.50 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 163.5, 148.9, 148.7, 139.0, 132.4, 131.4, 131.1, 130.7, 126.7, 124.5, 121.8, 114.0, 33.7, 32.4, 31.8, 29.6, 29.2, 29.0, 26.7, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3539, 3394, 2924, 2852, 1588, 1415, 1243, 1129, 754, 654; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}N_5O_2S$ is *m/z* 386.16452. Found: *m/z* 386.16465 ($C_{19}H_{24}N_5O_2S$).

**3-(Dec-9-en-1-yl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6j**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as a light yellow semi-solid with 67% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.42 (d, *J* = 8.85 Hz, 2H), 8.10 (d, *J* = 8.85 Hz, 2H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.14-3.17 (t, 2H), 2.01-2.05 (m, 2H), 1.90-1.96 (m, 2H), 1.25-1.48 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 163.5, 152.5, 149.9, 148.9, 139.0, 135.0, 128.0, 124.6, 114.1, 33.7, 29.6, 29.0, 28.8, 26.7, 25.0; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3539, 3394, 2924, 2852, 1588, 1415, 1243, 1129, 754; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}N_5O_2S$ is *m/z* 386.16452. Found: *m/z* 386.16515 ($C_{19}H_{24}N_5O_2S$).

**3-(Dec-9-en-1-yl)-6-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6k**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as an off-white semi-solid with 69% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.83 (d, *J* = 8.85 Hz, 2H), 7.03 (d, *J* = 8.85 Hz, 2H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.90 (s, 3H), 3.09-3.12 (t, 2H), 2.01-2.05 (m, 2H), 1.88-1.94 (m, 2H), 1.25-1.47 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 165.8, 162.9, 152.8, 148.4, 139.1, 128.7, 121.9, 114.7, 114.0, 55.5, 33.7, 29.6, 29.2, 29.07, 29.02, 28.8, 26.6, 25.0, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3530, 3393, 2924, 2853, 1588, 1466, 1242, 1129, 753, 654; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{20}H_{27}N_4OS$ is *m/z* 371.19001. Found: *m/z* 371.19002 ($C_{20}H_{27}N_4OS$).

**6-(3-Chlorophenyl)-3-(dec-9-en-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6l**

The crude compound was subjected to silica gel column chromatography and the required product was

eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off-white semi-solid with 78% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.75 (d, $J$ = 7.78 Hz, 1H), 7.55-7.58 (m, 1H), 7.47-7.50 (m, 1H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.12-3.15 (t, 2H), 2.01-2.05 (m, 2H), 1.89-1.94 (m, 2H), 1.25-1.48 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 168.7, 165.0, 152.7, 148.7, 139.1, 135.6, 132.6, 130.7, 126.9, 125.2, 114.1, 33.7, 31.8, 29.6, 29.2, 29.07, 28.8, 26.7, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ cm$^{-1}$): 3423, 2920, 2850, 1958, 1467, 1183, 1007, 771; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}N_4ClS$ is *m/z* 375.14047. Found: *m/z* 375.14077 ($C_{19}H_{24}N_4ClS$).

**3-(Dec-9-en-1-yl)-6-(thiophen-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6m**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as an off white semi-solid with 65% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.59-7.62 (m, 2H), 7.17-7.19 (m, 1H), 5.75-5.85 (m, 1H), 4.91-5.01 (m, 2H), 3.07-3.11 (t, 2H), 2.00-2.06 (m, 2H), 1.88-1.92 (m, 2H), 1.25-1.46 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 159.8, 152.4, 148.6, 139.0, 131.4, 131.0, 130.3, 128.2, 114.0, 33.7, 31.3, 29.6, 29.2, 28.9, 28.8, 26.6, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ cm$^{-1}$): 3075, 2925, 2854, 1679, 1589, 1469, 1399, 1216, 1012, 770, 666; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{17}H_{23}N_4S_2$ is *m/z* 347.13586. Found: *m/z* 347.13588 ($C_{17}H_{23}N_4S_2$).

**6-(4-Bromophenyl)-3-(dec-9-en-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6n**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off white semi-solid with 63% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, $J$ = 8.69 Hz, 1H), 7.69 (d, $J$ = 8.69 Hz, 1H), 5.76-5.84 (m, 1H), 4.91-5.00 (m, 2H), 3.11-3.14 (t, 2H), 2.01-2.05 (m, 2H), 1.89-1.95 (m, 2H), 1.25-1.48 (m, 10H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 166.8, 164.4, 151.9, 147.8, 138.3, 132.0, 130.7, 127.8, 113.5, 32.9, 31.1, 28.8, 28.2, 28.1, 25.9, 24.2, 21.8; IR ($CHCl_3$ $\nu_{max}$ cm$^{-1}$): 3412, 3012, 2926, 2854, 1679, 1589, 1470, 1399, 1295, 1215, 1012, 757, 667; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{19}H_{24}N_4SBr$ is *m/z* 419.08996. Found: *m/z* 419.08991 ($C_{19}H_{24}N_4SBr$).

**3-(Dec-9-en-1-yl)-6-(1H-pyrrol-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6o**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 70: 30, v/v) as a light brown semi-solid with 61% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.32 (s, 1H), 7.06 (m, 1H), 6.81 (m, 1H), 6.37 (m, 1H), 5.75-5.85 (m, 1H), 4.91-5.01 (m, 2H), 3.03-3.07 (t, 2H), 2.00-2.05 (m, 2H), 1.84-1.91 (m, 2H), 1.25-1.41 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 158.2, 152.3, 148.2, 139.0, 123.6, 121.3, 114.4, 114.1, 111.2, 33.7, 31.8, 31.3, 30.1, 29.6, 29.2, 29.1, 28.8, 26.7, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ cm$^{-1}$): 3423, 2919, 2850, 1958, 1586, 1467, 1183, 957, 772; HR-MS (ESI) *m/z* [M+H$^+$]: 330.17442.

**3-(Dec-9-en-1-yl)-6-(2-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6p**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as an off white semi-solid with 74% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.13-8.17 (t, 1H), 7.57-7.62 (m, 1H), 7.25-7.36 (m, 2H), 5.75-5.85 (m, 1H), 4.90-5.00 (m, 2H), 3.12-3.15 (t, 2H), 2.00-2.04 (m, 2H), 1.88-1.96 (m, 2H), 1.25- 1.41 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 167.2, 161.3, 159.3, 151.6, 139.1, 134.0, 128.7, 125.1, 116.8, 116.7, 114.1, 33.7, 31.8, 29.6, 29.0, 28.8, 26.7, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ cm$^{-1}$): 3423, 2920, 2850, 1958, 1561, 1467, 1275, 1183, 957, 771; HR-MS (ESI) *m/z*[M+H$^+$]: Calcd for $C_{19}H_{24}N_4FS$ is *m/z* 359.17002. Found: *m/z* 359.17029 ($C_{19}H_{24}N_4FS$).

**3-(Dec-9-en-1-yl)-6-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6q**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 80: 20, v/v) as an off white semi-solid with 72% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.07 (s, 1H), 5.75-5.83 (m, 1H), 4.91-5.00 (m, 2H), 3.97 (s, 6H), 3.93 (s, 3H), 3.11-3.14 (t, 1H), 2.01-2.05 (m, 2H), 1.90-1.96 (m, 2H), 1.25-1.49 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 169.2, 153.7, 153.0, 148.1, 139.0, 137.9, 129.5, 118.9, 114.0, 106.0, 60.8, 56.1, 56.0, 38.6, 33.6, 32.4, 31.8, 31.3, 30.1, 29.6, 29.2, 29.0, 26.6, 24.9, 22.5; IR ($CHCl_3$ $\nu_{max}$ cm$^{-1}$): 3412, 2924, 2854, 2674, 2557, 1679, 1589, 1469, 1295, 1216, 1012, 770; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{22}H_{31}O_3N_4S$ is *m/z* 431.21114. Found: *m/z* 431.21062 ($C_{22}H_{31}O_3N_4S$).

**3-(Dec-9-en-1-yl)-6-(3,4,5-trimethoxybenzyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6r**

The crude compound was subjected to silica gel column chromatography and the required product was

eluted in a solvent mixture (Hexane: EtOAc, 80: 20, v/v) as an off white semi-solid with 62% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 6.50 (s, 2H), 5.75-5.84 (m, 1H), 4.91-5.00 (m, 2H), 4.20 (s, 2H), 3.85-3.86 (m, 9H), 3.04-3.06 (t, 2H), 2.00-2.05 (m, 2H), 1.84-1.90 (m, 2H), 1.25-1.44 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 169.2, 153.7, 153.0, 148.1, 139.0, 137.9, 129.5, 118.9, 114.1, 106.2, 60.8, 56.1, 56.0, 38.6, 33.6, 31.8, 31.3, 30.1, 29.6, 29.2, 29.0, 28.9, 26.6, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3412, 2925, 2854, 2557, 1679, 1589, 1469, 1296, 1216, 1127, 1012, 770; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{23}H_{33}O_3N_4S$ is *m/z* 445.22679. Found: *m/z* 445.22724 ($C_{23}H_{33}O_3N_4S$).

**6-((1H-indol-3-yl)methyl)-3-(dec-9-en-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6s**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75: 25, v/v) as a light brown semi-solid with 65% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.62 (s, 1H), 7.54 (d, *J* = 7.21 Hz, 1H), 7.42 (d, *J* = 8.19 Hz, 1H), 7.23 (m, 1H), 7.12-7.15 (m, 1H), 5.76-5.86 (m, 1H), 4.92-5.02 (m, 2H), 4.43 (s, 2H), 3.05-3.09 (t, 2H), 2.01-2.06 (m, 2H), 1.85-1.91 (m, 2H), 1.25-1.41 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 171.5, 153.7, 147.9, 139.0, 136.4, 126.3, 123.9, 122.5, 119.8, 117.9, 114.0, 111.7, 108.0, 33.6, 31.8, 31.3, 30.1, 29.6, 29.0, 28.9, 28.8, 28.4, 26.7, 24.9, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3414, 3075, 2924, 2854, 2674, 1679, 1469, 1295, 1216, 1012, 770; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{22}H_{28}N_5S$ is *m/z* 394.20599. Found: *m/z* 394.20631 ($C_{22}H_{28}N_5S$).

**6-(2-(1H-indol-3-yl)ethyl)-3-(dec-9-en-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, 6t**

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Hexane: EtOAc, 75:25, v/v) as a light brown semi-solid with 70% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.60 (d, *J* = 7.93 Hz, 1H), 7.40 (d, *J* = 8.08 Hz, 1H), 7.20-7.23 (t, 1H), 7.12-7.15 (t, 1H), 7.03 (s, 1H), 5.76-5.83 (m, 1H), 4.91-5.00 (m, 1H), 3.37-3.39 (t, 2H), 3.27-3.30 (t, 2H), 3.02-3.04 (t, 2H), 2.01-2.05 (m, 2H), 1.80-1.85 (m, 2H), 1.25-1.42 (m, 10H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 169.0, 153.4, 148.0, 139.1, 136.3, 126.7, 122.3, 122.23, 119.5, 118.2, 114.1, 112.7, 111.4, 33.7, 33.0, 31.8, 29.6, 29.2, 29.0, 28.8, 26.6, 24.9, 24.1, 22.6; IR ($CHCl_3$ $\nu_{max}$ $cm^{-1}$): 3413, 3075, 2924, 2854, 2674, 1679, 1469, 1295, 1216, 1012, 770; HR-MS (ESI) *m/z* [M+H$^+$]: Calcd for $C_{23}H_{30}N_5S$ is *m/z* 408.22164. Found: *m/z* 408.22190 ($C_{23}H_{30}N_5S$).

## Biological Activities

### Antibacterial and antifungal assays

The antibacterial and antifungal activities of the synthesized compounds were determined using well diffusion method[34] against different pathogenic bacterial strains such as *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530 along with *Candida* strains such as *Candida albicans* MTCC 3017. Bacterial and fungal strains used in the present study were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The targeted compounds were dissolved in DMSO (10% at a dose range of 125-0.97 µg) and added in each well under sterile conditions. Standard antibiotic solutions of ciprofloxacin (for bacterial strains) and miconazole (for *Candida* strains) at a dose range of 125 - 0.97 µg well$^{-1}$, served as positive controls whereas the well containing only DMSO served as negative control. The plates used in experiment were incubated over 24 h period at 30°C temperature and the well containing the lowest concentration exhibiting the inhibition zone is considered to be the minimum inhibitory concentration. Total experiments were performed in duplicates and mean values of results are represented in Table I and Table II.

### Minimum bactericidal and concentration (MBC) assays

Bactericidal assay (NCCLS, 2000) was carried out against a panel of pathogenic bacterial strains, such as *S. aureus* MTCC 96, *S. aureus* MLS-16 MTCC 2940, *M. luteus* MTCC 2470, *B. subtilis* MTCC 121, *E. coli* MTCC 739, *P. aeruginosa* MTCC 2453 and *K. planticola*MTCC 530 which were cultured overnight in Mueller Hinton broth. Serial dilutions of compounds to be investigated were prepared with number of concentrations ranging from 0 to 150 µg mL$^{-1}$. To the test compounds, 100 µL of overnight cultured bacterial and *Candida* suspensions were added to reach a final concentration of $1.5 \times 10^8$cfu mL$^{-1}$ (equal to 0.5 McFarland) and incubated at 37 ºC temperature for 24 h period. After 24 h of incubation

Table I — Antimicrobial activity of undecenoic acid-based triazolothiadiazole derivatives

| Compd | Minimum inhibitory concentration (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* MTCC 96 | *Bacillus subtilis* MTCC 121 | *S. aureus* MLS16 MTCC 2940 | *Micrococcus luteus* MTCC 2470 | *Klebsiella planticola* MTCC 530 | *Escherichia coli* MTCC 739 | *Pseudomonas aeruginosa* MTCC 2453 | *Candida albicans* MTCC 3017 |
| **6d** | 7.8 | 3.9 | 3.9 | 7.8 | 3.9 | >125 | 3.9 | >125 |
| **6i** | 7.8 | >125 | >125 | >125 | 3.9 | >125 | 7.8 | >125 |
| **6k** | 7.8 | >125 | >125 | 3.9 | >125 | >125 | 3.9 | >125 |
| **6p** | 3.9 | >125 | >125 | >125 | 3.9 | >125 | 7.8 | >125 |
| **6q** | 3.9 | >125 | >125 | >125 | 7.8 | >125 | >125 | >125 |
| **6r** | 3.9 | >125 | 3.9 | >125 | 7.8 | >125 | >125 | >125 |
| **6s** | 3.9 | >125 | 3.9 | >125 | >125 | >125 | >125 | >125 |
| **6t** | 7.8 | >125 | 3.9 | >125 | >125 | >125 | >125 | >125 |
| Miconazole (Standard control) | – | – | – | – | – | – | – | 7.8 |
| Ciprofloxacin (Standard control) | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | – |

Table II — Minimum Bactericidal Concentration ofundecenoic acid-based triazolothiadiazole derivatives

| Compd | Minimum bactericidal concentration (μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *S. aureus* MTCC 96 | *Bacillus subtilis* MTCC 121 | *S. aureus* MLS16 MTCC 2940 | *Micrococcus luteus* MTCC 2470 | *Klebsiella planticola* MTCC 530 | *Escherichia coli* MTCC 739 | *Pseudomonas aeruginosa* MTCC 2453 |
| **6d** | 15.6 | 7.8 | 7.8 | 15.6 | 7.8 | >125 | 7.8 |
| **6i** | 15.6 | >125 | >125 | >125 | 7.8 | >125 | 15.6 |
| **6k** | 15.6 | >125 | >125 | 7.8 | >125 | >125 | 7.8 |
| **6p** | 7.8 | >125 | >125 | >125 | 7.8 | >125 | 7.8 |
| **6q** | 7.8 | >125 | >125 | >125 | 15.6 | >125 | >125 |
| **6r** | 7.8 | >125 | 7.8 | >125 | 15.6 | >125 | >125 |
| **6s** | 7.8 | >125 | 7.8 | >125 | >125 | >125 | >125 |
| **6t** | 15.6 | >125 | 7.8 | >125 | >125 | >125 | >125 |
| Miconazole (Standard control) | – | – | – | – | – | – | – |
| Ciprofloxacin (Standard control) | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |

time, the Minimum Bactericidal Concentration (MBC) was determined, the lowest concentration of test compound required to kill a certain bacterium. Entire assay experiments were carried out in duplicates.

## Biofilm inhibition assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates employing the modified biofilm inhibition assay[36]against a panel of pathogenic bacterial strains namely,*S. aureus* MLS-16 MTCC 2940, *K. planticola*MTCC 530 and *S. aureus* MTCC 96, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The set concentrations of synthesized test compounds ranging from 0 to 250 μg/mL were subjected to crystal violet assay. The inhibition data were elucidated from the dose-response curves, where $IC_{50}$ value is defined as the concentration of inhibitor needed to inhibit 50% of biofilm formation under the above said assay conditions. Total experiments were performed in triplicates and the values are depicted as mean ± S.D.

## Cytotoxicity assay

Cytotoxicity assay (MTT) was carried out for the synthesized compounds as per the method reported in the earlier study[35]. Four different cancer cell lines and one normal cell line namely, HeLa (Human cervix adenocarcinoma, ATCC No. CCL-2), B16-F10 (Mouse skin melanoma, ATCC No. CRL-6475), SKOV3 (Human Ovarian cancer, ATCC No. HTB-77), MCF7 (Human breast adenocarcinoma, ATCC No. HTB-22) and CHO-K1 (Chinese hamster ovary cells, Normal Cell line, ATCC No. CCL-61) were procured from the ATCC (Bethesda, MD, USA). The test compounds were investigated at different concentrations varying from 1 to 50 μM in triplicates and incubated for 24 h time. The cells were then

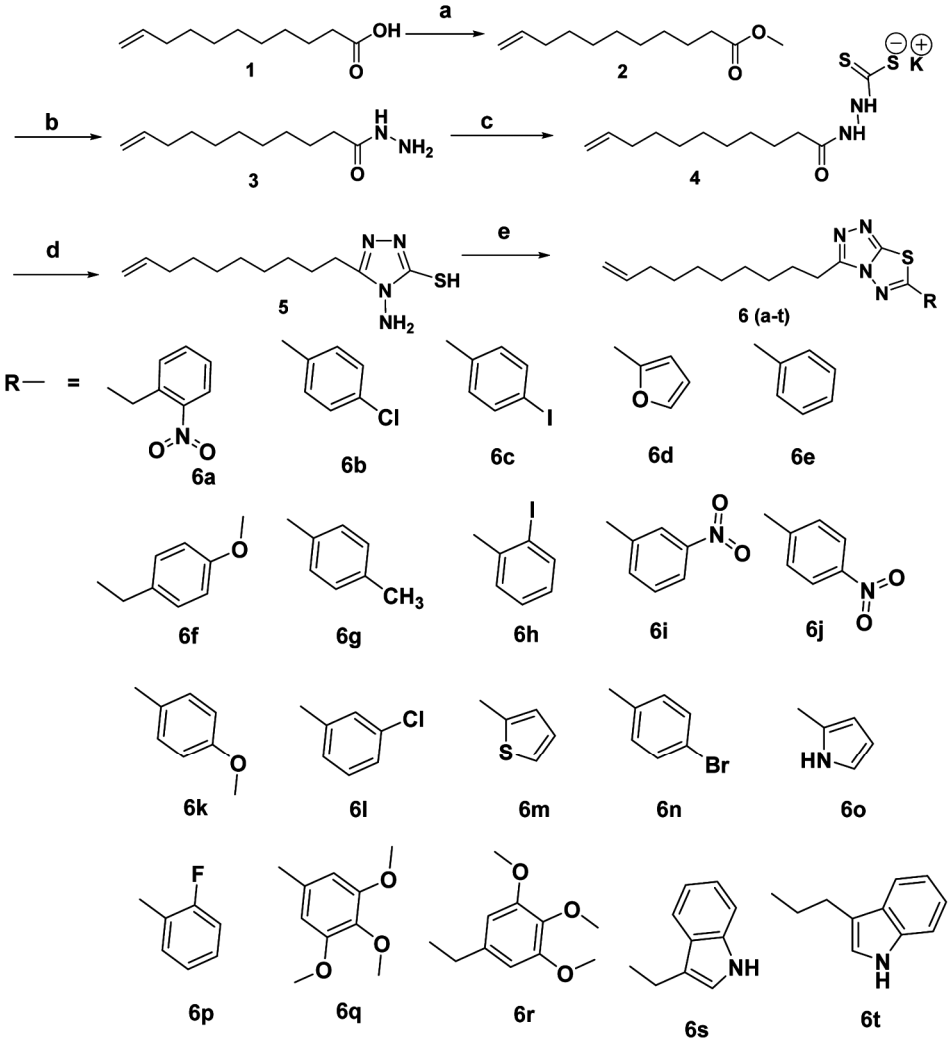
incubated with MTT (0.5 mg/mL) for 3 h period and to dissolve the insoluble formazan crystals 100 µL DMSO was added to each well. Finally, the absorbance of the plates was measured using a Synergy H1 multi-mode plate reader, USA. In this study, doxorubicin was employed as a positive control for the comparison of bioefficacy.

## Results and Discussion

### Synthesis and characterization

The target compounds were synthesized as outlined in Scheme I. Undec-10-enoic acid was converted to the methyl ester by using methanol and few drops of concentrated sulphuric acid. The product was identified by $^1$H NMR, the characteristic methoxy group protons appeared at δ 3.6. Undec-10-enoic acid methyl ester was treated with hydrazine hydrate to get undec-10-enehydrazide. The formation of product was identified by $^1$H NMR, the characteristic $NH_2$ group protons appeared at δ 2.6 - 3.0, which was further reacted with carbon disulfide in ethanolic potassium hydroxide to yield corresponding dithiocarbazinate salt in good yield. Dithiocarbazinate was directly reacted with hydrazine hydrate under refluxing conditions to yield triazole.

This cyclized triazole compound was identified by the TLC and ESI-MS, the characteristic mass peak observed as $[M+H]^+$ $m/z$ = 255. Condensation of triazole with various acids in presence of $POCl_3$ afforded triazolothiadiazoles (**6a-t)**. The structures of




**Reagents and conditions**: (a) MeOH, $H_2SO_4$, reflux, 10 h; (b) Hydrazine hydrate, EtOH, reflux, 10 h; (c) $CS_2$, KOH, EtOH, RT, 8 h; (d) Hydrazine hydrate, reflux, 5 h; (e) R-COOH, $POCl_3$, reflux, 6 h.

Scheme I

the newly synthesized compounds (**6a-t)** were confirmed by spectral data ($^{1}$H NMR, $^{13}$C NMR, IR, and ESI-MS). All the spectral data of the synthesized compounds were in full agreement with the proposed structures and also discussed for a representative compound such as 3-(Dec-9-en-1-yl)-6-(4-iodophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **(6c)**. For **6c** derivative, aromatic protons appeared as two distinct regions at 7.90 (d, *J* = 8.39Hz, 2H), 7.61 (d, *J* = 8.39Hz, 2H), where as terminal CH and $CH_2$protons of undecenoic group resonated at 5.76-5.84 (m, 1H) and 4.91-5.00 (m, 2H) respectively. $CH_2$ protons containing alkyl chain adjacent to triazole ring resonated as triplet at 3.10-3.13 (t, 2 H) and remaining aliphatic alkyl chain $CH_2$ protons appeared at 1.25-1.46 (m, 10H). From the HRMS data, molecular weight calculated for this compound as $C_{19}H_{24}IN_4S$ is 467.07609 and found as 467.07603 ($C_{19}H_{24}IN_4S$).

## Biology

### Antimicrobial activity

Total synthesized compounds (**6a–t)** were screened for their *in vitro* antimicrobial activity against different Gram-positive strains such as *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940, *Micrococcus luteus* MTCC 2470, and Gram-negative bacterial strains such as *Klebsiella planticola*MTCC 530, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, as well as *Candida albicans* MTCC 3017. Among the tested compounds, **6d** (against *B. subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940, *K. planticola*MTCC 530 and *P. aeruginosa* MTCC 2453 strains), **6i** (against *K. planticola*MTCC 530), **6k** (against *M. luteus* MTCC 2470 and *P. aeruginosa MTCC* 2453 strains), **6p** (against *S. aureus* MTCC 96 and *K. planticola*MTCC 530 strains), **6q** (against *S. aureus* MTCC 96), **6r** (against *S. aureus* MTCC 96 and *S. aureus* MLS16 MTCC 2940 strains), **6s** (against *S. aureus* MTCC 96 and *S. aureus* MLS16 MTCC 2940 strains) and **6t** (against *S. aureus* MLS16 MTCC 2940) exhibited promising activity with MIC value of 3.9 µg/mL. Further, compounds **6d** (against *S. aureus* MTCC 96 and *M. luteus* MTCC 2470 strains), **6i** (against *S. aureus* MTCC 96 and *P. aeruginosa* MTCC 2453 strains), **6k** (against *S. aureus* MTCC 96), **6p** (*P. aeruginosa* MTCC 2453), **6q** (against *K. planticola*MTCC 530), **6r** (*K. planticola*MTCC 530) and **6t** (*S. aureus* MTCC 96) exhibited good activity with MIC value of 7.8 µg/mL. The remaining compounds showed MIC value of >125 µg/mL. Further, it was interesting to note that none of the compounds exhibited antifungal activity against *Candida albicans*.

From a structure-activity relationship (SAR) perspective, it is noteworthy that in all the synthesized compounds, the 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole core was having undecenoic moiety at C-3 position and various substituted aryl, heteryl groups at C-6 position. The substituents on the aromatic ring of triazolothiadiazole at C-6 position played a crucial role in defining the degree of activity. Compound **6d** having 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole scaffold with furoic acid moiety at C-6 position and undecenoic moiety at C-3 position showed promising activities against the tested bacterial strains.

Further, **6i** (3-nitro group) and **6k** (4-methoxy group), **6p** (2-fluoro), **6q** (trimethoxy group), **6r** (phenyl trimethoxy group) and **6s** (indolyl acetic moiety) and **6t** (indolyl propionic moiety) compounds bearing undecenoic moiety at C-3 position displayed promising activities. This observation corroborates with our earlier studies [37]

Minimum bactericidal concentration assay was performed against all the three bacterial strains taking ciprofloxacin as standard. All the compounds exhibited promising activities with MBC values varying between 7.8 to 15.6 µg/mL.

Considering the promising minimum bactericidal concentration activities, anti-biofilm activity was investigated to understand the toxic effect of some compounds against the tested bacterial strains. In this regard, some of the promising compounds were screened for their anti-biofilm activity against nosocomial pathogens namely, *S. aureus* MTCC 96, *S. aureus* MLS-16 MTCC 2940, *M. luteus* MTCC 2470 and *K. planticola* MTCC 530. In the present study, the tested compounds exhibited low to moderate activities against all the examined strains. The results to this regard are depicted in Table III.

## Cytotoxicity

The cytotoxicity of all the synthesized compounds was screened against four human cancer cell lines namely, HeLa (Human cervix adenocarcinoma, ATCC No. CCL-2), B16-F10 (Mouse skin melanoma, ATCC No. CRL-6475), SKOV3 (Human ovarian cancer, ATCC No. HTB-77), MCF7 (Human breast adenocarcinoma, ATCC No. HTB-22) and CHO-K1 (Chinese Hamster ovary cells, Normal Cell line, ATCC No. CCL-61) using MTT assay. The

doxorubicin was employed as a positive control. $IC_{50}$ values of the test compounds for 24 h on each cell line was calculated and furnished in Table IV.

Most of the studied compounds exhibited significant cytotoxic activity against all the tested cancer cell lines. Prominent activities were exhibited by compounds **6c**, **6k, 6l**, **6n** and **6t** against HeLa cell line and compounds **6c** and **6h** against B16-F10 cell line with the $IC_{50}$ values ranging from 6.34 to 9.99 μM. Compounds **6e, 6f, 6g, 6h, 6i** and **6j** ($IC_{50}$ value range: 10.72 to 11.81 μM) and **6h, 6i** and **6j** ($IC_{50}$ value range: 10.74 to 13.64) showed promising activities against MCF-7 and SKOV3 cell lines, respectively. Remaining compounds showed good to moderate cytotoxic activity. Most of the tested compounds were non-toxic against the Chinese hamster ovary cells (CHO-K1).

It is worthy of mention that the cytotoxicity of tested compounds mainly depends on the nature of substituents on the phenyl ring. Compound **6k** having 4-methoxy group on aromatic ring showed prominent activity against HeLa cell line, while compounds **6n** (4-Bromo), **6t** (indolyl acetic moiety), **6c** (4-Iodo), **6e** (no substitution), **6f** (phenyl 4-Methoxy), **6g** (4-methyl), **6h** (2-Iodo), **6i** (3-nitro group) and **6j** (4-nitro group) showed promising activities against various cell lines.

Table III — Biofilm activity ofundecenoic acid-based triazolothiadiazole derivatives

| Compd | $IC_{50}$ values (μM) | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus* MTCC 96 | *S. aureus* MLS16 MTCC 2940 | *Micrococcus luteus* MTCC 2470 | *Klebsiella planticola* MTCC 530 |
| **6d** | – | – | 39.16 ± 0.1 | 45.45 ± 0.11 |
| **6i** | 49.31 ± 0.12 | 33.12 ± 0.1 | – | 38.9 ± 0.06 |
| **6k** | – | – | 48.6 ± 0.07 | – |
| **6p** | 29.65 ± 0.08 | – | – | 44.64 ± 0.09 |
| Ciprofloxacin (Standard) | 0.6 ± 0.08 | 0.7 ± 0.09 | 0.8 ± 0.11 | 0.8 ± 0.09 |

– = No activity

Table IV — Cytotoxicity of undecenoic acid-based triazolothiadiazole derivatives

| Compd | $IC_{50}$ values (μM) | | | | |
|---|---|---|---|---|---|
| | HeLa | B16-F10 | SKOV3 | MCF-7 | CHO-K1 |
| **6a** | 18.40 ± 0.40 | 19.75 ± 1.5 | 28.54 ± 1.4 | 34.96 ± 0.55 | NA |
| **6b** | 14.39 ± 0.96 | 36.57 ± 1.1 | 22.49 ± 1.13 | 18.61 ± 0.42 | NA |
| **6c** | 9.70 ± 0.58 | 8.42 ± 0.68 | 16.25 ± 0.66 | 16.63 ± 0.71 | 49.17 ± 1.5 |
| **6d** | 28.69 ± 0.34 | 11.53 ± 0.91 | 17.54 ± 1.4 | 13.56 ± 0.24 | 40.07 ± 0.66 |
| **6e** | 19.05 ± 1.4 | 21.83 ± 0.76 | 25.31 ± 1.19 | 11.76 ± 0.67 | 20.92 ± 0.72 |
| **6f** | 17.94 ± 1.1 | 21.55 ± 1.5 | 18.29 ± 1.6 | 11.48 ± 0.70 | 39.90 ± 1.6 |
| **6g** | 20.35 ± 0.63 | 19.38 ± 1.37 | 19.81 ± 1.0 | 11.30 ± 0.34 | 38.41 ± 0.82 |
| **6h** | 17.30 ± 0.62 | 9.99 ± 1.2 | 12.67 ± 0.39 | 10.72 ± 0.26 | NA |
| **6i** | 22.26 ± 0.71 | 18.61 ± 0.67 | 10.74 ± 1.4 | 11.11 ± 0.39 | NA |
| **6j** | 19.20 ± 1.6 | 12.54 ± 1.0 | 13.64 ± 0.31 | 11.81 ± 0.23 | NA |
| **6k** | 6.34 ± 0.40 | 20.66 ± 1.6 | NA | NA | NA |
| **6l** | 7.68 ± 0.23 | 39.32 ± 1.1 | NA | NA | NA |
| **6m** | 10.86 ± 0.39 | 17.11 ± 0.69 | NA | 40.58 ± 0.72 | NA |
| **6n** | 8.34 ± 0.31 | 40.98 ± 0.13 | 18.66 ± 0.91 | 44.29 ± 0.62 | NA |
| **6o** | 11.39 ± 0.52 | 27.12 ± 0.14 | 30.70 ± 0.87 | 35.62 ± 0.84 | NA |
| **6p** | 15.21 ± 0.99 | 20.03 ± 0.57 | 21.67 ± 1.3 | 24.51 ± 1.9 | NA |
| **6q** | 28.90 ± 1.2 | 15.95 ± 1.2 | 46.23 ± 0.21 | 27.09 ± 0.19 | NA |
| **6r** | 19.74 ± 0.44 | 27.45 ± 0.04 | 40.08 ± 0.76 | 25.35 ± 0.26 | NA |
| **6s** | 13.46 ± 0.64 | 25.47 ± 0.22 | 27.53 ± 0.90 | 30.27 ± 0.96 | NA |
| **6t** | 9.0 ± 1.5 | 29.59 ± 0.10 | 25.76 ± 1.63 | 28.79 ± 0.22 | NA |
| Doxorubicin(Standard ) | 0.8 ± 0.71 | 0.7 ± 0.56 | 0.8 ± 0.63 | 2.0 ± 0.81 | – |
| Mitomycin C (Standard) | – | – | – | – | 13.1 ± 0.68 |

NA = No activity, HeLa: *Homo sapiens* cervix adenocarcinoma (ATCC No. CCL-2), B16-F10: Mouse skin melanoma (ATCC No. CRL-6475), SKOV3: human ovarian cancer cell line (ATCC No. HTB-77), MCF-7: human breast adenocarcinoma cells (ATCC No. HTB-22), CHO-K1: Chinese hamster ovary cells, Normal Cell line (ATCC No. CCL-61).

## Conclusions

In conclusion, a series of novel undecenoic acid-based triazolothiadiazoles were synthesized and evaluated for their cytotoxic and antimicrobial activities. Furanoic, 3-nitro, 4-methoxy, 2-fluoro, trimethoxy phenyl, trimethoxy benzyl, methyl indole and propyl indole-based compounds exhibited promising activity against specific bacterial strains. Trimethoxy benzyl and methyl indole-based derivatives showed promising minimum bactericidal concentration activities. 3-Iodo, 4-methoxy, 3-chloro, 4-bromo and propyl indole-based derivatives and 3-iodo and 2-iodo-based compounds exhibited promising activity against HeLa and B16-F10 cell lines, respectively. Majority of the compounds were non-toxic against the normal cell line. Further analogues will be prepared based on the identified lead compounds and evaluated for the biological activities.

### Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/58776.

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### Conflict of interest

The authors declare that they have no competing interests.

## References

1 Zitoun G T, Kaplancikl Z A, Yildiz M T, Chevallet P & Kaya D, *Eur J Med Chem*, 40 (2005) 607.
2 Walczak K, Gondela A & Suwinsk J, *Eur J Med Chem*, 39 (2004) 849.
3 Holla B S, Veerendra B, Shivananda M K & Poojary B, *Eur J Med Chem*, 38 (2003) 759.
4 Amir M & Shikha K, *Eur J Med Chem*, 39 (2004) 535.
5 Ahmed S M, Ahmad F & Osman S M, *J Am Oil Chem Soc*, 62 (1985) 1578.
6 Thomas George DV, Mehta R, Tahilramani JD & Talwalker PK, *J Med Chem*, 14 (1971) 335.
7 Nawwar G A M, Haggag B M & Swellem R H, *Arch Pharmacol*, 326 (1993) 831.
8 Grant N, Mishriky N, Asaad F M & Fawzy N G, *Pharmazie*, 53 (1998) 543.
9 Shafik N A, Chabaka L M & Nawwar G A M, *Afinidad*, 63 (2006) 523.
10 Nawwar G A M, Chabaka L M, Shafik N A & Hany M S, *Afinidad*, 63 (2006) 153.
11 Karabasanagouda T, Adhikari A V & SuchethaShettey N, *Eur J Med Chem*, 42 (2007) 521.
12 Mathew V, Keshavayya J & Vaidya V P, *Eur J Med Chem*, 41 (2006) 1048.
13 Demirbas N, Demibras A, Karaoglu S A & Celik E, *Arkivoc*, 1 (2005) 75.
14 Zhang Z Y, Sun X W, Chu C H & Zhao L, *J Chin Chem Soc*, 44 (1997) 535.
15 Invidiata F P, Simoni D, Scintu F & Pinna N, *Il Farmaco*, 51 (1996) 659.
16 Imtiaz Hussain M & Kumar V, *Indian J Chem*, 31B (1992) 673.
17 Khawass S M E, Khalli M A, Hazza A A, Bassiouny A & Loutfy N F, *Il Farmaco*, 44 (1989) 703.
18 Rzeski W, Matysiak J & Kandefer-Szerszen M, *Bioorg Med Chem*, 15 (2007) 3201.
19 Srivastava V, Sen S & Shekar R, *Indian J Chem*, 33B (1994) 344.
20 Amir M, Harish K & Javed S A, *Eur J Med Chem*, 43 (2008) 2056.
21 Prasad A R, Ramlingam T, Bhaskar Rao A, Diwan P V & Sattur P B, *Indian J Chem*, 25B (1986) 566.
22 Ahmed S M, Ahmad F & Osman S M, *J Am Oil Chem Soc*, 62 (1985) 1578.
23 Khan M Y W, Ahmad F, Ahmad I & Osman S M, *J Am Oil Chem Soc*, 60 (1983) 949.
24 Pugliese P T & Pugliese P M, *US Patent*, (2002) US 6,448,251; *Chem Abstr* 137 (2002) 216818t.
25 Viklund F, Alander J & Hult K, *J Am Oil Chem Soc*, 80 (2003) 795.
26 Mallavadhani U V, Mahapatra A, Raja S S & Manjula C, *J Agric Food Chem*, 51 (2003) 1952.
27 Grunberg E & Titsworth E H, *Annu Rev Microbiol*, 27 (1973) 317.
28 Mod R R, Magne F C, Sumrell G & Novak A F, *J Am Oil Chem Soc*, 52 (1975) 455.
29 Takahashi H, Kosaka M, Watanabe Y, Nakade K & Fukuyama Y, *Bioorg Med Chem*, 11 (2003) 1781.
30 Rahman V P M, Mukhtar S, Ansari W H & Lemiere G, *Eur J Med Chem*, 40 (2005) 173.
31 Nagao Y, Mustafa J, Sano S, Ochiai M, Tazuko T & Shigeru T, *Med Chem Res*, 1 (1991) 295.
32 Lie Ken Jie M S F, Mustafa J & Pasha M K, *Chem Phys Lipids*, 100 (1999) 165.
33 Dolezalova M, Janis R, Svobodova H, Kasparkova V, Humpolıcek P & Krejci J, *Eur J Lipid Sci Technol*, 112 (2010) 1106.
34 Amsterdam D, 'Susceptibility testing of antimicrobials in liquid media' in, *Antibiotics in Laboratory Medicine*,4th edn edited by Loman V (Williams and Wilkins, Baltimore)(1996).
35 Vishnu Sravan B, Susheel Kumar N, Rama Krishna D, Nageshwara Rao S S, Misra S & Ranjan Patra C, *Nanotoxicology*, 10 (2016) 413.
36 Kamal A, Abdul R, Riyaz S, Poornachandra Y, Moku B, Kumar C G, Hussaini S M, Sridhar B & Machiraju P K, *Org Biomol Chem*, 13 (2015) 1347.
37 Venepally V, Sirisha K, Kumar C G, Krishna E V, Misra S & Jala R C R, *J Chem Sci*, 130 (2018) 23.