Solventless synthesis of new 4,5-disubstituted 1,2,3-selenadiazole derivatives and their antimicrobial studies

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Aditi A. Jadhav1, Vaishali P. Dhanwe1, Prasad G. Joshi1 and Pawan K. Khanna1*

Abstract: Two novel, namely 5-Phenyl-4-methyl-1, 2, 3-selenadiazole (5h) and 4-Phenyl-5-propyl-1, 2, 3-selenadiazole (5i) along with several other aliphatic and aromatic series of 1,2,3-selenadiazoles were synthesized at room temperature in one step under solventless conditions from the corresponding semicarbazones. All compounds were thoroughly characterized by various spectroscopic tools. The synthesized new and reported 1,2,3-selenadiazoles were found active against bacterial as well as fungal stains when screened for their antimicrobial activity against various pathogenic bacteria and fungi using agar disc diffusion as well as agar well diffusion method. Almost all selenadiazoles showed better antibacterial properties in comparison to established antibiotics like tetracycline. 4-ethyl-5-methyl-1,2,3-selenadiazole showed higher antimicrobial activity amongst the tested selenadiazoles.

Subjects: Biochemistry; Chemical Spectroscopy; Organic Chemistry

Keywords: organoselenium compounds; selenadiazoles; semicarbazones; solventless synthesis; antimicrobial studies

ABOUT THE AUTHOR
Pawan K. Khanna, the corresponding author, completed his PhD in Organometallic Chemistry of Se & Te from IIT-Bombay in 1989–90. Subsequently he did postdoctoral research with Chris Morely at the Queens’ University of Belfast and University of Wales at Swansea (UK). He worked in C-MET, Pune from 1993 to 1995. He was awarded the BOYSCAST fellowship of Govt of India during 1998–99 to work with David Cole-Hamilton at the Univesity of St. Andrews, Scotland. He moved to his current position of professor and head of Department of Applied Chemistry at Defence Institute of Advanced Technology (DIAT) in 2011 where he also served as dean of academics during 2011–2013. He has published over 150 research papers. He was awarded MRSI medal in 2010 and Researcher of the Year award at DIAT in 2014. Aditi Jadhav, Vaishali Dhanwe, Prasad Joshi are students of Pawan Khanna and are co-authors of the article. They are all developing organic and nanochemistry expertise.
1. Introduction

The current interest in the chemistry of 1,2,3-selenadiazoles is mainly due to their chemical reactivity owing to their soft transformations upon thermolysis (Jadhav, Dhanwe, Joshi, & Khanna, 2015; Junling et al., 2004) and or photolysis that derive free selenium via elimination of a nitrogen molecule leading to formation of alkyynes or new heterocycles (Jadhav, Dhanwe, et al., 2015; Meier & Voigt, 1972; Regitz & Krill, 1996; Jadhav & Khanna, 2015). Such new transformations make selenadiazoles and the end compounds thereof useful in pharmaceutical chemistry as well as precursors for coordination (Zhan, Liu, Fang, Pannecoque, & Clercq, 2009; Cervantes-Lee et al., 1998; Morley & Vaughan, 1993; Ford, Khanna, Morley & Vaira, 1999; Khanna & Morley, 1993) and materials chemistry including nanotechnology. (Khanna, 2005; Khanna, Gorte, & Morley, 2003; Khanna et al., 2009; Bhanoth, More, Jadhav, & Khanna, 2014; Jadhav, More, & Khanna, 2015).

The scope of 1,2,3-selenadiazole in pharmaceutical chemistry has widened due to resistance of micro-organisms against chemotherapeutic agents allowing infectious disease to become the second leading cause of death worldwide. Due to fast mutation process, many antibiotics become ineffective against the bacteria. As a result, bacterial resistance against antibiotic treatment is a common phenomenon. 1,2,3-selenadiazoles, in addition to their high-tech applications, have also been extensively studied for their pharmaceutical applications e.g. cytotoxicity and other biological activities. (Al-Smadi & Al-Momani, 2008; Pawar, Burungale, & Karale, 2009; Jalilian, Sattari, Bineshravarasti, Danesh talab, & Shafiee, 2003; El-Desoky, Badria, Abozeid, Kandeel, & Abdel-Rahman, 2013; Zhan et al., 2009; Xiao-Chun et al., 2012; Patil, Badami, & Puranik, 1994; Padmavathi, Sumathi, & Padmaja, 2002). Substituted 1,2,3-selenadiazoles and their derivatives have shown excellent antibacterial (Al-Smadi & Al-Momani, 2008; Pawar et al., 2009) antifungal (Jalilian et al., 2003), antitumor (El-Desoky et al., 2013; Xiao-Chun et al., 2012), and antiHIV properties (Zhan et al., 2009). Some of the 1,2,3-selenadiazole derivatives show antiahaemostatic activity (Patil et al., 1994) and insecticidal activities (Padmavathi et al., 2002). Specially it is worth mentioning that thioacetanilides derivatives of 1,2,3-selenadiazoles showed antiHIV activity against HIV-1 in MT-4 cells (Zhan et al., 2009). Antifungal study of 1,2,3-selenadiazoles e.g. sulphmoyl derivatives of 4,5-dihyronaphtho[1,2-d][1,2,3]selenadiazoles showed significant activity against Cryptococcus neoformans. Multiarm derivatives of 1,2,3-selenadiazoles were found highly active against E. coli, S. aureus, and P. aerugenosa bacteria (Al-Smadi & Al-Momani, 2008). Human melanoma cells (A375) growth was successfully inhibited by selenadiazole derivative i.e. 5-amino[1,2,5]selenadiazolo[3,4-d]pyrimidin-7-ol (El-Desoky et al., 2013). There are large number of such compounds useful as antimicrobial agents (Al-Smadi & Al-Momani, 2008; Pawar et al., 2009; Jalilian et al., 2003). Hence the researchers are continuously in the hunt for new molecules for controlling the bacterial infection timely and more effectively.

In view of excellent materials and biological applications of 1,2,3-selenadiazoles, it is warranted that meaningful studies should be conducted on such molecules to further enrich the knowledge bank so that their utility becomes more relevant. In order to tackle this issue, new 1,2,3-selenadiazole are required to be explored by conventional as well as non-conventional methods. We have recently reported solventless synthesis of cycloalkeno-1,2,3-selenadiazoles and tested their behavior towards several human pathogens (Jadhav, Dhanwe, et al., 2015). In our previous studies, a series of cyclic aliphatic 1,2,3-selenadiazoles were, for the first ever time, tested for their antibacterial activity and it was found that they act rather efficiently against number of microbes. To extend the feasibility of solventless synthesis and the effect of organic functionality via substitutions at 4 and 5 positions in the selenadiazole moiety on their antimicrobial activity, we herein report the synthesis of acyclic aliphatic and aromatic acetophenone derivatives of 1,2,3-selenadiazoles. Traditionally, 1,2,3-selenadiazoles have been synthesized from the corresponding semicarbazones via ring closure due to mild oxidation of semicarbazones by selenium dioxide (selenious acid)(Regitz & Krill, 1996; Labanauskas, Dudutiene, Matulis, & Urbelis, 2009; Lalezari, Shafiee, & Yalpani, 1971). Often the synthesis is based on solution method and except our last report (Jadhav, More, et al., 2015), there has not been any report described using solventless conditions for the preparation of 1,2,3-selenadiazoles. However, the solventless conditions have been occasionally mentioned for other types of
selenadiazoles (Junling et al., 2004). Photochemical sensitivity of reaction and the product alike, coupled with large solvent requirement and extended work-up process, warrants alternative approach to avoid pre-degradation of the compounds. Among the studied compounds, 5-Phenyl-4-methyl-1, 2, 3-selenadiazole (5h) and 4-Phenyl-5-propyl-1, 2, 3-selenadiazole (5i) have not been reported earlier. This article therefore deals with the two new selenadiazoles along with several other such compounds (5a-f) which have not been reported by solventless method. Additionally, their antibacterial properties against the chosen microbes are studied in the current work.

2. Experimental

2.1. Chemicals and methods

All chemicals and solvents (reagent or analytical grade) were purchased from Sigma–Aldrich Company and Merck India Ltd. and were used as received. The UV–visible spectra were recorded qualitatively at room temperature in the range of 200–800 nm using Analytik Jena SPECCORD 210 PLUS UV spectrophotometer. FTIR spectra were recorded at room temperature in the range of 4000–800 cm$^{-1}$ using FTIR Perkin Elmer spectrum two spectrometer. $^1$H and $^{13}$C NMR spectra (300 and 75 MHz, respectively) recorded on a Bruker DRX-300 instrument in CDCl$_3$, and the chemical shifts were reported relative to TMS as an internal standard. The high-resolution mass spectra were recorded on an Agilent Technologies 6540 UHD Accurate-Mass Q-TOF (LC/MS) spectrometer with electron spray ionization. The melting points were determined in open capillaries. The progress of reactions was monitored by TLC on Merck silica gel 60 F-254 aluminum sheets, eluent EtOAc, visualization UV light.

2.2. Synthesis

2-butanol semicarbazone (4a). The semicarbazone derivatives were synthesized using reported procedure (Al-Smadi & Ratrout, 2004). Yield 18.9 g (72%), white crystals, $R_f$ 0.58, mp 132–134°C (EtOH); Reported 144°C (Ibrahim & Al-Difar, 2011). UV spectrum (EtOH), $\lambda_{max}$ (nm): 273. IR spectrum, $\nu$ (cm$^{-1}$): 3473 (secondary N–H), 3190 (amide N–H), 1684 (C=O), 1587 (C = N), 1111 (NC=O), 2888 (C–H). $^1$H NMR spectrum, $\delta$ (ppm): 1.06–1.11 (3H, m, CH$_3$); 1.84 (3H, s, CH$_3$); 2.22–2.29 (2H, m, CH$_2$); 6.06 (2H, s, NH$_2$); 8.46 (1H, s, NH). $^{13}$C NMR spectrum, $\delta$ (ppm): 10.51 (CH$_3$); 15.22 (CH$_3$); 33.82 (CH$_2$); 151.69 (C = N); 158.36 (C=O). Found, m/z: 130.0997 [M]+. C$_5$H$_{12}$N$_3$O. Calculated, m/z: 130.0995

3-pentanone semicarbazone (4b) was synthesized analogously to compound 4a from 3-pentanone. Yield 21.20 g (85%), white crystals, $R_f$ 0.54, mp 102–105°C (EtOH) Reported 113°C (Ibrahim & Al-Difar, 2011). UV spectrum (EtOH), $\lambda_{max}$ (nm): 267. IR spectrum, $\nu$ (cm$^{-1}$): 3446 (secondary N–H), 3234 (amide N–H), 1673 (C=O), 1573 (C = N), 1112 (NC=O), 2955 (C–H). $^1$H NMR spectrum, $\delta$ (ppm): 1.06–1.10 (6H, m, 2CH$_3$); 2.22–2.29 (4H, m, 2CH$_2$); 6.00 (2H, s, NH$_2$); 8.74 (1H, s, NH). $^{13}$C NMR spectrum, $\delta$ (ppm): 9.60 (CH$_3$); 10.47 (CH$_3$); 22.39 (CH$_2$); 29.29 (CH$_2$); 155.95 (C = N); 158.86 (C=O). Found, m/z: 144.1131 [M + H]+. C$_6$H$_{14}$N$_3$O. Calculated, m/z: 144.1136

2-pentanone semicarbazone (4c) was synthesized analogously to compound 4a from 2-pentanone. Yield 22.44 g (90%), white crystals, $R_f$ 0.66, mp 106–108°C (EtOH). UV spectrum (EtOH), $\lambda_{max}$ (nm): 257. IR spectrum, $\nu$ (cm$^{-1}$): 3472 (secondary N–H), 3219 (amide N–H), 1676 (C=O), 1590 (C = N), 1130 (NC=O), 2962 (C–H). $^1$H NMR spectrum, $\delta$ (ppm): 0.90–0.95 (3H, m, CH$_3$); 1.49–1.64 (2H, m, CH$_2$), 1.84 (3H, s, CH$_3$); 5.94 (2H, br. s, NH$_2$); 8.35 (1H, s, NH). $^{13}$C NMR spectrum, $\delta$ (ppm): 16.5 (CH$_3$); 20.21 (CH$_3$); 33.45 (CH$_2$); 19.45 (CH$_3$); 150.00 (C = N); 158.12 (C=O). Found, m/z: 144.1137 [M + H]+. C$_6$H$_{14}$N$_3$O. Calculated, m/z: 144.1136

Isopropyl methyl semicarbazone (4d) was synthesized analogously to compound 4a from 2-butanol. Yield 19.45 g (78%), white crystals, $R_f$ 0.60, mp 110–115°C (EtOH). UV spectrum (EtOH), $\lambda_{max}$ (nm): 262. IR spectrum, $\nu$ (cm$^{-1}$): 3472 (secondary N–H), 3179 (amide N–H), 1667 (C=O), 1574 (C = N), 1127 (NC=O), 2877 (C–H). $^1$H NMR spectrum, $\delta$ (ppm): 1.06–1.11 (6H, m, 2CH$_3$); 1.84 (3H, s, CH$_3$); 2.17–2.22 (2H, m, CH$_2$); 5.94 (2H, br. s, NH$_2$); 8.69 (1H, s, NH). $^{13}$C NMR spectrum, $\delta$ (ppm): 18.01 (2CH$_3$); 22.23
(CH₃); 38.01 (CH); 148.05 (C = N); 158.41 (C=O). Found, m/z: 144.1132 [M + H]+. C₆H₁₄N₃O. Calculated, m/z: 144.1136.

Isobutyl methyl semicarbazone (4e) was synthesized analogously to compound 4a from isobutyl methyl ketone. Yield 14.54 g (61.78%), white crystals, R, 0.56, mp 130–135°C (EtOH). UV spectrum (EtOH), λₘₐₓ (nm): 259. IR spectrum, ν (cm⁻¹): 3424 (secondary N–H), 324 (amide N–H), 1675 (C=O), 1537 (C = N), 1134 (NC=O), 2871 (CH₂). 1H NMR spectrum, δ (ppm): 0.90–0.95 (6H, m, CH₃); 1.49–1.61 (1H, m, CH); 1.84 (3H, s, CH₃); 2.17–2.22 (2H, m, CH₂); 6.04 (2H, s, NH₂). 13C NMR spectrum, δ (ppm): 18.23 (2CH₃); 22.54 (CH₂); 38.21 (CH); 20.12 (CH₂); 150.11 (C = N); 157.31 (C=O). Found, m/z: 158.1302 [M + H]+. C₇H₁₆N₃O. Calculated, m/z: 158.1300.

Acetophone semicarbazone (4f) was synthesized analogously to compound 4a from acetophenone. Yield 18.14 g (82%), (Al-Smadi & Ratrout, 2004)

4-hydroxy acetophenone semicarbazone (4g) was synthesized analogously to compound 4a from 4-hydroxy acetophenone. Yield 14.9 g (70%), (Al-Smadi & Ratrout, 2004)

Valerophenone semicarbazone (4h) was synthesized analogously to compound 3a from 2-butanone. Yield 16.3 g (82%), white crystals, R, 0.58, mp 163–165°C (EtOH). UV spectrum (EtOH), λₘₐₓ (nm): 270. IR spectrum, ν, cm⁻¹: 3453 (secondary N–H), 3188 (amide N–H), 3135 (Ar. H) 1678 (C=O), 1602 (Ar. C=C), 1575 (C = N), 1198 (NC=O), 2975 (C–H). 1H NMR spectrum, δ (ppm): 0.91–0.93 (3H, m, CH₃); 1.21–1.26 (2H, m, CH₂); 1.58–1.64. (2H, m, CH₂); 2.70–2.73(2H, m, CH₂); 7.43–7.47(2H, m, Ar.CH); 7.72–7.75(3H, m, Ar.CH); 6.21(2H, s, NH₂); 8.90 (1H, s, NH). 13C NMR spectrum, δ (ppm): 10.41 (CH₃); 27.2 (CH₂); 28.3 (CH₂); 29.78 (CH₂); 123.27, 127.03, 140.80 (Ar Cs); 144.54 (C = N); 158.65 (C=O). Found, m/z: 220.1469 [M + H]+. C₁₂H₁₈N₃O. Calculated, m/z: 220.1467.

Propeophenone semicarbazone (4i) was synthesized analogously to compound 4a from propeophenone. Yield 11 g (77.19%), white crystals, R, 0.55, mp 165–168°C (EtOH). UV spectrum (EtOH), λₘₐₓ (nm): 260. IR spectrum, ν (cm⁻¹): 3451 (secondary N–H), 3199 (amide N–H), 3140 (Ar. H) 1689 (C=O), 1602 (Ar. C=C), 1575 (C = N), 1198 (NC=O), 2973 (C–H). 1H NMR spectrum, δ (ppm), J (Hz): 1.18–1.22 (3H, m, CH₃); 7.48–7.52 (2H, m, CH₂); 7.70–7.74 (3H, m, Ar.CH); 8.60 (1H, s, NH). 13C NMR spectrum, δ (ppm): 10.23 (CH₃); 20.12 (CH₂); 126.34, 128.43, 130.42 (ArCs); 150.16 (C = N); 158.60 (C=O). Found, m/z: 192.1134 [M + H]+. C₁₀H₁₄N₃O. Calculated, m/z: 192.1132.

4-ethyl-1,2,3-selenadiazole (5a) (General Method). 2-butanone semicarbazone (4a) (0.029 mol) and selenium dioxide (3.2 g, 0.029 mol) were ground together in a mortar pestle at room temperature for around 20 min. The process was monitored by TLC using hexane–AcOEt, 7:3, as solvent system. The crude product was dissolved in 100 ml toluene and filtered. The filtrate was evaporated using a rotary evaporator. The product was purified by column chromatography on silica gel (60–120 mesh), using petroleum ether (bp 60–80°C)—toluene, 7:3, as eluent. Yellow liquid product was collected as a final product which is characterized by UV–visible, FTIR, 1H and 13C NMR spectroscopy, and mass spectrometry. Yield 3.12 g (50%), UV spectrum (toluene), λₘₐₓ (nm): 341. IR spectrum, ν (cm⁻¹): 2921–2847 (C–H), 1620 (C=C), 1454 (C–H), 1302 (C–N). 1H NMR spectrum, δ (ppm): 1.45 (3H, J = 5, t, CH₃); 3.27 (2H, m, CH₂); 13C NMR spectrum, δ (ppm): 14.13 (CH₃); 29.99 (CH₂); 17.86 (CH₃); 153.35 (C=C–N); 161.06 (C=C–Se). Found, m/z: 162.9776 [M + H]+. C₄H₇N₂Se. Calculated, m/z: 162.9773.
4-propyl-1, 2, 3-selenadiazole (5c) was synthesized analogously to compound 5a. Yield 2.9 g (48%), yellow liquid, R, 0.78. UV spectrum (toluene), $\lambda_{max}$ (nm): 341. IR spectrum, $\nu$ (cm$^{-1}$): 2917–2850 (C–H), 1645 (C–H), 1300 (C–N). $^1$H NMR spectrum, $\delta$ (ppm), J (Hz): 0.87 (3H, t, J = 5.0, CH$_3$); 1.71–1.77 (2H, t, J = 5, CH$_2$); 3.06 (2H, t, CH$_2$); 8.80 (1H, s, selenadiazole ring H). $^{13}$C NMR spectrum, $\delta$ (ppm): 13.32 (CH$_3$); 22.71 (CH$_2$-CH$_3$); 31.06 (CH$_2$-CH$_2$); 137.59 (C=C–N); 163.14 (C=C–Se). Found, m/z: 176.9928 [M + H$^+$]. C$_5$H$_9$N$_2$Se. Calculated, m/z: 176.9926.

4-iso-propyl-1, 2, 3-selenadiazole (5d) was synthesized analogously to compound 5a. Yield 3.29 g (54%), yellow liquid, R, 0.65. UV spectrum, $\lambda_{max}$ (nm): 322. IR spectrum, $\nu$, cm $^{-1}$: 2923–2869 (C–H), 1620 (C=C), 1462 (C–H), 1300 (C–N). $^1$H NMR spectrum, $\delta$ (ppm), J (Hz): 1.35 (6H, m, CH$_3$); 3.45–3.53 (H, m, CH); (1H, S, heterocyclic ring H). $^{13}$C NMR spectrum, $\delta$ (ppm): 22.58 (CH$_3$-CH-CH$_3$); 29.42 (CH); 135.87 (C=C–N); 169.65 (C=C–Se). Found, m/z: 176.9930 [M + H$^+$]. C$_5$H$_9$N$_2$Se. Calculated m/z: 176.9929.

4-isobutyl-1, 2, 3-selenadiazole (5e) was synthesized analogously to compound 5a. Yield 3.12 g (52%), yellow liquid, R, 0.78. UV spectrum (toluene), $\lambda_{max}$ (nm): 325. IR spectrum, $\nu$ (cm$^{-1}$): 2923–2867 (C–H), 1633 (C=C), 1462 (C–H), 1292 (C–N). $^1$H NMR spectrum, $\delta$ (ppm), J (Hz): 0.80 (6H, t, J = 5, CH$_3$); 1.99–2.06 (H, m, CH); 2.90 (2H, t, CH$_2$); 8.71 (1H, s, selenadiazole ring H). $^{13}$C NMR spectrum, $\delta$ (ppm): 21.75 (CH$_3$-CH-CH$_3$); 28.66 (CH); 37.86 (CH$_2$); 138.24 (C=C–N); 161.98 (C=C–Se). Found, m/z: 191.0089 [M + H$^+$]. C$_6$H$_{11}$N$_2$Se. Calculated, m/z: 191.0087.

4-(4-hydroxyphenyl) -1, 2, 3-selenadiazole (5f) was synthesized analogously to compound 5a. Yield 2.61 g (45%), reddish solid, R, 0.42. (Al-Smadi & Ratrout, 2004)

5-phenyl-1, 2, 3-selenadiazole (5g) was synthesized analogously to compound 5a. Yield 2.81 g (48%), reddish solid, R, 0.57. (Al-Smadi & Ratrout, 2004)

5-phenyl-4-methyl-1, 2, 3-selenadiazole (5i) was synthesized analogously to compound 5a. Yield 2.07 g (45%), pinkish solid, R, 0.42. (Al-Smadi & Ratrout, 2004)

2.3. Antimicrobial study

The antimicrobial study was carried out at Kulkarni Laboratory and Quality Management Services at Pune, India. Microbial strains used in the study were clinical isolates of bacteria Staphylococcus aureus, Escherechia coli, Bacillus subtilis, and Pseudomonas aeruginosa, as well as fungi Aspergillus niger and Penicillium notatum. Muller Hinton agar media (gm/litre) was used for media preparation. For 1000 ml Muller Hinton agar preparation peptone 5 g, sodium chloride 8 gm, beef infusion 3 gm, and agar 16 gm were weighed and dissolved in 1000 ml of distilled water and maintained pH 7.3–7.4 which was sterilized by autoclaving at 121°C for 15 min at 15 psi pressure.

All synthesized compounds 5a-i, were screened for their antibacterial activity against Gram-positive and Gram-negative bacteria at concentration 0.0049 g/ml using two different methods involving agar disc diffusion and agar well method with Muller Hinton agar media (Table 1).
selected bacterial suspension was spread on the surface of Muller Hinton agar plates respectively. In case of agar disc diffusion method, the synthesized compounds were impregnated on Whatman No. 1 filter paper disc (6 mm diameter) at a concentration of 0.0049 g/ml. Each disc is coded with the name of the agent. Discs were placed on solidified media and allowed to diffuse for 5 min; the plates were kept for incubation at 37°C for 24 h for bacteria. Dimethylsulfoxide (DMSO) was used as the control. At the end of incubation, antibacterial activity was determined by measuring zone of inhibition in mm around each of the disc and compared with standard DMSO.

To check the antimicrobial activity of the samples against test organisms by well diffusion method, freshly prepared nutrient agar was seeded with 1% inoculums of each test organism. The 8-mm diameter wells were cut with the help of cork borer. Each well was then filled with sample (approximately 100 μl). All the plates were incubated at 37°C for 24 h. The zone of inhibition around each disc was measured after completion of incubation time.

Antifungal activity of the samples: Similar procedure was used to check the antifungal activity of the test samples. Fungal strains used for these studies are *Aspergillus niger* and *Penicillium notatum*. Potato Dextrose agar was used to check the activity. The fungal spores was dispersed in sterile saline with 1% Tween 80 and seeded to PDA media. The 8-mm diameter wells were cut with the help of cork borer. Each well was then filled with sample. All the plates were incubated at room temperature for five days. The zone of inhibition around each disc was measured after completion of incubation time.

### 3. Results and discussion

In the current work, we present the synthesis of two novel 1,2,3-selenadiazoles (5h,i) and comparative study of antimicrobial behavior of different series of 1,2,3-selenadiazoles. The synthesized compounds were tested against two Gram-positive and Gram-negative bacteria as well as fungi species by two different methods of antimicrobial screening. We herein present a solventless method for preparation of 1,2,3-selenadiazoles from the respective semicarbazones. Semicarbazones 4a-i can be easily synthesized by a reported procedure (Al-Smadi & Ratrout, 2004) from the respective ketones 3a-i and semicarbazide hydrochloride 1. We have earlier opined that a solventless cyclization

#### Table 1. Sensitivity of human pathogenic microbes to 1,2,3-selenadiazoles 5a–i using the agar disc and agar well diffusion method

| Sample codes | Conc. (g/ml) | Diameter of zone of inhibition (mm) |
|--------------|-------------|------------------------------------|
|              |             | S. aureus | P. aeruginosa | E. coli | B. subtilis | A. nigir | P. notatum |
|              | D.D.¹ | W.D.² | D.D. | WD | D.D. | W.D. | D.D. | W.D. | D.D. | W.D. |
| 5a           | 0.0049 | 17 | 22 | 12 | 20 | 12 | 15 | 20 | 20 | 23 |
| 5b           | 0.0049 | 16 | 23 | 15 | 22 | 13 | 15 | 23 | 27 | 22 | 24 |
| 5c           | 0.0049 | 17 | 21 | 12 | 20 | 17 | 18 | 23 | 27 | 22 | 24 |
| 5d           | 0.0049 | 15 | 20 | 11 | 15 | 10 | 17 | 20 | 25 | 18 | 23 |
| 5e           | 0.0049 | 14 | 17 | 11 | 15 | 9 | 16 | 17 | 22 | 17 | 20 |
| 5f           | 0.0049 | 15 | 18 | 12 | 17 | - | 15 | 16 | 20 | 10 | 15 |
| 5g           | 0.0049 | 11 | 17 | 12 | 12 | 12 | 15 | 15 | 17 | 10 | 14 |
| 5h           | 0.0049 | 12 | 16 | 10 | 16 | 10 | 18 | 11 | 17 | 9 | 10 |
| 5i           | 0.0049 | 10 | 15 | 10 | 15 | 10 | 17 | 10 | 17 | 8 | 9 |
| Tetracycline | 0.0049 | 20 | 12 | 12 | 23 | - | - | - | - | - | - |

¹D. D.: Disc diffusion method.
²W. D.: Well diffusion method; (-) not done.

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process is a rapid method in comparison to the solution chemistry and requires lesser time with reduced or negligible amount of solvent for the isolation of product. In our experiments, the solventless reaction of semicarbazones 4a–i with selenium dioxide requires hand grinding only and could be completed within 30–60 min producing 1,2,3-selenadiazoles 5a–i in moderate yields. Additionally, during synthesis it was observed that solventless method is more feasible for the synthesis of aliphatic derivatives of 1,2,3-selenadiazoles 5a–e compared to aromatic derivatives 5f–i. It is further noted that compounds 5f–i need longer grinding (reaction) time with low yield compared to compounds 5a–e.

The structures of various semicarbazones 3a–i and selenadiazoles 5a–i were confirmed by UV–visible, FTIR, $^1$H and $^{13}$C NMR spectroscopy, and mass spectrometry. UV–visible spectra of 1,2,3-selenadiazoles 5a–i showed absorption bands $\lambda_{\text{abs}}$ at ~300–350 nm due to $\pi\rightarrow\pi^*$ electronic transition of C=C in conjugation with N = N in which the N and C are attached to the Se atom in heterocyclic ring. The longer wavelength absorption was observed due to extensive conjugation along with the presence of Se in heterocyclic ring.

FTIR spectra of compounds 5a–i were recorded as KBr pellets. Figure SI 1 shows IR spectrum of 4-phenyl-5-propyl-1, 2, 3-selenadiazole. Generally, peaks at 2951–2848 cm$^{-1}$ are due to C–H stretching mode of vibrations and at 1648–1627 cm$^{-1}$ for C=C stretching mode of vibrations (due to Se-C=C-N moiety in 1,2,3-selenadiazoles). The IR transmission band at 1482–1436 cm$^{-1}$ was assigned to C–H deformation mode vibrations, and the band at 1303–1286 cm$^{-1}$ is assigned to C–N stretching mode of vibrations. Whereas, compounds having aromatic moieties showed additional bands at 3130–3080 cm$^{-1}$ and 1580–1600 cm$^{-1}$ which can be assigned to Ar C–H and C=C stretch, respectively.

FTIR spectra show obvious variations in Ar C–H, C=C stretch along with other stretching modes of vibrations because of varying organic substituents at R and R’ groups. The $^1$H NMR spectra of 1,2,3-selenadiazoles 5a–i in CDCl$_3$ showed different chemical shifts for CH, CH$_2$, and CH$_3$ protons in the range of $\delta$ 1.10–3.25 ppm for alkyl groups. For protons associated with the heterocyclic ring chemical shifts are observed in the range of $\delta$ 8.70–9.50 ppm. Similarly, compounds having aromatic moieties showed chemical shifts in the range of $\delta$ 6.90–8.3 ppm for aromatic ring protons. Likewise, the $^{13}$C NMR spectrum of 1,2,3-selenadiazoles 5a–i showed the expected number of signals due to different carbon atoms in the molecules. Chemical shift at around $\delta$ 130–137 ppm and $\delta$ 157–165 ppm are assigned to (C=C–N) and (C=C–Se) heterocyclic ring carbon atoms, respectively. For typical understanding the $^1$H NMR and $^{13}$C NMR spectrum of 4-phenyl-5-propyl-1, 2, 3-selenadiazole is shown in Figure S12 and 3. Mass spectra of 1, 2, 3-selenadiazoles showed peaks with a set of isotopic components, characteristics of the presence of selenium which has 8 naturally occurring isotopes with atomic mass 72–82 out of which only 6 isotopes are stable. The m/z value of all the compounds

![Scheme 1](image-url)
corresponded to the respective protonated molecular ions. To study the fragmentation pattern through MS–MS, analysis of some samples were carried out. The mass fragmentation pattern of 4-Phenyl-5-propyl-1, 2, 3-selenadiazole is shown in scheme 2 (Figure SI 4a,b). In a typical fragmentation analysis, molecular ion peak undergoes initial breakdown by loss of selenium followed by elimination of a nitrogen molecule to form asymmetric alkynes. Alkyne so generated may further dissociate stepwise by loss of a methyl group to eventually result in the formation of toluene molecule.

3.1. Antimicrobial studies

The activity of synthesized selenadiazoles 5a–i was tested against some human pathogenic microbes. For studying their activity, two Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and two Gram-positive (Staphylococcus aureus and Bacillus subtillis) species were selected. For more authentication antibacterial activity of compounds were tested by two different methods i.e. agar disc diffusion method and agar well diffusion method. All the compounds described in this text were also screened for antifungal activity against Aspergillus niger and Penicillium notatum. From the results obtained by these methods, it was found that agar well method compared to agar disc diffusion method gives better results and thus can be considered more suitable for selenadiazoles. Encouraged with the suitability of agar well method for antibacterial screening, antifungal activity testing was also performed following the same method. Some of the tested compounds were highly active even at concentrations as low as 4.9 mg/ml (Table 1, Figure 1). For example, compound 5a showed good inhibition against the highly resistant Pseudomonas aeruginosa. Generally, the

![Figure 1. Sensitivity of 1,2,3-selenadiazoles 5a–i against human pathogenic microbes.](image)
extracts of all selenadiazoles 5a–i in dimethyl sulfoxide (DMSO) were active against all the tested pathogens, in the range of 8–25 mm diameter inhibition zone. DMSO used as control which showed no activity against any of the tested pathogens. On the basis of the presented data, it can be concluded that the tested 1,2,3- selenadiazoles 5a–i possess good antimicrobial properties compared to tested antibiotic drugs such as tetracycline. Tetracycline is well-known antibiotic for the inhibition of microbial cell growth as it suppresses protein synthesis. Tetracycline showed zone of inhibition against tested bacteria in the range of (20–23 mm) where many of the selenadiazoles showed better zones inhibition than the drug. It is further observed that 1,2,3-selenadiazoles 5a–i showed maximum activity against Gram-positive Bacillus subtillis and minimum activity against Gram-negative E.Coli. Amongst various selenadiazoles tested, compound 5b showed maximum inhibition against all the pathogens. Selenadiazoles in which R and R’ both groups are aliphatic showed better zone of inhibition in comparison to selenadiazoles in which R is an aromatic group. Within aliphatic series compounds, it was observed that compounds having branched chain alkyl groups showed lesser zone of inhibition compared to compounds having straight chain alkyl groups. The probable reason for variation in activity among selenadiazoles could be due to the variation in their stability and solubility. Since aliphatic series compounds are less stable, release of Se from such compounds is more efficient, thus making them more effective. However due to higher stability and less solubility aromatic series compounds in DMSO, they show marginally poor activity against the tested pathogens.

3.2. Mechanism of action
It has been reported that the cause of suppression of bacterial growth in presence of antibacterial agents could be due to several factors e.g. interference of compounds with cell wall synthesis, inhibition of protein synthesis by binding to various ribosomal subunits, suppression of nucleic acid synthesis, disturbances to metabolic pathway, and disruption of bacterial membrane structure. It is opined that selenadiazoles may show good antimicrobial activity due to the presence of diazole fragment along with selenium. There are a few selenium-containing organic compounds with a reported antibacterial activity (Radhakrishna et al., 2010). It can be postulated that selenium might partially replace sulfur in sulfur-containing amino acids present in the bacterial cells (Figure 2) and inhibit their growth due to toxicity of selenium compounds and metabolic disturbances to the microorganisms (Li, Liu, Wu, Liang, & Qu, 2002; Bemheim & Klein, 1941). In the present series of compounds, initial elimination of selenium from 1,2,3-selenadiazoles may interact with microbes to alter their bioactivities. Such studies therefore, clearly indicate that selenium plays an important role for these compounds to enhance their antimicrobial activity.

4. Conclusions
In conclusion, synthesis and characterization of different series of other 1,2,3-selenadiazoles by solventless method are presented. Fragmentation pathway for some new molecules was ascertained by m/z spectroscopy. All the selenadiazoles were tested for their antimicrobial activity to highlight...
that they showed excellent antimicrobial activity against wide range of bacteria and fungi. The instant formation of such organoselenium compounds will likely promote their application in pharmaceutical chemistry, biochemistry, microbiology.

Supplementary Material
Supplementary material for this article can be accessed here http://dx.doi.org/10.1080/23312009.2016.1144670.

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Author details
Aditi A. Jadhav
E-mail: aditijadhav@yahoo.com
Vaishali P. Dhanwe
E-mail: vdhane@gmail.com
Prasad G. Joshi
E-mail: joshi.p13@gmail.com
Pawan K. Khanna
E-mail: pawankhanna2002@yahoo.co.in

1 Nanochemistry Laboratory, Department of Applied Chemistry, Defence Institute of Advanced Technology (DIAT), Ministry of Defence, Govt. of India, Girinagar, Pune 411 025.

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