# 5-Nitroimidazole Derivatives and their Antimicrobial Activity

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**Summary:** 5-Nitroimidazole derivatives **2-8** were synthesized from secnidazole. The syntheses were accomplished in two steps which start from the oxidation of secnidazole to the secnidazolone **1**. Secnidazolone **1** was converted into its hydrazone derivative **2-8** by treating with different substituted acid hydrazide. Compounds **2-8** were evaluated for their antimicrobial activity against Gram-positive and Gram-negative bacteria, compounds **3** and **4** showed the significant activity against *Staphylococcus epidermidis*, however, compound **2** showed good inhibitions against *Corynebacterium diphtheria* when compared with the standard. Compound **3** showed good inhibitory potential against tested Gram-negative bacterial strains *i.e. Enterobacter aerogene, Escherichia coli, Salmonella typhi, Salmonella paratyphi A, Shigella flexeneri* and *Vibrio choleriae*. All synthetic derivatives were also tested against eight fungal stains, however, they were weekly active against *Aspergillus flavus* and *Candida albican.* The synthesized compounds were characterized by different spectroscopy techniques.

*Keywords*: Secnidazole, Secnidazolone; Anti-bacterial activity; Anti-fungal activity

## Introduction

Infections caused by pathogenic microorganisms like bacteria can bring human morbidity and mortality [1-2]. Even though the efforts regarding research for antibiotics has somehow improved mankind's health condition, but still the emergence of bacterial resistance has become a serious problem for the clinical management worldwide [3-6]. Infections are usually treated by utilizing antimicrobials chemotherapy [7]. Nitroimidazoles (such as secnidazole, metronidazole) and their derivatives have been extensively used as antimicrobial drugs [8-10] and has been accepted as drug of choice for anti-infectious therapy to kill microbials in host tissues and organs [11]. Most importantly, the metabolism and toxicology of nitroimidazoles have been well documented [12]. Particularly, metronidazole has been used as the most preferred treatment of choice worldwide [13], despite of its several side effects [14]. Therefore, the research regarding development of antibacterial drugs are oriented towards the design of new and efficient antibacterial agents with lesser side effects [15-21].

Secnidazole could be an important alternative for metronidazole [22], due to its longer half-life (17-29 h), rapid and complete absorbtion after oral administration [23], with fewer side effects [22]. More importantly, secnidazole has been extensively used as single-dose therapy in amebiasis, giardiasis, trichomoniasis, and bacterial vaginosis [24]. In this regards, the treatment with secnidazole is shorter, more effective and better choice of clinicians than other imidazole drugs [25].

The aim of our study was to further evaluate the antimicrobial activity of secnidazole by doing its structure modifications. It is evident that the nitro group is playing a very crucial role in the metabolic activation [26] as it produces the toxic nitro radicals after activation by low redox potential reactions in anaerobes which eventually cause death of the anaerobic organism after covalently bonding with DNA of the microorganism and ultimately brings about the lethal effect [27,28]. Nitroso, nitroxide, amine and hydroxylamine are the potential reactive intermediates reveals by the literature [29]. Thus the structural modification at the hydroxyl group of alkyl chain of secnidazole has received our attention and then we synthesized the representative hydrazones of nitroimidazole. A total of seven hydrazones of nitroimidazole (**2-8**) were synthesized and evaluated for their antimicrobial activities. To the best of our

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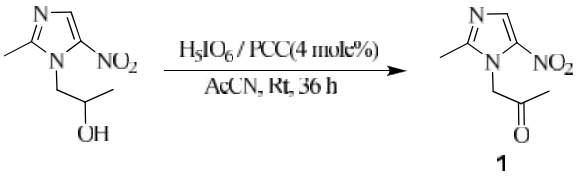
knowledge, all the synthesized derivatives in this study are new.

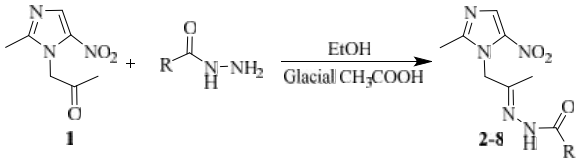
## Results and Discussion

*Chemistry*

Secnidazolone **1** was first time synthesized from secnidazole by our research group (Scheme-1) [30]. Keeping in mind, the critical structural modification of secnidazole with numerous evidences as well as the multi dimensional activities of Schiff bases [31-46], we have synthesized different phenyl hydrazones of secnidazolone by condensing with different acid hydrazides in ethanol in the presence of catalytic amount of glacial acetic acid under reflux for 2 to 4 h in satisfactory yields (Scheme-2). The crude products were further crystallized from ethanol to get pure products. The structures of phenyl hydrazones of secnidazolone **2-8** were deduced by using different spectroscopic techniques including EIMS, IR, and NMR. All compounds gave acceptable elemental analyses and in good concurrence with calculated values.




Scheme-1: Synthesis of Secnidazolone from Secnidazole

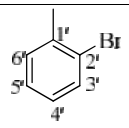
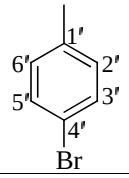
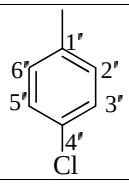
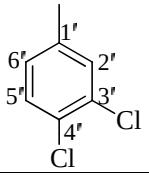
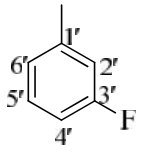
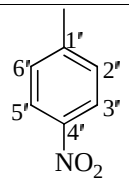
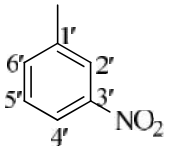



Scheme-2: Synthesis of Substituted Phenyl hydrazones of Secnidazolone

*In vitro Antimicrobial Activity*

Literature reveals that one of the major factors plays a very vital role in antibacterial activity is the lipophilicity means that only lipid soluble materials can easily passed through the cell membrane of microorganism [47,48]. This key factor as well as the crucial role of nitro group in secnidazole was our major focus throughout the synthetic work. Therefore, it is worth mentioning that we have avoided the hydroxyl group in our synthesized compounds by keeping in mind the major factor of lipophilicity. So, all the synthesized compounds are much lipophilic with no hydroxyl groups, having halogen substituent and two derivatives **7** and **8** are nitro derivatives as the nitro group was our main focus too in this study.

Table-1: Structures of Substituted Phenyl hydrazones of Secnidazolone **2-8**

| Compound No. | R | Yield (%) |
|---|---|---|
| **2** |  | **67** |
| **3** |  | **73** |
| **4** |  | **69** |
| **5** |  | **72** |
| **6** |  | **74** |
| **7** |  | **70** |
| **8** |  | **68** |

Seven newly synthesized compounds **2**, **3**, **4**, **5**, **6**, **7**, and **8** were evaluated in order to check their antibacterial activity against both Gram-positive and Gram-negative bacterial strains by following standard disc diffusion method [49] while DMSO was utilized as a negative control. *Bacillus subtilis*, *Corynebacterium diphtheria*, *Corynebacterium*

*xerosis, Staphylococcus aureus, Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus faecalis* and *Streptococcus pyogenes* were used as Gram-positive bacterial strains. The results in zone diameter of growth inhibition (mm) showed that all the new synthetic compounds have demonstrated weak to significantly good inhibitory potential against Gram-positive bacteria when compare to the standard gentamicin Table-2. Fortunately, all the synthesized compounds specifically gave the better activity against *Staphylococcus saprophyticus* even better than the standard. Compound **3** and **4** displayed notably good activity against *Staphylococcus epidermidis* comparable with the standard. Similarly, compound **2** also exhibited significantly good activity against *Corynebacterium diphtheria*, while remaining compounds showed weak to good activity against tested Gram-positive bacteria.

Table-3 showed the results of the inhibitory activity of compounds **2**, **3**, **4**, **5**, **6**, **7**, and **8** against Gram-negative bacterial strains. *Enterobacter aerogene, Escherichia coli, Escherichia coli (MDR), Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi B, Shigella flexeneri, Shigella dysenteriae, Vibrio choleriae* and *Aeromonas* were used as Gram-negative bacterial strains. Compound **3** out of all the synthetic derivatives exhibited significantly good inhibitory potential against *Enterobacter aerogene, Escherichia coli, Salmonella typhi, Salmonella paratyphi A, Shigella flexeneri* and *Vibrio choleriae*, while compounds **6**, **7**, and **8** showed good activities against *Salmonella paratyphi A.* Compounds **4** and **7** also showed sound activity against *Shigella flexeneri.* Furthermore, rest of the compounds showed weak to moderate activity against tested Gram-negative bacteria.

Ketoconazole used as standard for the antifungal bioassay. The structure of the ketoconazole have halogen (two chloro substituents), imidazole ring and amide linkage. These key structural features are also the part of our synthesized derivatives and were taking into account while hoping that our compounds will also show the antifungal activity.

*In vitro* inhibitory antifungal activity of all compounds **2**, **3**, **4**, **5**, **6**, **7**, and **8** was evaluated against eight species *i.e. Aspergillus flavus, Aspergillus niger, Penicillium spp., Rhizopus sp., Mucor, Candida albican, Candida tropicalis* and *Saccharomyces cerevisiae* using disc diffusion method [49]. The linear growth of the all fungus was obtained by measuring the diameter of the fungal colony after one week. Table-4 reveals the results as zone diameter of growth inhibition (mm). All the compounds showed weak activity against *Aspergillus flavus* and *Candida albican.* Whereas compounds **2**, **3**, **4** and **5** as well as compounds **2**, **3**, **4**, **5**, **6** and **7** showed weak activities against *Aspergillus niger* and *Saccharomyces cerevisiae*, respectively.

Table-2: *In vitro* antibacterial activity of compounds **2-8** against Gram-positive bacteria (Inhibition zones in mm using the disc diffusion method).

| Gram-positive bacteria | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Gentamicin |
|---|---|---|---|---|---|---|---|---|
| ***Bacillus subtilis*** | **17** | **19** | **19** | **17** | **18** | **19** | **19** | **22** |
| ***Corynebacterium diphtheria*** | **22** | **19** | **20** | **20** | **17** | **20** | **20** | **25** |
| ***Corynebacterium xerosis*** | **14** | **9** | **8** | **15** | **8** | **8** | **15** | **22** |
| ***Staphylococcus aureus*** | **16** | **19** | **19** | **18** | **19** | **19** | **20** | **25** |
| ***Staphylococcus aureus* (MRSA)** | **10** | **15** | **14** | **12** | **15** | **15** | **15** | **20** |
| ***Staphylococcus epidermidis*** | **15** | **25** | **23** | **15** | **20** | **19** | **19** | **28** |
| ***Staphylococcus saprophyticus*** | **15** | **20** | **18** | **15** | **17** | **17** | **17** | **09** |
| ***Streptococcus faecalis*** | **11** | **14** | **13** | **12** | **15** | **15** | **15** | **25** |
| ***Streptococcus pyogenes*** | **14** | **14** | **14** | **12** | **15** | **15** | **16** | **25** |

Keys: - = No zone of inhibition, Key: 8-10 mm= weakly active, 12-14 mm = moderately active, >15 = good activity

Table-3: *In vitro* antibacterial activity of compounds **2-8** against Gram-negative bacteria (Inhibition zones in mm using the disc diffusion method)

| Gram-negative bacteria | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Gentamicin |
|---|---|---|---|---|---|---|---|---|
| ***Enterobacter aerogene*** | **11** | **16** | **15** | **13** | **15** | **14** | **14** | **22** |
| ***Escherichia coli*** | **14** | **16** | **15** | **14** | **14** | **15** | **15** | **29** |
| ***Escherichia coli(MDR)*** | **12** | **14** | **13** | **11** | **12** | **12** | **13** | **20** |
| ***Pseudomonas aeruginosa*** | **11** | **14** | **14** | **11** | **13** | **12** | **14** | **22** |
| ***Salmonella typhi*** | **14** | **17** | **15** | **15** | **15** | **15** | **15** | **25** |
| ***Salmonella paratyphi* A** | **15** | **16** | **15** | **15** | **16** | **18** | **17** | **25** |
| ***Salmonella paratyphi* B** | **13** | **14** | **13** | **14** | **13** | **14** | **15** | **25** |
| ***Shigella flexeneri*** | **18** | **19** | **19** | **15** | **15** | **16** | **15** | **28** |
| ***Shigella dysenteriae*** | **15** | **14** | **14** | **14** | **14** | **15** | **15** | **23** |
| ***Vibrio choleriae*** | **12** | **19** | **15** | **13** | **15** | **15** | **14** | **25** |
| ***Aeromonas*** | **12** | **14** | **13** | **11** | **12** | **12** | **13** | **27** |

Keys: - = No zone of inhibition, Key: 8-10 mm= weakly active, 12-14 mm = moderately active, >15 = good activity

Table-4: *In vitro* antifungal activity (Inhibition zones in mm using the disc diffusion method).

| Names of Fungus | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Ketoconazole |
|---|---|---|---|---|---|---|---|---|
| ***Aspergillus flavus*** | **8** | **11** | **11** | **10** | **9** | **11** | **8** | **24** |
| ***Aspergillus niger*** | **8** | **11** | **8** | **10** | **-** | **-** | **-** | **24** |
| ***Penicillium* spp.** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **22** |
| ***Rhizopus* sp.** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **22** |
| ***Mucor*** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **24** |
| ***Candida albican*** | **11** | **10** | **12** | **10** | **12** | **14** | **12** | **22** |
| ***Candida tropicalis*** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **22** |
| ***Saccharomyces cerevisiae*** | **8** | **9** | **9** | **9** | **8** | **8** | **-** | **22** |

Keys: - = No zone of inhibition, Key: 8-10 mm= weakly active, 12-14 mm = moderately active, >15 = good activity

## Experimental

*General Information*

Thin layer chromatography (TLC) was performed on silica gel aluminum plates (pre-coated, Kieselgel 60 F-254, 0.20 mm, Merck, Darmstadt, Germany). Chromatograms were visualized on a handhold UV lamp at (254 and 365 nm) or iodine vapors. Electron impact mass spectra (EI-MS) were recorded by using Finnigan MAT-311A, Germany (70eV) spectrophotometers and the data were tabulated as *m/z*. IR spectroscopic analysis was carried out on Shimadzu-IR-460 as KBr pellets and the values are reported in $cm^{-1}$. $^{1}$H-NMR spectra were recorded on Avance Bruker AM spectrometers (300, 400 and 500 MHz, respectively) and signals were reported as s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shifts are given in $\delta$ (ppm) and coupling constants are reported in Hz. CHN analysis was conducted on a Carlo Erba Strumentazione-Mod-1106, Italy. All solvents and reagents were of reagent grade and used directly without purification.

*Bioassay*

*Antibacterial Assay*

The antibacterial activity of compound was determined by using the disc diffusion method [49]. The concentration of stock solution (100 mg/mL) was prepared by dissolving compounds in dimethyl sulphoxide (DMSO), whereas stock solution of concentration (50 mg/mL) was prepared by dissolving in DMSO. For the purpose of screening 10 $\mu$L of stock solution were used in sterile filter discs. The Mueller Hinton agar (Oxoid) plates were used for seeding with 24 h. Mueller Hinton broth (Oxoid) was used for old culture grown. The plates were incubated at 37 $^{\circ}$C for 24 hours after placing the prepared discs on to the surfaces at different positions. Results were recorded thrice by measuring the zone of inhibitions in mm. Antibacterial activity of all synthesized compounds was performed by using gentamicin as positive control. DMSO was used as negative control for antibacterial activity.

*Antifungal Assay*

The antifungal activity was also determined by using the disc diffusion method [49] as above. Briefly, a small amount of culture was taken in 2-3 mL distilled water or normal saline in a screw capped tube with few glass beads of 1 mm diameter. Vortexes used for 5-10 minutes in order to homogeny the suspension of fungal culture. These suspensions were seeded in Sabouraud dextrose agar (SDA) plates. All the plates were incubated for one week at room temperature after placing the sterile filter discs containing 10 $\mu$L of stock solution on to the surfaces at different positions. Results were recorded thrice by measuring the zone of inhibitions in mm. Antifungal activity of all synthetic compounds was performed using ketoconazole as positive control.

*General Procedure for the Syntheses of Hydrazones of Secnidazolone* **(2-8)**

Acid hydrazide (0.5 mmol) and secnidazolone **1** (0.5 mmol) were taken in ethanol (15 mL) with catalytic volume of glacial acetic acid into a 100 mL round-bottommed flask. Reaction mixture was refluxed for 2 to 4 h and completion of reaction was monitored by TLC (6:4 = EtOAc:Hexane). Reaction mixture was poured into cold distilled water (100 mL) and precipitates were formed which were filtered and dried. The precipitates were crystallized from ethanol. Product was characterized by spectroscopic techniques (EIMS and NMR) and elemental analysis was also performed.

*Spectroscopic Data*

*(E)-1-(2-(2-(2-Bromophenyl)hydrazono)propyl)-2-methyl-5-nitro-1H-imidazole* **(2)**

Yield: 67%; $^{1}$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 11.12 (s, 1H, NH), 7.50 (m, 4H, H-Ar), 6.96 (s, 1H, H-4$'$), 4.92 (s, 2H, $CH_2$), 2.06 (s, 3H, 2$'$-$CH_3$), 1.98 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 155.5 (C=N, C), 153.0 (C, C-2), 140.2 (C, C-1$'$), 137.5 (C, C-2$'$), 138.3 (C, C-5), 132.7 (CH, C-6$'$), 132.1 (CH, C-4), 128.4 (CH, C-3$'$), 126.2 (C, C-4$'$), 115.2 (CH, C-5$'$), 48.0 ($CH_2$, C), 12.5 ($CH_3$, C), 12.1

($CH_3$, C); EI-MS: *m/z* (rel. abund. %), 333 ($M^+$-$NO_2$, 1), 335 (M+2-$NO_2$, 1), 316 (6), 294 (7), 239 (16), 185 (100), 155 (22), 134 (10); Anal. Calcd for $C_{14}H_{14}BrN_5O_3$: C, 44.23; H, 3.71; N, 18.42; O, 12.62; Found: C, 44.26; H, 3.75; N, 18.40; O, 12.65; IR (KBr, $cm^{-1}$): 3354 (NH), 1702 (C=O), 1645 (C=N), 1640 (C=N), 1635 (C=C), 1600 (C=C).

*(E)-1-(2-(2-(4-Bromophenyl)hydrazono)propyl)-2-methyl-5-nitro-1H-imidazole* **(3)**

Yield: 73%; $^1$H-NMR: (500 MHz, DMSO-$d_6$): $\delta$ 10.77 (s, 1H, NH), 8.04 (s, 1H, H-4′), 7.72 (bd.s, 2H, H-3″,5″), 7.66 (bd.s, 2H, H-2″,6″), 5.21 (s, 2H, $CH_2$), 2.40(s, 3H, 2′-$CH_3$), 2.01 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 155.5 (C=N, C), 153.0 (C, C-2), 142.1 (C, C-1'), 138.3 (C, C-5), 132.5 (CH, C-3'), 132.5 (CH, C-5'), 132.1 (CH, C-4), 117.1 (CH, C-2'), 117.1 (CH, C-6'), 116.6 (C, C-4'), 48.0 ($CH_2$, C), 12.5 ($CH_3$, C), 12.1 ($CH_3$, C);.EI-MS: *m/z* (rel. abund. %), 333 ($M^+$-$NO_2$, 2), 335 (M+2-$NO_2$, 3), 316 (9), 294 (5), 239 (13), 185 (100), 155 (24), 134 (11); Anal. Calcd for $C_{14}H_{14}BrN_5O_3$: C, 44.23; H, 3.71; N, 18.42; O, 12.62; Found: C, 44.27; H, 3.74; N, 18.39; O, 12.66; IR (KBr, $cm^{-1}$): 3345 (NH), 1710 (C=O), 1638 (C=N), 1630 (C=N), 1627 (C=C), 1612 (C=C).

*(E)-1-(2-(2-(4-Chlorophenyl)hydrazono)propyl)-2-methyl-5-nitro-1H-imidazole* **(4)**

Yield: 69%; $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.75 (s, 1H, NH), 8.03 (s, 1H, H-4′), 7.79 (bd.s, 2H, H-3″,5″), 7.52 (bd.s, 2H, H-2″, 6″), 5.21 (s, 2H, $CH_2$), 2.39 (s, 3H, 2′-$CH_3$), 2.02 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 155.5 (C=N, C), 153.0 (C, C-2), 141.2 (C, C-1'), 138.4 (C, C-5), 132.2 (CH, C-4), 129.5 (CH, C-3'), 129.5 (CH, C-5'), 127.6 (C, C-4'), 117.8 (CH, C-2'), 117.8 (CH, C-6'), 48.0 ($CH_2$, C), 12.6 ($CH_3$, C), 12.1 ($CH_3$, C); EI-MS: *m/z* (rel. abund. %), 336 ($M^+$, 1), 289 (7), 248 (17), 195 (35), 139 (100), 111 (71); Anal. Calcd for $C_{14}H_{14}ClN_5O_3$: C, 50.08; H, 4.20; N, 20.86; O, 14.30; Found: C, 50.05; H, 4.23; N, 20.84; O, 14.33; IR (KBr, $cm^{-1}$): 3350 (NH), 1698 (C=O), 1652 (C=N), 1644 (C=N), 1630 (C=C), 1615 (C=C).

*(E)-1-(2-(2-(3,4-Dichlorophenyl)hydrazono)propyl)-2-methyl-5-nitro-1H-imidazole* **(5)**

Yield: 72%; $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.82 (s, 1H, NH), 8.02 (m, 4H, H-Ar, H-4′), 5.22 (s, 2H, $CH_2$), 2.40 (s, 3H, 2′-$CH_3$), 2.03 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 155.6 (C=N, C), 153.0 (C, C-2), 142.6 (C, C-1'), 138.3 (C, C-5), 132.1 (CH, C-4), 131.7 (C, C-3'), 129.2 (CH, C-5'), 123.5 (C, C-4'), 118.2 (CH, C-2'), 115.7 (CH, C-6'), 48.2 ($CH_2$, C), 12.5 ($CH_3$, C), 12.1 ($CH_3$, C); EI-MS: *m/z* (rel. abund. %), 323 ($M^+$-$NO_2$, 7), 325 (M+2-$NO_2$, 5), 282 (21), 229 (20), 173 (100), 145 (33), 134 (14); Anal. Calcd for $C_{14}H_{13}Cl_2N_5O_3$: C, 45.42; H, 3.54; N, 18.92; O, 12.97; Found: C, 45.45; H, 3.57; N, 18.95; O, 12.95; IR (KBr, $cm^{-1}$): 3335 (NH), 1720 (C=O), 1647 (C=N), 1637 (C=N), 1630 (C=C), 1625 (C=C).

*(E)-1-(2-(2-(3-Fluorophenyl)hydrazono)propyl)-2-methyl-5-nitro-1H-imidazole* **(6)**

Yield: 74%; $^1$H-NMR: (500 MHz, DMSO-$d_6$): $\delta$ 12.12 (s, 1H, NH), 10.66 (s, 1H, NH), 8.15 (s, 1H, H-4′), 7.78 (m, 5H, H-Ar, H-1), 3.29 (s, 3H, 2′-$CH_3$), 2.49 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 163.3 (C, C-3'), 155.6 (C=N, C), 153.0 (C, C-2), 152.5 (C, C-1'), 138.4 (C, C-5), 132.3 (CH, C-4), 130.7 (CH, C-5'), 110.8 (C, C-4'), 108.7 (CH, C-6'), 97.9 (CH, C-2'), 48.0 ($CH_2$, C), 12.5 ($CH_3$, C), 12.1 ($CH_3$, C); EI-MS: *m/z* (rel. abund. %), 273 ($M^+$-$NO_2$, 16), 123.0 (100), 95 (80), 75 (44); Anal. Calcd for $C_{14}H_{14}FN_5O_3$: C, 52.66; H, 4.42; N, 21.93; O, 15.03; Found: C, 52.63; H, 4.45; N, 21.96; O, 15.00; IR (KBr, $cm^{-1}$): 3345 (NH), 1730 (C=O), 1648 (C=N), 1636 (C=N), 1630 (C=C), 1609 (C=C).

*(E)-2-Methyl-5-nitro-1-(2-(2-(4-nitrophenyl)hydrazono)propyl)-1H-imidazole* **(7)**

Yield: 70%; $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 11.12 (s, 1H, NH), 8.29 (d, $J_{3''-2''/5''-6'}$ = 7.2 Hz, 1H, H-3″,5″), 8.02 (d, $J_{2''-3''/6''-5''}$ = 8.4 Hz, 2H, H-2″,6″), 7.41 (s, 1H, H-4′), 5.24 (s, 2H, $CH_2$), 2.41(s, 3H, 2′-$CH_3$), 2.04 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 155.5 (C=N, C), 153.0 (C, C-2), 149.2 (C, C-1'), 138.3 (C, C-5), 137.8 (C, C-4'), 124.6 (CH, C-3'), 124.6 (CH, C-5'), 132.1 (CH, C-4), 113.1 (CH, C-2'), 113.3 (CH, C-6'), 48.2 ($CH_2$, C), 12.5 ($CH_3$, C), 12.0 ($CH_3$, C); EI-MS: *m/z* (rel. abund. %), 300 ($M^+$-$NO_2$, 7), 259 (28), 206 (15), 150 (100), 134 (10), 120 (9), 104 (28); Anal. Calcd for $C_{14}H_{14}N_6O_5$: C, 48.56; H, 4.08; N, 24.27; O, 23.10; Found: C, 48.54; H, 4.05; N, 24.30; O, 23.14; IR (KBr, $cm^{-1}$): 3340 (NH), 1700 (C=O), 1645 (C=N), 1635 (C=N), 1627 (C=C), 1605 (C=C).

*(E)-2-Methyl-5-nitro-1-(2-(2-(3-nitrophenyl)hydrazinyl)prop-1-enyl)-1H-imidazole* **(8)**

Yield: 68%; $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 12.39 (s, 1H, NH), 8.72 (m, 6H, H-Ar, H-4′, H-1), 2.49 (s, 3H, 2′-$CH_3$), 2.03 (s, 3H, 2-$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): ¢ 155.5 (C=N, C), 153.0

(C, C-2), 151.8 (C, C-1'), 148.3 (C, C-3'), 138.3 (C, C-5), 132.1 (CH, C-4), 130.2 (CH, C-5'), 119.4 (CH, C-6'), 114.4 (C, C-4'), 104.7 (CH, C-2'), 48.2 ($CH_2$, C), 12.6 ($CH_3$, C), 12.1 ($CH_3$, C); EI-MS: *m/z* (rel. abund. %), 300 ($M^+$-$NO_2$, 4), 259 (18), 206 (7), 150 (100), 134 (7), 120 (2), 104 (18); Anal. Calcd for $C_{14}H_{14}N_6O_5$: C, 48.56; H, 4.08; N, 24.27; O, 23.10; Found: C, 48.53; H, 4.06; N, 24.31; O, 23.13; IR (KBr, $cm^{-1}$): 3346 (NH), 1715 (C=O), 1640 (C=N), 1632 (C=N), 1624 (C=C), 1606 (C=C).

## Conclusions

Halogens and nitro substituted hydrazone derivatives of secnidazolone **2-8** were synthesized in two steps. Newly synthesized compounds **2, 3, 4, 5, 6, 7** and **8** were evaluated for their antimicrobial inhibitory potential which showed weak to significantly good activity.

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