

# Synthesis and Biological Activity Evaluation of Some New Heterocyclic Spirocompounds with Imidazolinone and Pyrazoline Moieties

Omar A. Miqdad (Corresponding author) & Nada M. Abunada

Department of Chemistry, Faculty of Applied Sciences, Al-Aqsa University  
76888, Gaza, Palestine

E-mail: miqdadomar@hotmail.com; nadanadannrs@yahoo.com

Hamdi M. Hassaneen

Department of Chemistry, Faculty of Science, Cairo University, Egypt

Ahmed S. M. Abu Samaha

Department of Biology, Faculty of Applied Sciences, Al-Aqsa University  
76888, Gaza, Palestine

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## Abstract

The synthesis of antimicrobial activity spirocompounds was achieved via the reaction of hydrazoneoyl halides **1** with exocyclic 4-arylidene-2-methylimidazolin-5-one **3** in benzene in the presence of triethylamine. Correct elemental analyses and spectral data (IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS) confirm the structure of the synthesized spirocompounds. All the synthesized compounds were evaluated in vitro for their antimicrobial activity against five gram-positive and two gram-negative bacteria using well diffusion method in Mueller-Hinton agar. Most of them exhibited significant antibacterial activity compared with selected standard drugs.

**Keywords:** Spirocompound, Hydrazonoyl halide, Cycloaddition reaction

## 1. Introduction

Spirocompounds form a group of generally less investigated compounds. However, recently growing efforts have been made to synthesize, characterize and investigate the biological activities of these compounds. Many spirocompounds possess very promising biological activities as anticancer agents (Young-Won et al., 2003; Wen-Liang et al., 2007), antibacterial agents (van-der-Sar et al., 2006; Hyeong-Beom et al., 2007), anticonvulsant agents (Jolanta & Krzysztof, 2006; Krzysztof et al., 2008; Jolanta et al., 2006), anti tuberculosis agents (Chande et al., 2005), anti-Alzheimer's agents (Masakazu et al., 2001), pain-relief agents (Frank et al., 2003; Hans et al., 2006), anti-dermatitis agents (Nakao et al., 2008) and antimicrobial agents (Pawar et al., 2009; Thadhaney et al., 2010). In addition to their medical uses, some spirocompounds have found other uses in the agricultural and industrial fields. For example, they are used as antifungal agents (Hejiao et al., 2006), pesticides (Lindell et al., 2001), laser dyes (Kreuder et al., 1999) and electroluminescent devices (Lupo et al., 2008). Spiro compounds have also been recently used as antioxidants (Sarma et al., 2010; Shimakawa et al., 2003). Also, the pyrazoline derivatives are very interesting compounds due to their important role for the antifungal (Korgaokar et al., 1996; Abunada et al., 2009), antidepressant (Palaska et al., 2003; Rajendra et al., 2005; Ozdemir et al., 2007; Ruhoglu et al., 2005), anticonvulsant (Ozdemir et al., 2007; Ruhoglu et al., 2005), anti-inflammatory (Karabasanagouda et al., 2009), antibacterial (Abunada et al., 2009; Nauduri & Reddy, 1998) and anti-tumor (Taylor & Patel, 1992) activity. Besides, it is known that the imidazolinone derivatives are associated with a wide range of therapeutic activities such as anticonvulsant (Godefroi & Platje, 1972), potent CNS depressant, (Harfenist et al., 1978). Recently some new imidazolinone derivatives have been reported as antimicrobial (Desai et al., 2009; Solankee et al., 2004), anticancer (Solankee et al., 2004) and L-DOPA prodrugs in the treatment of Parkinson's disease (Giorgonia et al., 2010). In view of these results and as continuation of our

recent work on the synthesis of unreported hitherto spirocompounds (Miqdad et al., 2011), it was therefore considered worthwhile to synthesize some new spirocompound derivatives with pyrazoline residue attach to imidazolinone one in order to find new biologically active molecules. Thus, the synthesis of new tetraspiro[4,4]nona-2,8-dien-6-one derivatives has been achieved.

## 2. Results and discussion

Tetraspiro[4,4]nona-2,8-dien-6-one derivatives **4a-w** were produced via the cycloaddition reaction of nitrilimines **2**, generated in situ by the action of triethylamine on hydrazoneoyl halides **1a-f** in dry benzene at reflux temperature with exocyclic double bond of 4-arylidene-1-aryl-2-methyl-1H-imidazol-5(4H)-one **3a-f** (Scheme 1). The structures of compounds **4a-w** were established based on the basis of their elemental analyses and spectral data. Their IR spectra showed absorption bands in the region 1705.0 - 1759.0 and 1627.8 - 1651.0  $\text{cm}^{-1}$  assignable to the stretching bands of C=O and C=N bonds. Their  $^1\text{H}$  NMR spectra revealed, besides aromatic protons at 6.88 - 8.29 ppm, imidazolinone methyl at 1.52 - 1.72 ppm and the pyrazoline ring proton resonates at 5.30 - 5.63 ppm. Comparison of this chemical shift of the 4-H-pyrazoline ring residue proton of **4** with those of the related compounds we recently prepared (Miqdad et al., 2011) showed similarity between their chemical shifts. Since, in the case of cycloaddition took place in the opposite direction to give **5**, the expected chemical shift of 5-CH proton would shift downfield due to the deshielding effect of the more electronegative nitrogen atom attached directly to the carbon atom bearing to the hydrogen one. The chemical shift of 5-CH proton of pyrazoline has appeared at 5.9 - 6.0 ppm (Hassaneen et al., 1995; Shawali et al., 1992). The  $^{13}\text{C}$  NMR spectra of some selected compounds showed all the signals corresponding to the proposed structures, especially 4-CH and C-5 (spiro carbons) were found to resonate at 62.42 - 63.75 and 89.55 - 91.13 ppm respectively, and the carbonyl carbon were found to resonate at 178.43 - 179.57 ppm. Also, the electron impact (EI) mass spectra displayed the correct molecular ions in accordance with the suggested structures. It is worthy to mention that all attempts to isolate products from the reaction of **3** with hydrazoneoyl halides bearing carbonyl group (RCOC(X)=NNHAr) at the carbon atom failed.

## 3. In vitro antimicrobial activity evaluation

An evaluation of the antibacterial activity using five Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus Species*, *Salmonella Species*, *Klebsiella Species*) and two Gram-positive bacteria (*Viridans Streptococci* and *Coagulase negative Staphylococcus*) which were isolated from clinical samples and was assessed for the twenty three newly synthesized compounds by well diffusion method (Atta-ur-Rahman et al., 2001). Based upon the antimicrobial evaluation data of the synthesized compounds it can be seen that compounds **4m**, **4q**, **4r**, **4s** and **4u** showed significant activity against all the tested microorganisms around 16 - 25 mm. Compounds **4r** and **4u** are the most effective the compounds in the entire series, although they are not effective against C.s. The highest antibacterial activity of 25 mm and 24 mm against E.c. was observed for compounds **4r** and **4u** respectively. Also, compounds **4i**, **4j**, **4r** and **4u** possess maximum activity of 23, 24, 20 and 22 mm respectively, against V.s. **4m** and **4s** showed highest inhibitory activity of 24 and 22 mm respectively against C.s. and least activity observed against V.s. Compounds **4m**, **4v** and **4j**, **4l** and **4r** showed highest activity of 24 and 22 mm against P.s. Also, compounds **4k**, **4r** and **4n** possess the highest activity of 24 - 20 mm against S.s. Finally, **4c**, **4r** and **4u** exhibited highest inhibitory activity against K.s. of 23 - 22 mm. Although, compound **4c** was inactive against P.a., V.s., C.s. and P.s. and possess low activity against E.c. and moderate activity against S.s., it exhibit the highest inhibitory activity of 23 mm against K.s. The antibacterial activities of most of the tested compounds were found higher than the activity of most selected reference antibiotics.

## 4. Experimental

Melting points were measured on Stuart Scientific electrothermal melting point apparatus SMP1 and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a Pye Unicam SP 3-300 and Shimadzu FT-IR 8101 PC infrared spectrophotometer. The  $^1\text{H}$  NMR (300.07 MHz) and  $^{13}\text{C}$  NMR (75.45 MHz) spectra were recorded in DMSO-d<sub>6</sub> on a Mercury-300BB NMR using TMS as the internal reference. Mass spectra were measured on a GCMS-QP 1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. Hydrazoneoyl halides **1a**, **1b** (Wolkoff, 1975), **1c** (Shawali & Hassaneen, 1972), **1d** (Hegarty & Scott, 1966), **1e** (Aylward & Scott, 1969), and **1f** (Shawali et al., 1990), and the imidazolinone **3** (Islam et al., 1973; Bhattacharjya et al., 2005) were prepared according to the methods reported in the literature.

### 4.1 Reaction of 3a-f with Hydrazoneoyl Halides 1a-f (General Procedure)

Triethylamine (0.7 ml, 5 mmol) was added to a solution of the appropriate imidazolinone **3a-f** (5 mmol) and hydrazoneoyl halides **1a-f** (5 mmol) in dry benzene (50 ml) and the resulting mixture was refluxed for 6 h. The

reaction mixture was filtered while hot to remove triethylamine hydrochloride salt and evaporated under reduced pressure till dryness and the remaining residue was triturated with ethanol (10 ml). The separated solid compound was filtered off and crystallized from a given solvent.

**8-Methyl-1,3,4,7-tetraphenyl-1,2,7,9-tetrazaspiro[4, 4]nona-2,8-dien-6-one **4a**.** White crystals, yield 94%; mp 220 - 222 °C (benzene); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3039.6 (aromatic CH), 2931.6, 2866.0 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.65 (s, 3H, CH<sub>3</sub>-imidazolinone), 5.62 (s, 1H, 4H-pyrazole), 7.16-8.26 (m, 20H, ArH's); MS m/z (%): 428 (M<sup>+</sup>-28, 24), 336 (42), 323 (30), 249 (21), 213 (23), 172 (18), 118 (70), 91 (39), 77 (100); Anal. Calcd. for C<sub>30</sub>H<sub>24</sub>N<sub>4</sub>O: C, 78.92; H, 5.30; N, 12.27. Found: C, 78.63; H, 5.25; N, 12.04%.

**3-(4-Chlorophenyl)-8-methyl-1,4,7-triphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4b**.** Pale yellow crystals, yield 93%; mp 202 - 204 °C (benzene/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3039.6 (aromatic CH), 2931.6, 2866.0 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.66 (s, 3H, CH<sub>3</sub>-imidazolinone), 5.42 (s, 1H, 4H-pyrazole), 7.08-7.59 (m, 19H, ArH's);  $^{13}\text{C}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  15.368 (CH<sub>3</sub>-imidazolinone), 62.717 (CH-pyrazole), 91.133 (C-spiro), 115.200, 121.521, 127.125, 127.873, 128.021, 128.285, 128.487, 128.781, 129.143, 129.196, 129.727 (11C, CHArC's), 129.902, 132.553, 132.576, 133.518, 143.376, 148.713, 161.255 (7C, C=N, ArC's), 179.030 (CO); MS m/z (%): 490 (M<sup>+</sup>, 23), 492 (8.5), 372 (12), 118 (97), 91 (48), 77 (100); Anal. Calcd. for C<sub>30</sub>H<sub>23</sub>ClN<sub>4</sub>O: C, 73.39; H, 4.72; Cl, 7.22; N, 11.41. Found: C, 73.40; H, 4.67; N, 11.45%.

**3-(2,4-Dichlorophenyl)-8-methyl-1-(4-nitrophenyl)-4,7-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4c**.** Yellow crystals, yield 55%; mp 272 - 274 °C (dimethylformamide/methanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3035.7 (aromatic CH), 2923.9, 2839.0 (aliphatic CH), 1751.2 (C=O), 1651.0 (C=N), 1593.1 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.66 (s, 3H, CH<sub>3</sub>-imidazolinone), 5.39 (s, 1H, 4H-pyrazole), 6.93-7.94 (m, 17H, ArH's); MS m/z (%): 570 (M<sup>+</sup>, 9.5), 572 (3), 574 (0.8), 541 (21), 540 (19), 449 (20), 448 (16), 118 (100), 90 (23), 77 (92); Anal. Calcd. for C<sub>30</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>3</sub>: C, 63.17; H, 3.71; Cl, 12.43; N, 12.28. Found: C, 63.38; H, 3.67; N, 12.30%.

**3-(2-Furyl)-8-methyl-1-(4-nitrophenyl)-4,7-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4d**.** Yellow crystals, yield 56%; mp 276 - 278 °C (dioxane); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3058.9 (aromatic CH), 2962.5, 2873.7 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1593.1 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.64 (s, 3H, CH<sub>3</sub>-imidazolinone), 5.42 (s, 1H, 4H-pyrazole), 7.02-7.71 (m, 17H, ArH's); MS m/z (%): 491 (M<sup>+</sup>, 30), 463 (17), 371 (14), 370 (13), 118 (100), 77 (75); Anal. Calcd. for C<sub>28</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>: C, 68.42; H, 4.31; N, 14.25. Found: C, 69.01; H, 4.29; N, 14.08%.

**8-methyl-7-(4-methylphenyl)-1,3,4-triphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4e**.** Off white crystals, yield 65%; mp 214 - 216 °C (tetrahydrofuran); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3058.9, 3035.7 (aromatic CH), 2970.2, 2923.9, 2862.2 (aliphatic CH), 1747.4 (C=O), 1647.1 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.65 (s, 3H, CH<sub>3</sub>-imidazolinone), 2.37 (s, 3H, CH<sub>3</sub>-Ph), 5.62 (s, 1H, 4H-pyrazole), 7.15-7.82 (m, 19H, ArH's); MS m/z (%): 470 (M<sup>+</sup>, 37), 336 (56), 132 (69), 91 (100); Anal. Calcd. for C<sub>31</sub>H<sub>26</sub>N<sub>4</sub>O: C, 79.12; H, 5.57; N, 11.91. Found: C, 79.08; H, 5.60; N, 12.00%.

**3-(4-chlorophenyl)-8-methyl-7-(4-methylphenyl)-1, 4-diphenyl-1, 2, 7, 9-tetrazaspiro[4, 4]nona-2,8-dien-6-one **4f**.** Off white crystals, yield 85%; mp 186 - 188 °C (benzene/pet.ether 40 - 60 °C); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3031.9 (aromatic CH), 2923.9, 2862.2 (aliphatic CH), 1755.1 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.67 (s, 3H, CH<sub>3</sub>-imidazolinone), 2.38 (s, 3H, CH<sub>3</sub>-Ph), 5.56 (s, 1H, 4H-pyrazole), 7.09-7.61 (m, 18H, ArH's); MS m/z (%): 507 (M<sup>+</sup>+2, 4), 505 (M<sup>+</sup>, 12), 504 (31), 370 (46), 132 (75), 91 (100), 77 (21); Anal. Calcd. for C<sub>31</sub>H<sub>25</sub>ClN<sub>4</sub>O: C, 73.73; H, 4.99; Cl, 7.02; N, 11.09. Found: C, 37.71; H, 4.95; N, 11.10%.

**3-(4-Fluorophenyl)-8-methyl-7-(4-methylphenyl)-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4g**.** Canarian yellow crystals, yield 52%; mp 260 - 262 °C (benzene/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3062.7 (aromatic CH), 2920.0, 2877.6 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.72 (s, 3H, CH<sub>3</sub>-imidazolinone), 2.38 (s, 3H, CH<sub>3</sub>-Ph), 5.63 (s, 1H, 4H-pyrazole), 7.13-8.27 (m, 17H, ArH's);  $^{13}\text{C}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  15.440 (CH<sub>3</sub>-imidazolinone), 20.659 (CH<sub>3</sub>-Ph), 63.266 (CH-pyrazole), 89.550 (C-spiro), 113.258, 115.791-115.501 (d, J = 21.7 Hz, C-o-F), 125.889, 126.698, 127.228, 128.128-128.082 (d, J = 3.5 Hz, C-p-F), 128.212, 129.543-129.429 (d, J = 8.6 Hz, C-m-F), 129.74 (9C, ArCH's), 129.894, 130.154, 132.271, 139.126, 140.057, 148.018, 152.108, 162.781, 164.483-161.187 (d, J = 248.6 Hz, C-F) (9C, C=N, ArC's), 178.437 (CO); MS m/z (%): 533 (M<sup>+</sup>, 47), 505 (38), 504 (37), 399 (40), 353 (21), 132 (100), 107 (65), 91 (82); Anal. Calcd. for C<sub>31</sub>H<sub>24</sub>FN<sub>5</sub>O<sub>3</sub>: C, 69.78; H, 4.53; N, 13.13. Found: C, 69.83; H, 4.55; N, 13.05%.

8-Methyl-7-(4-methylphenyl)-1-(4-nitrophenyl)-3,4-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4h**. Canarian yellow crystals, yield 87%; mp 280 - 282 °C (tetrahydrofuran); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3062.7, 3035.7 (aromatic CH), 2862.2 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1589.2 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.70 (s, 3H, CH<sub>3</sub>-imidazolinone), 2.39 (s, 3H, CH<sub>3</sub>-Ph), 5.54 (s, 1H, 4H-pyrazole), 7.12-8.29 (m, 18H, ArH's); MS m/z (%): 515 (M<sup>+</sup>, 46), 514 (39), 487 (46), 486 (39), 381 (40), 380 (36), 335 (22), 132 (99), 91 (100); Anal. Calcd. for C<sub>31</sub>H<sub>25</sub>N<sub>5</sub>O<sub>3</sub>: C, 72.22; H, 4.89; N, 13.58. Found: C, 71.94; H, 4.85; N, 13.59%.

3-(2-Furyl)-8-Methyl-7-(4-methylphenyl)-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4i**. Orange crystals, yield 62%; mp 270 - 272 °C (dioxane); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3112.9, 3062.7 (aromatic CH), 2966.3, 2869.9 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1589.2 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.69 (s, 3H, CH<sub>3</sub>-imidazolinone), 2.37 (s, 3H, CH<sub>3</sub>-Ph), 5.62 (s, 1H, 4H-pyrazole), 7.14-8.19 (m, 16H, ArH's); MS m/z (%): 505 (M<sup>+</sup>, 41), 504 (41), 476 (29), 371 (20), 370 (15), 132 (100), 107 (43), 91 (82); Anal. Calcd. for C<sub>29</sub>H<sub>23</sub>N<sub>5</sub>O<sub>4</sub>: C, 68.90; H, 4.59; N, 13.85. Found: C, 69.00; H, 4.60; N, 13.78%.

7-(2-methoxyphenyl)-8-methyl-1,3,4-triphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4j**. White crystals, yield 80%; mp 202 - 204 °C (tetrahydrofuran/pet.ether 40 - 60 °C); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3058.9 (aromatic CH), 2943.2, 2893.0 (aliphatic CH), 1747.4 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.59 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.89 (s, 3H, CH<sub>3</sub>O-Ph), 5.40 (s, 1H, 4H-pyrazole), 6.94-7.60 (m, 19H, ArH's);  $^{13}\text{C}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  14.555 (CH<sub>3</sub>-imidazolinone), 56.174 (OCH<sub>3</sub>), 63.750 (CH-pyrazole), 90.526 (C-spiro), 112.701, 114.723, 120.823, 121.239, 126.701, 127.518, 27.720, 127.834, 128.334, 128.784, 128.914, 129.036, 129.185 (13C, CHArC's), 130.249, 131.096, 132.565, 143.296, 149.07, 154.629, 161.873, (7C, C=N, ArC's), 179.574 (CO); MS m/z (%): 486 (M<sup>+</sup>, 54), 427 (27), 336 (77), 148 (71), 91 (76), 77 (100); Anal. Calcd. for C<sub>31</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>: C, 76.52; H, 5.39; N, 11.51. Found: C, 76.61; H, 5.41; N, 11.50%.

3-(4-Chlorophenyl)-7-(2-methoxyphenyl)-8-methyl-1,4-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4k**. White crystals, yield 97%; mp 234 - 236 °C (benzene/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3066.6, 3031.9 (aromatic CH), 2954.7, 2846.7 (aliphatic CH), 1751.2 (C=O), 1647.1 (C=N), 1600.8 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.64 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.88 (s, 3H, CH<sub>3</sub>O-Ph), 5.43 (s, 1H, 4H-pyrazole), 7.14-7.65 (m, 18H, ArH's); MS m/z (%): 523 (M<sup>+</sup>+2, 6), 521 (M<sup>+</sup>, 20), 461 (28), 370 (65), 148 (84), 91 (84), 77 (100); Anal. Calcd. for C<sub>31</sub>H<sub>25</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 71.46; H, 4.48; Cl, 6.80; N, 10.75. Found: C, 71.52; H, 4.51; Cl, 6.80; N, 10.83%.

3-(2,4-Dichlorophenyl)-7-(2-methoxyphenyl)-8-methyl-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4l**. Yellow crystals, yield 58%; mp 268 - 270 °C (dioxane/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3058.9 (aromatic CH), 2927.7, 2835.2 (aliphatic CH), 1755.1 (C=O), 1643.2 (C=N), 1593.1 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.62 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.87 (s, 3H, CH<sub>3</sub>O-Ph), 5.41 (s, 1H, 4H-pyrazole), 7.02-7.91 (m, 16H, ArH's); MS m/z (%): 602 (M<sup>+</sup>+2, 7), 600 (M<sup>+</sup>, 15), 570 (20), 540 (24), 449 (17), 403 (13), 123 (100), 77 (56); Anal. Calcd. for C<sub>31</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>4</sub>: C, 62.01; H, 3.86; Cl, N, 11.81. Found: C, 62.00; H, 3.91; Cl, N, 11.76%.

3-(2-Furyl)-7-(2-methoxyphenyl)-8-methyl-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4m**. Yellow crystals, yield 57%; mp 214 - 216 °C (tetrahydrofuran/pet.ether 40 - 60 °C); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3082.0, 3043.5 (aromatic CH), 2939.3, 2842.9 (aliphatic CH), 1751.2 (C=O), 1627.8 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.64 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.73 (s, 3H, CH<sub>3</sub>O-Ph), 5.40 (s, 1H, 4H-pyrazole), 6.88-7.74 (m, 16H, ArH's); MS m/z (%): 522 (M<sup>+</sup>+1, 40), 461 (33), 370 (58), 148 (92), 91 (78), 77 (100); Anal. Calcd. for C<sub>29</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>: C, 66.79; H, 4.45; N, 13.43. Found: C, 66.81; H, 4.48; N, 13.44%.

7-(3-Methoxyphenyl)-8-methyl-1,3,4-triphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4n**. Off white crystals, yield 94%; mp 232 - 234 °C (tetrahydrofuran/methanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3058.9, 3028.0 (aromatic CH), 2962.5, 2835.2 (aliphatic CH), 1759.0 (C=O), 1647.1 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.68 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.81 (s, 3H, CH<sub>3</sub>O-Ph), 5.49 (s, 1H, 4H-pyrazole), 7.14-7.63 (m, 19H, ArH's); MS m/z (%): 487 (M<sup>+</sup>+1, 34), 381 (40), 193 (14), 132 (83), 91 (100), 77 (49); Anal. Calcd. for C<sub>31</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>: C, 76.52; H, 5.39; N, 11.51. Found: C, 76.66; H, 5.40; N, 11.55%.

3-(4-Chlorophenyl)-7-(3-methoxyphenyl)-8-methyl-1,4-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4o**. Off white crystals, yield 86%; mp 198 - 200 °C (tetrahydrofuran/pet.ether 40 - 60 °C); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3043.5 (aromatic CH), 2935.5, 2835.5 (aliphatic CH), 1751.2 (C=O), 1639.4 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  1.69 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.82 (s, 3H, CH<sub>3</sub>O-Ph), 5.39 (s, 1H, 4H-pyrazole), 7.08-7.60 (m, 18H, ArH's);  $^{13}\text{C}$  NMR (DMSO-d<sub>6</sub>)  $\delta$  15.242 (CH<sub>3</sub>-imidazolinone), 55.346 (OCH<sub>3</sub>), 62.423 (CH-pyrazole), 91.049 (C-spiro), 112.869, 114.571, 115.169, 119.099, 121.418, 127.731, 127.876, 127.921, 128.124, 128.357, 129.044, 129.780 (12C, CHArC's), 130.398, 132.519, 133.377, 133.457, 143.261, 148.682, 159.863, 161.183 (8C, C=N, ArC's), 178.708 (CO); MS m/z (%): 522 (M<sup>+</sup>+2, 25), 521, (41), 520 (M<sup>+</sup>, 73), 492 (42), 491 (41), 370

(79), 148 (97), 107 (51), 91 (95), 77 (100); Anal. Calcd. for  $C_{31}H_{25}ClN_4O_2$ : C, 71.46; H, 4.84; Cl, 6.80; N, 10.75. Found: C, 71.52; H, 4.86; Cl, 6.80; N, 10.75%.

**3-(4-Fluorophenyl)-7-(3-methoxyphenyl)-8-methyl-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4p**.** Yellow crystals, yield 59%; mp 239 - 241 °C (tetrahydrofuran/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3078.2 (aromatic CH), 2842.9 (aliphatic CH), 1751.2 (C=O), 1651.0 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.69 (s, 3H,  $\text{CH}_3$ -imidazolinone), 3.82 (s, 3H,  $\text{CH}_3\text{O-Ph}$ ), 5.39 (s, 1H, 4H-pyrazole), 7.08-7.60 (m, 17H, ArH's); MS m/z (%): 549 ( $M^+$ , 50), 521 (48), 399 (45), 353 (22), 148 (100), 90 (38), 77 (100); Anal. Calcd. for  $C_{31}H_{24}FN_5O_4$ : C, 67.75; H, 4.40; N, 12.74. Found: C, 67.80; H, 4.41; N, 12.75%.

**7-(4-Ethoxyphenyl)-8-methyl-1,3,4-triphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4q**.** Off white crystals, yield 74%; mp 220 - 222 °C (benzene/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3077.5 (aromatic CH), 2977.9, 2873.7 (aliphatic CH), 1747.4 (C=O), 1647.1 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.33 (t, 3H, J = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 1.64 (s, 3H,  $\text{CH}_3$ -imidazolinone), 4.05 (q, 2H, J = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 5.39 (s, 1H, 4H-pyrazole), 6.95-7.66 (m, 19H, ArH's); MS m/z (%): 500 ( $M^+$ , 44), 399 (46), 336 (44), 323 (44), 192 (24), 148 (68), 91 (68), 77 (100); Anal. Calcd. for  $C_{32}H_{28}N_4O_2$ : C, 76.78; H, 5.64; N, 11.19. Found: C, 76.82; H, 5.66; N, 11.22%.

**3-(4-Chlorophenyl)-7-(4-ethoxyphenyl)-8-methyl-1,4-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4r**.** Off white crystals, yield 94%; mp 191 - 193 °C (tetrahydrofuran/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3039.6 (aromatic CH), 2985.6, 2923.9, 2869.9 (aliphatic CH), 1751.2 (C=O), 1647.1 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.33 (t, 3H, J = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 1.63 (s, 3H,  $\text{CH}_3$ -imidazolinone), 4.06 (q, 2H, J = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 5.41 (s, 1H, 4H-pyrazole), 6.96-7.61 (m, 18H, ArH's);  $^{13}\text{C}$  NMR (DMSO-d<sub>6</sub>) δ 14.475 (CH<sub>3</sub>-ethyl), 15.257 (CH<sub>3</sub>-imidazolinone), 62.789 (CH-pyrazole), 63.499 (CH<sub>2</sub>-ethyl), 91.030 (C-spiro), 115.166, 115.372, 121.452, 124.886, 127.850, 127.983, 128.269, 128.399, 128.456, 128.492 (10C, CHARC's), 129.177, 129.925, 132.591, 133.526, 143.425, 148.632, 158.886, 161.744 (8C, C=N, ArC's), 179.310 (CO); MS m/z (%): 535 ( $M^+$ , 24), 534 ( $M^+-1$ , 55), 506 (39), 370 (75), 162 (83), 137 (85), 91 (100), 77 (48); Anal. Calcd. for  $C_{32}H_{27}ClN_4O_2$ : C, 71.83; H, 5.09; Cl, 6.63; N, 10.47. Found: C, 71.90; H, 5.12; Cl, 6.61; N, 10.51%.

**3-(2,4-Dichlorophenyl)-7-(4-ethoxyphenyl)-8-methyl-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4s**.** Yellow crystals, yield 56%; mp 298 - 300 °C (dimethylsulfoxide/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3074.3 (aromatic CH), 2981.7, 2935.5, 2885.3 (aliphatic CH), 1751.2 (C=O), 1647.1 (C=N), 1589.2 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.34 (t, 3H, J = 7.1 Hz,  $\text{OCH}_2\text{CH}_3$ ), 1.65 (s, 3H,  $\text{CH}_3$ -imidazolinone), 4.03 (q, 2H, J = 7.1 Hz,  $\text{OCH}_2\text{CH}_3$ ), 5.48 (s, 1H, 4H-pyrazole), 7.01-7.93 (m, 16H, ArH's); MS m/z (%): 616 ( $M^++2$ , 8), 614 (12), 584 (24), 448 (14), 403 (10), 162 (50), 137 (100), 90 (18); Anal. Calcd. for  $C_{32}H_{25}Cl_2N_5O_4$ : C, 62.55; H, 4.10; Cl, 11.54; N, 11.40. Found: C, 62.58; H, 4.08; Cl, 11.57; N, 11.43%.

**7-(4-ethoxyphenyl)-3-(2-furyl)-8-methyl-1-(4-nitrophenyl)-4-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4t**.** Orange crystals, yield 53%; mp 227 - 229 °C (benzene); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3066.6 (aromatic CH), 2908.5, 2869.9 (aliphatic CH), 1751.2 (C=O), 1639.4 (C=N), 1593.1 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.32 (t, 3H, J = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 1.64 (s, 3H,  $\text{CH}_3$ -imidazolinone), 4.05 (q, 2H, J = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 5.42 (s, 1H, 4H-pyrazole), 7.03-7.71 (m, 16H, ArH's); MS m/z (%): 535 ( $M^+$ , 46), 534 (40), 507 (33), 162 (70), 137 (100), 134 (27), 65 (39); Anal. Calcd. for  $C_{30}H_{25}N_5O_5$ : C, 67.28; H, 4.71; N, 13.08. Found: C, 67.30; H, 4.73; N, 13.10%.

**7-(2-Methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-1,3-diphenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4u**.** Off white crystals, yield 95%; mp 192 - 194 °C (tetrahydrofuran/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3062.0, 3035.7 (aromatic CH), 2974.0, 2939.3, 2839.0 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.64 (s, 3H,  $\text{CH}_3$ -imidazolinone), 3.73, 3.80 (two s, 6H, two  $\text{CH}_3\text{O-Ph}$ ), 5.30 (s, 1H, 4H-pyrazole), 6.88-7.60 (m, 18H, ArH's);  $^{13}\text{C}$  NMR (DMSO-d<sub>6</sub>) δ 14.635 (CH<sub>3</sub>-imidazolinone), 54.828, 55.606 (2C,  $\text{OCH}_3$ ), 62.427 (CH-pyrazole), 90.366 (C-spiro), 113.033, 114.525, 114.849, 120.735, 120.922, 124.161, 124.579, 126.499, 128.117, 128.670, 128.822, 128.933 (12C, CHARC's), 129.063, 131.054, 131.218, 143.185, 149.105, 154.965, 158.661, 160.897 (8C, C=N, ArC's), 179.459 (CO); MS m/z (%): 516 ( $M^+$ , 79), 488 (38), 456 (28), 365 (39), 366 (52), 148 (91), 91 (87), 77 (100); Anal. Calcd. for  $C_{32}H_{28}N_4O_3$ : C, 74.40; H, 5.46; N, 10.85. Found: C, 74.42; H, 5.49; N, 11.00%.

**3-(4-Chlorophenyl)-7-(2-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-1-phenyl-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4v**.** Off white crystals, yield 82%; mp 134 - 136 °C (benzene/ethanol); IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3066.6, 3039.6 (aromatic CH), 2966.3, 2953.5, 2839.0 (aliphatic CH), 1751.2 (C=O), 1643.2 (C=N), 1600.8 (C=C);  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) δ 1.64 (s, 3H,  $\text{CH}_3$ -imidazolinone), 3.74, 3.82 (two s, 6H, two  $\text{CH}_3\text{O-Ph}$ ), 5.32 (s, 1H, 4H-pyrazole), 6.93-7.59 (m, 17H, ArH's); MS m/z (%): 551 ( $M^+$ , 6), 322 (56), 148 (100), 91 (30), 77 (65);

Anal. Calcd. for  $C_{32}H_{27}ClN_4O_3$ : C, 69.75; H, 4.94; Cl, 6.43; N, 10.17. Found: C, 70.00; H, 5.00; Cl, 6.44; N, 10.21%.

3-(2-Furyl)-7-(2-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-1-(4-nitrophenyl)-1,2,7,9-tetrazaspiro[4,4]nona-2,8-dien-6-one **4w**. Canarian yellow crystals, yield 54%; mp 160 - 162 °C (tetrahydrofuran); IR (KBr)  $\nu_{max}/cm^{-1}$  3070.5, 3004.9 (aromatic CH), 2974.0, 2912.3, 2839.0 (aliphatic CH), 1705.0 (C=O), 1643.2 (C=N), 1596.9 (C=C);  $^1H$  NMR (DMSO-d<sub>6</sub>) δ 1.66 (s, 3H, CH<sub>3</sub>-imidazolinone), 3.75, 3.83 (two s, 6H, two CH<sub>3</sub>O-Ph), 5.36 (s, 1H, 4H-pyrazole), 6.91-7.62 (m, 15H, ArH's); MS m/z (%): 551 (M<sup>+</sup>, 24), 391 (53), 390 (41), 374 (27), 267 (28), 200 (20), 123 (100), 92 (29), 77 (17); Anal. Calcd. for  $C_{30}H_{25}N_5O_6$ : C, 65.33; H, 4.57; N, 12.70. Found: C, 65.36; H, 4.60; N, 12.73%.

#### 4.2 In Vitro Antimicrobial Activity Screening

For the in vitro antimicrobial activity, a stock solution of the synthesized compound (1000 µg/mL) in DMSO was prepared and 30 µl of graded dilutions (Table 1) of the tested compounds were incorporated in a cavity (depth 3 mm, diameter 4 mm) made in the center of the petri plate which corresponding to 30 µg, 15 µg, 7.5 µg and 3.75 µg. The plates were allowed to stand at room temperature for two hours for diffusion and then incubated. The organisms were grown on Muller Hinton's agar with sheep blood (5 %) at 37 °C for 24 hours. After incubation period, the clear zone around the wells was carefully measured in mm as inhibition zones. The absence of a clear zone around the well was taken as inactivity. The antibiotics Amoxycillin (10 µg/disc), Amikacin (10 µg/disc), Ceftriazone (10 µg/disc), and Ciprofloxacin (30 µg/disc) were used as references and dimethylsulphoxide (DMSO) as solvent and control. The investigation results are listed in Table 1.

#### References

- Abunada, N. M., Hassaneen, H. M., M.Abusamaha, A. S., & Miqdad, O. A. (2009). Synthesis and antimicrobial evaluation of some new pyrazole, pyrazoline and chromeno[3, 4-c]pyrazole derivatives. *J. Braz. Chem. Soc.*, 20(5), 975-87. <http://dx.doi.org/10.1590/S0103-50532009000500024>
- Atta-ur-Rahman, Choudhary, M. I., & Thomsen, W. J. (2001). Bioassay techniques for drug development, harwood academic publishers. *The Netherlands*, 16, 22.
- Aylward, J. B., & Scott, F. L. (1969). Preparation and solvolysis of N-arylbenzohydrazonyl bromides. *J. Chem. Soc. (B)*, 1080-84. <http://dx.doi.org/10.1039/j29690001080>
- Bhattacharjya, G., Savitha, G., & Tamanathan, G. (2005). C-H...O interactions are favoured in the crystal structures of imidazolin-5-ones. *J. Mol. Stru.*, 752(1-3), 98-103. <http://dx.doi.org/10.1016/j.molstruc.2005.05.044>
- Chande, M. S., Verma, R. S., Barve, P. A., Khanwelkar, R. R., Vaidya, R. B., & Ajaikumar, K. B. (2005). Facile synthesis of active antitubercular, cytotoxic and antibacterial agents: a Michael addition approach. *Eur. J. Med. Chem.*, 40(11), 1143-48. <http://dx.doi.org/10.1016/j.ejmech.2005.06.004>
- Desai, N. C., Bhavsar, A. M., & Baldaniya, B. B. (2009). Synthesis and antimicrobial activity of 5-imidazolinone derivatives. *Indian J. Pharm. Sci.*, 71(1), 90-94. <http://dx.doi.org/10.4103/0250-474X.51953>
- Frank, R., Reich, M., Jostock, R., Bahrenberg, G., Schick, H., Henkel, B., & Sonnenschein, H. (2008). *Substituted spiro compounds and their use for producing pain-relief medicaments*. US Pat. 20080269271 (App USPTO: 514278).
- Giorgioni, G., Claudi, F., Ruggieri S., Ricciutelli, M., F.Palmieri, G., Di-Stefano, A., Sozio, P., S.Cerasa, L., Chiavoroli, A., Ferrante, C., Orlando, G., & R. A. Glennon. (2010). Design, synthesis, and preliminary pharmacological evaluation of new imidazolinones as L-DOPA prodrugs. *Bioorg. Med. Chem.*, 18(5), 1834-43. <http://dx.doi.org/10.1016/j.bmc.2010.01.041>
- Godefroi, E. F., & Platje, J. T. J. (1972). DL-1-(.alpha.-Methylbenzyl)-2-methylimidazole-5-carboxylate esters. Synthesis and pharmacological properties. *J. Med. Chem.*, 15(3), 336-37. <http://dx.doi.org/10.1021/jm00273a035>
- Hans, S., Robert, F., Reich, M., Ruth, O., Gregor, B., Fritz, T., & Henkel, B. (2006). *Substituted spiro compounds and their use for producing pain-relief drugs*. Int. Pat. WO/2006/122769 (App No: PCT/EP2006/004651).
- Harfenist, M., Soroko, E. F., & Mckenzie, G. M. (1978). 2-(Alkoxyaryl)-2-imidazoline monoamine oxidase inhibitors with antidepressant activity. *J. Med. Chem.*, 21(4), 405-9. <http://dx.doi.org/10.1021/jm00202a021>
- Hassaneen, H. M., Daboun, H. A., Abdelhadi, H. A., & Abdel-Reheim, N. A. (1995). Site selectivity and regiochemistry of nitrilimines. Cycloadditions to 1,3-diphenyl-2-thiono-4-imidazolidinone and its

5-phenylmethylene derivatives. *Phosphorus Sulfur and Silicon*, 107(1-4), 269-73.  
<http://dx.doi.org/10.1080/10426509508027942>

Hegarty, A. F., & Scott, F. L. (1966). The kinetics of bromination of hydrazones. *J. Chem. Soc., (B)*, 672-75.  
<http://dx.doi.org/10.1039/j29660000672>

Hejiao, H., Huijuan, G., Erwei, L., Xingzhong, L., Yuguang, Z., Yongsheng, C., & Decaspirones, F.-I. (2006). Bioactive secondary metabolites from the saprophytic fungus *Helicoma viridis*. *J. Nat. Prod.*, 69(12), 1672-75.  
<http://dx.doi.org/10.1021/np0603830>

Hyeong-Beom, P., Hyun, J. N., Hee, H. J., Hoon, C. J., Hoon, C. J., Jung-Hyuck, C., Ho, Y. K., & Chang-Hyun, O. (2007). Synthesis and In-Vitro activity of novel 1 $\beta$ -methylcarbapenems having spiro[2, 4]heptane moieties. *Arch Pharm*, 340(10), 530-37. <http://dx.doi.org/10.1002/ardp.200700060>

Islam, A. M., Khalil, A. M., & AbdEl-Gawad, I. I. (1973). Reaction of 2-Aryl-4-arylmethylene-2-oxazolin-5-ones with amines. *Aust. J. Chem.*, 26(4), 827-30.  
<http://dx.doi.org/10.1071/CH9730827>

Jolanta, O., & Krzysztof, K. (2006). Synthesis and anticonvulsant properties of new N-phenylamino derivatives of 2-azaspiro[4, 4]nonane, 2-azaspiro[4,5]decane-1,3-dione and 3-cyclohexylpyrrolidine-2,5-dione. Part IV. *Acta Pol. Pharm.*, 6(2) 3, 101-8.

Jolanta, O., Krzysztof, K., & Ewa, T. (2006). Impact of aromatic substitution on the anticonvulsant activity of new N-(4-arylpiperazin-1-yl)-alkyl-2-azaspiro[4,5]decane-1,3-dione derivatives. *Pharmacol Rep.*, 58(2), 207-14.

Karabasanagouda T., Airody, V. A., & Girisha, M. (2009). Synthesis of some new pyrazolines and isoxazoles carrying 4-methylthiophenyl moiety as potential analgesic and anti-inflammatory agents. *Indian J. Chem.*, 48B, 430-437.

Korgaokar, S. S., Patil, P. H., Shah, M. J., & Parekh, H. H. (1996). Studies on pyrazolines: preparation and antimicrobial activity of 3-(p-chlorophenylsulphonamidophenyl)-5 aryl- 1H/acetyl pyrazolines. *Indian J. Pharm. Sci.*, 58(6), 222-25.

Kreuder, W., Yu, N., & Salbeck, J. (1999). Use of spiro compounds as LASER dyes. Int. Pat. WO/1999/040655 (App PCT/EP1999/000441).

Krzysztof, K., Jolanta, O., & Małgorzata, D. (2008). Synthesis, physicochemical and anticonvulsant properties of new N-phenylamino derivatives of 2-azaspiro[4,4]nonane- and [4,5]decane-1,3-diones: Part V. *Eur. J. Med. Chem.*, 43(1), 53-61.

Lindell, S., Sanft, U., & Thönen, M.-T. (2001). *Heterocyclic spiro compounds as pesticides*. Int. Pat. WO/2001/011968 (App PCT/EP2000/ 007851).

Lupo, D., J. Salbeck, Schenk, H., Stehlin, T., Stern, R., & Wolf, A. (2008). *Spiro compounds and their use as electroluminescence materials*. US Pat. 5840217 (App USPTO: 08/417390).

Masakazu, F., Kenji, H., & Jiro, K. (2001). *Spiro compound, process for preparing the same and use thereof as drugs*. Int. Pat. WO/2001/066546 (App PCT/JP2001/001793).

Miqdad, O. A., Abunada, N. M., & Hassaneen, H. M. (2011). Regioselectivity of nitrilimines 1,3-dipolar cycloaddition: Novel synthesis of spiro[4,4]nona-2,8-dien-6-one derivatives. *Heteroatom chemistry*, 22(2), 131-36. <http://dx.doi.org/10.1002/hc.20666>

Nakao, K., Ikeda, K., Kurokawa, T., Togashi, Y., Umeuchi, H., Honda, T., Okano, K., & Mochizuki, H. (2008). A selective kappa opioid receptor agonist, on scratching behavior in an animal model of atopic dermatitis. *Nihon Shinkei Seishin Yakurigaku Zasshi Japanese Journal of Psychopharmacology*, 28(2), 75-83.

Nauduri, D., & B. Reddy, G. (1998). Antibacterial and antimycotics: part 1: Synthesis and activity of 2-pyrazoline derivatives. *Chem. Pharm. Bull. (Tokyo)*, 46(8), 1254-60.

Ozdemir, Z., Kandilici, H. B., Gumusel, B., Calis, U., & ABilgin, A. (2007). Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. *Eur. J. Med. Chem.*, 42(3), 373-79. <http://dx.doi.org/10.1016/j.ejmecm.2006.09.006>

Palaska, E., Aytemir, M., Uzbay, I., & Erol, D. (2001). Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines. *Eur. J. Med. Chem.*, 36(6), 539-43.  
[http://dx.doi.org/10.1016/S0223-5234\(01\)01243-0](http://dx.doi.org/10.1016/S0223-5234(01)01243-0)

- Pawar, M. J., Burungale, A. B., & Karale, B. K. (2009). Synthesis and antimicrobial activity of spiro(chromeno[4,3-d][1,2,3]thiadiazole-4,1'-cyclohexanes), spiro(chromeno-[4,3-d][1,2,3]-selenadiazole-4,1'-cyclohexanes) and (spiro-chroman-2,1'-cyclohexan-4-one)-5-spiro-4-acetyl-2-(acetylamino)- $\Delta$ 2-1,3,4-thiadiazoline compounds. *ARKIVOC*, (XIII), 97-107.
- Rajendra P. Y., Lakshmana, R. A., Prasoona, L., Murali, K., & Ravi, K. P. (2005). Synthesis and antidepressant activity of some 1,3,5-triphenyl-2-pyrazolines and 3-(2"-hydroxy naphthalen-1"-yl)-1,5-diphenyl-2-pyrazolines. *Bioorg. Med. Chem. Lett.*, 15(22), 5030-34. <http://dx.doi.org/10.1016/j.bmcl.2005.08.040>
- Ruhoglu, O., Ozdemir, Z., Calis, U., Gumusel, B., & Bilgin, A. A. (2005). Synthesis of and pharmacological studies on the antidepressant and anticonvulsant activities of some 1,3,5-trisubstituted pyrazolines. *Arzneimittelforschung*, 55(8), 431-36.
- Sarma, B. K., Manna, D., Minoura, M., & G. Mugesh. (2010). Synthesis, structure, spirocyclization mechanism and Glutathione Peroxidase-like antioxidant activity of stable spirodiazaselenurane and spirodiazatellurane. *J. Am. Chem. Soc.*, 132(15), 5364-74. URL: <http://pubs.acs.org/doi/abs/10.1021/ja908080u>
- Shawali, A. S., Ezmirly, S. T., & Bukhari, A. M. (1992). Nuclear magnetic resonance spectroscopy and the structures of the regioisomeric products of the cycloaddition of C-ethoxycarbonyl-N-arylnitrilimines to  $\alpha$ ,  $\beta$ -unsaturated ketones. *Spectrochim. Acta., Part A*, 48(8), 1165-71. [http://dx.doi.org/10.1016/0584-8539\(92\)80127-I](http://dx.doi.org/10.1016/0584-8539(92)80127-I)
- Shawali, A. S., & Hassaneen, H. M. (1973). Reaction of carbanions of  $\beta$ -diketones and  $\beta$ -keto esters with hydrazidic bromides. *Tetrahedron*, 29(1), 121-24. [http://dx.doi.org/10.1016/S0040-4020\(01\)99385-8](http://dx.doi.org/10.1016/S0040-4020(01)99385-8)
- Shawali, A. S., Hassaneen, H. M., & Ibrahim, H. A. (1990). Synthesis and cycloaddition reactions of N-aryl-2-furohydrazone chlorides. *Arch Pharm Res*, 13(2), 126-31. <http://dx.doi.org/10.1007/BF02857788>
- Shimakawa, S., Yoshida, Y., & Niki, E. (2003). Antioxidant action of lipophilic nitroxyl radical, cyclohexane-1-spiro-2'-(4'-oxyimidazolidine-1'-oxyl)-5'-spiro-1"-cyclohexane against peroxidation under hypoxic conditions. *Lipids*, 38(3), 225-31. <http://dx.doi.org/10.1007/s11745-003-1055-3>
- Solankee, A., Kapadiya, K., Thakor, I., & Patel, J. (2004). Synthesis and Antimicrobial Activity of 1-(4'-Trifluoro methylphenyl)-2-phenyl-4-(benzylidene/substituted Benzylidene/2'furylidene/2'-thienylidene)-imidazolin-5-ones. *Asian J. Chem.*, 16(2), 917-20.
- Taylor, E. C., & Patel, H. H. (1992). Synthesis of pyrazolo[3,4-d]pyrimidine analogues of the potent agent N-4-2-2-amino-4(3H)-oxo-7H-pyrrolo [2,3-d]pyrimidin-5-yl)ethylbenzoyl-L-glutamic acid (LY231514). *Tetrahedron*, 48(37), 8089-100. [http://dx.doi.org/10.1016/S0040-4020\(01\)80479-8](http://dx.doi.org/10.1016/S0040-4020(01)80479-8)
- Thadhaney, B., Sain, D., Pernawat, G., & Talesara, G. L. (2010). Synthesis and antimicrobial evaluation of ethoxyphthalimide derived from spiro[indole-3,5'-(1,3)thiazole(4,5-c)isoxazol]-2(1H)-ones via ring closure metathesis. *Indian J. Chem.*, 49B, 368.
- Van-Der-Sar, S., Blunt, J., & Munro, M. (2006). Spiro-mamakone A: A unique relative of the spirobisnaphthalene class of compounds. *Org. Lett.*, 8(10), 2059-61. <http://dx.doi.org/10.1021/o1060434k>
- Wen-Liang, W., Tian-Jiao, Z., Hong-Wen, T., Zhen-Yu, L., Yu-Chun, F., Qian-Qun, G., & Wei Ming, Z. (2007). Three novel, structurally unique spirocyclic alkaloids from the halotolerant b-17 fungal strain of aspergillus variecolor. *Chem. Biodivers*, 4(12), 2913-19. <http://dx.doi.org/10.1002/cbdv.200790240>
- Wolkoff, P. (1975). A new method of preparing hydrazone halides. *Can. J. Chem.*, 53(9), 1333-35. <http://dx.doi.org/10.1139/v75-183>
- Young-Won, C., Angela, S., Bao-Ning, S., Quiwen, M., Hee-Byung, C., Soedarsono, R., Leonardus, K., Agus, R., Norman, F., Steven, S., & Douglas, K. (2008). Potential anticancer activity of naturally occurring and semisynthetic derivatives of aculeatins a and b from amomum aculeatum. *J. Nat. Prod.*, 71(3), 390-95. <http://dx.doi.org/10.1021/np070584j>

Table 1. Antibacterial activity results of the synthesized compounds 4a-w

Comp.	Conc. µg/mL	Microorganisms <sup>a</sup>						
		Inhibition Zone diameter (mm) <sup>d</sup>						
E.c.	P.a.	V.s..	C.s.	P.s.	S.s.	K.s.		
4a	1000	15	14	-	12	7	17	19
	500	10	11	-	8	-	12	16
	250	7	7	-	-	-	8	14
	125	-	-	-	-	-	-	11
4b	1000	20	18	17	20	18	19	22
	500	15	16	11	17	14	15	16
	250	10	11	-	14	9	12	13
	125	8	-	-	10	-	10	9
4c	1000	12	-	-	-	-	17	23
	500	10	-	-	-	-	13	19
	250	8	-	-	-	-	10	17
	125	-	-	-	-	-	8	14
4d	1000	15	20	16	20	17	14	19
	500	14	18	10	17	12	11	16
	250	10	12	-	13	9	8	11
	125	8	8	-	9	-	-	8
4e	1000	10	-	-	19	9	13	16
	500	8	-	-	14	-	9	12
	250	-	-	-	11	-	6	7
	125	-	-	-	9	-	-	-
4f	1000	-	13	20	20	14	-	12
	500	-	9	14	14	10	-	9
	250	-	-	9	10	7	-	6
	125	-	-	-	8	-	-	-
4g	1000	18	10	10	21	13	14	17
	500	16	8	7	17	9	10	11
	250	12	-	-	10	-	7	8
	125	8	-	-	7	-	-	-
4h	1000	12	18	-	19	10	9	18
	500	10	12	-	16	7	6	16
	250	9	9	-	14	-	-	11
	125	9	6	-	9	-	-	8

	1000	20	18	23	20	16	14	10
4i	500	16	15	16	15	14	11	7
	250	12	11	12	13	11	8	-
	125	7	-	9	10	9	6	-
	1000	12	24	24	20	22	12	20
4j	500	10	20	21	18	16	8	17
	250	7	10	17	11	12	-	13
	125	-	-	12	9	8	-	11
	1000	15	12	9	18	17	24	18
4k	500	10	8	-	15	13	20	14
	250	8	-	-	10	10	17	10
	125	-	-	-	5	7	14	8
	1000	21	19	18	18	22	22	20
4l	500	18	16	15	14	18	19	17
	250	15	11	9	11	15	17	15
	125	10	7	7	9	10	13	10
	1000	20	14	12	24	24	20	16
4m	500	17	12	8	19	20	18	14
	250	12	8	-	13	17	14	11
	125	8	-	-	11	12	10	7
	1000	16	14	10	22	19	15	20
4n	500	14	9	6	17	15	11	17
	250	8	-	-	13	10	7	11
	125	7	-	-	8	7	-	8
	1000	20	20	12	14	17	18	19
4o	500	15	15	6	11	14	16	15
	250	10	-	-	-	10	12	13
	125	15	-	-	-	-	9	10
	1000	20	12	14	12	21	13	10
4p	500	15	-	11	10	15	11	8
	250	10	-	-	-	13	10	-
	125	7	-	-	-	7	7	-
	1000	20	20	19	17	19	20	21
4q	500	15	17	16	12	10	16	18
	250	-	12	11	9	7	14	16
	125	-	9	6	7	-	9	12

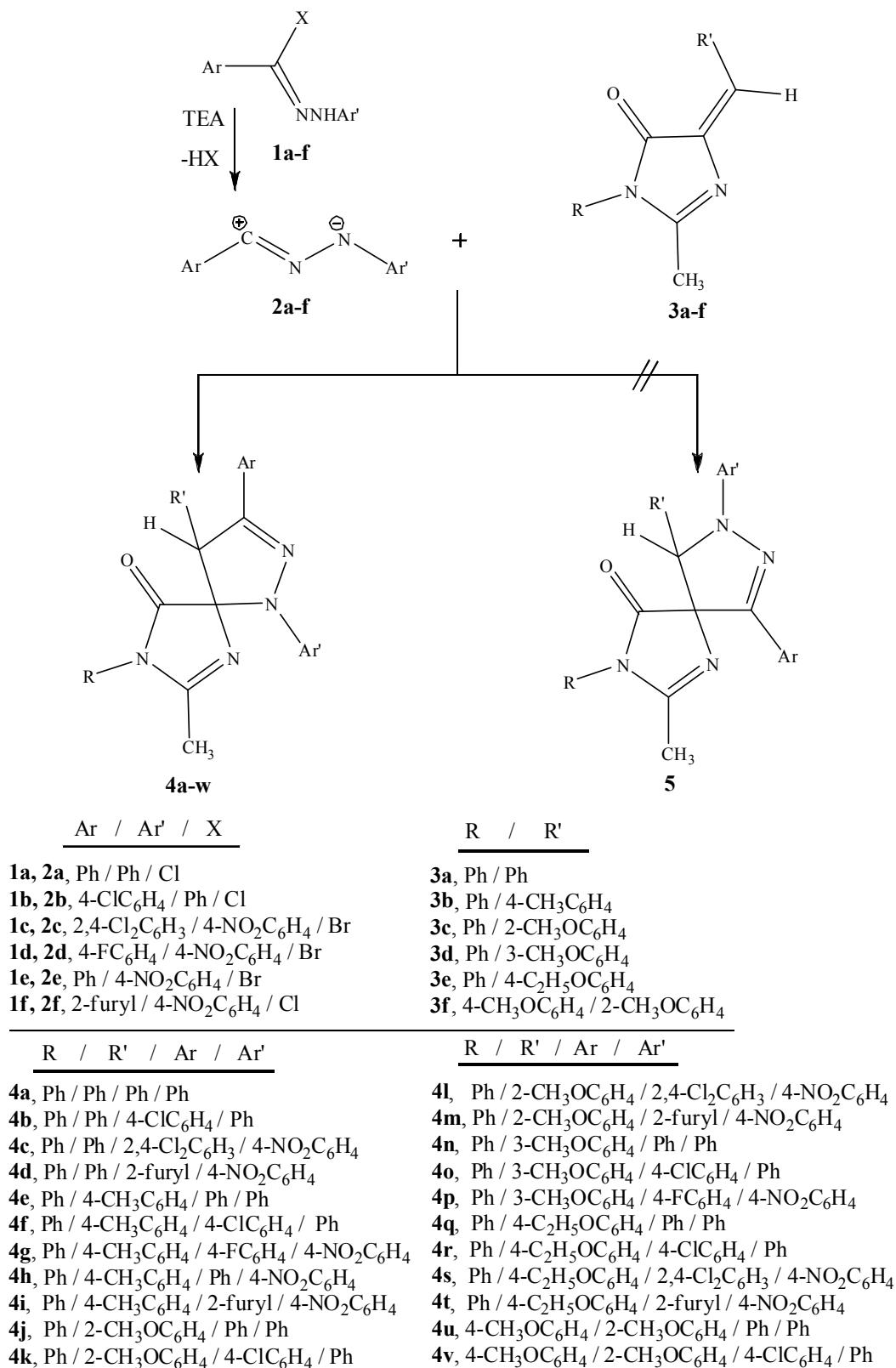
	1000	25	21	20	12	22	22	23
4r	500	18	19	17	9	19	19	20
	250	15	14	11	-	15	15	19
	125	12	10	8	-	11	11	14
4s	1000	22	21	12	22	16	18	20
	500	17	15	9	17	12	13	17
	250	12	13	-	11	8	9	13
	125	8	9	-	9	-	7	11
4t	1000	12	14	-	17	7	14	-
	500	11	10	-	15	-	11	-
	250	8	8	-	11	-	8	-
	125	-	-	-	7	-	-	-
4u	1000	24	22	22	10	21	20	22
	500	20	22	18	8	18	16	18
	250	16	18	14	-	12	13	15
	125	10	14	10	-	9	9	9
4v	1000	18	19	17	14	24	17	19
	500	16	13	13	12	19	15	15
	250	15	10	10	7	15	10	13
	125	12	-	8	-	12	7	11
4w	1000	15	8	-	18	13	20	-
	500	14	-	-	14	9	17	-
	250	8	-	-	11	7	15	-
	125	8	-	-	8	-	12	-
AMX <sup>b</sup>	10 µg/disc	11	10	19	12	20	18	10
AK <sup>b</sup>	10 µg/disc	18	17	11	19	20	18	10
CTR <sup>b</sup>	10 µg/disc	8	9	16	13	17	16	9
CIP <sup>b</sup>	30 µg/disc	13	11	17	22	21	23	12
DMSO <sup>c</sup>		5	5	0	5	6	0	6

Abbreviations: <sup>(a)</sup>Microorganisms, E.c.: *Escherichia Coli*; P.a.: *Pseudomonas Aeruginosa*; V.s.: *Viridans Streptococci*; C.s.: *Coagulase negative Staphylococcus*; P.s.: *Proteus Species*; S.s.: *Salmonella Species*; K.s.: *Klebsiella Species*; <sup>(b)</sup>Antibiotics, AMX: Amoxycillin; AK: Amikacin; CTR: Ceftriazone; CIP: Ciprofloxacin.

<sup>c</sup>DMSO used as the control.

<sup>d</sup>The data represents the mean values of two replicates

6-10 mm, low activity; 11-15 mm, moderate activity; 16-19 mm, high activity; more than 20 mm, very high activity.



Scheme 1