**SUPPLEMENTARY MATERIAL (Tables S1-S3)**

***In vitro* trichomonacidal activity and preliminary *in silico* chemometric studies of 5-nitroindazolin-3-one and 3-alkoxy-5-nitroindazole derivatives**

Alexandra Ibáñez-Escribano1,2,\*, Juan José Nogal-Ruiz1,2, Alicia Gómez-Barrio1,2, Vicente J. Arán1,3, José Antonio Escario1,2,\*

1 *Moncloa Campus of International Excellence, UCM-UPM & CSIC, Madrid, Spain*

2 *Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid (UCM), Pza. Ramón y Cajal s/n, 28040 Madrid, Spain*

3 *Instituto de Química Médica (IQM), CSIC, c/ Juan de la Cierva 3, 28006 Madrid, Spain*

\* Corresponding autors. A. Ibáñez-Escribano, J.A. Escario, Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid, Pza. Ramón y Cajal s/n, 28040 Madrid, Spain. Tel.: + 34 913941817; fax: + 34 913941815. *E-mail addresses*: [alexandraibanez@ucm.es](mailto:alexandraibanez@ucm.es) (A. Ibáñez-Escribano), [escario@ucm.es](mailto:escario@ucm.es) (J.A. Escario)

Table S1. *In silico* determination of mutagenic (Mut.), tumorigenic (Tum.), irritative (Irrit.) and reproductive (Rep.) risks and drug-score values of 1,2-disubstituted indazolinones **1**-**19** (series A), 1-substituted 3-alkoxyindazoles **20**-**24** (series B) and 2-substituted 3-alkoxyindazoles **25**-**39** (series C) using OSIRIS Property Explorer (Organic Chemistry Portal, 2014).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **Mut.** | **Tum.** | **Irrit.** | **Rep.** | **Drug-score** |  | **Compound** | **Mut.** | **Tum.** | **Irrit.** | **Rep. Effect** | **Drug-Score** |
| **1** | Non-risk | Non-risk | Non-risk | Non-risk | 0.50 |  | **20** | Non-risk | Non-risk | Non-risk | Non-risk | 0.47 |
| **2** | Non-risk | Non-risk | Non-risk | Non-risk | 0.51 |  | **21** | Non-risk | Non-risk | Non-risk | Non-risk | 0.43 |
| **3** | Non-risk | Non-risk | Non-risk | Non-risk | 0.50 |  | **22** | Non-risk | Non-risk | Non-risk | Non-risk | 0.40 |
| **4** | Non-risk | Non-risk | Non-risk | Non-risk | 0.26 |  | **23** | Non-risk | Non-risk | Non-risk | Non-risk | 0.39 |
| **5** | Non-risk | Non-risk | Non-risk | Non-risk | 0.49 |  | **24** | Non-risk | Non-risk | Non-risk | Non-risk | 0.29 |
| **6** | Non-risk | Non-risk | Non-risk | Non-risk | 0.29 |  | **25** | Risk | Risk | Non-risk | Non-risk | 0.19 |
| **7** | Non-risk | Non-risk | Non-risk | Non-risk | 0.47 |  | **26** | Risk | Risk | Non-risk | Non-risk | 0.17 |
| **8** | Non-risk | Non-risk | Non-risk | Non-risk | 0.45 |  | **27** | Risk | Risk | Non-risk | Non-risk | 0.17 |
| **9** | Non-risk | Non-risk | Non-risk | Non-risk | 0.49 |  | **28** | Risk | Risk | Non-risk | Non-risk | 0.40 |
| **10** | Non-risk | Non-risk | Non-risk | Non-risk | 0.93 |  | **29** | Risk | Risk | Non-risk | Non-risk | 0.18 |
| **11** | Non-risk | Non-risk | Non-risk | Non-risk | 0.46 |  | **30** | Risk | Risk | Non-risk | Non-risk | 0.15 |
| **12** | Non-risk | Non-risk | Non-risk | Non-risk | 0.42 |  | **31** | Risk | Risk | Non-risk | Non-risk | 0.19 |
| **13** | Non-risk | Non-risk | Non-risk | Non-risk | 0.40 |  | **32** | Risk | Risk | Non-risk | Non-risk | 0.16 |
| **14** | Non-risk | Non-risk | Non-risk | Non-risk | 0.40 |  | **33** | Risk | Risk | Non-risk | Non-risk | 0.16 |
| **15** | Non-risk | Non-risk | Non-risk | Non-risk | 0.38 |  | **34** | Risk | Risk | Risk | Non-risk | 0.08 |
| **16** | Non-risk | Non-risk | Non-risk | Non-risk | 0.49 |  | **35** | Risk | Risk | Non-risk | Non-risk | 0.12 |
| **17** | Non-risk | Non-risk | Non-risk | Non-risk | 0.38 |  | **36** | Risk | Risk | Non-risk | Non-risk | 0.12 |
| **18** | Non-risk | Non-risk | Non-risk | Non-risk | 0.34 |  | **37** | Risk | Risk | Non-risk | Non-risk | 0.12 |
| **19** | Non-risk | Non-risk | Non-risk | Non-risk | 0.33 |  | **38** | Risk | Risk | Non-risk | Non-risk | 0.10 |
| **Metronidazole** | Non-risk | Non-risk | Non-risk | Risk | 0.51 |  | **39** | Risk | Risk | Non-risk | Non-risk | 0.10 |

Table S2. In silico determined physicochemical molecular properties of 1,2-disubstituted 5-nitroindazolin-3-ones 1–19 (series A) using Molinspiration software (Molinspiration Cheminformatics, 2014).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | | **CLogP** | **MW** | **Ha** | **Hd** | **Lipinski’s violation** | **TPSA** | **Atoms** | **Rotb** | **Volume** | **% Abs** |
| **1** | 1.144 | | 207.19 | 6 | 0 | 0 | 72.763 | 15 | 1 | 174.209 | 84.79 |
| **2** | 2.022 | | 235.24 | 6 | 0 | 0 | 72.763 | 17 | 3 | 207.813 | 86.80 |
| **3** | 1.883 | | 235.24 | 6 | 0 | 0 | 72.763 | 17 | 2 | 207.598 | 86.80 |
| **4** | 2.418 | | 269.26 | 6 | 0 | 0 | 72.763 | 20 | 2 | 229.057 | 89.24 |
| **5** | 2.739 | | 283.28 | 6 | 0 | 0 | 72.763 | 21 | 3 | 245.859 | 90.24 |
| **6** | 2.418 | | 269.26 | 6 | 0 | 0 | 72.763 | 20 | 2 | 229.057 | 96.40 |
| **7** | 3.297 | | 297.31 | 6 | 0 | 0 | 72.763 | 22 | 4 | 262.660 | 91.25 |
| **8** | 3.157 | | 297.31 | 6 | 0 | 0 | 72.763 | 22 | 3 | 262.445 | 91.25 |
| **9** | 2.739 | | 283.28 | 6 | 0 | 0 | 72.763 | 21 | 3 | 245.859 | 90.24 |
| **10** | 2.804 | | 238.28 | 3 | 0 | 0 | 26.939 | 18 | 2 | 222.524 | 87.02 |
| **11** | 3.617 | | 311.34 | 6 | 0 | 0 | 72.763 | 23 | 5 | 279.462 | 92.25 |
| **12** | 3.478 | | 311.34 | 6 | 0 | 0 | 72.763 | 23 | 4 | 279.247 | 92.25 |
| **13** | 4.176 | | 325.36 | 6 | 0 | 0 | 72.763 | 24 | 6 | 296.264 | 93.26 |
| **14** | 4.682 | | 339.39 | 6 | 0 | 0 | 72.763 | 25 | 7 | 313.066 | 94.26 |
| **15** | 4.333 | | 359.38 | 6 | 0 | 0 | 72.763 | 27 | 5 | 317.508 | 95.69 |
| **16** | 2.948 | | 297.31 | 6 | 0 | 0 | 72.763 | 22 | 4 | 262.220 | 91.25 |
| **17** | 3.922 | | 333.34 | 6 | 0 | 0 | 72.763 | 25 | 3 | 289.850 | 93.83 |
| **18** | 4.801 | | 361.39 | 6 | 0 | 0 | 72.763 | 27 | 5 | 323.454 | 95.83 |
| **19** | 4.661 | | 361.39 | 6 | 0 | 0 | 72.763 | 27 | 4 | 323.239 | 95.83 |
| **Metronidazole** | 0.468 | | 171.15 | 6 | 1 | 0 | 83.878 | 12 | 3 | 146.771 | 76.75 |
| **Acceptable range** | ≤ 5 | | ≤ 500 | ≤ 10 | ≤ 5 | ≤ 1 | <140 | 20-70 | ≤ 10 | - | - |
| CLogP: calculated partition coefficient; MW: molecular weight; Ha: hydrogen bond acceptors; Hd: hydrogen bond donors; TPSA: topological polar surface area; Rotb: number of rotatable bonds; %Abs: percentage of absorption calculated following the formula %Abs = 90-3.34Ha-5.56Hd+0.0716MW (Zhao *et al.* 2002). | | | | | | | | | | | |

Table S3: *In silico* determined physicochemical molecular properties of 1-substituted 3-alkoxyindazoles **20**-**24** (series B) and 2-substituted 3-alkoxyindazoles **25**-**39** (series C) using Molinspiration software (Molinspiration Cheminformatics, 2014).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **CLogP** | **MW** | **Ha** | **Hd** | **Lipinski’s**  **violation** | **TPSA** | **Atoms** | **Rotb** | **Volume** | **% Abs** |
| **20** | 1.809 | 207.19 | 6 | 0 | 0 | 72.884 | 15 | 2 | 174.687 | 84.79 |
| **21** | 3.083 | 269.26 | 6 | 0 | 0 | 72.884 | 20 | 3 | 229.534 | 89.24 |
| **22** | 3.404 | 283.28 | 6 | 0 | 0 | 72.884 | 21 | 4 | 246.336 | 90.24 |
| **23** | 3.613 | 297.31 | 6 | 0 | 0 | 72.884 | 22 | 5 | 263.138 | 91.25 |
| **24** | 4.587 | 333.34 | 6 | 0 | 0 | 72.884 | 25 | 4 | 290.328 | 93.83 |
| **25** | 1.809 | 207.19 | 6 | 0 | 0 | 72.884 | 15 | 2 | 174.687 | 84.79 |
| **26** | 2.688 | 235.24 | 6 | 0 | 0 | 72.884 | 17 | 4 | 208.290 | 86.80 |
| **27** | 2.548 | 235.24 | 6 | 0 | 0 | 72.884 | 17 | 3 | 208.076 | 86.80 |
| **28** | 3.404 | 283.28 | 6 | 0 | 0 | 72.884 | 21 | 4 | 246.336 | 90.24 |
| **29** | 3.083 | 269.26 | 6 | 0 | 0 | 72.884 | 20 | 3 | 229.534 | 89.24 |
| **30** | 3.823 | 297.31 | 6 | 0 | 0 | 72.884 | 22 | 4 | 262.923 | 91.25 |
| **31** | 3.404 | 283.28 | 6 | 0 | 0 | 72.884 | 21 | 4 | 246.336 | 90.24 |
| **32** | 4.283 | 311.34 | 6 | 0 | 0 | 72.884 | 23 | 6 | 279.940 | 92.25 |
| **33** | 4.143 | 311.34 | 6 | 0 | 0 | 72.884 | 21 | 5 | 279.725 | 92.25 |
| **34** | 4.842 | 325.36 | 6 | 0 | 0 | 72.884 | 24 | 7 | 296.742 | 93.26 |
| **35** | 5.347 | 339.39 | 6 | 0 | 1 | 72.884 | 25 | 8 | 313.543 | 94.26 |
| **36** | 4.999 | 359.38 | 6 | 0 | 0 | 72.884 | 27 | 6 | 317.986 | 95.69 |
| **37** | 4.587 | 333.34 | 6 | 0 | 0 | 72.884 | 25 | 4 | 290.328 | 93.83 |
| **38** | 5.466 | 361.39 | 6 | 0 | 1 | 72.884 | 27 | 6 | 323.932 | 95.84 |
| **39** | 5.326 | 361.39 | 6 | 0 | 1 | 72.884 | 27 | 5 | 323.717 | 95.84 |
| **Metronidazole** | 0.468 | 171.15 | 6 | 1 | 0 | 83.878 | 12 | 3 | 146.771 | 76.75 |
| **Acceptable range** | ≤ 5 | ≤ 500 | ≤ 10 | ≤ 5 | ≤ 1 | <140 | 20-70 | ≤ 10 | - | **-** |
| CLogP: calculated partition coefficient; MW: molecular weight; Ha: hydrogen bond acceptors; Hd: hydrogen bond donors; TPSA: topological polar surface area; Rotb: number of rotatable bonds; %Abs: percentage of absorption calculated following the formula %Abs = 90-3.34Ha-5.56Hd+0.0716MW (Zhao *et al.* 2002). | | | | | | | | | | |