

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

ALEXANDRA IBÁÑEZ-ESCRIBANO^{1,2*}, JUAN JOSÉ NOGAL-RUIZ^{1,2},
ALICIA GÓMEZ-BARRIO^{1,2}, VICENTE J. ARÁN^{1,3} and JOSÉ ANTONIO ESCARIO^{1,2*}

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

(Received 15 June 2015; revised 7 September 2015; accepted 28 September 2015; first published online 4 November 2015)

SUMMARY

A selection of 1,2-disubstituted 5-nitroindazolin-3-ones (1–19) and 3-alkoxy-5-nitroindazoles substituted at positions 1 (20–24) or 2 (25–39) from our in-house compound library were screened *in vitro* against the most common curable sexually transmitted pathogen, *Trichomonas vaginalis*. A total of 41% of the studied molecules (16/39) achieved a significant activity of more than 85% growth inhibition at the highest concentration assayed (100 µg mL⁻¹). Among these compounds, 3-alkoxy-5-nitroindazole derivatives 23, 24, 25 and 27 inhibited parasite growth by more than 50% at 10 µg mL⁻¹. In addition, the first two compounds (23, 24) still showed remarkable activity at the lowest dose tested (1 µg mL⁻¹), inhibiting parasite growth by nearly 40%. Their specific activity towards the parasite was corroborated by the determination of their non-specific cytotoxicity against mammalian cells. The four mentioned compounds exhibited non-cytotoxic profiles at all of the concentrations assayed, showing a fair antiparasitic selectivity index (SI > 7.5). *In silico* studies were performed to predict pharmacokinetic properties, toxicity and drug-score using Molinspiration and OSIRIS computational tools. The current *in vitro* results supported by the virtual screening suggest 2-substituted and, especially, 1-substituted 3-alkoxy-5-nitroindazoles as promising starting scaffolds for further development of novel chemical compounds with the main aim of promoting highly selective trichomonacidal lead-like drugs with adequate pharmacokinetic and toxicological profiles.

Key words: *Trichomonas vaginalis*, rule of five, nitroindazoles, chemotherapy, trichomonosis, pharmacokinetic properties, toxicity, Molinspiration, OSIRIS software.

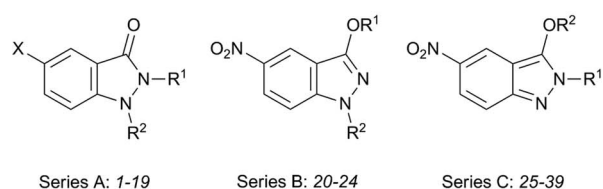
INTRODUCTION

Trichomonas vaginalis is the aetiological causative agent of more than half of curable sexually transmitted infections (STI) with an estimated 256 million cases every year (WHO, 2012). Notwithstanding this high prevalence, which accounts for more cases than syphilis, chlamydia and gonorrhoea combined, trichomonosis is still a non-reportable disease receiving low attention from public health organisms (Van der Pol, 2007). The infection has high rates of asymptomatic cases in women and especially in men (Lewis, 2014) and is associated with severe consequences including adverse outcomes during pregnancy (Cotch *et al.* 1997) and increased risk of acquisition of other dangerous STI as human papillomavirus (HPV) (Lazenby *et al.* 2014) or human immunodeficiency virus (HIV) (McClelland *et al.* 2007). Trichomonosis is also linked to pelvic inflammatory disease (Moodley *et al.* 2002) and increases the risk of cervical

(El-Gayar and Rashwan, 2007) and prostatic neoplasia (Sutcliffe *et al.* 2012) development. Furthermore, different studies have demonstrated a significant reduction of HIV-1 RNA in vaginal secretions after treatment for *T. vaginalis* in HIV+ patients with a corresponding reduction in vaginal shedding (Wang *et al.* 2001; Kissinger *et al.* 2009).

Metronidazole is the reference drug approved by the Food and Drug Administration for trichomonosis treatment since the 1960s (Helms *et al.* 2008). Although this 5-nitroimidazole has supported excellent pharmacokinetic and antiparasitic profiles, clinical cases of metronidazole resistance were reported a few years after its introduction (Dunne *et al.* 2003). Not until 2004 was an alternative drug, tinidazole, also approved for this sexually transmitted disease (Bachmann *et al.* 2011). Moreover, diverse side effects, cross-resistance between both related drugs and their possible mutagenic and teratogenic effects (Cudmore *et al.* 2004) evidence the necessity of discovering novel molecules with trichomonacidal properties and different structural patterns. The proposed aim of reinforcing the 5-nitroimidazole pharmacological arsenal would thus follow the guidelines of the World Health Organization,

* Corresponding author. Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid, Pza. Ramón y Cajal s/n, 28040 Madrid, Spain. E-mail: alexandraibanez@ucm.es, escario@ucm.es



Series A: 1-19 Series B: 20-24 Series C: 25-39

Series A	Series B	Series C	X	R ¹	R ²
1	20	25	NO ₂	Me	Me
2	-	26	NO ₂	Me	Pr
3	-	27	NO ₂	Me	iPr
4	21	-	NO ₂	Me	Ph
5	-	28	NO ₂	Me	Bn
6	-	29	NO ₂	Ph	Me
7	-	-	NO ₂	Ph	Pr
8	-	30	NO ₂	Ph	iPr
9	22	31	NO ₂	Bn	Me
10	-	-	H	Bn	Me
11	-	32	NO ₂	Bn	Pr
12	-	33	NO ₂	Bn	iPr
13	-	34	NO ₂	Bn	Bu
14	-	35	NO ₂	Bn	Pn
15	-	36	NO ₂	Bn	Bn
16	23	-	NO ₂	Phe	Me
17	24	37	NO ₂	Nm	Me
18	-	38	NO ₂	Nm	Pr
19	-	39	NO ₂	Nm	iPr

Bn: benzyl; Bu: butyl; Me: methyl; Nm: 2-naphthylmethyl; Ph: phenyl;
Phe: phenethyl; Pn: pentyl; Pr: propyl; iPr: isopropyl

Fig. 1. Indazole derivatives studied in the current work.

directed to accomplish diverse millennium development goals (WHO, 2007).

Accordingly, during the last decade our research group has been working on the design, synthesis and biological activity of novel potential scaffolds, especially nitroheterocycles, against trichomonosis and other protozoan diseases. In this context, trichomonacidal activity of many indazol-3-ol/indazolin-3-one derivatives has previously been reported (Arán *et al.* 2005; Marrero-Ponce *et al.* 2005, 2006). More recently, several 1,2-disubstituted 5-nitroindazolin-3-ones (1–19; Fig. 1, series A) and 3-alkoxy-5-nitroindazoles substituted at positions 1 (20–24; Fig. 1, series B) and 2 (25–39; Fig. 1, series C), have been synthesized and tested against *Trypanosoma cruzi* (Vega *et al.* 2012; Fonseca-Berzal *et al.* 2014; Muro *et al.* 2014). In order to investigate novel compounds with interesting trichomonacidal activity, the molecules mentioned above have been subjected to *in vitro* screening against well-established cultures of *T. vaginalis* following a previously validated fluorometric method (Ibáñez-Escribano *et al.* 2012). To determine their

non-specific cytotoxicity profiles, the most active compounds were also screened against mammalian cells according to the sequential flow-chart method used in our laboratory (Ibáñez-Escribano *et al.* 2014). Furthermore, the evaluated compounds were submitted to computational prediction of their toxicity risks including reproductive side effects and drug-like scores by using OSIRIS Property Explorer (Organic Chemistry Portal, 2014). The compounds were also tested for compliance with Lipinski's 'rule of five' (RO5) (Lipinski *et al.* 1997) with the main aim of studying their bioavailability properties using the Molinspiration online software (Molinspiration Cheminformatics, 2014). The design of viable new drug candidates should consider molecules with specific activity but also with acceptable ADME (absorption, distribution, metabolism, and excretion) properties, which include a chemical structure which does not present difficulties in oral bioavailability. These *in silico* calculations permit the removal of molecular entities that would probably be discarded in the later stages of discovery and development due to their low oral bioavailability or toxic features.

METHODS

Chemistry: preparation of the studied compounds

Compounds 1–19 (series A), 20–24 (series B) and 25–39 (series C) were prepared by alkylation of the corresponding 2-substituted indazolin-3-ones or 1-substituted indazol-3-ols with the required alkyl halides (Vega *et al.* 2012; Muro *et al.* 2014).

Biological assays

In vitro determination of trichomonacidal activity.

Trichomonas vaginalis isolate JH31A4 from the American Culture Type Collection (ATCC) was grown *in vitro* in modified TYM medium at pH 6 with 10% inactivated foetal bovine serum (FBS), 100 IU penicillin mL⁻¹ and 100 µg mL⁻¹ streptomycin, in a humidified chamber at 37 °C and sub-cultured every 48–72 h.

In vitro trichomonacidal activity assays were carried out in glass tubes containing 10⁵ trophozoites mL⁻¹. After 5 h, log-phase cultures were incubated with the different nitroindazole derivatives at 100, 10 and 1 µg mL⁻¹ for 24 h at 37 °C and 5% CO₂. Stock solutions of chemical compounds were dissolved in dimethyl sulfoxide (DMSO) prior to use. Metronidazole was used as a reference drug at 4 µg mL⁻¹ and prepared in a similar fashion to the rest of the chemical derivatives. The final concentration of DMSO in cultures never exceeded 0.2%. Afterwards, cultures were seeded in 96-well microtitre plates and washed with PBS supplemented with 0.1% glucose. The trichomonacidal effect was determined by fluorometry after 1 h of incubation with

resazurin dye (stock solution 3 mM) following the methodology previously described (Ibáñez-Escribano *et al.* 2012). Activity values were calculated from the percentage reduction of resazurin in treated and untreated wells. Each concentration was evaluated in triplicate and values were obtained from the average of two separate determinations.

In vitro determination of non-specific cytotoxicity in mammalian cells. Cytotoxicity against Vero cells was determined following the sequential procedure reported by our research group (Ibáñez-Escribano *et al.* 2014). The monkey kidney epithelial cell line Vero CCL-81 (from ATCC) was grown in RPMI-1640 medium (Sigma) supplemented with 10% heat-inactivated FBS and antibiotics in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C. Cells were seeded at a density of 5×10^4 cells/well in 96-well flat-bottom microplates (Nunc). After cell attachment, the drugs dissolved in RPMI medium were added at the same concentrations as for the evaluation against *T. vaginalis*. The plates were incubated for 24 h at 37 °C in 5% CO₂ and the cytotoxic activity was revealed by adding 20 μ L of 1 mM resazurin solution. After 3 h in contact with the redox dye, the fluorescence readings were carried out in a fluorometer (Infinite 200, TECAN) as described previously (Ibáñez-Escribano *et al.* 2015) with minor modifications. The selectivity index (SI) was determined as the ratio between the concentration of the compound needed to reduce cell viability to 50% (CC₅₀) and the concentration that showed a 50% growth inhibitory effect (GI₅₀) on the parasitic culture. Only those compounds showing an antiparasitic profile, defined as having a significant trichomonacidal activity without non-specific toxicity, were tested again at six different serial 2-fold dilutions and their SI was determined.

Computational analysis: molecular properties, bioavailability parameters and prediction of potential biological risks

The molecular properties derived from the chemical structure were calculated using online bioinformatics tools. The different properties related to molecular permeability such as physicochemical features involved in the Lipinski's RO5 (Lipinski *et al.* 1997) as well as topological polar surface area (TPSA), rotatable bonds and molecular volume were computed using free online software (Molinspiration Cheminformatics, 2014). The percentage absorption was estimated following the criteria of Zhao *et al.* (2002). On the other hand, the online program OSIRIS (Organic Chemistry Portal, 2014) was employed for the prediction of potential risks including mutagenic, tumorigenic, irritative and reproductive side effects by comparing

the chemical structures of the studied compounds with those of commercial drugs.

RESULTS AND DISCUSSION

Compounds of series A, B and C (Fig. 1) as well as the reference drug metronidazole were analysed for their *in vitro* antiparasitic activity against a well-established isolate of *T. vaginalis* following a previously reported procedure (Ibáñez-Escribano *et al.* 2012). As shown in Table 1, 16 of the synthesized compounds exhibited a significant trichomonacidal profile with more than 85% growth inhibition after 24 h in contact with the trophozoites. When compared with the reference drug, none of the compounds reached a minimum inhibitory concentration (MIC₁₀₀) lower than that of metronidazole (MIC_{100-MTZ} = 4 μ g mL⁻¹); nevertheless, compounds 23, 24, 25 and 27 from series B and C, inhibited parasite growth by more than 55% at 10 μ g mL⁻¹ (30–48 μ M) and, in addition, the first two compounds (23, 24) still showed a noticeable activity at the lowest dose tested (1 μ g mL⁻¹, ca. 3 μ M), inhibiting parasite growth by nearly 40%. From the observed results we can conclude that 1,2-disubstituted 5-nitroindazolin-3-ones (1–19, series A), which were very efficient against *T. cruzi* (Vega *et al.* 2012; Fonseca-Berzal *et al.* 2014) show very low trichomonacidal activity, while 3-alkoxy-5-nitroindazoles containing simple substituents at positions 1 (20–24, series B) or 2 (25–29, series C) display moderate activity. We have not been able to establish a general structure-activity relationship; however, it is clear that among compounds of series B, the best compounds (23, 24) show bulky lipophilic substituents (phenethoxy and 2-naphthylmethoxy, respectively) at position 3 of the indazole ring. Conversely, for series C the best compounds (25, 27) support small substituents (methoxy and isopropoxy, respectively) at the same position. Accordingly, series B and C scaffolds could be promising for further chemical modifications. In fact, we have previously reported that for compounds of series B, activity increases considerably after the introduction of complex ω -(dialkylamino) alkyl chains at position 1 (Arán *et al.* 2005).

On the other hand, a comparison of the activities of compound 9 and its denitro analogue 10 at 100 μ g mL⁻¹ shows that the 5-nitro group of the indazole ring plays a relevant role in trichomonacidal activity. In fact, a similar effect has previously been noticed for 1-substituted indazol-3-ols (Marrero-Ponce *et al.* 2006).

With the results gathered in the present article, we complete the exploration of trichomonacidal activity of indazol-3-ol/indazolin-3-one tautomeric system derivatives substituted at the pyrazole ring. In fact, previous studies carried out for monosubstituted derivatives have shown that 1-substituted

Table 1. *In vitro* trichomonacidal effect 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)

Compound	Conc. (μM) ^a	% Growth inhibition ^b			Compound	Conc. (μM) ^a	% Growth inhibition ^b		
		100 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	1 $\mu\text{g mL}^{-1}$			100 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	1 $\mu\text{g mL}^{-1}$
1	482.6	94.7	41.2	0	20	482.6	100	35.1	25.7
2	425.1	80.7	26.5	10.8	21	371.4	92.4	29.2	1.0
3	425.1	24.3	14.1	0	22	353.0	100	37.1	0
4	371.4	11.4	8.9	3.1	23	336.3	97.6	64.2	43.1
5	353.0	89.2	10.3	1.2	24	300.0	69.2	60.6	37.8
6	371.4	39.9	5.42	9.1	25	482.6	100	58.0	0
7	336.3	52.3	35.0	0	26	425.1	93.9	31.7	11.2
8	336.3	37.6	0	0	27	425.1	90.4	67.8	6.6
9	353.0	87.5	22.1	5.5	28	353.0	19.6	31.9	21.9
10	419.7	31.3	8.2	36.9	29	371.4	67.8	41.4	0.3
11	321.2	90.1	27.8	37.9	30	363.3	31.2	0	0
12	321.2	52.3	35.0	0	31	353.0	78.1	22.3	6.9
13	307.4	63.3	0	0	32	321.2	89.4	27.0	17.0
14	294.6	84.2	0	0	33	321.2	73.6	16.5	2.9
15	278.3	46.9	0	0	34	307.4	90.2	22.6	0
16	336.3	12.0	26.2	0.2	35	294.6	87.0	4.8	13.4
17	300.0	93.4	27.1	10.5	36	278.3	50.4	0	2.5
18	276.7	95.3	14.7	1.0	37	300.0	82.6	0	0
19	276.7	47.9	25.1	24.5	38	276.7	80.2	23.6	8.3
Metronidazole	584.2	100.0	100.0	76.5	39	276.7	2.5	7.8	1.0

^a Micromolar concentration (μM) corresponding to the highest tested concentration ($100 \mu\text{g mL}^{-1}$) is indicated for each compound.

^b Results are expressed as a percentage. All molecules were assayed in triplicate in at least two independent experiments. All the results displayed a standard deviation of less than 10%.

5-nitroindazol-3-ols are very efficient (Marrero-Ponce *et al.* 2005, 2006), while 2-substituted 5-nitroindazolin-3-ones and 3-alkoxy-5-nitroindazoles, although there are few studied cases, seem to display very low activity (Marrero-Ponce *et al.* 2005).

The metronidazole and tinidazole mode of action is based on the nitro group located at position 5 of the imidazole ring. These compounds are in fact prodrugs, which enter into the parasite by passive diffusion and are activated inside the hydrogenosome by redox parasitic enzymes. It is usually accepted that reduction of the nitro group generates cytotoxic radicals and intermediates that are highly reactive towards DNA and proteins, leading to the formation of covalent adducts with essential parasite biomolecules (Leitsch *et al.* 2009). A similar mode of action associated with the generation of NO_2 radicals inducing oxidative stress could explain the importance of the nitro group in a remarkable number of nitro derivatives showing trichomonacidal activity (Adagu *et al.* 2002; Navarrete-Vázquez *et al.* 2006; Hernández-Núñez *et al.* 2009; Kumar *et al.* 2010, 2011, 2012). Keeping in mind, however, the recent advances on the reductive metabolism and mechanism of action of other nitroheterocyclic prodrugs such as the antichagasic drugs nifurtimox (Hall *et al.* 2011) and benznidazole (Hall and Wilkinson, 2012; Trochine *et al.* 2014), related mechanistic

studies in the field of trichomonacidal nitroheterocycles are required.

With the main aim of studying the specific anti-protozoal activity of the synthetic compounds, the four most active molecules 23, 24, 25 and 27, were evaluated again against the parasite at six different concentrations and a non-specific cytotoxicity test on VERO cells was simultaneously performed to determine the SI following a previously described method (Ibáñez-Escribano *et al.* 2014). Two of the four compounds (23 and 25) displayed a remarkable GI_{50} (*T. vaginalis* 50% growth inhibition) calculated from log-probit analyses using linear regression (SPSS, IBM v.22). Compound 25 exhibited a GI_{50} of $6.69 \mu\text{g mL}^{-1}$ ($18.51 \mu\text{M}$) while 23 showed a GI_{50} of $11.63 \mu\text{g mL}^{-1}$ ($39.12 \mu\text{M}$).

The screening assays revealed no non-specific cytotoxic effects against Vero cells at the assayed concentrations ($1\text{--}100 \mu\text{g mL}^{-1}$), displaying a reduction of cellular growth of $<10\%$ at the highest concentration studied. Moreover, no difference was observed between cells grown with $100 \mu\text{g mL}^{-1}$ of compounds 23 and 24 during 24 h and growth controls. Only a slight percentage reduction of 6.68 ± 4.97 and 9.44 ± 3.22 was detected for 25 and 27, respectively. Based on this *in vitro* screening profile, compounds 23, 24, 25 and 27 show a remarkable SI of more than 7.5. The absence of non-specific cytotoxic activity against mammalian cells is in

agreement with previous studies conducted by our research group with other 3-alkoxy-5-nitroindazole derivatives (Arán *et al.* 2005; Vega *et al.* 2012; Muro *et al.* 2014).

Furthermore, these *in vitro* non-specific cytotoxicity results are in agreement with the virtual prediction of several risks made by a fragment-based method using the OSIRIS software (Supplementary data, Table S1). It was found that all the synthesized 1,2-disubstituted 5-nitroindazolin-3-one derivatives (1–19) exhibit non-toxic features in terms of mutagenic (Mut.), tumorigenic (Tum.), irritative (Irrit.) or reproductive (Reprod.) risks. These results increase the interest in compounds of series A, taking in account the fact that some of its members, especially 11–13, have shown to be very effective against *T. cruzi* (Vega *et al.* 2012; Fonseca-Berzal *et al.* 2014). On the other hand, the results shown in Supplementary Table S1 suggest that substitution at position 2 of the 3-alkoxy-5-nitroindazole moiety (25–39) induces tumorigenic and mutagenic effects, but no risks were predicted for molecules with substituents at position 1 (20–24). Fortunately no irritative or detrimental effects on mammalian reproduction were predicted for the evaluated molecules with minor exceptions. In fact, only compound 34 (2-benzyl-3-butoxy-5-nitro-2*H*-indazole) was classified as potentially irritative also having the lowest calculated drug-score (0.08; see below). The risk of detrimental reproductive effects from metronidazole was also corroborated by the virtual screening as summarized in Supplementary Table S1.

The drug-score gives information related to the overall potential of a compound to be classified as a drug, taking into consideration different parameters related to physicochemical, risk and drug likeness calculations. Thus, its estimation allows compounds that will probably be poor drugs to be discarded at an early stage, according to the balance of their overall structure and molecular properties. In our case, the values calculated by the OSIRIS software for compounds of series A and B reflect the suitability of these structures for biological studies. As shown in Supplementary Table S1, 5-nitroindazolin-3-ones (1–9 and 11–19, series A) and 1-substituted 3-alkoxy-5-nitroindazoles (20–24, series B) display remarkable drug-scores in the range of 0.26–0.51, close in many cases to that of metronidazole (0.51). In the case of 2-substituted 3-alkoxy-5-nitroindazoles (25–39, series C), drug-scores are, except for compound 28, very low (0.08–0.19).

In relation to Lipinski's 'rule of five' (RO5) (Lipinski *et al.* 1997) and further studies conducted by other research groups (Ertl *et al.* 2000; Veber *et al.* 2002), several physicochemical properties such as molecular weight (MW), hydrogen-bond acceptors (Ha) and donors (Hd), volume, number

of atoms, calculated coefficient of partition (CLogP), TPSA and rotatable bonds (Rotb) have been empirically inferred (Supplementary data, Tables S2 and S3). Neither indazolin-3-one derivatives (1–19) nor 3-alkoxy-5-nitroindazoles (20–39) violate Lipinski's rule. The rule states that compounds with more than 5 Hd, 10 Ha, a MW > 500 and a CLogP > 5 are more likely to exhibit poor absorption and permeation (Lipinski *et al.* 1997). Regarding the rest of the physicochemical properties, all the evaluated compounds were adequately sized between 15 and 27 atoms, and had less than 8 Rotb which correlates with a suitable molecular flexibility (Veber *et al.* 2002). Their TPSA values were 72.763 and 72.884 for series A and series B/C, respectively. These values predict good oral bioavailability since they can be described as excellent oral absorption parameters (Palm *et al.* 1997), defined as a suitable percentage of absorption >84% for the 39 studied compounds, calculated according to the method of Zhao *et al.* (2002). The TPSA values (c. 73 Å²) and the number of H-bond acceptors (Ha = 6) classified these entities as inadequate for trespassing the blood-brain barrier by passive-diffusion (Lipinski, 2004). In agreement with these results, no undesirable side effects associated with the central nervous system should be expected.

In conclusion, the *in silico* and *in vitro* results obtained in the current study suggest 2-substituted (series C) and, especially, 1-substituted 3-alkoxy-5-nitroindazoles (series B) as promising lead-like scaffolds for further chemical modifications. The evaluated compounds of series B presented interesting trichomonocidal activity and specificity as well as remarkable bioavailability and safety profiles. This is the case, in particular, for 3-phenethoxy- (23) and 3-(2-naphthylmethoxy) (24) indazole derivatives. Novel structures should be developed, however, with the aim of identifying new highly selective trichomonocidal drugs.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0031182015001419>.

ACKNOWLEDGMENTS

The Research Group 'Antiparasitic Therapy' (Ref. 911120) of the Universidad Complutense de Madrid and CEI Moncloa (cluster of i-Medicine) is gratefully acknowledged by all the authors.

FINANCIAL SUPPORT

A. Ibáñez-Escribano thanks Moncloa Campus of International Excellence (UCM-UPM & CSIC) for a PICATA predoctoral fellowship.

REFERENCES

- Adagu, I. S., Nolder, D., Warhurst, D. C. and Rossignol, J.-F. (2002). *In vitro* activity of nitazoxanide and related compounds against isolates of *Giardia intestinalis*, *Entamoeba histolytica* and *Trichomonas vaginalis*. *Journal of Antimicrobial Chemotherapy* **49**, 103–111.
- Arán, V. J., Ochoa, C., Boiani, L., Buccino, P., Cerecetto, H., Gerpe, A., González, M., Montero, D., Nogal, J. J., Gómez-Barrio, A., Azqueta, A., López de Ceráin, A., Piro, O. E. and Castellano, E. E. (2005). Synthesis and biological properties of new 5-nitroindazole derivatives. *Bioorganic & Medicinal Chemistry* **13**, 3197–3207.
- Bachmann, L. H., Hobbs, M. M., Seña, A. C., Sobel, J. D., Schwabke, J. R., Krieger, J. N., Scott McClelland, R. and Workowski, K. A. (2011). *Trichomonas vaginalis* genital infections: progress and challenges. *Clinical Infectious Diseases* **53**, S160–S172.
- Cotch, M. F., Pastorek, J. G., Nugent, R. P., Hillier, S. L., Gibbs, R. S., Martin, D. H., Eschenbach, D. A., Edelman, R., Carey, J. C., Regan, J. A., Krohn, M. A., Klebanoff, M. A., Rao, A. V., Rhoads, G. G., Yaffe, S. J., Catz, C. S., McNellis, D., Berendes, H. W., Blackwelder, W. C., Kaslow, R. A., Reed, G. F., Greenberg, E. M., Williams, S. and Rettig, P. J. (1997). *Trichomonas vaginalis* associated with low birth weight and preterm delivery. *Sexually Transmitted Diseases* **24**, 353–360.
- Cudmore, S. L., Delgaty, K. L., Hayward-McClelland, S. F., Petrin, D. P. and Garber, G. E. (2004). Treatment of infections caused by metronidazole-resistant *Trichomonas vaginalis*. *Clinical Microbiology Reviews* **17**, 783–793.
- Dunne, R. L., Dunn, L. A., Upcroft, P., O'Donoghue, P. J. and Upcroft, J. A. (2003). Drug resistance in the sexually transmitted protozoan *Trichomonas vaginalis*. *Cell Research* **13**, 239–249.
- El-Gayar, E. K. and Rashwan, M. F. (2007). Cervical intraepithelial neoplasia (CIN) and *Trichomonas vaginalis* infection as revealed by polymerase chain reaction. *Journal of the Egyptian Society of Parasitology* **37**, 623–630.
- Ertl, P., Rohde, B. and Selzer, P. (2000). Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *Journal of Medicinal Chemistry* **43**, 3714–3717.
- Fonseca-Berzal, C., Escario, J. A., Arán, V. J. and Gómez-Barrio, A. (2014). Further insights into biological evaluation of new anti-*Trypanosoma cruzi* 5-nitroindazoles. *Parasitology Research* **113**, 1049–1056.
- Hall, B. S. and Wilkinson, S. R. (2012). Activation of benzimidazole by trypanosomal type I nitroreductases results in glyoxal formation. *Antimicrobial Agents and Chemotherapy* **56**, 115–123.
- Hall, B. S., Bot, C. and Wilkinson, S. R. (2011). Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. *Journal of Biological Chemistry* **286**, 13088–13095.
- Helms, D. J., Mosure, D. J., Secor, W. E. and Workowski, K. A. (2008). Management of *Trichomonas vaginalis* in women with suspected metronidazole hypersensitivity. *American Journal of Obstetrics and Gynecology* **198**, 370.e1–370.e7.
- Hernández-Núñez, E., Tlahuext, H., Moo-Puc, R., Torres-Gómez, H., Reyes-Martínez, R., Cedillo-Rivera, R., Nava-Zuazo, C. and Navarrete-Vázquez, G. (2009). Synthesis and *in vitro* trichomonacidal, giardicidal and amebicidal activity of *N*-acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles. *European Journal of Medicinal Chemistry* **44**, 2975–2984.
- Ibáñez-Escribano, A., Meneses Marcel, A., Machado Tugores, Y., Nogal Ruiz, J. J., Arán Redó, V. J., Garcia-Trevijano, J. A. and Gómez Barrio, A. (2012). Validation of a modified fluorimetric assay for the screening of trichomonacidal drugs. *Memórias do Instituto Oswaldo Cruz* **107**, 637–643.
- Ibáñez-Escribano, A., Meneses-Marcel, A., Marrero-Ponce, Y., Nogal-Ruiz, J. J., Arán, V. J., Gómez-Barrio, A. and Escario, J. A. (2014). A sequential procedure for rapid and accurate identification of putative trichomonacidal agents. *Journal of Microbiological Methods* **105**, 162–167.
- Ibáñez-Escribano, A., Reviriego, F., Nogal-Ruiz, J. J., Meneses-Marcel, A., Gómez-Barrio, A., Escario, J. A. and Arán, V. J. (2015). Synthesis and *in vitro* and *in vivo* biological evaluation of substituted nitroquinoxalin-2-ones and 2,3-diones as novel trichomonacidal agents. *European Journal of Medicinal Chemistry* **94**, 276–283.
- Kissinger, P., Amedee, A., Clark, R. A., Dumestre, J., Theall, K. P., Myers, L., Hagensee, M. E., Farley, T. A. and Martin, D. H. (2009). *Trichomonas vaginalis* treatment reduces vaginal HIV-1 shedding. *Sexually Transmitted Diseases* **36**, 11–16.
- Kumar, L., Sarswat, A., Lal, N., Sharma, V. L., Jain, A., Kumar, R., Verma, V., Maikhuri, J. P., Kumar, A., Shukla, P. K. and Gupta, G. (2010). Imidazole derivatives as possible microbicides with dual protection. *European Journal of Medicinal Chemistry* **45**, 817–824.
- Kumar, L., Sarswat, A., Lal, N., Jain, A., Kumar, S., Kumar, S. T. V. S. K., Maikhuri, J. P., Pandey, A. K., Shukla, P. K., Gupta, G., Sharma, V. L. (2011). Design and synthesis of 3-(azol-1-yl)phenylpropanes as microbicidal spermicides for prophylactic contraception. *Bioorganic & Medicinal Chemistry Letters* **21**, 176–181.
- Kumar, L., Jain, A., Lal, N., Sarswat, A., Jangir, S., Kumar, L., Singh, V., Shah, P., Jain, S. K., Maikhuri, J. P., Siddiqi, M. I., Gupta, G. and Sharma, V. L. (2012). Potentiating metronidazole scaffold against resistant *Trichomonas*: design, synthesis, biology and 3D-QSAR analysis. *ACS Medicinal Chemistry Letters* **3**, 83–87.
- Lazenby, G. B., Taylor, P. T., Badman, B. S., McHaki, E., Korte, J. E., Soper, D. E. and Young Pierce, J. (2014). An association between *Trichomonas vaginalis* and high-risk human papillomavirus in rural Tanzanian women undergoing cervical cancer screening. *Clinical Therapeutics* **36**, 38–45.
- Leitsch, D., Kolarich, D., Binder, M., Stadlmann, J., Altmann, F. and Duchêne, M. (2009). *Trichomonas vaginalis*: metronidazole and other nitroimidazole drugs are reduced by the flavin enzyme thioredoxin reductase and disrupt the cellular redox system. Implications for nitroimidazole toxicity and resistance. *Molecular Microbiology* **72**, 518–536.
- Lewis, D. (2014). Trichomoniasis. *Medicine (Avingdon)* **42**, 369–371.
- Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today Technologies* **1**, 337–341.
- Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* **23**, 3–25.
- Marrero-Ponce, Y., Machado-Tugores, Y., Montero Pereira, D., Escario, J. A., Gómez Barrio, A., Nogal-Ruiz, J. J., Ochoa, C., Arán, V. J., Martínez-Fernández, A. R., García Sánchez, R. N., Montero-Torres, A., Torrens, F. and Meneses-Marcel, A. (2005). A computer-based approach to the rational discovery of new trichomonacidal drugs by atom-type linear indices. *Current Drug Discovery Technologies* **2**, 245–265.
- Marrero-Ponce, Y., Meneses-Marcel, A., Castillo-Garit, J. A., Machado-Tugores, Y., Escario, J. A., Gómez Barrio, A., Montero Pereira, D., Nogal-Ruiz, J. J., Arán, V. J., Martínez-Fernández, A. R., Torrens, F., Rotondo, R., Ibarra-Velarde, F. and Alvarado, Y. J. (2006). Predicting antitrichomonal activity: a computational screening using atom-based bilinear indices and experimental proofs. *Bioorganic & Medicinal Chemistry* **14**, 6502–6524.
- McClelland, R. S., Sangare, L., Hassan, W. M., Lavreys, L., Mandaliya, K., Kiarie, J., Ndinya-Achola, J., Jaoko, W. and Baeten, J. M. (2007). Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. *Journal of Infectious Diseases* **195**, 698–702.
- Molinspiration Cheminformatics (2014). Slovak Republic Free online molecular descriptor calculations. Available from: <http://www.molinspiration.com/services/properties.html>.
- Moodley, P., Wilkinson, D., Connolly, C., Moodley, J. and Sturm, A. W. (2002). *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clinical Infectious Diseases* **34**, 519–522.
- Muro, B., Reviriego, F., Navarro, P., Marín, C., Ramírez-Macias, I., Rosales, M. J., Sánchez-Moreno, M. and Arán, V. J. (2014). New perspectives on the synthesis and antichagasic activity of 3-alkoxy-1-alkyl-5-nitroindazoles. *European Journal of Medicinal Chemistry* **74**, 124–134.
- Navarrete-Vázquez, G., Rojano-Vilchis, M. M., Yépez-Mulia, L., Meléndez, V., Gerena, L., Hernández-Campos, A., Castillo, R. and Hernández-Luis, F. (2006). Synthesis and antiprotozoal activity of some 2-(trifluoromethyl)-1*H*-benzimidazole bioisosteres. *European Journal of Medicinal Chemistry* **41**, 135–141.
- Organic Chemistry Portal (2014). OSIRIS Property Explorer. Free online software. Available from: <http://www.organic-chemistry.org/prog/peo/>.
- Palm, K., Stenberg, P., Luthman, K. and Artursson, P. (1997). Polar molecular surface properties predict the intestinal absorption of drugs in human. *Pharmaceutical Research* **14**, 568–571.
- Sutcliffe, S., Neace, C., Magnuson, N. S., Reeves, R. and Alderete, J. F. (2012). Trichomonosis, a common curable STI, and prostate carcinogenesis – a proposed molecular mechanism. *PLoS Pathogens* **8**, e1002801.
- Trochine, A., Creek, D. J., Faral-Tello, P., Barrett, M. P. and Robello, C. (2014). Benzimidazole biotransformation and multiple targets in *Trypanosoma cruzi* revealed by metabolomics. *PLoS Neglected Tropical Diseases* **8**, e2844.
- Van der Pol, B. (2007). *Trichomonas vaginalis* infection: the most prevalent nonviral sexually transmitted infection receives the least public health attention. *Clinical Infectious Diseases* **44**, 23–25.

- Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W. and Kopple, K. D.** (2002). Molecular properties that influence the oral bio-availability of drug candidates. *Journal of Medicinal Chemistry* **45**, 2615–2623.
- Vega, M. C., Rolón, M., Montero-Torres, A., Fonseca-Berzal, C., Escario, J. A., Gómez-Barrio, A., Gálvez, J., Marrero-Ponce, Y. and Arán, V. J.** (2012). Synthesis, biological evaluation and chemometric analysis of indazole derivatives. 1,2-Disubstituted 5-nitroindazolinones, new prototypes of antichagasic drug. *European Journal of Medicinal Chemistry* **58**, 214–227.
- Wang, C. C., McClelland, R. S., Reilly, M., Overbaugh, J., Emery, S. R., Mandaliya, K., Chohan, B., Ndinya-Achola, J., Bwayo, J. and Kreiss, J. K.** (2001). The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. *Journal of Infectious Diseases* **183**, 1017–1022.
- WHO (World Health Organization)** (2007). *Global Strategy for the Prevention and Control of Sexually Transmitted Infections: 2006–2015*, Geneva, Switzerland.
- WHO (World Health Organization)** (2012). *Global Incidence and Prevalence of Selected Curable Sexually Transmitted Infections-2008*, Geneva, Switzerland.
- Zhao, Y. H., Abraham, M. H., Le, J., Hersey, A., Luscombe, C. N., Beck, G., Sherborne, B. and Cooper, I.** (2002). Rate-limited steps of human oral absorption and QSAR studies. *Pharmaceutical Research* **19**, 1446–1457.