Acta Pharm. **71** (2021) 485–495
https://doi.org/10.2478/acph-2021-0032

Short communication

Esters of quinoxaline-7-carboxylate-1,4-di-*N*-oxide as *Trichomonas vaginalis* triosephosphate isomerase inhibitors

ISIDRO PALOS[1]
ROSA MOO-PUC[2]
JOSÉ LUIS VIQUE-SÁNCHEZ[3,6]
CLAUDIA G. BENÍTEZ-CARDOZA[3]
ANTONIO MONGE[4]
JUAN CARLOS VILLALOBOS-ROCHA[5]
ALMA D. PAZ-GONZALEZ[5]
GILDARDO RIVERA[5,*]

[1] *Unidad Académica Multidisciplinaria Reynosa-Rodhe, Universidad Autónoma de Tamaulipas, 88779 Reynosa, México*

[2] *Unidad de Investigación Médica Yucatán, Unidad Médica de Alta Especialidad, Centro Médico Ignacio García Téllez, Instituto Mexicano del Seguro Social Col. Industrial, 97150 Mérida, México*

[3] *Laboratorio de Investigación Bioquímica Escuela Nacional de Medicina y Homeopatía Instituto Politécnico Nacional, Guillermo Massieu Helguera No. 239, La Escalera Ticoman 07320 Ciudad de México, México*

[4] *Neglected Diseases Section, Drug R & D Unit Center for Applied Pharmacobiology Research University of Navarra, C/Irunlarrea, 31008 Pamplona, Spain*

[5] *Laboratorio de Biotecnología Farmacéutica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, 88710 Reynosa, México*

[6] *Facultad de Medicina, Universidad Autónoma de Baja California, Mexicali, México*

Accepted October 9, 2020
Published online November 10, 2020

Trichomoniasis is a public health problem worldwide. Trichomoniasis treatment consists of the use of nitroimidazole derivatives; however, therapeutic ineffectiveness occurs in 5 to 20 % of the cases. Therefore, it is essential to propose new pharmacological agents against this disease. In this work, esters of quinoxaline-7-carboxylate-1,4-di-*N*-oxide (EQX-*NO*) were evaluated in *in vitro* assays as novel trichomonicidal agents. Additionally, an *in vitro* enzyme assay and molecular docking analysis against triosephosphate isomerase of *Trichomonas vaginalis* to confirm their mechanism of action were performed. Ethyl (compound **12**) and *n*-propyl (compound **37**) esters of quinoxaline-7-carboxylate-1,4-di-*N*-oxide derivatives showed trichomonicidal activity comparable to nitazoxanide, whereas five methyl (compounds **5**, **15**, **19**, **20** and **22**), four isopropyl (compounds **28**, **29**, **30** and **34**), three ethyl (compounds **4**, **13** and **23**) and one *n*-propyl (compound **35**) ester derivatives displayed activity comparable to albendazole. Compounds **6** and **20** decreased 100 % of the enzyme activity of recombinant protein triosephosphate isomerase.

Keywords: quinoxaline 1,4-di-*N*-oxide, trichomoniasis, triosephosphate isomerase inhibitor

Trichomonas vaginalis (*T. vaginalis*) is a protozoon that poses an important public health challenge (1–3). It colonizes the human urogenital tract by adhering to the epithelium. *T. vaginalis* infection is often asymptomatic but can produce inflammation of the urinary

* Correspondence; e-mail address: gildardors@hotmail.com

tract. Trichomoniasis is a non-viral sexually diffused infection that affects approximately 276 million people per year (4, 5).

The drug of choice for trichomoniasis is metronidazole, a 5-nitroimidazole derivative (5); however, it causes severe adverse effects since it is carcinogenic and teratogenic (6). These effects have been associated with prolonged treatment or high doses (7, 8). Additionally, the expansion of resistant *T. vaginalis* strains makes new antitrichomoniasis agents necessary.

Quinoxaline (QX) is a privileged structure for the design and development of new antibacterial (9), anticancer (10), and antiparasitic (11) agents with some derivatives in the clinical phase studies (12, 13). Interestingly, quinoxaline 1,4-di-*N*-oxide (QX-*N*O) derivatives show biological effects against diverse protozoa (14–17); however, QX-*N*O has not been evaluated against *T. vaginalis*. Therefore, in this work, the esters of quinoxaline-7-carboxylate-1,4-di-*N*-oxide (EQX-*N*O) derivatives were evaluated as novel antitrichomoniasis agents. Furthermore, *in silico* and *in vitro* analyses were done against *T. vaginalis* triosephosphate isomerase to elucidate their mechanism of action.

EXPERIMENTAL

Syntheses

All EQX-*N*O derivatives were synthesized using the method reported by Gomez-Caro *et al*. (18). All molecules were identified by IR spectroscopy, ^{1}H NMR and elemental analysis of C, H and N (see Table SI, Supplementary material), as in previous research (14, 15, 17, 18).

In vitro *evaluation against* T. vaginalis

T. vaginalis strain GT3 (a wild strain isolated in Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico) was used. EQX-*N*O derivatives, albendazole (ABZ), metronidazole (MTZ), and nitazoxanide (NTZ) were tested against *T. vaginalis* strain GT3 *in vitro* using the previously reported method (19). Dimethylsulfoxide (DMSO) was used as a negative control. The half-maximal inhibitory concentration (IC_{50}) was calculated using the Probit analysis.

In silico *procedures*

Protein structure. – The atomic coordinates of triosephosphate isomerase (TIM) from *T. vaginalis* (TvTIM) from the Protein Data Bank (PDB, code 3QSR) were used with Molecular Operating Environment (MOE) software and CHARMM27 force field (20, 21). This structure was used to predict binding free energy (ΔG_{bind}) values between TvTIM and EQX-*N*O derivatives. Site Finder implemented in the MOE was used to determine potential binding sites (22, 23).

Molecular docking. – For molecular docking, the receptor (TIM, 3QSR) was kept inflexible, while examining twenty conformations for each of the ligands. All crystallographic water molecules were deleted from the initial structures. Molecular docking was done

with the MOE software using the previously reported procedure (24, 25). The molecules were analyzed on twenty-three potential binding sites from TvTIM (Table SII, Supplementary material) to propose a potential interaction site that explains the effect on enzyme activity.

To describe the interaction of the ligands, the binding affinity of each complex was estimated with the generalized Born/volume integral ratio (GB/VI) using parameters in MOE (26). The generalized Born or non-bonded interaction energies comprised van der Waals, Coulomb electrostatic interactions, and implied solvent interaction energies.

In vitro *assays against TvTIM*

Stock solutions of EQX-*N*O derivatives were prepared in DMSO (1 %) and further diluted with media to obtain 50 and 100 μg μL^{-1} of each compound tested. Recombinant TvTIM was obtained as previously described (27, 28). The conversion of glyceraldehyde 3-phosphate (GAP) into dihydroxyacetone phosphate was followed to determine enzyme activity following a previous procedure (29–35).

RESULTS AND DISCUSSION

Methyl (m-), ethyl (e-), isopropyl (*i*-), and *n*-propyl (*n*p-) EQX-*N*O derivatives were obtained (see Supplementary material) (18) and evaluated *in vitro* against *T. vaginalis*. Table I shows their trichomonicidal activity.

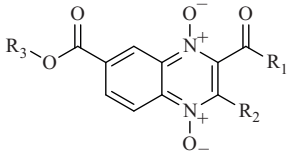
Trichomonicidal activity

Among m- and e-EQX-*N*O derivatives, twelve compounds had an IC_{50} between 1 and 10 μmol L^{-1}, and seven compounds had a higher IC_{50} value than ABZ (Table I). However, none of the m- and e-EQX-*N*O derivatives had trichomonicidal activity greater than MTZ and NTZ. Only compound **12**, an ethyl methyl-3-methyl-quinoxaline-2,7-dicarboxylate-1,4-di-*N*- oxide, had IC_{50} of 0.97 μmol L^{-1}, a value comparable or equal to both reference drugs (MTZ and NTZ). In general, the structure-activity relationship (SAR) analysis showed that aliphatic, ethoxy and methoxy groups at R1-position on the QX ring enhance biological effects. Additionally, the steric effect at R1-position is a key factor in the activity. A trifluoromethyl and a methyl group at R2- and R3-position, resp., also showed trichomonicidal activity.

None of the five *n*p-EQX-*N*O derivatives showed trichomonicidal activity, but two compounds had IC_{50} values from 5.0 to 6.2 μmol L^{-1}, whereas two more had an IC_{50} value < 3 μmol L^{-1} (Table I). Compounds **35** (*n*-propyl methyl-3-methylquinoxaline-2,7-dicarboxylate-1,4-di-*N*-oxide) and **37** (*n*-propyl ethyl-3-methylquinoxaline-2,7-dicarboxylate-1,4-di--*N*-oxide) showed better trichomonicidal activity than ABZ. Both compounds have an ester and methyl group at R1- and R2-position, resp., on the QX-*N*O ring. Additionally, compound **37** showed activity comparable to NTZ.

From *i*-EQX-*N*O derivatives, nine compounds showed no trichomonicidal activity (IC_{50} > 10 μmol L^{-1}) and eight compounds had an IC_{50} value from 2.9 to 6.4 μmol L^{-1}.

Table I. The half-maximal inhibitory concentration (IC_{50}) *of EQX-NO derivatives against* T. vaginalis



Compd.	R_1	R_2	R_3	IC_{50} (μmol L^{-1})[a]
1	C_6H_5	CH_3	CH_3	> 10
2	C_6H_5	CH_3	CH_3CH_2	> 10
3	CH_3	CF_3	CH_3CH_2	> 10
4	$C_{10}H_7$	CF_3	CH_3CH_2	3.52 ± 0.07
5	C_4H_3S	CF_3	CH_3	2.63 ± 0.01
6	C_4H_3S	CF_3	CH_3CH_2	> 10
7	NHC_6H_5	CH_3	CH_3	> 10
8	OCH_2CH_3	CH_3	CH_3	> 10
9	OCH_2CH_3	CH_3	CH_3CH_2	5.93 ± 0.23
10	C_6H_5	CF_3	CH_3	5.32 ± 0.12
11	OCH_3	CH_3	CH_3	6.19 ± 0.32
12	OCH_3	CH_3	CH_3CH_2	0.97 ± 0.04
13	CH_2CH_3	CF_3	CH_3CH_2	2.51 ± 0.09
14	C_6H_5Cl	CF_3	CH_3CH_2	> 10
15	$C_{10}H_7$	CF_3	CH_3	4.29 ± 0.12
16	C_4H_3O	CF_3	CH_3	> 10
17	CH_3	CH_3	CH_3	> 10
18	OCH_2CH_3	$CH_2COOCH_2CH_3$	CH_3	> 10
19	CH_2CH_3	CF_3	CH_3	3.25 ± 0.05
20	$CH(CH_3)_2$	CF_3	CH_3	4.01 ± 0.11
21	$OCH_2C_6H_5$	CH_3	CH_3	> 10
22	OCH_2CH_3	C_6H_5	CH_3	2.55 ± 0.03
23	OCH_2CH_3	C_6H_5	CH_3CH_2	2.92 ± 0.01
24	NH_2	CH_3	CH_3CH_2	6.00 ± 0.04
25	OCH_3	CH_3	$(CH_3)_2CH$	> 10
26	OCH_2CH_3	CH_3	$(CH_3)_2CH$	> 10
27	$OC(CH_3)_3$	CH_3	$(CH_3)_2CH$	> 10
28	OCH_2CH_3	$CH_2COOCH_2CH_3$	$(CH_3)_2CH$	3.50 ± 0.12
29	C_4H_3S	CF_3	$(CH_3)_2CH$	3.68 ± 0.04

Compd.	R_1	R_2	R_3	IC_{50} (μmol L^{-1})[a]
30	CH_3	CF_3	$(CH_3)_2CH$	3.77 ± 0.02
31	C_6H_5	CF_3	$(CH_3)_2CH$	5.25 ± 0.01
32	$C_{10}H_7$	CF_3	$(CH_3)_2CH$	5.41 ± 0.01
33	C_4H_3O	CF_3	$(CH_3)_2CH$	> 10
34	$CH(CH_3)_2$	CF_3	$(CH_3)_2CH$	3.21 ± 0.07
35	OCH_3	CH_3	$CH_3CH_2CH_2$	2.56 ± 0.11
36	C_4H_3S	CF_3	$CH_3CH_2CH_2$	5.04 ± 0.12
37	OCH_2CH_3	CH_3	$CH_3CH_2CH_2$	1.46 ± 0.01
38	$C(CH_3)_3$	CH_3	$CH_3CH_2CH_2$	> 10
39	NHC_6H_5	CH_3	$(CH_3)_2CH$	> 10
40	C_6H_5	CH_3	$(CH_3)_2CH$	6.46 ± 0.08
41	$C(CH_3)_3$	$C(CH_3)_3$	$(CH_3)_2CH$	> 10
42	NH_2	CH_3	$(CH_3)_2CH$	> 10
43	C_4H_3O	CF_3	$CH_3CH_2CH_2$	> 10
44	OCH_2CH_3	CF_3	$(CH_3)_2CH$	2.98 ± 0.07
45	$C(CH_3)_3$	$CF_2CF_2CF_3$	$(CH_3)_2CH$	> 10
46	CF_2CF_3	CF_3	$(CH_3)_2CH$	> 10
47	OCH_2CH_3	CF_3	$CH_3CH_2CH_2$	> 10
48	NHC_6H_5	CH_3	$CH_3CH_2CH_2$	> 10
49	CH_3	CH_3	$CH_3CH_2CH_2$	6.24 ± 0..41
50	NH_2	CH_3	$CH_3CH_2CH_2$	> 10
51[b]	OCH_3	CH_3	CH_2CH_3	> 10
52[b]	C_6H_5	CF_3	CH_3	4.94 ± 0.12
53[b]	$CH(CH_3)_2$	CF_3	CH_3	> 10
ABZ				4.08 ± 0.21
MTZ				0.69 ± 0.01
NTZ				1.00 ± 0.05

ABZ – albendazole, MTZ – metronidazole, NTZ – nitazoxanide

[a] Mean ± SD of triplicate determinations.

[b] Quinoxaline derivatives without *N*-oxide groups.

Although five compounds had lower IC_{50} values (< 4.0 μmol L^{-1}) than ABZ, none showed better trichomonicidal activity than MTZ and NTZ (Table I).

Finally, the *N*-oxide groups on the QX ring from some EQX-*N*O derivatives were eliminated (by reduction with sodium dithionite) to determine their effect on trichomonicidal activity (Table I). EQX-*N*O derivatives **10**, **12**, and **20** with trichomonicidal activity below

6.0 μmol L^{-1} were selected to obtain QX derivatives **51**, **52**, and **53** (see Supplementary material), resp. These new compounds showed no or low biological activity, except compound **52** (methyl 2-benzoyl-3-trifluoromethyl quinoxaline-7-carboxylate), which had an antiprotozoal effect against *T. vaginalis*. These results show that the *N*-oxide groups are the key factor for the trichomonicidal activity and suggest that compound **52** has a different mechanism of action than EQX-*N*O derivatives. Still, the mechanism of action of QX-*N*O is currently not clear, as reported by Cheng *et al*. (12).

Enzymatic activity of TvTIM

Previously, Guzman *et al.* (32), reported that QX-*N*O derivatives affect the enzymatic activity of *Trypanosoma cruzi* TIM. Therefore, in this work, TvTIM was considered a potential drug target. All EQX-*N*O derivatives were evaluated in enzyme activity assays, but only four compounds (**6**, **10**, **20** and **42**) decreased TvTIM activity to some degree. The effect of compounds **6** (ethyl 2-(thiophene-2-carbonyl)-3-trifluoromethylquinoxaline-7-carboxylate-1,4-di-*N*-oxide), **10** (methyl 2-benzoyl-3-trifluoromethylquinoxaline-7-carboxyl-

Table II. Effect of EQX-NO derivatives against the enzymatic activity of TvTIM, predictive binding free energy value and main residues in the interaction on the active site

Compd.	TvTIM enzymatic activity (%)[a]		ΔG_{bind} (kcal mol^{-1})	Main amino acids in the interactions
	50 g L^{-1}	100 g L^{-1}		
6	49.6 ± 4.1	0	–0.6	Glu166
10	69.0 ± 4.5	38.3 ± 3.2	–7.3	Lys11, Gly172, and Ser212
20	49.6 ± 2.5	0	–3.2	Lys11, Ile171, and Gly172
42	51.6 ± 4.1	30.3 ± 4.0	–4.7	Lys11, Ala170, Ile171, and Gly172

ΔG_{bind} – binding free energy

[a] Mean ± SD of triplicate determinations.

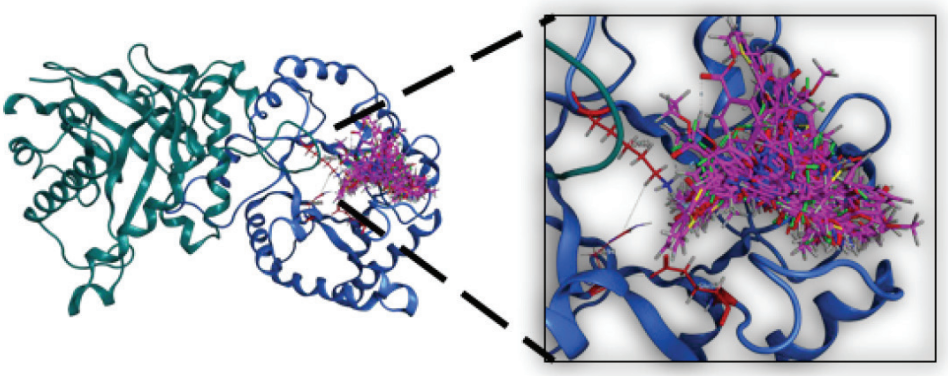


Fig. 1. The main interaction site on TvTIM for the compounds **6**, **10**, **20**, and **42**. Residues Lys11, His94, and Glu166 (red color) and seven conformers of each compound (pink color).

ate-1,4-di-*N*-oxide), **20** (ethyl methyl-3-methyl-quinoxaline-2,7-dicarboxylate-1,4-di-*N*-oxide), and **42** (isopropyl 2-amide-3-methyl-quinoxaline-7-carboxylate-1,4-di-*N*-oxide) on the activity of TvTIM was determined in two concentrations (50 and 100 μg μL^{-1}). The results are displayed in Table II. Three of the abovementioned EQX-*N*O derivatives (**6**, **20**, and **42**) showed similar enzyme activity inhibition (50 % approx.) at 50 μg μL^{-1}, and only compounds **6** and **20** showed 100 % enzyme inhibition at 100 μg μL^{-1}. These compounds caused a decrease in the function of TvTIM at higher concentrations than the other reported compounds (34–36). Nevertheless, this is the first report that shows that EQX-*N*O derivatives have an inhibitory effect against TvTIM. Previously, Cheng *et al*. (12) mentioned two sulfoxide QX-NO derivatives with a better trichonomicidal activity than metronidazole, however, the mechanism of action was not explored. Therefore, QX-NO derivatives are a new option to develop new TvTIM inhibitors.

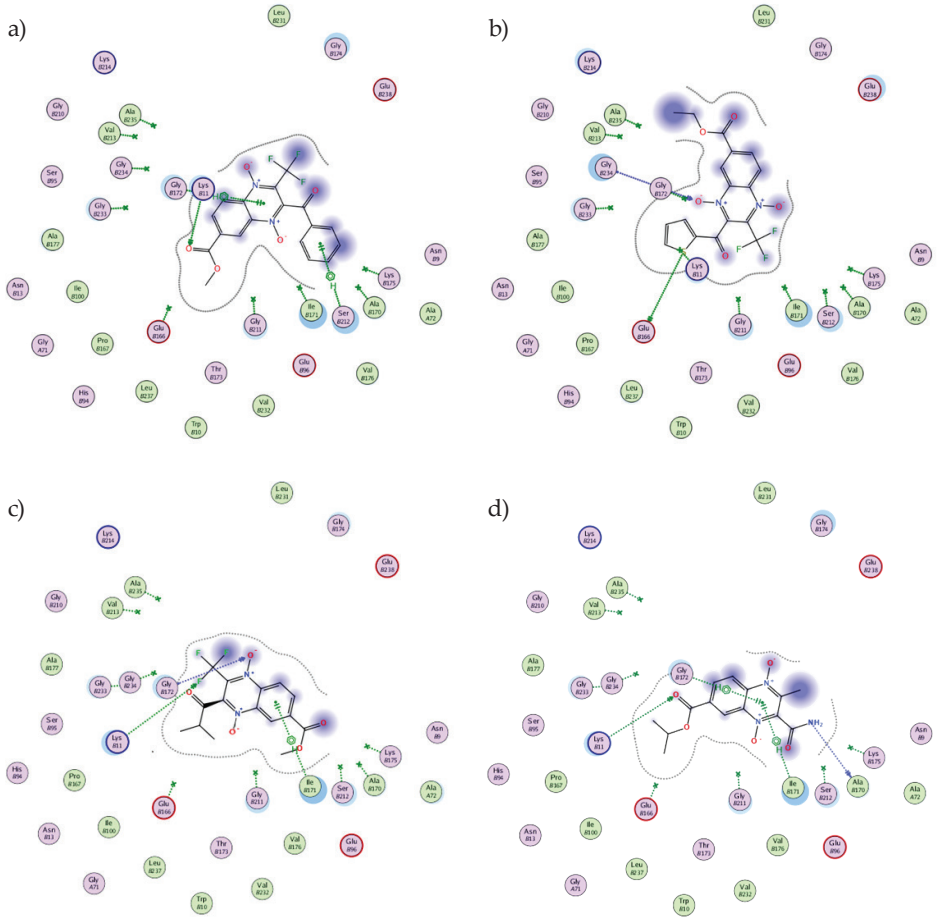


Fig. 2. Interaction site between EQX-*N*O derivatives and the potential active site of TvTIM. a) compound **6**, b) compound **10**, c) compound **20**, and d) compound **42**.

Molecular docking analysis on TvTIM

Molecular docking analysis was done to describe the molecular interaction of the EQX-*N*O derivatives on 23 potential TvTIM binding sites (Table SII, Supplementary material).

The compounds that affected the enzymatic activity (**6**, **10**, **20**, and **42**) showed the best interactions with Lys11, His94, and Glu166, among other residues, near the active site (Fig. 1). The best interactions for the four compounds were determined in the region of residues near Lys11, His94, Glu166, Ala170, Ile171, Gly172, and Ser212 (Fig. 2). Additionally, predictive ΔG_{bind} was determined (Table II). Compound **10** showed the lowest ΔG_{bind} value (–7.3 kcal mol^{-1}); however, this compound showed the lowest enzyme inhibition (31 %) at 50 μg μL^{-1}. The other three compounds (**6**, **20**, and **42**) showed very different ΔG_{bind} values but comparable enzyme inhibition. These results suggest no correlation between *in vitro* and *in silico* analysis. Therefore, compounds **6**, **10**, **20**, and **42** could be interacting on another binding site of TvTIM, causing an effect on the enzyme activity assays. The molecular docking results from the other QX-*N*O derivatives showed a probable interaction in the interface and core of TvTIM, which can be related to not affecting the enzymatic activity of TvTIM.

CONCLUSIONS

Four different types of EQX-*N*O derivatives were tested as novel agents against *T. vaginalis*. e-EQX-*N*O derivatives showed a better trichomonicidal effect than BZN. In particular, compound **12** (ethyl methyl-3-methyl-quinoxaline-2,7-dicarboxylate-1,4-di-*N*-oxide) *in vitro* assays show a better trichomonicidal activity than ABZ and NTZ. Additionally, four compounds **6** (ethyl 2-(thiophene-2-carbonyl)-3-trifluoromethylquinoxaline-7-carboxylate-1,4-di--*N*-oxide), **10** (methyl 2-benzoyl-3-trifluoromethylquinoxaline-7-carboxylate-1,4-di-*N*-oxide), **20** (ethyl methyl-3-methyl-quinoxaline-2,7-dicarboxylate-1,4-di-*N*-oxide), and **42** (isopropyl 2-amide-3-methyl-quinoxaline-7-carboxylate-1,4-di-*N*-oxide) reduced the enzymatic activity of recombinant protein TvTIM. SAR analysis showed that methyl and ethyl groups at R3-position and *N*-oxide groups on the QX ring are the key moieties for the biological activity. Molecular docking analysis revealed that the main interaction site of these compounds is near to the active site where they interact with Lys11, Glu166, Ala170, Ile171, and Gly172. Therefore, these types of compounds could help in the development of new and more effective TvTIM inhibitors to combat the *T. vaginalis* resistance.

Acknowledgments. – Supplementary materials available upon request.

REFERENCES

1. D. Leitsch, Drug resistance in the microaerophilic parasite *Giardia lamblia*, *Curr. Trop. Med. Rep.* **2** (2015) 128–135; https://doi.org/10.1007/s40475-015-0051-1
2. B. R. Ansell, M. J. McConville, S. Y. Ma'ayeh, M. J. Dagley, R. B. Gasser, S. G. Svärd and A. R. Jex, Drug resistance in *Giardia duodenalis*, *Biotechnol. Adv.* **33** (2015) 888–901; https://doi.org/10.1016/j.biotechadv.2015.04.009
3. C. B. Menezes, A. P. Frasson and T. Tasca, Trichomoniasis – are we giving the deserved attention to the most common non-viral sexually transmitted disease worldwide?, *Microb. Cell* **3** (2016) 404–419; https://doi.org/10.15698/mic2016.09.526

4. D. Leitsch, Recent advances in the *Trichomonas vaginalis* field, *F1000Res.* **5** (2016) Article ID 162 (7 pages); https://doi.org/10.12688/f1000research.7594.1
5. P. Kissinger, *Trichomonas vaginalis*: a review of epidemiologic, clinical and treatment issues, *BMC Infect. Dis.* **15** (2015) Article ID 307 (8 pages); https://doi.org/10.1186/s12879-015-1055-0
6. P. Upcroft and J. A. Upcroft, Drug targets and mechanisms of resistance in the anaerobic protozoa, *Clin. Microbiol. Rev.* **14** (2001) 150–164; https://doi.org/10.1128/CMR.14.1.150-164.2001
7. P. A. Cano, A. Islas-Jácome, J. González-Marrero, L. Yépez-Mulia, F. Calzada and R. Gámez-Montaño, Synthesis of 3-tetrazolylmethyl-4*H*-chromen-4-ones via Ugi-azide and biological evaluation against *Entamoeba histolytica*, *Giardia lamblia* and *Trichomona vaginalis*, *Bioorg. Med. Chem.* **22** (2014) 1370–1376; https://doi.org/10.1016/j.bmc.2013.12.069
8. S. Chaturvedi, M. Y. Malik, M. Rashid, S. Singh, V. Tiwari, P. Gupta, S. Shukla, S. Singh and M. Wahajuddin, Mechanistic exploration of quercetin against metronidazole induced neurotoxicity in rats: possible role of nitric oxide isoforms and inflammatory cytokines, *Neurotoxicology* **79** (2020) 1–10; https://doi.org/10.1016/j.neuro.2020.03.002
9. J. Jampilek, Recent advances in design of potential quinoxaline anti-infectives, *Curr. Med. Chem.* **21** (2014) 4347–4373; https://doi.org/10.2174/09298673216661410111948 25
10. I. Balderas-Renteria, P. Gonzalez-Barranco, A. Garcia, B. K. Banik and G. Rivera, Anticancer drug design using scaffolds of β-lactams, sulfonamides, quinoline, quinoxaline and natural products. Drugs advances in clinical trials, *Curr. Med. Chem.* **19** (2012) 4377–4398; https://doi.org/10.2174/092986712803251593
11. N. B. Patel, J. N. Patel, A. C. Purohit, V. M. Patel, D. P. Rajani, R. Moo-Puc, J. C. Lopez-Cedillo, B. Nogueda-Torres and G. Rivera, *In vitro* and *in vivo* assessment of newer quinoxaline-oxadiazole hybrids as antimicrobial and antiprotozoal agents, *Int. J. Antimicrob. Agents* **50** (2017) 413–418; https://doi.org/10.1016/j.ijantimicag.2017.04.016
12. G. Cheng, W. Sa, C. Cao, L. Guo, H. Hao, Z. Liu, X. Wang and Z. Yuan, Quinoxaline 1,4-di-*N*-oxides: Biological activities and mechanisms of actions, *Front. Pharmacol.* **7** (2016) Article ID 64 (21 pages); https://doi.org/10.3389/fphar.2016.00064
13. R. El Aissi, J. Liu, S. Besse, D. Canitrot, O. Chavignon, J. M. Chezal, E. Miot-Noirault and E. Moreau, Synthesis and biological evaluation of new quinoxaline derivatives of ICF01012 as melanoma-targeting probes, *ACS Med. Chem. Lett.* **5** (2014) 468–473; https://doi.org/10.1021/ml400468x
14. J. C. Villalobos-Rocha, L. Sánchez-Torres, B. Nogueda-Torres, A. Segura-Cabrera, C. A. García-Pérez, V. Bocanegra-García, I. Palos, A. Monge and G. Rivera, Anti-*Trypanosoma cruzi* and antileishmanial activity by quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives, *Parasitol. Res.* **113** (2014) 2027–2035; https://doi.org/10.1007/s00436-014-3850-8
15. K. F. Chacón-Vargas, S. Andrade-Ochoa, B. Nogueda-Torres, D. C. Juárez-Ramírez, E. E. Lara-Ramírez, R. Mondragón-Flores, A. Monge and G. Rivera, L. E. Sánchez-Torres, Isopropyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives induce regulated necrosis-like cell death on *Leishmania (Leishmania) mexicana*, *Parasitol. Res.* **117** (2018) 45–58; https://doi.org/10.1007/s00436-017-5635-3
16. M. Quiliano, A. Pabón, G. Ramirez-Calderon, C. Barea, E. Deharo, S. Galiano and I. Aldana, New hydrazine and hydrazide quinoxaline 1,4-di-*N*-oxide derivatives: *In silico* ADMET, antiplasmodial and antileishmanial activity. *Bioorg. Med. Chem. Lett.* **27** (2017) 1820–1825; https://doi.org/10.1016/j.bmcl.2017.02.049
17. B. E. Duque-Montaño, L. C. Gómez-Caro, M. Sanchez-Sanchez, A. Monge, E. Hernández-Baltazar, G. Rivera and O. Torres-Angeles, Synthesis and *in vitro* evaluation of new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide against *Entamoeba histolytica*, *Bioorg. Med. Chem.* **21** (2013) 4550–4558; https://doi.org/10.1016/j.bmc.2013.05.036

18. L. C. Gómez-Caro, M. Sánchez-Sánchez, V. Bocanegra-García, A. Monge and G. Rivera, Synthesis of quinoxaline 1,4-di-*N*-oxide derivatives on solid support using room temperature and microwave-assisted solvent-free procedures, *Quim. Nova* **34** (2011) 1147–1151; https://doi.org/10.1590/S0100-40422011000700008
19. E. Hernández-Núñez, H. Tlahuext, R. Moo-Puc, H. Torres-Gómez, R. Reyes-Martínez, R Cedillo-Rivera, C. Nava-Zuazo and G. Navarrete-Vazquez, Synthesis and *in vitro* trichomonicidal, giardicidal and amebicidal activity of *N*-acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles, *Eur. J. Med. Chem.* **44** (2009) 2975–2984; https://doi.org/10.1016/j.ejmech.2009.01.005
20. B. R. Brooks, C. L. Brooks, A. D. Mackerell, L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch, A. Caflisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York and M. Karplus, CHARMM: The biomolecular simulation program, *J. Comput. Chem.* **30** (2009) 1545–1614; https://doi.org/10.1002/jcc.21287
21. P. R. Gerber and K. Müller, MAB, a generally applicable molecular force field for structure modelling in medicinal chemistry, *J. Comput. Aided Mol. Des.* **9** (1995) 251–268; https://doi.org/10.1007/bf00124456
22. C. A. Del Carpio, Y. Takahashi and S.-i. Sasaki, A new approach to the automatic identification of candidates for ligand receptor sites in proteins: (I) Search for pocket regions, *J. Mol. Graph.* **11** (1993) 23–29; https://doi.org/10.1016/0263-7855(93)85003-9
23. A. Miranker and M. Karplus, Functionality maps of binding sites: A multiple copy simultaneous search method, *Proteins: Struct. Funct. Genet.* **11** (1991) 29–34; https://doi.org/10.1002/prot.34011010
24. S. Thangapandian, S. John, Y. Lee, S. Kim and K. W. Lee, Dynamic structure-based pharmacophore model development: A new and effective addition in the histone deacetylase 8 (HDAC8) inhibitor discovery, *Int. J. Mol. Sci.* **12** (2011) 9440–9462; https://doi.org/10.3390/ijms12129440
25. A. Wadood, M. Ghufran, S. F. Hassan, H. Khan, S. S. Azam and U. Rashid, *In silico* identification of promiscuous scaffolds as potential inhibitors of 1-deoxy-D-xylulose 5-phosphate reductoisomerase for treatment of *Falciparum* malaria, *Pharm. Biol.* **55** (2017) 19–32; https://doi.org/10.1080/13880209.2016.1225778
26. A. M. Clark and P. Labute, 2D depiction of protein–ligand complexes, *J. Chem. Inf. Model.* **47** (2007) 1933–1944; https://doi.org/10.1021/ci7001473
27. S. Lara-González, P. Estrella, C. Portillo, M. E. Cruces, P. Jiménez-Sandoval, J. Fattori, A. C. Migliorini-Figueira, M. López-Hidalgo, C. Díaz-Quezada, M. López-Castillo, C. H. Trasviña-Arenas, E. Sánchez-Sandoval, A. Gómez-Puyou, J. Ortega-López, R. Arroyo, C. G. Benítez-Cardoza and L. G. Brieba, Substrate-induced dimerization of engineered monomeric variants of triosephosphate isomerase from *Trichomonas vaginalis*, *PLoS ONE* **10** (2015) e0141747; https://doi.org/10.1371/journal.pone.0141747
28. P. Jiménez-Sandoval, J. L. Vique-Sanchez, M. L. Hidalgo, G. Velazquez-Juarez, C. Díaz-Quezada, L. F. Arroyo-Navarro, G. M. Morán, J. Fattori, A. J. Diaz-Salazar, E. Rudiño-Pinera, R. Sotelo-Mundo, A. C. Migliorini-Figueira, S. Lara-Gonzalez, C. G. Benítez-Cardoza and L. G. Brieba, A competent catalytic active site is necessary for substrate induced dimer assembly in triosephosphate isomerase, *Biochim. Biophys. Acta – Prot. Proteom.* **1865** (2017) 1423–1432; https://doi.org/10.1016/j.bbapap.2017.07.014
29. G. Álvarez, J. Martínez, B. Aguirre-López, N. Cabrera, L. Pérez-Díaz, M. T. de Gómez-Puyou, A. Gómez-Puyou, R. Pérez-Montfort, B. Garat, A. Merlino, M. González and H. Cerecetto, New chemotypes as *Trypanosoma cruzi* triosephosphate isomerase inhibitors: a deeper insight into the mechanism of inhibition, *J. Enzyme Inhib. Med. Chem.* **29** (2014) 198–204; https://doi.org/10.3109/14756366.2013.765415

30. A. Gómez-Puyou, E. Saavedra-Lira, I. Becker, R. A. Zubillaga, A. Rojo-Dominguez and R. Perez-Montfort, Using evolutionary changes to achieve species-specific inhibition of enzyme action — studies with triosephosphate isomerase, *Chem. Biol.* **2** (1995) 847–855; https://doi.org/10.1016/1074-5521(95)90091-8

31. M. de N. C. Soeiro and S. L. Castro, Screening of potential anti-*Trypanosoma cruzi* candidates: *In vitro* and *in vivo* studies, *Open Med. Chem. J.* **5** (2011) 21–30; https://doi.org/10.2174/1874104501105010021

32. G. Álvarez, B. Aguirre-López, J. Varela, M. Cabrera, A. Merlino, G. V. López, M. L. Lavaggi, W. Porcal, R. Di Maio, M. González, H. Cerecetto, N. Cabrera, R. Pérez-Montfort, M. Tuena de Gómez-Puyou and A. Gómez-Puyou, Massive screening yields novel and selective *Trypanosoma cruzi* triosephosphate isomerase dimer-interface-irreversible inhibitors with anti-trypanosomal activity, *Eur. J. Med. Chem.* **45** (2010) 5767–5772; https://doi.org/10.1016/j.ejmech.2010.09.034

33. C. G. Benítez-Cardoza, D. A. Fernández-Velasco and J. L. Vique-Sánchez, Triosephosphate isomerase inhibitors as potential drugs against *Clostridium perfringens*, *Chem. Sel.* **5** (2020) 2365–2370; https://doi.org/10.1002/slct.201904632

34. J. L. Vique-Sánchez, L. A. Caro-Gómez, L. G. Brieba and C. G. Benítez-Cardoza, Developing a new drug against trichomoniasis, new inhibitory compounds of the protein triosephosphate isomerase, *Parasitol. Int.* **76** (2020) Article ID 102086; https://doi.org/10.1016/j.parint.2020.102086

35. A. Téllez-Valencia, S. Avila-Ríos, R. Pérez-Montfort, A. Rodríguez-Romero, M. Tuena de Gómez-Puyou, F. López-Calahorra and A. Gómez-Puyou, Highly specific inactivation of triosephosphate isomerase from *Trypanosoma cruzi*, *Biochem. Biophys. Res. Commun.* **295** (2002) 958–963; https://doi.org/10.1016/s0006-291x(02)00796-9

36. B. Hernández-Ochoa, G. Navarrete-Vázquez, C. Nava-Zuazo, A. Castillo-Villanueva, S. T. Méndez, A. Torres-Arroyo, S. Gómez-Manzo, J. Marcial-Quino, M. Ponce-Macotela, Y. Rufino-González, M. Martínez-Gordillo, G. Palencia-Hernández, N. Esturau-Escofet, E. Calderon-Jaimes, J. Oria-Hernández and H. Reyes-Vivas, Novel giardicidal compounds bearing proton pump inhibitor scaffold proceeding through triosephosphate isomerase inactivation, *Sci. Rep.* **7** (2017) Article ID 7810; https://doi.org/10.1038/s41598-017-07612-y