Computational POM evaluation of experimental in vitro Trypanosoma cruzi *and* Mycobacterium tuberculosis *inhibition of heterocyclic-2-carboxylic acid (3-cyano-1,4-di-noxidequinoxalin-2-yl)amide derivatives*

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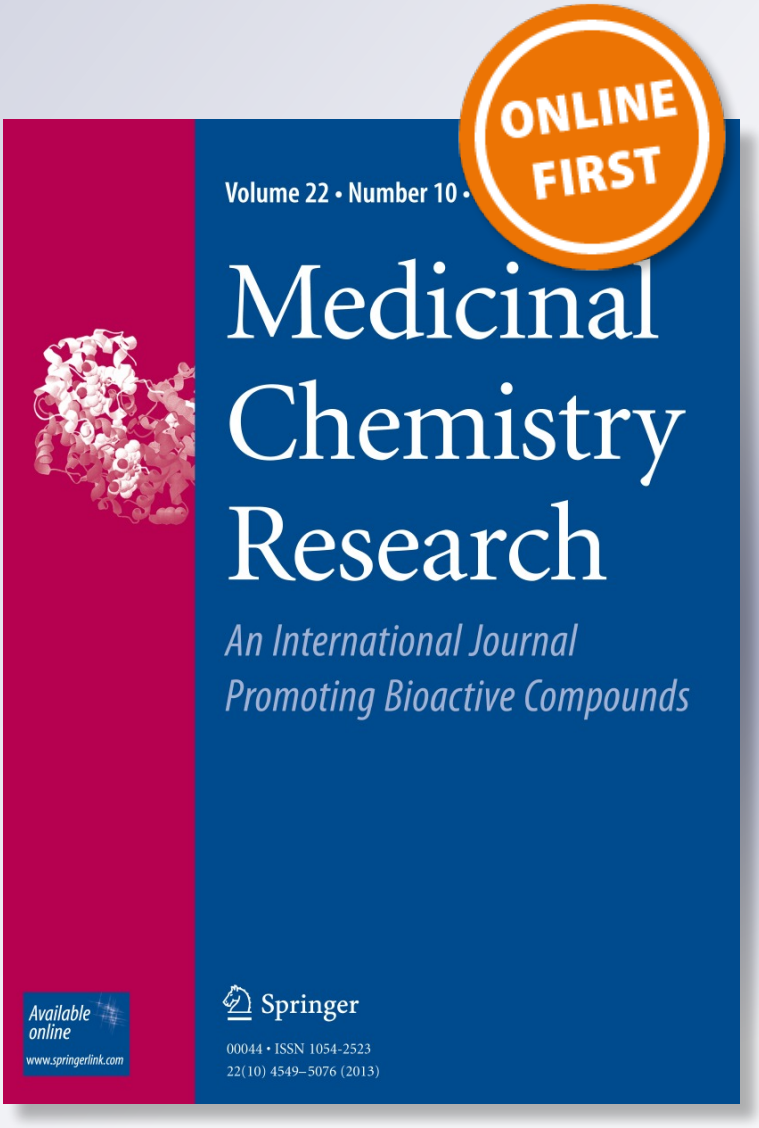

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Computational POM evaluation of experimental in vitro *Trypanosoma cruzi* and *Mycobacterium tuberculosis* inhibition of heterocyclic-2-carboxylic acid (3-cyano-1,4-di-noxidequinoxalin-2-yl)amide derivatives

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Abstract A computation model has been developed for the rational design of bioactive pharmacophore sites as anti-*Mycobacterium tuberculosis* and anti-*Trypanosoma cruzi* (TC) candidates. The 40 compounds **1–40** analyzed have been previously screened for their antitubercular and antitrypanosomal activity. The highest anti-TC activity is obtained for compounds **8** and **18** which exhibited low IC_{50} values (9.2 and 10.8 μM), almost equal to clinical drug, nifurtimox (7.7 μM; 100 % Inhib.). This could be attributed to the existence of two synergic ($O^{\delta-}$–$N^{\delta-}$) and ($O^{\delta-}$–$O^{\delta-}$) antitrypanosomal pharmacophore sites. In contrast to compounds **8** and **18** which contain electro-attractor groups (R^1, R^2 = F), analog compounds **1** and **13** with electro-donor or only hydrogen (R^1, R^2 = CH_3, H) show best antibacterial activity (MIC = 0.977 and 1.190 μg/mL) very close to antitubercular activity of Rifampicin (MIC = 0.125 μg/mL). This could be attributed to the existence of ($O^{\delta-}$–$NH^{\delta+}$) antibacterial pharmacophore site.

Keywords *Mycobacterium tuberculosis* (MBT) · *Trypanosoma cruzi* (TC) · Carboxylic acid quinoxaline 1 · 4-Di-*N*-oxides (CAQDO) · Combined antiparasitic and antibacterial activity · POM analyses

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Introduction

Trypanosoma cruzi (TC) and *Mycobacterium tuberculosis* (MBT) diseases represent a major health problem. It is estimated that one-third of the world population is infected with tuberculosis (TB). Besides TB, Chagas disease, affects approximately 20 million people (Ancizu *et al.*, 2009). Benznidazole (Bdz) and nifurtimox (Nfx) (Scheme 1) have been two of the few most widely used anti-TC drugs. The success of Bdz was mainly due to its outstanding clinical efficacy, and the slow speed at which resistance developed to this drug. But the side effects and the final arrival of resistance and the alarming spread of Bdz-resistant TC on a global scale created an urgent need for the development of novel TC drugs (Castro and de Diaz Toranzo, 1988; Moll *et al.*, 2008; Viotti *et al.*, 2009). Hence, the novel anti-*Trypanosoma* drugs were strongly and urgently needed in a goal to be used as new drugs without side effects and multi-drug resistance.

Our laboratory focuses on new spiro-oxazoline-thio-chromanones (STIO) and all series containing hits with combined pharmacophore sites. Our library of spiroheterocycle derivatives (Akkurt *et al.*, 2006, 2010; Al Houari *et al.*, 2008a, b; Badri *et al.*, 1999; Bennani *et al.*, 2002, 2007a, b; Hadda *et al.*, 2008; Orhan *et al.*, 2009) provided

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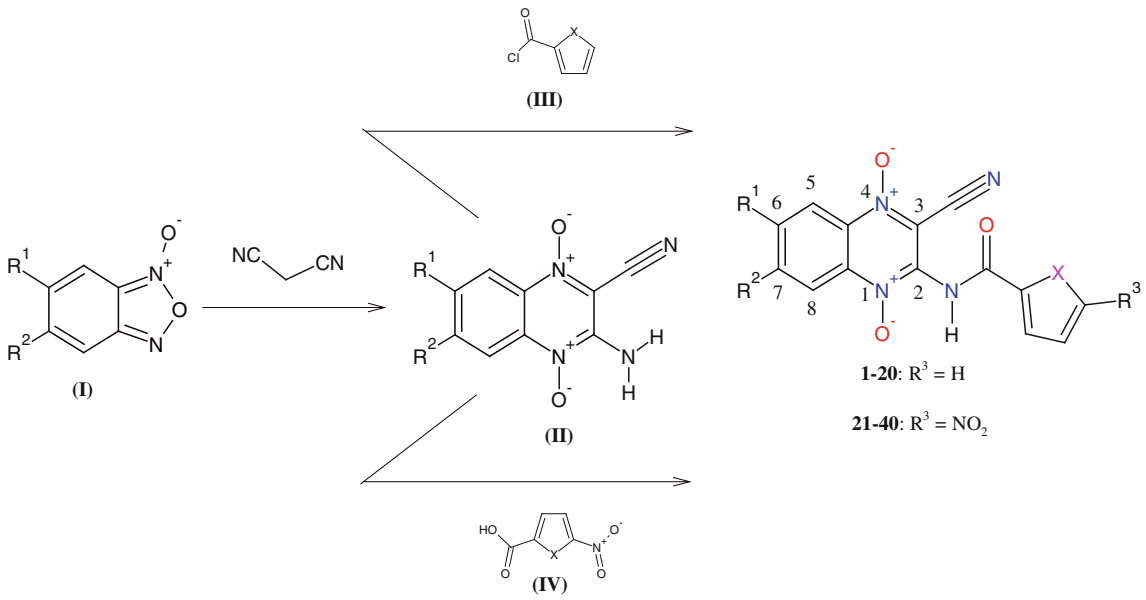

Scheme 1 Structures of previously described CAQDO compound **1–40** (Ancizu *et al.*, 2009). All substituents (R^1, R^2) and heteroatom X are given in Table 1

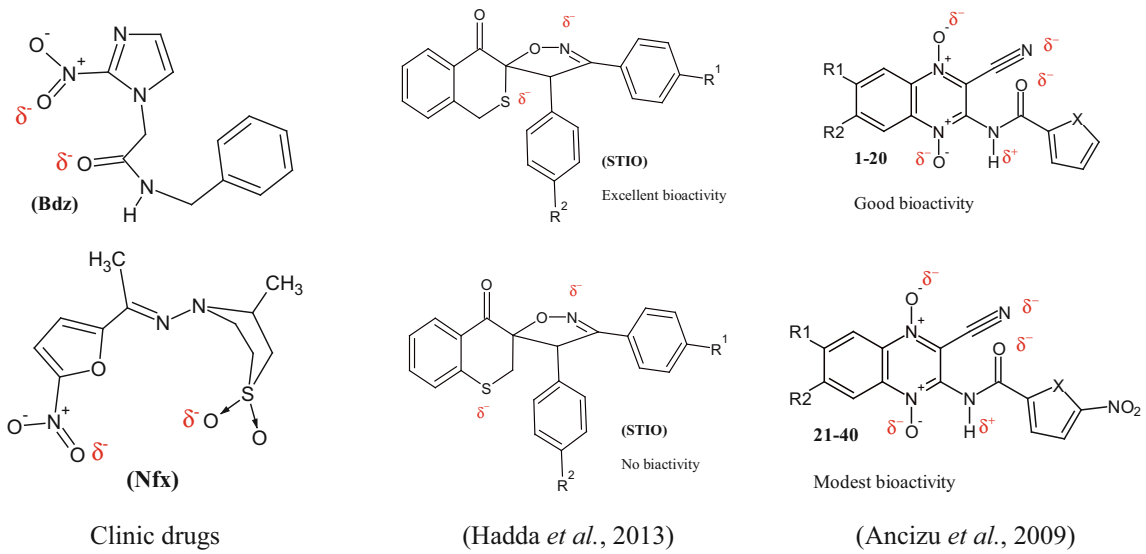

Fig. 1 Structures of candidate CAQDO compounds **1–40** and some active antiparasitic agents and their hypothetical O/O; S/N; and O/N-pharmacophore sites

compounds with similar pharmacophore site of clinical drugs Bdz and Nfx (Fig. 1).

We have previously reported a study concerning one series of spiroheterocycle derivatives (STIO) as antiviral drugs (Orhan *et al.*, 2009). Among them, ten spiro-compounds displayed less toxicity than Bdz and Nfx, and six of them have two combined antiviral pharmacophore sites; (O–C–C–O–N) and (S–C–O–N) (Fig. 1).

More recently, Ancizu *et al.* (2009) reported the preparation of 40 quinoxaline 1,4-di-*N*-oxide derivatives and tested them against *M. tuberculosis* and *T. cruzi*. Carboxylic acid quinoxaline 1,4-di-*N*-oxides (CAQDOs) **1** and **13** showed MIC values in same order as the reference anti-tuberculosis drug, Rifampicin. Meanwhile, CAQDO **8** and **18** presented IC50 values in same order as the anti-chagasic drug, Nfx.

For all these reasons, compounds **1–40** could represent potential antiparasitic activity for further optimization as anti-*Trypanosoma* cruzi candidates with supplementary antitubercular bioactivity. Pharmacological studies

conducted on compounds suggested not only a Bdz-like mode of action but also the involvement of additional antibacterial mechanism (Anaflous *et al.*, 2004). Bioinformatics studies (Bennani *et al.*, 2007b) also suggest the existence of additional combined pharmacophore sites, possibly involving an original mechanism of drug/target interaction.

Considering good pharmacological results obtained in this family, and taking into account some previously established structure–activity relationship, we further decided to broaden virtual screening in order to get more SAR information of these known compounds (CAQDO) in a hope to improve activity/cytotoxicity ratio. Firstly, considering compound Bdz as a reference drug, we decided to introduce geometrical diversity taking place between different moieties (Fig. 1). Secondly, taking into account the important flexibility of rotamer structure of candidates **1–40**, a notable antiparasitic activity is predicted. In this series, we decided to identify both flexible pharmacophore sites of candidates **1–40**.

Chemistry

Heterocyclic compounds **1–40** (Scheme 1) were obtained by the condensation of 3-amine-1,4-di-*N*-oxide quinoxaline-2-carbonitrile derivatives (**II**) with various furyl and thienyl derivatives (Ancizu *et al.*, 2009). Compounds **1–40** are stable at ambient temperature. Their structures have been determined by IR, elemental analyses, and ^{1}H-NMR spectroscopy.

Pharmacology

Origin of dual antiprotozoal and antibacterial activity of compounds (1–40)

All the compounds **1–40** were tested against TC using the Bdz-resistant strain and they were tested for their antitubercular bioactivity. The results were compared with those of the standard drugs Bdz and Nfx (Table 1).

All the synthesized compounds exhibited varying degree of inhibitory effect on the growth of two different tested strains. A significant activity was observed by two compounds **8** and **18** against Trypomastigotes. Compounds **21–40** showed moderate activity, whereas compounds **1**, **13**, **17**, **19**, and **39** showed significant activity against MBT $H_{37}Rv$ bacteria. So although most of the compounds showed moderate to significant activity against bacteria or parasite strains but no compound of series **1–40** is active against both strains (Table 1).

Results and discussion

Molecular properties calculations

In series **1–40** the introduction of a variety of fragments by means of the terminal aryl substituted at positions C-6 and C-7 provided compounds with a broad range of anti-TC and antitubercular activities. The highest anti-TC activities were obtained for compounds **8** and **18** which exhibited low IC_{50} values between 19.2 (53 % Inhib) and 10.80 μM (92 % Inhib), near equal to Nfx (MIC = 7.7 μM; 100 % Inhib).

POM virtual screening

Nfx and Bdz are generally well tolerated by children, particularly in the acute phase of the disease, but relatively frequent and severe gastrointestinal or dermatological adverse reactions may be observed. Recurrence of the disease is a significant problem, and as such these drugs are considered generally ineffective. The main limitations of both drugs are their long courses of administration and the occurrence of adverse side effects. For these reasons, it is crucial to design new more efficient drugs with minimum or no side effects. Here we analyze series of compounds **1–40** by using POM suite-2012 which is a combination of Petra, Osiris, and Molinspiration programs.

Pi-charges calculations

The series **1–40** of CAQDO have been subjected to delocalized charge calculations (Table 1) using Petra method of non-hydrogen common atoms, obtained from the partial π-charge of the heteroatoms, and have been used to model the bioactivity against TC.

It is found that the negative charges of the oxygen of central amide moiety and nitrogen atom of nitrile and one oxygen atom of N–O group contribute positively in favor of antitrypanosomal activity, more, and this is in good agreement with the hypothetical mode of antitrypanosomal action of the compounds bearing ($X^{\delta-}/Y^{\delta-}$) pharmacophore site (X, Y = O, N, S) (Fig. 1).

On the other hand, it was previously hypothesized that difference in charges between two heteroatoms of the same dipolar pharmacophore site ($X^{\delta-}/Y^{\delta+}$) may facilitate the inhibition of bacteria, more than viruses and funguses (Anaflous *et al.*, 2004; Bennani *et al.*, 2007b). It is further found that the activity increases with increase in negative charge of one heteroatom of the common pharmacophore fragment of the hits. The presence of π-delocalisation phenomena in rigid pharmacophore sites was presented previously (Bennani *et al.*, 2007b).

Table 1 In vitro bioactivity of compounds **1–40** against MBT and TC (Ancizu *et al.*, 2009)

Compounds	R^1	R^2	R^3	X	Bioactivity[a]		Combined bioactivity	
					MBT[b]	TC[c]	Anti-MBT	Anti-TC
1	H	H	H	O	0.977	3.1	+++	−
2	CH_3	H	H	O	8.843	11.6	+	−
3	CH_3	CH_3	H	O	NT	13.1	NT	−
4	OCH_3	H	H	O	NT	9.5	NT	−
5	Cl	H	H	O	15.36	NT	+	NT
6	Cl	Cl	H	O	NT	31.6	NT	−
7	F	H	H	O	NT	19.8	NT	−
8	F	F	H	O	4.700	53	+	++
9	H	CF_3	H	O	17.90	NT	+	NT
10	CF_3	H	H	O	24.22	NT	+	NT
11	H	H	H	S	5.38	19.0	++	NT
12	CH_3	H	H	S	2.08	16.4	++	−
13	CH_3	CH_3	H	S	1.19	13.5	+++	−
14	OCH_3	H	H	S	48.91	15.1	++	−
15	Cl	H	H	S	2.47	NT	+	NT
16	Cl	Cl	H	S	13.61	NT	−	NT
17	F	H	H	S	1.47	27.5	++	−
18	F	F	H	S	NT	92	NT	+++
19	H	F	H	S	4.58	NT	+	NT
20	CF_3	H	H	S	9.18	NT	+	NT
21	H	H	NO_2	O	NT	0.7	NT	−
22	CH_3	H	NO_2	O	NT	0.0	NT	−
23	CH_3	CH_3	NO_2	O	NT	0.0	NT	−
24	OCH_3	H	NO_2	O	14.49	NT	+	NT
25	Cl	H	NO_2	O	5.81	NT	+	NT
26	Cl	Cl	NO_2	O	13.57	NT	+	NT
27	F	H	NO_2	O	21.68	NT	+	NT
28	F	F	NO_2	O	22.16	NT	+	NT
29	H	CF_3	NO_2	O	12.82	NT	+	NT
30	CF_3	H	NO_2	O	14.41	NT	+	NT
31	H	H	NO_2	S	2.88	0.0	++	−
32	CH_3	H	NO_2	S	NT	11.4	NT	−
33	CH_3	CH_3	NO_2	S	NT	36.4	NT	−
34	OCH_3	H	NO_2	S	9.54	NT	+	NT
35	Cl	H	NO_2	S	NT	15.0	NT	−
36	Cl	Cl	NO_2	S	NT	29.8	NT	−
37	F	H	NO_2	S	NT	2.5	NT	−
38	F	H	NO_2	S	NT	9.2	NT	−
39	H	CF_3	NO_2	S	1.70	NT	+++	NT
40	CF_3	H	NO_2	S	6.00	NT	+	NT
Nfx[c]	−	−	−	−	−	100	−	+++
RIF[c]	−	−	−	−	0.125	−	++++	−

++++, super active; +++, highly active; ++, moderately active; +, slightly active; −, not active

[a] From Ancizu et al (2009), *NT* not tested, Standard drugs: *Bdz* benznidazole, *Nfx* nifurtimox

[b] Minimum inhibitory concentration (μg/mL) against *M. tuberculosis* H37Rv

[c] Percentage of growth inhibition at 25 μM doses in *T. cruzi* Tulahuen 2 strain

On the basis of this rigid S/N-pharmacophore analog system described above (Hadda *et al.*, 2013), in compound **1–40**, sets of distribution of pharmacophore sites in series **1–20** and **21–40** could be considered as probably active in presence of TC and MBT. This synergistic and streamlined working procedure led to highly combined active flexible/selective ($X^{\delta-}/Y^{\delta-}$) and ($X^{\delta-}/Y^{\delta+}$) receptor ligands (Figs. 2, 3; Table 2).

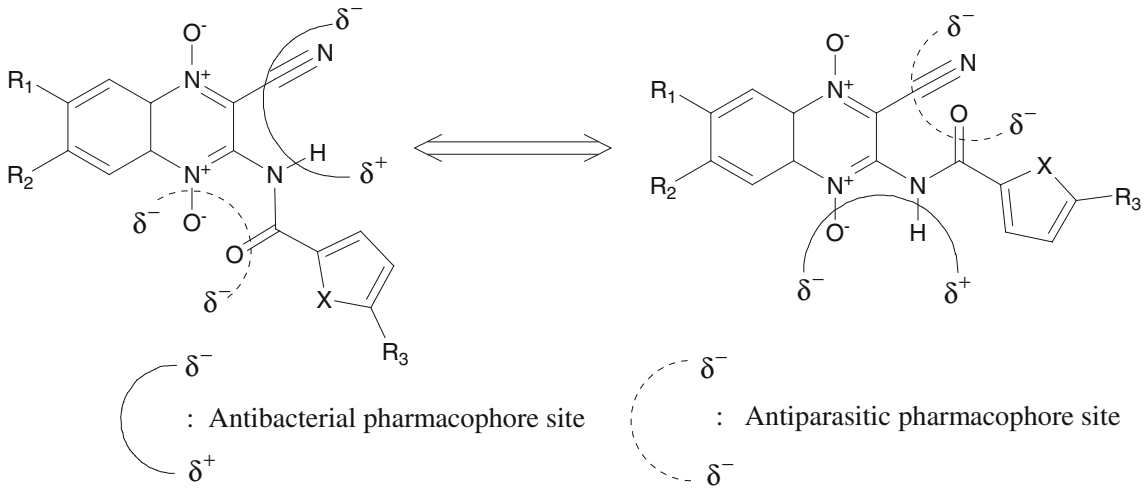

Fig. 2 Identification of antitrypanosomal cruzi and antibacterial pharmacophore sites

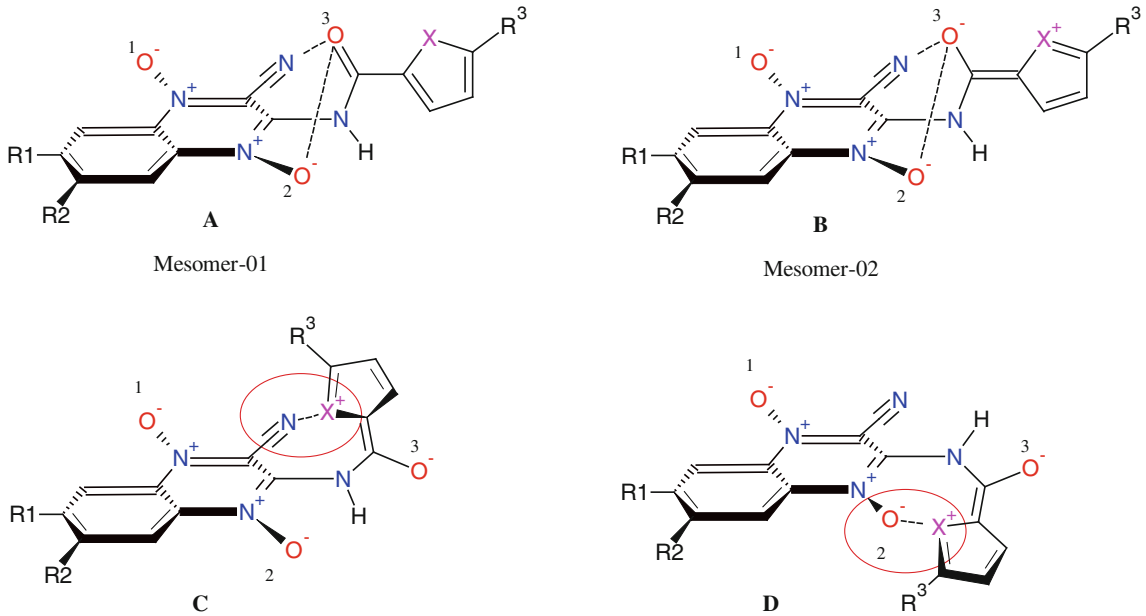

Fig. 3 Most favorable mesomeric and geometric forms of compounds **1–40**

Osiris calculations

Structure-based design is now fairly routine but many potential drugs fail to reach the clinic because of ADMET liabilities. One very important class of enzymes, responsible for many ADMET problems, is the cytochromes P450. Inhibition of these or production of unwanted metabolites can result in many adverse drug reactions.

Of the most important program, Osiris is already available online (Ali *et al.*, 2012; Chohan *et al.*, 2010, 2011; Parvez *et al.*, 2010; Rauf *et al.*, 2010; Sheikh *et al.*, 2011) and it is used to assess the possible toxicity risks associated with the compounds **1–40** (Table 3).

With our recent publications on drug design combination of various pharmacophore sites by using spiro-heterocyclic structure, it is now possible to predict activity and/or inhibition with increasing success in two targets (bacteria and HIV) (Bennani *et al.*, 2007b). This is done using a combined electronic/structure docking procedure and an example will be given here (Table 3). The

Table 2 Calculation of π-charge of heteroatoms of **1–40**

Compounds	X	Substituents			π-charge of O (in e)			π-charge of N & X (in e)				
		R^1	R^2	R^3	O_1	O_2	O_3	N_1	N_2	N_3	N_4	X
1	O	H	H	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.02
2	O	CH_3	H	H	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	−0.02
3	O	CH_3	CH_3	H	−0.06	−0.06	0.01	0.00	0.01	0.12	0.03	−0.02
4	O	OCH_3	H	H	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	−0.02
5	O	Cl	H	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.02
6	O	Cl	Cl	H	−0.06	−0.06	0.01	0.01	0.00	0.12	0.03	−0.02
7	O	F	H	H	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	−0.02
8	O	F	F	H	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	−0.02
9	O	H	CF_3	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.02
10	O	CF_3	H	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.02
11	S	H	H	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.04
12	S	CH_3	H	H	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	−0.04
13	S	CH_3	CH_3	H	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	−0.04
14	S	OCH_3	H	H	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	−0.04
15	S	Cl	H	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.04
16	S	Cl	Cl	H	−0.06	−0.06	0.01	0.01	0.00	0.12	0.03	−0.04
17	S	F	H	H	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	−0.04
18	S	F	F	H	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	−0.04
19	S	H	F	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.04
20	S	CF_3	H	H	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	−0.04
21	O	H	H	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.02
22	O	CH_3	H	NO_2	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	0.02
23	O	CH_3	CH_3	NO_2	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	0.02
24	O	OCH_3	H	NO_2	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	0.02
25	O	Cl	H	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.02
26	O	Cl	Cl	NO_2	−0.06	−0.06	0.01	0.01	0.00	0.12	0.03	0.02
27	O	F	H	NO_2	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	0.02
28	O	F	F	NO_2	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	0.02
29	O	H	CF_3	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.02
30	O	CF_3	H	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.02
31	S	H	H	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.01
32	S	CH_3	H	NO_2	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	0.01
33	S	CH_3	CH_3	NO_2	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	0.01
34	S	OCH_3	H	NO_2	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	0.01
35	S	Cl	H	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.01
36	S	Cl	Cl	NO_2	−0.06	−0.06	0.01	0.01	0.00	0.12	0.03	0.01
37	S	F	H	NO_2	−0.06	−0.06	0.01	0.00	0.00	0.12	0.03	0.01
38	S	F	H	NO_2	−0.06	−0.06	0.01	0.00	−0.01	0.12	0.03	0.01
39	S	H	CF_3	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.01
40	S	CF_3	H	NO_2	−0.05	−0.06	0.01	0.01	0.00	0.12	0.03	0.01

remarkably well behaved mutagenicity of divers synthetic molecules classified in the data base of CELERON Company of Swiss can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA.

Prediction results are valued and symbol coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown by symbol (−). Whereas a symbol (+++) indicates drug-conform behavior.

Table 3 Osiris calculations of compounds **1–40**

Compounds	MW	Toxicity risks				Osiris calculations			
		MUT	TUM	IRRI	REP	cLog P	Sol	DL	DS
1	296	+++	+++	+++	+++	1.48	−8.71	−4.97	0.24
2	310	+++	+++	+++	+++	1.80	−9.05	−6.01	0.24
3	324	+++	+++	+++	+++	2.11	−9.39	−4.96	0.23
4	311	+++	+++	+++	+++	1.19	−8.81	−6.00	0.24
5	315	+++	+++	+++	+++	1.90	−9.53	−5.70	0.24
6	364	+++	+++	+++	+++	2.71	−10.18	−4.53	0.22
7	314	+++	+++	+++	+++	1.54	−9.02	−6.22	0.24
8	332	+++	+++	+++	+++	1.60	−9.33	−6.24	0.23
9	364	+++	+++	+++	+++	2.24	−9.48	−11.38	0.23
10	364	+++	+++	+++	+++	2.24	−9.48	−11.38	0.23
11	312	+++	+++	+++	+++	2.22	−9.03	−4.31	0.24
12	326	+++	+++	+++	+++	2.53	−9.38	−5.35	0.23
13	340	+++	+++	+++	+++	2.85	−9.72	−4.30	0.23
14	342	+++	+++	+++	+++	2.11	−9.05	−3.96	0.24
15	346	+++	+++	+++	+++	2.83	−9.77	−3.68	0.23
16	380	+++	+++	+++	+++	3.44	−10.5	−3.91	0.21
17	330	+++	+++	+++	+++	2.28	−9.35	−5.57	0.23
18	348	+++	+++	+++	+++	2.34	−9.66	−5.59	0.23
19	380	+++	+++	+++	+++	2.98	−9.81	−10.79	0.21
20	380	+++	+++	+++	+++	2.98	−9.81	−10.79	0.21
21	341	+++	+++	+++	+++	1.83	−9.69	−6.40	0.23
22	355	+++	+++	+++	+++	2.14	−10.03	−7.47	0.23
23	369	+++	+++	+++	+++	2.46	−10.30	−6.42	0.22
24	371	+++	+++	+++	+++	1.72	−9.71	−6.19	0.23
25	411	+++	+++	+++	+++	2.44	−10.43	−5.85	0.22
26	409	+++	+++	+++	+++	3.05	−11.16	−6.07	0.21
27	359	+++	+++	+++	+++	1.89	−10.00	−7.66	0.23
28	377	+++	+++	+++	+++	1.95	−10.30	−7.68	0.22
29	409	+++	+++	+++	+++	2.59	−10.43	−12.93	0.21
30	409	+++	+++	+++	+++	2.59	−10.43	−12.93	0.21
31	357	+++	+++	+++	+++	2.31	−9.64	−8.72	0.23
32	371	+++	+++	+++	+++	2.63	−9.99	−9.74	0.22
33	385	+++	+++	+++	+++	2.94	−10.33	−8.70	0.21
34	387	+++	+++	+++	+++	2.20	−9.66	−8.29	0.22
35	391	+++	+++	+++	+++	2.92	−10.38	−8.04	0.21
36	425	+++	+++	+++	+++	3.54	−11.1	−8.28	0.19
37	375	+++	+++	+++	+++	2.37	−9.96	−9.98	0.22
38	393	+++	+++	+++	+++	2.43	−10.23	−10.00	0.22
39	425	+++	+++	+++	+++	3.07	−10.43	−15.15	0.20
40	425	+++	+++	+++	+++	3.07	−10.43	−15.15	0.20
Bdz	260	−	+++	+++	−	260	0.6	−1.6	−3.3
Nfx	301	+++	+++	+++	+++	301	0.92	−3.4	−2.6

+++, not toxic; −, highly toxic

MUT mutagenic, *TUM* tumorigenic, *IRRIT* irritant, *RE* reproductive effective, *Sol* solubility, *DL* druglikness, *DS* drug-score, *Bdz* benznidazole, *Nfx* nifurtimox, the reference drugs

Table 4 Molinspiration calculations of compounds **1–40**

Compounds	Molinspiration calculations				Drug-likeness					
	TPSA	NONI	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
1	117	1	0	238	−0.03	−0.36	−0.06	−0.60	−0.29	−0.11
2	117	1	0	255	−0.06	−0.40	−0.07	−0.57	−0.30	−0.15
3	117	1	0	271	−0.05	−0.39	−0.07	−0.53	−0.27	−0.15
4	126	1	0	264	−0.05	−0.40	−0.03	−0.51	−0.29	−0.13
5	117	1	0	251	−0.01	−0.30	−0.06	−0.58	−0.29	−0.12
6	117	1	0	265	0.02	−0.28	−0.06	−0.54	−0.25	−0.11
7	117	1	0	243	0.24	−0.31	0.02	−0.51	−0.26	−0.10
8	117	1	0	248	0.04	−0.28	−0.00	−0.49	−0.19	−0.08
9	117	1	0	269	0.05	−0.20	0.04	−0.33	−0.16	−0.08
10	117	1	0	269	0.05	−0.20	0.04	−0.33	−0.16	−0.08
11	104	1	0	247	−0.02	−0.39	0.06	−0.46	−0.16	−0.04
12	104	1	0	263	−0.05	−0.39	0.06	−0.46	−0.16	−0.04
13	104	1	0	280	−0.04	−0.37	0.06	0.42	−0.13	−0.04
14	113	1	0	273	−0.04	−0.38	0.10	−0.41	−0.15	−0.02
15	104	1	0	261	0.02	−0.28	0.08	−0.47	−0.15	−0.01
16	104	1	0	274	0.03	−0.26	0.07	−0.43	−0.12	−0.00
17	104	1	0	252	0.05	−0.29	0.15	−0.40	−0.12	0.01
18	104	1	0	257	0.05	−0.27	0.13	−0.38	−0.05	0.02
19	104	1	0	278	0.06	−0.19	0.16	−0.24	−0.03	0.02
20	104	1	0	278	0.06	−0.19	0.16	−0.24	−0.03	0.02
21	104	1	0	261	−0.26	−0.70	−0.09	−0.91	−0.41	−0.19
22	163	1	0	278	−0.29	−0.73	−0.11	−0.89	−0.44	−0.23
23	163	1	0	294	−0.28	−0.71	−0.11	−0.84	−0.43	−0.22
24	172	1	0	287	−0.28	−0.71	−0.07	−0.83	−0.44	−0.21
25	163	1	0	275	−0.23	−0.36	−0.06	−0.60	−0.29	−0.11
26	163	1	0	288	−0.22	−0.61	−0.10	−0.85	−0.41	−0.19
27	163	1	0	266	−0.21	−0.65	−0.03	−0.83	−0.41	−0.18
28	163	1	0	271	−0.20	−0.61	−0.05	−0.80	−0.35	−0.17
29	163	1	0	293	−0.17	−0.52	−0.01	−0.65	−0.33	−0.16
30	163	1	0	293	−0.17	−0.52	−0.01	−0.65	−0.33	−0.16
31	150	1	0	270	−0.21	−0.45	−0.05	−0.52	−0.24	−0.11
32	150	1	0	287	−0.24	−0.48	−0.07	−0.52	−0.28	−0.15
33	150	1	0	304	−0.23	−0.47	−0.07	−0.48	−0.27	−0.14
34	159	1	0	296	−0.23	−0.48	−0.03	−0.47	−0.29	−0.13
35	150	1	0	294	−0.18	−0.39	−0.06	−0.52	−0.52	−0.12
36	150	1	0	298	−0.17	−0.37	−0.06	−0.49	−0.26	−0.11
37	150	1	0	275	−0.16	−0.40	0.01	−0.46	−0.25	−0.10
38	150	1	0	280	−0.15	−0.38	−0.02	−0.45	−0.20	−0.09
39	150	1	0	302	−0.13	−0.30	0.02	−0.31	−0.18	−0.09
40	150	1	0	302	−0.13	−0.30	0.02	−0.31	−0.18	−0.08
Bdz	93	1	0	225	−0.39	−0.69	−0.84	−1.55	–	–
Nfx	109	0	0	246	−1.24	−1.46	−0.92	−1.49	–	–

TPSA total molecular polar surface area, *N viol*. number of violation of five Lipinsky rules, *Vol* volume, *GPCRL* GPCR ligand, *ICM* ion channel modulator, *KI* kinase inhibitor, *NRL* nuclear receptor ligand, *PI* protease inhibitor, *EI* enzyme inhibitor, *Bdz* benznidazole, *Nfx* nifurtimox, the reference drugs

Molinspiration calculations

CLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors (Table 3). The method is very robust and is able to process practically all organic, and most organometallic molecules. Molecular polar surface area TPSA is calculated based on the methodology published by Ertl *et al*. (2000) as a sum of fragment contributions. O– and N– centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood–brain barrier penetration. Prediction results of compounds **1–40** molecular properties (TPSA, GPCR ligand and ICM) are valued (Table 4).

Conclusion

This work provided with additional structure–activity and structure–cytotoxicity information in the CAQDOs family. Indeed, this study proved that a simple control of nature of few numbers of substituents leads to compounds with high activities and reduced cytotoxicities of standard drugs (Pinazo *et al.*, 2010). POM analyses of the CAQDO compounds **1–40** showed that lipophilic substituents bearing electro-donor groups could be introduced in quinoxaline moiety maintaining a high antiparasitic activity. Introduction of sulfur atom (furyl) instead oxygen atom (pyrole) on the CAQDO template provided three additional compounds **13**, **17**, and **39** with no antiparasital activity. On other hand, there are just 2/40 compounds showing anti-TC activity; compounds **8** and **18** with 53 and 92 % as percentage of growth inhibition at 25 μM doses in *T. cruzi* Tulahuen 2 strain. The CAQDO-molecules bearing ($O^{\delta-}/O^{\delta-}$) and ($N^{\delta-}/O^{\delta-}$) pharmacophore sites in equilibrium, constitute potential antitrypanosomal drugs. A similar ($O^{\delta-}/O^{\delta-}$) and ($N^{\delta-}/S^{\delta-}$) pharmacophores are found respectively in curcuminoid analogs (Changtam *et al.*, 2010) and STIO (Hadda *et al.*, 2013), with potent activity against *Trypanosoma* and *Leishmania* species.

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