New salicylamide and sulfonamide derivatives of quinoxaline 1,4-di-N-oxide with antileishmanial and antimalarial activities

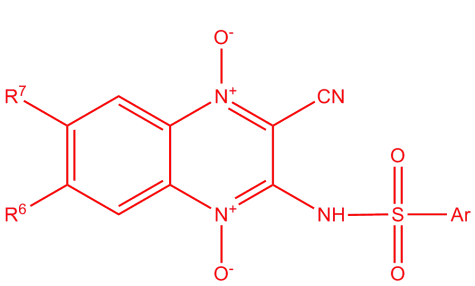
ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · JUNE 2011

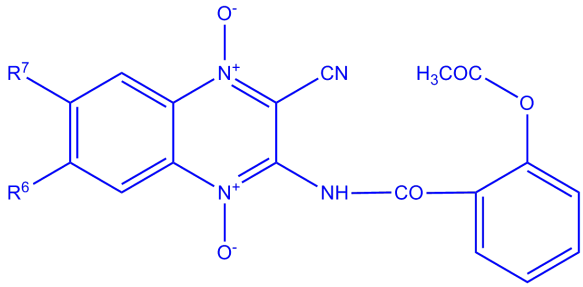
Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2011.05.125 · Source: PubMed

Graphical Abstract

New salicylamide and sulfonamide derivatives of quinoxaline 1,4-di-*N*-oxide with antileishmanial and antimalarial activities

Carlos Barea[a], Adriana Pabón[b], Denis Castillo[c], Mirko Zimic[d], Miguel Quiliano[d], Silvia Galiano[a], Silvia Pérez-Silanes[a], Antonio Monge[a], Eric Deharo[e,f] and Ignacio Aldana[a] *





New salicylamide and sulfonamide derivatives of quinoxaline 1,4-di-*N*-oxide with antileishmanial and antimalarial activities

Carlos Barea[a], Adriana Pabón[b], Denis Castillo[c], Mirko Zimic[d], Miguel Quiliano[d], Silvia Galiano[a], Silvia Pérez-Silanes[a], Antonio Monge[a], Eric Deharo[e,f] and Ignacio Aldana[a] *.

[a]*Unidad en Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, Pamplona, Spain.*
[b]*Grupo Malaria, Universidad de Antioquia, Medellín, Colombia.*
[c]*Unidad de Parasitología Celular, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú.*
[d]*Bioinformatics Unit - Drug Design Group. Laboratorios de Investigación y Desarrollo. Facultad de Ciencias y Filosofía. Universidad Peruana Cayetano Heredia. Lima, Perú.*
[e]*Université de Toulouse ; UPS ; UMR 152 (PHARMA-DEV, Pharmacochimie et Pharmacologie pour le Développement), 118, rte de Narbonne, F-31062 Toulouse cedex 9, France.*
[f]*Institut de Recherche pour le Développement; UMR-152; Mission IRD Casilla 18-1209 Lima, Perú.*

*Corresponding author : Prof. Ignacio Aldana Moraza. Centro de Investigación en Farmacobiología Aplicada. Universidad de Navarra. E-31008 Pamplona. SPAIN. +34 948 425653 (Telephone) ; +34 948 425652 (Fax). e-mail : ialdana@unav.es

ABSTRACT

Continuing with our efforts to identify new active compounds against malaria and leishmaniasis, fourteen new 3-amino-1,4-di-*N*-oxide quinoxaline-2-carbonitrile derivatives were synthesized and evaluated for their *in vitro* antimalarial and antileishmanial activity against *Plasmodium falciparum* Colombian FCR-3 strain and *Leishmania amazonensis* strain MHOM/BR/76/LTB-012A. Further computational studies were carried out in order to analyze graphic SAR and ADME properties. The results obtained indicate that compounds with one halogenous group substituted in position 6 and 7 provide an efficient approach for further development of antimalarial and antileishmanial agents. In addition, interesting ADME properties were found.

Keywords:Quinoxaline; N-oxides; Malaria; Leishmaniasis

Malaria is by far the world's most important tropical parasitic disease and one of the oldest diseases known to mankind. For nearly half a century, chloroquine (CQ) has been the primary therapy of choice. However, CQ-resistant *Plasmodium falciparum* is now observed in nearly all of the malaria-endemic regions and causes the most deadly form of malaria [1]. Mortality, currently estimated at over a million people per year, has risen in recent years, probably due to increasing resistance. Therefore, it is necessary to develop cheaper and more effective drugs against the parasite [2,3]. Leishmaniasis is generally recognized for its cutaneous form which causes non-fatal disfiguring lesions, although epidemics of the potentially fatal visceral form cause thousands of deaths. Today, this disease threatens about 350 million people, and 12 million people are believed to be currently infected, with about 1–2 million estimated new cases occurring every year. Most available drugs against Leishmaniasis are costly, require long treatment regimes and are becoming more and more ineffective, requiring the discovery of new drugs [4]. Moreover, since the introduction of miltefosine at the beginning of this century, no new antileishmanial compounds have been approved for human treatment [5].

Many classes of organic compounds have been tested, with special attention being paid to nitrogen heterocycles, five- and six-membered rings. Quinoxalines display diverse pharmacological activities as antibacterial, antiviral, anticancer and antiparasitic agents and more specifically, their 1,4-di-*N*-oxides, which increase the biological properties enormously [6]. Our research project led to the publication of several papers, in which both synthesis and biological activity assessments have been described for a large number of quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives with a variety of substituents in positions 2, 3, 6 and 7 of the quinoxaline ring [7-10]. In an attempt to exalt antiparisitic activity of quinoxaline derivatives, our group has synthesized different series with promising results, one of them being the introduction of a carbonitrile group in position 2, which increases the antiparasitic activity, and an amine group in position 3, with the aim of linking together new molecules with interesting activities. We synthesized and evaluated *in vitro* fourteen new

3-amino-1,4-di-*N*-oxide quinoxaline-2-carbonitrile analogues against *Plasmodium falciparum* Colombian FCR-3 strain and *Leishmania amazonensis* MHOM/BR/76/LTB-012A strain. The ADME properties of all molecules were calculated *in silico*.

We have prepared fourteen new quinoxaline 1,4-di-*N*-oxide derivatives. The benzofuroxane starting compounds (BFX, **I**, Scheme 1-2) have been prepared using previously described methods [11,12]. The 3-amino-1,4-di-*N*-oxide quinoxaline-2-carbonitrile derivatives (cyanoamines, **II**) were obtained by the Beirut reaction from the corresponding BFX with malononitrile using *N,N*-dimethylformamide (DMF) as solvent and triethylamine as catalyst [13].

With regard to scheme 1, the next stage consists of reacting 3-amino-1,4-di-*N*-oxide quinoxaline-2-carbonitrile derivatives with aromatic sulfonyl chloride derivatives in order to synthesize sulfonamides which have shown interesting antileishmanial activity [14].

These quinoxaline derivatives were tested for their activity against two parasites: *Plasmodium falciparum* (responsible for malaria) and *Leishmania amazonensis* (responsible for cutaneous New World leishmaniasis), and against non-tumorigenic cell line (Murine Peritoneal Macrophages: MPM). Biological activities and graphical representation of the structure-activity relationship for series 1 and 2 are presented in **Table 1, Table 2 and figure 1 (a,b).** None of the sulfonamides (compounds **1-7**) showed interesting activity against malaria parasite, being 60 to >80 times less active than chloroquine. Similarly, most of acetoxybenzamides were scarcely active, being 90 to >100 times less active than chloroquine. Only compounds **9** and **14** showed some activity, with an IC_{50} of approximately 10 μM (although they were almost 30 times less active than CQ). Interestingly, a Cl (**9)** or a F (**14)** in R^7 position increased the activity 4-7 times when compared to a H or a CH_3 in the same position. As shown in figure 1(b), the position of one electronegative atom (F, Cl or O), represented as a blue field, is clearly important in order for the atoms to act as possible hydrogen bond acceptors. This contrasts with the groups CH_3 and H, which projected hydrophobic regions (represented as golden fields).

Compound **9**, with an acetoxybenzamide in position 3, is almost twice as active as compound **4,** the most active compound of the first series. From a spatial point of view, according to figure 1 (a,b), this could mean that a strong concentration of electronegative atoms (potential hydrogen bond acceptors) in one same plane is a good feature for acetoxybenzamides. Therefore, we can say that o-nitrophenyl, p-nitrophenyl and 2-naphtyl sulfonamides lower the activity. The most active compound against malaria was compound **14** (IC_{50} 7,4 μM), followed by compound **9** (10.8 μM).

With regard to Leishmania, compounds **4** and **5** inhibited 50% of parasite growth at approximately 3 μM, being almost 10-15 times less active than the reference drug amphotericin B. As we mentioned before, in order to be active, the R^7 position must be occupied by one electronegative atom Cl, because when missing (**7**), the activity drops dramatically (86 μM). The paranitrophenyl substituent is also responsible for the activity in association with Cl in R^7 position, because once it is charged by a naphtyl group (**1**), the activity lowers ten times. Interestingly, the presence of withdrawing groups, such as o-nitro and p-nitro, in the sulfonamide region is correlated to the activity (see figure 1b). Nevertheless, compounds **4** and **5** showed a low Selectivity Index (2-4), almost ten times lower than the SI of amphotericin B. Although compound **2** and **6** were almost 5 times less active than compounds **4** and **5**, we decided to test them against Leishmania-infected macrophages, because these products were not toxic against non-infected macrophages (> 245 and 89 μM respectively).

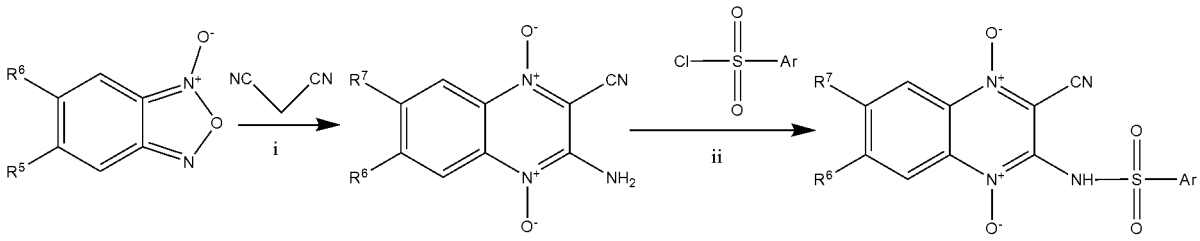
In infected macrophages, compound **2** was 7 times more active than **6** and showed the best Selectivity Index (>15), being very close to that of amphotericin B. In the second series, the most active compound against *Leishmania* (**14**) was 2-3 times less active than compounds **4** and **5**. It appears that in order to be leishmanicidal, the R^7 position must be occupied by a CH_3 or a F. The activity drops when Cl or CH_3O are in

R^7 position. Nevertheless, when the active compounds were tested on non-infected macrophage, they showed some toxicity, being almost as toxic on macrophage as on Leishmania.

A computational study designed to predict the ADME properties of our salicylamide and sulfonamide derivatives of quinoxaline was performed (results are presented in Table 3). Topological polar surface area (TPSA) is a good indicator of drug absorption in the intestines, Caco-2 monolayer penetration, and blood–brain barrier crossing [15]. TPSA was used to calculate the percentage of absorption (%ABS) following the equation: %ABS = 109 - 0.345 x TPSA, as reported [16]. In addition, the number of rotable bonds (n-ROTB), and Lipinski's rule of five were also calculated [17]. The most active compounds found for antileishmanial and antimalarial bioassays (compounds 4, 5, 9 and 14) showed medium percentages of intestinal absorption, with mean values of 58%, and an excellent n-ROTB, ranging only from 3 to 4 [18]. None of these compounds violated any of the Lipinski's parameters, an important characteristic for future drug-development.

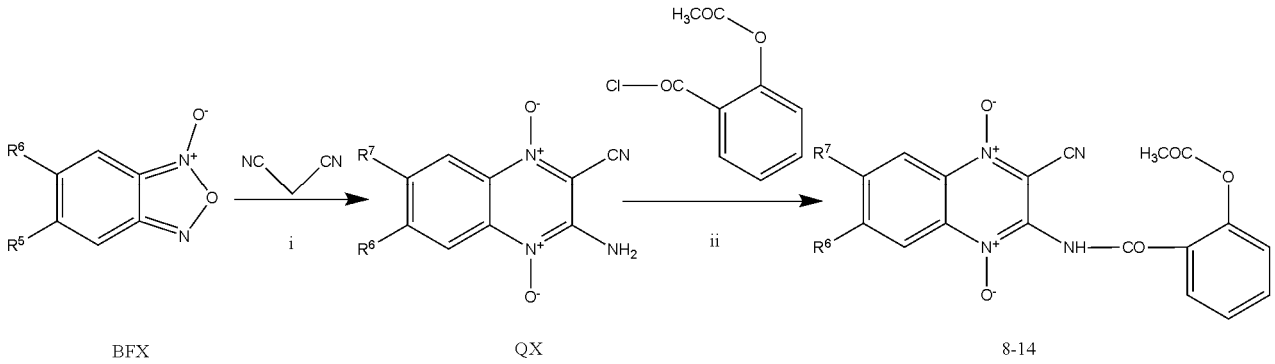
On the other hand, correlations between molecular descriptors such as molecular weight, LogP and activities of acetoxybenzamides and sulfonamides derivatives were found. Simple Pearson correlation values, which are useful for measuring the association between two variables, were calculated between biological activities reported in Table 1 and Table 2, and physical chemical properties in Table 3. In contrast to previously reported results [8,9], whose derivatives only correlate between LogP and activities, in our case, an important element for the activity was also conferred by MW of the derivatives, an important parameter that is directly related with the size of the molecule, which is useful to illustrate the influence of the shape and structural features of a molecule. In the second series, the reported values showed a correlation between MW and antimalarial activity and antileishmanial activity respectively, expressed as Log ($1/IC_{50}$), in which higher values exponentially indicate greater potency (the logarithm of inverse of the IC_{50} is classically used in QSAR studies). Compounds in the second series followed a positive linear relationship (r = 0.47 for Malaria, r=0.64 for Leishmania and undetermined for cytotoxicity in murine peritoneal macrophages) while the first series only followed a negative linear relationship between LogP and antimalarial activity (r = -0.56), also expressed as Log ($1/IC_{50}$).

The seven new sulfonamides presented in this paper were prepared through the synthetic route illustrated in **Scheme 1**.



Scheme 1. General synthesis of sulfonamides. Reagents and conditions: i) malononitrile, DMF, triethylamine, 15-90%; ii) pyridine, 0º C, 13-15%.

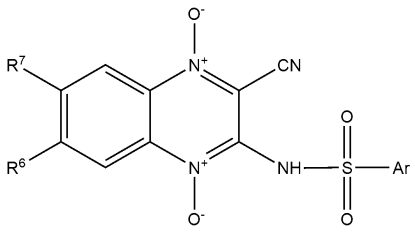
With regard to **Scheme 2**, the final synthetic route consists in combining 3-amino-1,4-di-*N*-oxide quinoxaline-2-carbonitrile derivatives with o-acetylsalicyloyl chloride, used for the treatment of infections caused by protozoans, bacteria and viruses [19].



Scheme 2. General synthesis of acetoxybenzamides. Reagents and conditions: i) malononitrile, DMF, triethylamine, 15-90%; ii) dry THF, 25%.

In the last stage of the synthesis of sulfonamides, the temperature is critical and must remain below 0ºC, whereas the last stage of synthesis of acetoxybenzamides is carried out at room temperature. In addition, there is a loss of yield due to the purification by column chromatography, decreasing from 50% to 25% (acetoxybenzamides) and 13-15% (sulfonamides).

Table 1. Biological characterization of sulfonamides

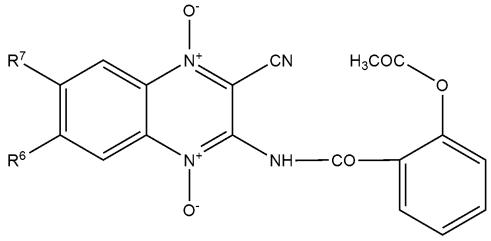


Cpd.	MW	R^6	R^7	Ar	IC_{50} (µM)[a]	CQ Index[b]	IC_{50} amas (µM)[c]	Anf Index[d]	CC_{50} Mø (µM)[e]	SI[f]
1	426.6	H	Cl	2-naphtyl	>23.4	>78	20 ± 1.4	100	88.6 ± 5.6	4.4
2	406.1	H	CH_3	2-naphtyl	>24.6	>82	16.3 ± 0.8	81.5	>245.6	> 15.1
3	420.1	CH_3	CH_3	2-naphtyl	>23.8	>79	>100	>100	NT	NT
4	421.6	H	Cl	o-nitrophenyl	17.4 ± 0.5	58	3.1 ± 0.1	15.5	6.8 ± 0.8	2.2
5	421.6	H	Cl	p-nitrophenyl	>23.7	>79	2.1 ± 0.1	10.5	7.9 ± 0.3	3.8
6	456.1	Cl	Cl	p-nitrophenyl	18.7 ± 1	62	15.9 ± 1.3	-15	89.1 ± 6.4	5.6
7	387.1	H	H	p-nitrophenyl	20 ± 2	67	86.3 ± 8.5	430	NT	-
CQ	320				0.2 ± 0.1	1	20 ± 1.4	100	88.6 ± 5.6	4.4
Anf B	320						0.2	1	4.4	22

[a] IC_{50} against *P. falciparum* FCR-3.
[b] CQ Index: IC_{50} drug/ IC_{50} CQ.
[c] IC_{50} against axenic amastigotes of *L. amazonensis*.
[d] Anf Index: IC_{50} of compounds /IC_{50} amphotericin B.
[e] Cytotoxicity in murine peritoneal macrophages.

[f] Selectivity Index (SI): CC_{50} drug/ IC_{50} drug.
MW molecular weight; NT: Not tested.

Table 2. Biological characterization of acetoxybenzamides



Cpd.	MW	R^6	R^7	IC_{50} (µM)[a]	CQ Index[b]	IC_{50} amas (µM)[c]	Anf Index[d]	CC_{50} Mø (µM)[e]	SI[f]
8	364	H	H	41.4 ± 1.1	138	111.8 ± 3.2	559	NT	-
9	398.5	H	Cl	10.8 ± 1.8	36	33.6 ± 1.3	168	NT	-
10	378	H	CH_3	52.8 ± 1.4	176	18.8 ± 0.3	94	21.7 ± 1.9	1.1
11	394	H	CH_3O	27 ± 1.7	90	42.4 ± 1.7	212	NT	-
12	400	F	F	36 ± 0.9	120	14.8 ± 0.1	74	10.5 ± 0.7	0.7
13	392	CH_3	CH_3	59.4 ± 2.0	198	17.6 ± 0.6	88	12.5 ± 1.1	0.7
14	382	H	F	7.4 ± 0.6	25	7.3 ± 0.1	36.5	13.6 ± 1.6	1.8
CQ	320			0.3 ± 0.01	1				
Anf B						0.2 ± 0.01	1	4.4 ± 0.1	22

[a] IC_{50} against *P. falciparum* FCR-3.
[b] CQ Index: IC_{50} drug/ IC_{50} CQ.
[c] IC_{50} against axenic amastigotes of *L. amazonensis*.
[d] Anf Index: IC_{50} of compounds /IC_{50} amphotericin B.
[e] Cytotoxicity in murine peritoneal macrophages.
[f] Selectivity Index (SI): CC_{50} drug/ IC_{50} drug.
MW molecular weight; NT: Not tested.

Table 3.Physical Chemical Properties of quinoxaline derivatives [a]

ID	%ABS	TPSA (Å)	*n*-RT	Molecular weight	miLog*P*	Lipophilicity descriptor ($\Sigma\pi$)[b]	n-OHNH donors	n-NO acceptors	Lipinski's violations
rule		≤140		<500	<5		≤5	≤10	≤1
1	67.28	120.9	3	426.84	1.19	0.65	1	8	0
2	67.27	120.8	3	406.42	0.96	0.43	1	8	0
3	67.27	120.8	3	420.45	1.34	0.80	1	8	0
4	51.48	166.7	4	421.79	-0.08	0.65	1	11	1
5	51.48	166.7	4	421.79	-0.03	0.65	1	11	1
6	51.48	166.7	4	456.22	0.57	1.25	1	11	1
7	51.48	166.7	4	387.33	-0.68	0	1	11	1
8	69.98	113.1	4	364.32	-0.47	0	1	9	0
9	64.11	130.1	4	398.76	0.18	0.65	1	9	0
10	64.11	130.1	4	378.34	-0.04	0.43	1	9	0
11	60.94	139.3	5	394.34	-0.43	0.04	1	10	0
12	64.11	130.1	4	400.29	-0.23	0.24	1	9	0
13	64.11	130.1	4	392.37	0.33	0.80	1	9	0
14	64.11	130.1	4	396.33	-0.33	0.14	1	9	0

[a] %ABS, percentage of absorption, calculated by: %ABS = 109 – (0.345 x TPSA); TPSA, topological polar surface area; n-RT, number of rotable bonds; miLog*P* , logarithm of compounds partition coefficient between *n*-octanol and water.

[b]Hydrophilic-lipophilic fragments determined for changes in R^7 and R^6, calculated by the sum of $\Sigma\pi$ values, using compounds 7 and 8, and the fragment constant method as reference [20].

A computational study for prediction of ADME properties of all the molecules was performed using Molinspiration online property calculation toolkit (MolinspirationCheminformatics). Parameters such as solubility (miLogP), Topological Polar Surface Area (TPSA) [15], and absorption (%ABS) were calculated using the formula: %ABS = 109 – (0.345 x TPSA) [16]. Violations of Lipinski's rule-of-five were evaluated [17].

Analysis of structure-activity relationship was performed using Cresset's field technology (http://www.cresset-group.com/) which condenses the molecular fields down to a set of points around the molecule, termed "Field Points" [25]. Field Points are the local extrema of the electrostatic, van der Waals and hydrophobic potentials of the molecule. Throughout FielView® software, the Field Points are colored as follows: Blue; negative field points (like to interact with positives/H-bond donors on a protein), red; positive field points (like to interact with negatives/H-bond acceptors on a protein), yellow; van der Waals surface field points (describing possible surface/vdW interactions), or gold/orange; Hydrophobic field points (describe regions with high polarizability/hydrophobicity).

Quinoxaline derivatives harboring a halogenous group in positions 6 and 7 present interesting activity against *Plasmodium* and *Leishmania* parasite and show interesting *in silico* ADME characteristics. These products should be the starting point for the synthesis of antiprotozoal compounds.

Acknowledgements

The authors gratefully acknowledge the ''Oficina de Cooperación de la Embajada de Bélgica" for funding a Master Scholarship to Denis Castillo. Carlos Barea is indebted to the University of Navarra (Spain) for PhD scholarship.

References and Notes

1. Vicente, E.; Charnaud, S.; Bongard, E.; Villar, R.; Burguete, A.; Solano, B.; Ancizu, S.; Perez-Silanes, S.; Aldana, I.; Vivas, L.; Monge, A. *Molecules*, **2008**, *13*, 69.
2. Biot, C.; Chibale, K. *Infect. Disord. Drug Targets*, **2006**, *2*, 173.
3. WHO: http://www.who.int/malaria/world_malaria_report_2010/en/index.html
4. *WHO*: http://www.who.int/leishmaniasis/en/. Accessed March 27, **2011**.
5. Kedzierski, L.; Sakthianandeswaren, A.; Curtis, J. M.; Andrews, P. C.; Junk, P. C.; Kedzierska, K. *Curr. Med. Chem.* **2009**, *16*, 599.
6. Carta, A.; Corona, P.; Loriga, M. *Curr. Med. Chem.* **2005**, *12*, 2259.
7. Monge, A.; Palop, J. A.; Piñol, A.; Martínez-Crespo, F. J.; Narro, S.; González, M.; Sáinz, Y.; López de Ceráin, A. *J. Heterocycl.. Chem.* **1994**, *31*, 1135.
8. Burguete, A.; Estevez, Y.; Castillo, D.; González, G.; Villar, R.; Solano, B.; Vicente, E.; Pérez-Silanes, S.; Aldana, I.; Monge, A.; Sauvain, M.; Deharo, E. *Memorias do Oswaldo* Cruz, **2008**, *103*, 778.
9. Estevez, Y.; Quiliano, M.; Burguete, A.; Zimic, M.; Málaga, E.; Verástegui, M.; Pérez-Silanes, S.; Aldana, I.; Monge, A.; Castillo, D.; Deharo, E. *Experimental Parasitology,* **2011**, *127*, 745.
10. Ancizu, S.; Moreno, E.; Torres, E.; Burguete, A.; Perez-Silanes, S.; Benitez, D.; Villar, R.; Solano, B.; Marin, A.; Aldana, I.; Cerecetto, H.; Gonzalez, M.; Monge, A. *Molecules,* **2009***, 14,* 2256.
11. Ortega, M.A.; Sainz, Y.; Montoya, M.E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Arzneim.-Forsch.* **2002**, *52*, 113.
12. González, M.; Cerecetto, H. *Springer*, **2007**, *10*, 265.
13. Ley, K.; Seng. F. *Synthesis,* **1975**, 415.
14. Dea-Ayuela, M. A.; Castillo, E.; González-Álvarez, M.; Vega, C.; Rolón, M.; Bolás-Fernández, F.; Borrás, J.; González-Rosende, M. E. *Bioorg. Med.. Chem.* **2009**, *17*, 7449.
15. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
16. Zhao, Y. H.; Abraham, M. H.; Le, J.; Hersey, A.; Luscombe, C. N.; Beck, G.; Sherborne, B.; Cooper, I. *Pharm. Res.* **2002**, *19*, 1446.
17. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
18. Veber D. F.; Johnson S. R.; Cheng H. Y.; Smith B. R.; Ward K. W.; Kopple K. D. *J. Med. Chem.* **2002**, *45*, 2615.
19. De Carvalho, L. P. S.; Lin, G.; Jiang, X.; Nathan, C. *J. Med. Chem.* **2009**, *52*, 5789.
20. Gordon, L.F. *J. Pharm. Sci.* **1980**, *69*, 1109.
21. Trager, W.; Jensen, J. B. *Science*, **1976**, *193*, 673.
22. Desjardins, R.E.; Canfield, C.J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
23. Sereno, D.; Lemesre, J. L. *Parasitol. Res.* **1997b,** *83*, 401.
24. Castillo, D.; Arevalo, J.; Herrera, F.; Ruiz, C.; Rojas, R.; Rengifo, E.; Vaisberg, A.; Lock, O.; Lemesre, J. L.; Gornitzka, H.; Sauvain, M. *J. Ethnopharmacol.* **2007,** *112*, 410.
25. Cheeseright, T.; Mackey, M.; Rose, S.; Vinter, J. G. *J. Chem. Inf. Model.* **2006**, *46*, 665.