SAR Studies and Biological Characterization of a Chromen-4-one Derivative as an Anti-*Trypanosoma brucei* Agent

Chiara Borsari,^{‡,†} Nuno Santarem,[§] Sara Macedo,[§] María Dolores Jiménez-Antón,^{||} Juan J. Torrado,^{||} Ana Isabel Olías-Molero,^{||} María J. Corral,^{||} Annalisa Tait,[‡] Stefania Ferrari,[‡] Luca Costantino,[‡] Rosaria Luciani,[‡] Glaucio Ponterini,[‡] Sheraz Gul,[⊥] Maria Kuzikov,[⊥] Bernhard Ellinger,[⊥] Birte Behrens,[⊥] Jeanette Reinshagen,[⊥] José María Alunda,^{||} Anabela Cordeiro-da-Silva,^{§, #} and Maria Paola Costi^{*,‡,†}

[‡]University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy

[§]IBMC and Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4150-180 Porto, Portugal

^{||}Complutense University of Madrid, 28040 Madrid, Spain

[⊥]Fraunhofer Institute for Molecular Biology and Applied Ecology Screening Port, 22525 Hamburg, Germany

[#]Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal

S Supporting Information

ABSTRACT: Chemical modulation of the flavonol 2-(benzo[d][1,3]-dioxol-5-yl)-chromen-4-one (**1**), a promising anti-*Trypanosomatid* agent previously identified, was evaluated through a phenotypic screening approach. Herein, we have performed structure–activity relationship studies around hit compound **1**. The pivaloyl derivative (**13**) showed significant anti-*T. brucei* activity ($EC_{50} = 1.1 \mu M$) together with a selectivity index higher than 92. The early *in vitro* ADME-tox properties (cytotoxicity, mitochondrial toxicity, cytochrome P450 and hERG inhibition) were determined for compound **1** and its derivatives, and these led to the identification of some liabilities. The 1,3-benzodioxole moiety in the presented compounds confers better *in vivo* pharmacokinetic properties than those of classical flavonols. Further studies using different delivery systems could lead to an increase of compound blood levels.

KEYWORDS: *Trypanosoma brucei*, flavonol-like compounds, SAR studies, ADME-tox properties, neglected tropical diseases

Neglected tropical diseases (NTDs) are a group of infections that affect more than 1.4 billion people worldwide and mainly thrive among the poorest populations in tropical and subtropical areas.¹ Kinetoplastid parasites are responsible for the potentially fatal insect-borne diseases, namely Chagas disease, Human African Trypanosomiasis (HAT), and Leishmaniasis.² HAT, also known as sleeping sickness, is caused by infection with the *gambiense* and *rhodesiense* subspecies of the extracellular protozoan parasite *Trypanosoma brucei* (*T. brucei*).³ The tsetse fly, *Glossina spp.*, is the vector of the sleeping sickness disease.⁴ According to the World Health Organization (WHO), HAT continues to be a public health issue with an estimated number of new cases per year around 20000 and an estimated population at risk of 65 million people.⁵ Despite the serious health, economic, and social consequences of *T. brucei* infections, effective vaccines are lacking and the limited existing drug therapy presents drawbacks including toxicity, poor efficacy, and serious side effects. Most of the available drugs have been used for over half a century; thus, problems of drug resistance are emerging. Therefore, there is an urgent need for new, safe and effective

drugs.⁶ A phenotypic approach is a useful tool for drug discovery with the advantage of identifying compounds which are active against the whole cell. Membrane permeability, cell uptake, and cell efflux are taken into account in the selection of new hits through phenotypic screening.⁷ Phenotypic approaches to drug discovery have been successfully used in the field of neglected diseases, particularly for the treatment of HAT.^{8,9} Two compounds discovered through phenotypic screening have recently been progressed into clinical trials by DNDi (Drugs for Neglected Diseases initiative): fexinidazole, a nitroimidazole and SCYX-7158, an oxaborole.¹⁰ A wide range of chemical structures, including flavonols (3-hydroxy-2-phenylchromen-4-one), have been investigated in drug discovery programs with the aim of identifying novel antileishmanial and antitrypanosomatid agents.^{11–15} Very

Special Issue: Highlighting Medicinal Chemistry in Italy

Received: November 21, 2018

Accepted: January 29, 2019

Published: January 29, 2019

recently, we had replaced the phenyl ring of classical flavonols with heteroaromatic rings and biphenyl rings and we had synthesized a series of flavonol-like compounds with improved antiparasitic activity with respect to classical flavonols (Figure 1). Compound **1** bearing a 1,3-benzodioxole was identified as

Figure 1. SAR studies on flavonol-like compounds and identification of compound **1**.

the most active and selective molecule toward *T. brucei* ($EC_{50} = 0.4 \mu\text{M}$, Selectivity Index (SI) = 250) (Figure 1).¹⁶ According to the biological activity profile, compound **1** was suitable for progression in the drug discovery path. Moreover, the 1,3-benzodioxole represents a crucial pharmacophore with diverse biological activities and has been exploited in bioactive compounds with a wide range of medical applications, including cancer,^{17,18} tuberculosis,¹⁹ hepatitis B,²⁰ fungal infections,²¹ and parasitic diseases.^{22,23}

The aims of our study were to validate compound **1** through structure activity relationship (SAR) studies, discover follow-up hits, and characterize their biological profile for potential liabilities identifications. The synthetic procedure followed for the synthesis of the compounds (**1–21**) is shown in Scheme 1, and the chemical structures are depicted in Tables 1–3. The chalcones (**22–34**) were synthesized by Claisen–Schmidt condensation using substituted acetophenones and benzaldehydes in the presence of NaOH as base. The reaction was carried out in ethanol as previously reported.¹⁵ The chalcones were converted into the corresponding flavonol-like com-

pounds (**1–10**, **19–21**) using the Flynn–Algar–Oyamada method for epoxidation and subsequent intramolecular cyclization of the open-chain structure (Scheme 1A). For the synthesis of esters (**11–15**) and carbamate **16**, compound **1** was treated with an excess of acyl chloride in dry DCM and in the presence of triethylamine. The reaction was carried out at room temperature overnight. For the synthesis of ethers **17** and **18**, alkyl halide was added to a solution of compound **1** in dry DMF and in the presence of K_2CO_3 . The reaction was carried out under microwave irradiation (Scheme 1B).

The novel library of flavonol-like compounds (**2–21**) was evaluated toward *T. brucei* bloodstream form. The series was assessed for cytotoxicity on THP1 macrophage-like cells to estimate the CC_{50} . For compounds showing a percentage of parasite growth inhibition higher than 70%, the dose–response curve (DRC) was performed. The percentages of parasite growth inhibition at $10 \mu\text{M}$ are reported in Table S1 of the Supporting Information.

We started the SAR investigation of this scaffold by modifying the substituents on ring A (Table 1). Nine compounds (**2–10**) were synthesized introducing different substituents in position 6 and 7 of ring A. Five compounds (**2**, **4**, **8–10**) showed a significant activity toward *T. brucei* with EC_{50} lower than $5 \mu\text{M}$. When the OCH_3 in position 7 of compound **1** was replaced with a methyl group and a chlorine or fluorine (**8**, **9**, and **10**, respectively), the compounds maintained a meaningful anti-*T. brucei* activity. Moving the methoxy group from position 7 to 6 (compound **3**), we observed a huge drop of the antiparasitic activity. Compound **2**, bearing unsubstituted ring A, and compound **4**, with a methyl group in position 6 showed activity toward *T. brucei*, while compounds bearing halogen in position 6 (5-bromide; **6**-chlorine; **7**-fluorine) did not significantly inhibit *T. brucei* cells growth. Compound **8** ($EC_{50} = 0.4 \mu\text{M}$) displayed a potency

Scheme 1. (A) Synthesis of the Compounds **1–10** and **19–21**.^a (B) Synthesis of the Compounds **11–18**.^b

^aReaction conditions: (i) NaOH (3 M), EtOH, r.t.; (ii) H_2O_2 , NaOH (1 M), EtOH, r.t. ^bReaction conditions: (iii) acyl chloride, dry DCM, N_2 , r.t.; (iv) carbamoyl chloride, dry DCM, r.t.; (v) alkyl halide, dry DMF, MW 80°C , 0.5 h.

Table 1. SAR Study on Ring A of the Cromen-4-one Scaffold

Comp.	R ₃	R ₆	R ₇	EC ₅₀ ± SD (μM)	CC ₅₀ (μM)	SI
1	OH	H	OCH ₃	0.4 ± 0.1	>100	250
2	OH	H	H	2.9 ± 0.4	12.5 < CC ₅₀ < 25	4
3	OH	OCH ₃	H		<12.5	
4	OH	CH ₃	H	4.1 ± 2.1	<12.5	3 ^a
5	OH	Br	H		12.5 < CC ₅₀ < 25	
6	OH	Cl	H		<12.5	
7	OH	F	H		12.5 < CC ₅₀ < 25	
8	OH	H	CH ₃	0.4 ± 0.1	12.5 < CC ₅₀ < 25	31
9	OH	H	Cl	3.8 ± 4.0	<12.5	3 ^a
10	OH	H	F	2.4 ± 0.3	<12.5	8 ^a

^aOnly estimations as the lower threshold of toxicity were not determined, EC₅₀ > 10 μM. The reference compound for *T. brucei* was pentamidine (IC₅₀ = 1.55 ± 0.24 nM). The synthesis of compounds 1,²⁸ 2,²⁹ 3,³⁰ 4,³¹ 5,³⁰ 6,³⁰ 7,³⁰ 8,³² and 9³³ has been already published in the literature. Compound 10 is a novel structure and has not been previously reported in the literature.

Table 2. SAR Study on the Hydroxyl Group in Position 3 of the CROMEN-4-one Scaffold

Comp.	R ₃	EC ₅₀ ± SD (μM)	CC ₅₀ (μM)	SI
11		0.3 ± 0.3	<12.5	46*
12		0.5 ± 0.1	<12.5	24*
13		1.1 ± 0.2	>100	>92
14		0.6 ± 0.2	<12.5	22*
15		0.5 ± 0.1	12.5 < CC ₅₀ < 25	25
16		-	>100	-
17		-	50 < CC ₅₀ < 100	-
18		-	12.5 < CC ₅₀ < 25	-

*Only estimations, as the lower threshold of toxicity was not determined, EC₅₀ > 10 μM. The reference compound for *T. brucei* was pentamidine (IC₅₀ = 1.55 ± 0.24 nM). Compounds 11–18 are novel structures and have not been previously reported in the literature.

comparable to that of the starting hit 1; however, it presented a reduced selectivity index (SI = 31).

Following this, our SAR was focused on modifications of the hydroxyl group in position 3 of the chromen-4-one scaffold (Table 2). The presence of an ester instead of a hydroxyl group in position 3 (11–15) led to significant activity on *T. brucei* (EC₅₀ < 1.1 μM) together with a SI > 20. Among the esters, the 3-pivaloyl derivative of compound 1 (13) showed the most

Table 3. SAR Study Modifying the 1,3-Benzodioxole Ring of Compound 1^a

Comp.	R ₂	EC ₅₀ ± SD (μM)	CC ₅₀ (μM)	SI
19		-	>100	-
20		-	50 < CC ₅₀ < 100	-
21		3.1 ± 0.5	25 < CC ₅₀ < 50	8

^aEC₅₀ > 10 μM. The reference compound for *T. brucei* was pentamidine (IC₅₀ = 1.55 ± 0.24 nM). Compounds 19–21 are novel structures and have not been previously reported in the literature.

interesting profile with an EC₅₀ toward *T. brucei* of 1.1 μM and SI > 92. On the contrary, the presence of a carbamate (16) or an ether (17 and 18) led to inactivity toward *T. brucei*. These data suggested that the hydroxyl group in position 3 should be free in order to have a meaningful anti-*T. brucei* activity. The activity of esters can be related to an easier hydrolysis with respect to ethers and carbamates. We enlarged the SAR study modifying the 1,3-benzodioxole ring of compound 1 (compounds 19–21, Table 3). Compound 19, with two fluorine atoms instead of two hydrogens linked to the dioxolane ring, was less active than the starting compound 1. The anti-*T. brucei* activity decreased by replacing the dioxolane ring of 1 with a dioxane (compound 20), while it was maintained in compound 21, bearing a tetrahydrofuran. Compound 21 presented an EC₅₀ toward *T. brucei* equal to 3.1 μM, but SI = 8. Overall, six compounds (8, 11–15) showed a low micromolar EC₅₀ and SI > 20. Compound 13, the 3-pivaloyl derivative of 1, was the most selective among the novel synthesized molecules.

The synthesized library was assessed at 10 μM in a panel of early *in vitro* ADME-tox assays including cytotoxicity (A549 cell line), mitochondrial toxicity, cytochrome P450 (CYP1A2,

CYP2C9, CYP2C19, CYP2D6, and CYP3A4 isoforms) and hERG inhibition. The data are reported in Figure 2 using a

Entry	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
% in. hERG																					
% in. CYP1A2																					
% in. CYP2C9																					
% in. CYP2C19																					
% in. CYP2D6																					
% in. CYP3A4																					
% cell growth A549																					
% tox. Mitochondria																					

Figure 2. Early *in vitro* ADME-tox properties of compounds 1–21. All the assays were performed at 10 μ M. The data are reported as a traffic light system. An ideal compound would be expected to be associated with a green color (yielding <30% effect). For CYP450, hERG, and mitochondrial toxicity, the cell is colored green when the value is 0–30%, yellow for values 31–60%, and red for values $\geq$ 61%. Compounds are noncytotoxic (green) when the A549 cell growth value is 60–100%, cytostatic (yellow) for values 0–59%, and cytotoxic (red) for values <0%.

traffic light system. Compound 1 and all its derivatives exhibited no liability toward hERG and mitochondrial toxicity. Some compounds were shown to be cytostatic, with two compounds (9 and 12) being cytotoxic (<0% A549 cell growth). Most compounds displayed varying degrees of CYP450 liability. The IC_{50} s toward hERG and CYP isoforms were measured for compound 1. The hERG IC_{50} (>100 μ M) was over 250-fold higher than the EC_{50} toward the parasite, thus in accord with the Target Product Profile (TPP) for hit prioritization. Compound 1 IC_{50} values toward CYP1A2 and CYP2D6 were 0.4 and 0.05 μ M, respectively, whereas for CYP2C9, CYP2C19, and CYP3A4 the IC_{50} values were equal to 1.6, 1.5, and 6.0 μ M, respectively. Compound 1 was the best for its antitrypanosomatid activity and ADME-tox profile and progressed to *in vivo* pharmacokinetic studies.

In vivo bioavailability and half-life were evaluated in BALB/c mice treated IV with 1 mg/kg and orally with 20 mg/kg. Compound 1 displayed a half-life of 19 h after iv administration and of 45 h after oral (os) administration (Table 4). Both AUC and C_{max} values were similar despite the much higher dose administered per os. T_{max} for IV administration was reached after 1 h, this suggesting the possible intravascular aggregation of compound 1 given its low solubility.

The aggregation behavior of compound 1 in aqueous solution was investigated spectroscopically and the albumin sequestration assay was performed. As compound 1 concentration increases, both the absorption and the emission spectra show an increase of bands due to aggregates relative to the monomer bands (Figure 3). The absorption data were well fitted in terms of a monomer/dimer equilibrium, with a $1.8 (\pm 0.3) \times 10^5 M^{-1}$ equilibrium constant at 20 $^{\circ}C$ (see the Supporting Information). The fact that the aggregate absorption band is found at shorter wavelengths and its emission band at longer wavelengths than the corresponding bands of the monomeric form indicates the aggregates to be of

Figure 3. Absorption (left) and fluorescence emission spectra of compound 1 in phosphate buffer at pH 8 in the absence (top) and in the presence of human serum albumin (HSA). Top: effect of increasing concentration of compound 1: 1.25, 2.5, 3.75, 5, 6.25, 7.5, 8.75, 10, 11.25 μ M. Bottom: the arrows indicate the effect of the subsequent additions of HSA (1.68, 2.72, 4.11, 6.65, 9.65, 13.86 μ M) to the 11.25 μ M solution of compound 1. Absorption maxima: free and HSA-complexed monomer, $\approx$ 360 nm; aggregate, 325 nm. Emission maxima: free monomer, 475 nm; aggregate, 560 nm, HSA-complexed monomer, 540 nm. λ_{exc} = 320 nm. The emission spectra were normalized to their maximum values to facilitate presentation.

H-type (as opposed to a J-type), i.e., with the monomers stacked on top of each other with a small slip angle.^{24,25} Subsequent additions of human serum albumin (HSA) caused a progressive recovery of the monomer absorption band and the replacement of both aggregate and free monomer emission bands with a single new band that we assign to a compound 1/HSA complex. Therefore, the latter represents a stable state with respect to the monomeric and dimeric states. Emission data analysis provided in the Supporting Information allowed us to estimate the 1/HSA binding equilibrium constant, $2.5 (\pm 1) \times 10^5 M^{-1}$. These results indicate that compound 1 has a tendency to aggregate in aqueous solution that can be reverted by albumin binding. We expect this behavior to occur in blood where albumin binding should help compound solubilization. Chemical changes enhancing solubility are expected to avoid aggregate formation and increase the blood levels of compound 1, thus producing an acceptable pharmacokinetic behavior for infected animal testing.

Although removal of systemic infection may be beneficial to host survival, in the second stage HAT (which represents 90% of the total cases), the parasites colonize the central nervous system. To understand the suitability of compound 1 to pass the BBB, we evaluated molecular descriptors, such as lipophilicity (cLogP), molecular weight (MW), and polar surface area (PSA) that provide insight into the factors governing BBB penetration. Compound 1 fulfills the requirements for BBB penetration, i.e. cLogP in the range 1.5–2.7 (2.19 for compound 1), MW < 400 (312.3 for compound 1)

Table 4. Pharmacokinetic Parameters of Compound 1

Comp.	Dose (mg) and route	C_{max} (ng/mL)	C_{max} (μ M)	T_{max} (h)	AUC _{tot} (ng/mL h)	AUC _{tot} (nmol/mL h)	Half life (h)
1	1 (IV)	340	1.08	1.00	3120	9.99	19.8
1	20 (per os)	290	0.91	0.50	2700	8.65	45.4

and PSA < 90 Å² (74.22 Å² for compound **1**). Additionally, the 10⁵ order of magnitude of the 1/HSA binding equilibrium constant is consistent with that of CNS drugs that do cross the BBB (6 × 10⁴ M⁻¹). Therefore, we expect compound **1** to be sufficiently lipophilic to be transported by HSA and pass the CNS barrier.²⁶

In summary, we have validated compound **1** bearing a 1,3-benzodioxole moiety as a potent anti-Trypanosomatid agent *in vitro*.¹⁶ SAR studies around compound **1** have confirmed its profile as a valuable hit to progress to animal studies. We have synthesized 20 derivatives (**2**–**21**); compounds **10**–**21** are novel structures and have not been previously reported in the literature. The pivaloyl derivative (**13**) was the best compound of the hit-to-lead optimization process. Compound **13** has significant anti-*T. brucei* activity (EC₅₀ = 1.1 μM) together with SI > 92 and a reduced toxicity, thus showing a biological profile similar to **1**. The pharmacokinetic (PK) studies on **1** have demonstrated the ability of the 1,3-benzodioxole flavonol derivative to reach plasma concentrations > EC₅₀ for *T. brucei* with oral administration, thus increasing classical flavonols half-life.¹⁵ Compound **1** blood exposure was probably limited due to its low solubility and sequestration by albumin, as shown in aqueous solution experiments. Compound **1** is an interesting scaffold for anti-Trypanosomatid drug development that can be further exploited using drug delivery systems such as β-cyclodextrins, which have a proven capacity to improve solubility of flavonoids.²⁷

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00565.

Antiparasitic activity toward *Trypanosoma brucei* (Table S1); Early ADME-tox data (Table S2); General information and experimental data of synthesized compounds (pp S6–S16) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*M.P.C.: phone, 0039-059-205-8579; E-mail, mariapaola.costi@unimore.it.

ORCID

Chiara Borsari: 0000-0002-4688-8362

Stefania Ferrari: 0000-0003-1149-5953

Maria Paola Costi: 0000-0002-0443-5402

Present Address

†(C.B.) Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This project has received funding from the European Union's Seventh Framework Programme for research, technological development, and demonstration under grant agreement no. 603240 (NMTrypI - New Medicine for Trypanosomatid Infections).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the COST Action CM1307, http://www.cost.eu/COST_Actions/cmst/CM1307 for the contribution to the discussion of the research results.

■ ABBREVIATIONS

ADME-tox, Absorption, Distribution, Metabolism, and Excretion, toxicology; A549, human lung adenocarcinoma epithelial cell line; CC₅₀, half maximal cytotoxicity concentration; DCM, dichloromethane; DMF, dimethylformamide; DRC, dose–response curve; EC₅₀, half maximal effective concentration; EtOH, ethanol; HAT, Human African trypanosomiasis; hERG, human ether-a-go-go-related gene; HAS, human serum albumin; NaOH, sodium hydroxide; SI, selectivity index; *T. brucei*, *Trypanosoma brucei*; THP1, human monocytic cell line

■ REFERENCES

- (1) Soeiro, M. N.; Werbovetz, K.; Boykin, D. W.; Wilson, W. D.; Wang, M. Z.; Hemphill, A. Novel amidines and analogues as promising agents against intracellular parasites: a systematic review. *Parasitology* **2013**, *140* (8), 929–951.
- (2) Nussbaum, K.; Honek, J.; Cadmus, C. M.; Efferth, T. Trypanosomatid parasites causing neglected diseases. *Curr. Med. Chem.* **2010**, *17* (15), 1594–1617.
- (3) Morrison, L. J. Parasite-driven pathogenesis in *Trypanosoma brucei* infections. *Parasite Immunol.* **2011**, *33* (8), 448–455.
- (4) Stein, J.; Mogk, S.; Mudogo, C. N.; Sommer, B. P.; Scholze, M.; Meiwes, A.; Huber, M.; Gray, A.; Duszenko, M. Drug development against sleeping sickness: old wine in new bottles? *Curr. Med. Chem.* **2014**, *21* (15), 1713–1727.
- (5) Squarre, D.; Kabongo, I.; Munyeme, M.; Mumba, C.; Mwasinga, W.; Hachaambwa, L.; Sugimoto, C.; Namangala, B. Human African Trypanosomiasis in the Kafue National Park, Zambia. *PLoS Neglected Trop. Dis.* **2016**, *10* (5), No. e0004567.
- (6) Reddy, M.; Gill, S. S.; Kalkar, S. R.; Wu, W.; Anderson, P. J.; Rochon, P. A. Oral drug therapy for multiple neglected tropical diseases: a systematic review. *JAMA* **2007**, *298* (16), 1911–1924.
- (7) Gilbert, I. H. Drug discovery for neglected diseases: molecular target-based and phenotypic approaches. *J. Med. Chem.* **2013**, *56* (20), 7719–7726.
- (8) Sykes, M. L.; Avery, V. M. Approaches to protozoan drug discovery: phenotypic screening. *J. Med. Chem.* **2013**, *56* (20), 7727–7740.
- (9) Borsari, C.; Santarem, N.; Torrado, J.; Olías, A. I.; Corral, M. J.; Baptista, C.; Gul, S.; Wolf, M.; Kuzikov, M.; Ellinger, B.; Witt, G.; Gribbon, P.; Reinshagen, J.; Linciano, P.; Tait, A.; Costantino, L.; Freitas-Junior, L. H.; Moraes, C. B.; Bruno Dos Santos, P.; Alcântara, L. M.; Franco, C. H.; Bertolacini, C. D.; Fontana, V.; Tejera Nevado, P.; Clos, J.; Alunda, J. M.; Cordeiro-da-Silva, A.; Ferrari, S.; Costi, M. P. Methoxylated 2'-hydroxychalcones as antiparasitic hit compounds. *Eur. J. Med. Chem.* **2017**, *126*, 1129–1135.
- (10) Éperon, G.; Balasegaram, M.; Potet, J.; Mowbray, C.; Valverde, O.; Chappuis, F. Treatment options for second-stage gambiense human African trypanosomiasis. *Expert Rev. Anti-Infect. Ther.* **2014**, *12* (11), 1407–1417.
- (11) Tasdemir, D.; Kaiser, M.; Brun, R.; Yardley, V.; Schmidt, T. J.; Tosun, F.; Rüedi, P. Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrob. Agents Chemother.* **2006**, *50* (4), 1352–1364.
- (12) Singh, N.; Mishra, B. B.; Bajpai, S.; Singh, R. K.; Tiwari, V. K. Natural product based leads to fight against leishmaniasis. *Bioorg. Med. Chem.* **2014**, *22* (1), 18–45.
- (13) da Silva, E. R.; Maquiaveli-Cdo, C.; Magalhães, P. P. The leishmanicidal flavonols quercetin and quercitrin target *Leishmania*

- (Leishmania) amazonensis arginase. *Exp. Parasitol.* **2012**, *130* (3), 183–188.
- (14) Arioka, S.; Sakagami, M.; Uematsu, R.; Yamaguchi, H.; Togame, H.; Takemoto, H.; Hinou, H.; Nishimura, S. Potent inhibitor scaffold against *Trypanosoma cruzi* trans-sialidase. *Bioorg. Med. Chem.* **2010**, *18* (4), 1633–1640.
- (15) Borsari, C.; Luciani, R.; Pozzi, C.; Pöhner, I.; Henrich, S.; Trande, M.; Cordeiro-da-Silva, A.; Santarém, N.; Baptista, C.; Tait, A.; Di Pisa, F.; DelloIacono, L.; Landi, G.; Gul, S.; Wolf, M.; Kuzikov, M.; Ellinger, B.; Reinshagen, J.; Witt, G.; Gribbon, P.; Kohler, M.; Keminer, O.; Behrens, B.; Costantino, L.; Tejera Nevado, P.; Bifeld, E.; Eick, J.; Clos, J.; Torrado, J.; Jiménez-Antón, M. D.; Corral, M. J.; Alunda, J. M.; Pellati, F.; Wade, R. C.; Ferrari, S.; Mangani, S.; Costi, M. P. Profiling of flavonol derivatives for the development of antitypanosomatidic drugs. *J. Med. Chem.* **2016**, *59* (16), 7598–7616.
- (16) Ph.D. thesis Borsari, C. Drug discovery and delivery approaches for the identification and optimization of novel agents for neglected tropical diseases and tuberculosis.
- (17) Wei, P. L.; Tu, S. H.; Lien, H. M.; Chen, L. C.; Chen, C. S.; Wu, C. H.; Huang, C. S.; Chang, H. W.; Chang, C. H.; Tseng, H.; Ho, Y. S. J. The in vivo antitumor effects on human COLO 205 cancer cells of the 4,7-dimethoxy-5-(2-propen-1-yl)-1,3-benzodioxole (apirole) derivative of 5-substituted 4,7-dimethoxy-5-methyl-1,3-benzodioxole (SY-1) isolated from the fruiting body of *Antrodia campthorae*. *Cancer Res. Ther.* **2012**, *8* (4), 532–536.
- (18) Goodarzi, S.; Hadjiakhoondi, A.; Yassa, N.; Khanavi, M.; Tofighi, Z. New Benzodioxole Compounds from the Root Extract of *Astrodaucuspersicus*. *Iran J. Pharm. Res.* **2016**, *15* (4), 901–906.
- (19) Deshpande, S. R.; Nagrale, S. N.; Patil, M. V.; Chavan, P. S. Novel 3,4-Methylenedioxybenzene Scaffold Incorporated 1,3,5-Trisubstituted-2-pyrazolines: Synthesis, Characterization and Evaluation for Chemotherapeutic Activity. *Indian J. Pharm. Sci.* **2015**, *77* (1), 24–33.
- (20) Huber, R.; Hockenjos, B.; Blum, H. E. DDB treatment of patients with chronic hepatitis. *Hepatology* **2004**, *39* (6), 1732–1733.
- (21) Moon, Y. S.; Choi, W. S.; Park, E. S.; Bae, I. K.; Choi, S. D.; Paek, O.; Kim, S. H.; Chun, H. S.; Lee, S. Antifungal and Antiaflatoxigenic Methylenedioxy-Containing Compounds and Piperine-Like Synthetic Compounds. *Toxins* **2016**, *8* (8), E240.
- (22) dos Santos Filho, J. M.; Moreira, D. R.; de Simone, C. A.; Ferreira, R. S.; McKerrow, J. H.; Meira, C. S.; Guimarães, E. T.; Soares, M. B. Optimization of anti-*Trypanosoma cruzi* oxadiazoles leads to identification of compounds with efficacy in infected mice. *Bioorg. Med. Chem.* **2012**, *20* (21), 6423–6433.
- (23) Mariz Gomes da Silva, L. M.; de Oliveira, J. F.; Silva, W. L.; da Silva, A. L.; de Almeida Junior, A. S. A.; Barbosa Dos Santos, V. H.; Alves, L. C.; Brayner Dos Santos, F. A.; Costa, V. M. A.; Aires, A. L.; de Lima, M. D. C. A. Albuquerque MCPA New 1,3-benzodioxole derivatives: Synthesis, evaluation of in vitro schistosomicidal activity and ultrastructural analysis. *Chem.-Biol. Interact.* **2018**, *283*, 20–29.
- (24) Baraldi, I.; Caselli, M.; Momicchioli, F.; Ponterini, G.; Vanossi, D. Dimerization of green sensitizing cyanines in solution. A spectroscopic and theoretical study of the bonding nature. *Chem. Phys.* **2002**, *275*, 149–165.
- (25) Caselli, M.; Latterini, L.; Ponterini, G. Consequences of H-dimerization on the photophysics and photochemistry of oxacarbo-cyanines. *Phys. Chem. Chem. Phys.* **2004**, *6*, 3857–3863.
- (26) Zheng, X.; Li, Z.; Podariu, M. I.; Hage, D. S. Determination of Rate Constants and Equilibrium Constants for Solution-Phase Drug-Protein Interactions by Ultrafast Affinity Extraction. *Anal. Chem.* **2014**, *86*, 6454–6460.
- (27) Tommasini, S.; Raneri, D.; Ficarra, R.; Calabro, M. L.; Stancanelli, R.; Ficarra, P. Improvement in solubility and dissolution of flavonoids by complexation rate with β -cyclodextrin. *J. Pharm. Biomed. Anal.* **2004**, *35*, 379–387.
- (28) Williams, A. C.; Camp, N. Product class 4: benzopyranones and benzopyranthiones. *Science of Synthesis* **2003**, *14*, 347–638.
- (29) Das, S.; Mitra, I.; Batuta, S.; Niharul Alam, M.; Roy, K.; Begum, N. A. Design, synthesis and exploring the quantitative structure-activity relationship of some antioxidant flavonoid analogues. *Bioorg. Med. Chem. Lett.* **2014**, *24* (21), 5050–5054.
- (30) Chang-yong, H.; Tae-sik, P.; Young-kwan, K.; Jin-ho, L.; Jong-hyun, K.; Dong-myung, K.; Ho-sun, S.; Sang-woong, K.; Eunice Eun-kyeong, K. Preparation of novel CDK inhibitors having flavone structure. PCT Int. Appl. WO 2000012496 A1 20000309, 2000.
- (31) Wu, B.; Morrow, J. K.; Singh, R.; Zhang, S.; Hu, M. Three-dimensional quantitative structure-activity relationship studies on UGT1A9-mediated 3-O-glucuronidation of natural flavonols using a pharmacophore-based comparative molecular field analysis model. *J. Pharmacol. Exp. Ther.* **2011**, *336* (2), 403–413.
- (32) Marathe, M. G.; Naik, V. G.; Gore, K. G. Benzopyrone series. VII. Synthesis of 3',4'-methylenedioxyflavones. *Journal of the University of Poona, Science and Technology* **1959**, *16*, 41–49.
- (33) Zhang, L.; Fourches, D.; Sedykh, A.; Zhu, H.; Golbraikh, A.; Ekins, S.; Clark, J.; Connelly, M. C.; Sigal, M.; Hodges, D.; Guiguemde, A.; Guy, R. K.; Tropsha, A. Discovery of novel antimalarial compounds enabled by QSAR-based virtual screening. *J. Chem. Inf. Model.* **2013**, *53* (2), 475–492.