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SAR Studies and Biological Characterization of a Chromen-4-one Derivative as an Anti-Trypanosoma brucei Agent / Borsari, Chiara; Santarem, Nuno; Macedo, Sara; Jiménez-Antón, María Dolores; Torrado, Juan J.; Olías-Molero, Ana Isabel; Corral, María J.; Tait, Annalisa; Ferrari, Stefania; Costantino, Luca; Luciani, Rosaria; Ponterini, Glauco; Gul, Sheraz; Kuzikov, Maria; Ellinger, Bernhard; Behrens, Birte; Reinshagen, Jeanette; Alunda, José María; Cordeiro-da-Silva, Anabela; Costi, Maria Paola. - In: ACS MEDICINAL CHEMISTRY LETTERS. - ISSN 1948-5875. - 10:4(2019), pp. 528-533. [10.1021/acsmmedchemlett.8b00565]

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

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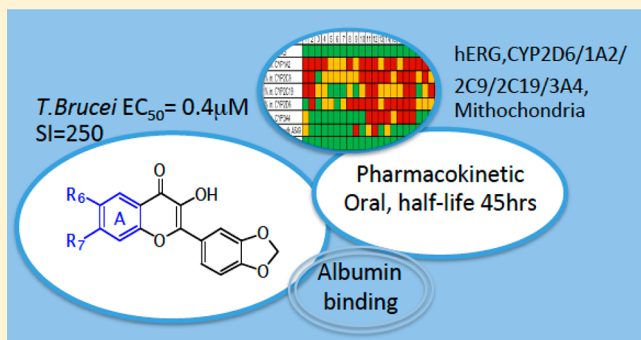
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Supporting Information

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

Special Issue: Highlighting Medicinal Chemistry in Italy

Received: November 21, 2018

Accepted: January 29, 2019

Published: January 29, 2019



discovery programs with the aim of identifying novel antileishmanial and antitrypanosomatid agents.^{11–15} Very 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

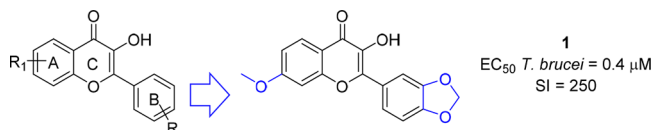


Figure 1. SAR studies on flavonol-like compounds and identification of compound **1**.

the most active and selective molecule toward *T. brucei* ($EC_{50} = 0.4 \mu M$, 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}

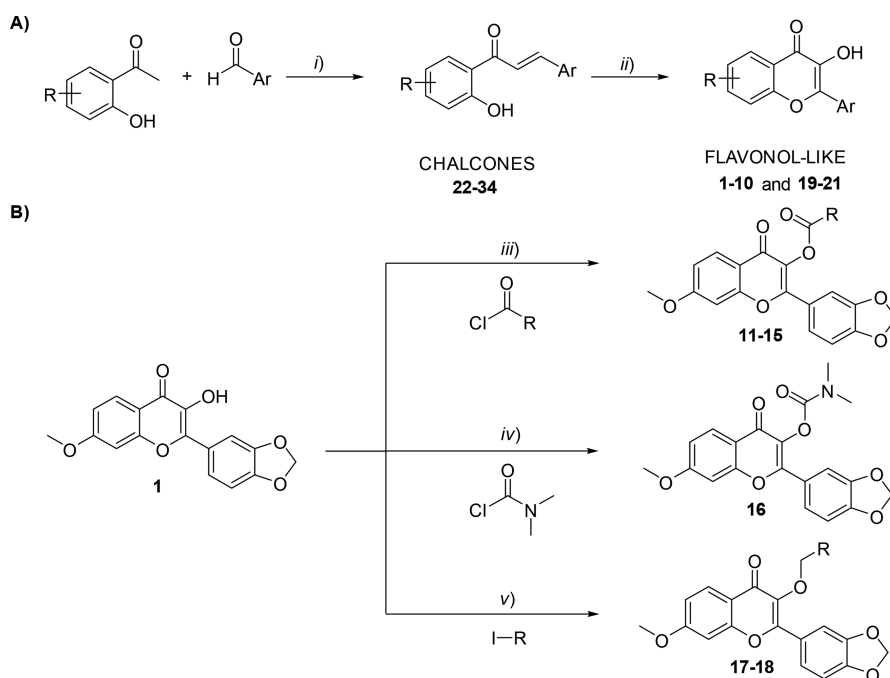
The aims of our study were to validate compound **1** through structure activity relationship (SAR) studies, discover follow-up hits, and characterize their biological profile for potential liabilities identifications. The synthetic procedure followed for the synthesis of the compounds (**1–21**) is 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 compounds (**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 $10 \mu M$ are reported in Table S1 of the Supporting Information.

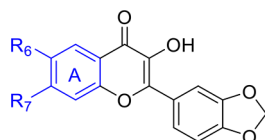
We started the SAR investigation of this scaffold by modifying the substituents on ring A (Table 1). Nine compounds (**2–10**) were synthesized introducing different substituents in position 6 and 7 of ring A. Five compounds (**2**, **4**, **8–10**) showed a significant activity toward *T. brucei* with EC_{50} lower than $5 \mu M$. 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**-

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 $80^\circ C$, 0.5 h.

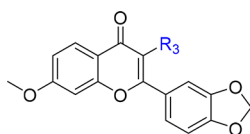
Table 1. SAR Study on Ring A of the Cromen-4-one Scaffold



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

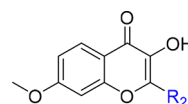
^aOnly estimations as the lower threshold of toxicity were not determined, EC₅₀ > 10 μM. The reference compound for *T. brucei* was pentamidine (IC₅₀ = 1.55 ± 0.24 nM). The synthesis of compounds 1,²⁸ 2,²⁹ 3,³⁰ 4,³¹ 5,³⁰ 6,³⁰ 7,³⁰ 8,³² and 9³³ has been already published in the literature. Compound 10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

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.

(EC₅₀ < 1.1 μM) together with a SI > 20. Among the esters, 130 the 3-pivaloyl derivative of compound 1 (13) showed the most 131 interesting profile with an EC₅₀ toward *T. brucei* of 1.1 μM and 132 SI > 92. On the contrary, the presence of a carbamate (16) or 133 an ether (17 and 18) led to inactivity toward *T. brucei*. These 134 data suggested that the hydroxyl group in position 3 should be 135 free in order to have a meaningful anti-*T. brucei* activity. The 136 activity of esters can be related to an easier hydrolysis with 137 respect to ethers and carbamates. We enlarged the SAR study 138 modifying the 1,3-benzodioxole ring of compound 1 139 (compounds 19–21, Table 3). Compound 19, with two 140 fluorine atoms instead of two hydrogens linked to the 141 dioxolane ring, was less active than the starting compound 1. 142 The anti-*T. brucei* activity decreased replacing the dioxolane 143 ring of 1 with a dioxane (compound 20), while it was 144 maintained in compound 21, bearing a tetrahydrofuran. 145 Compound 21 presented an EC₅₀ toward *T. brucei* equal to 146 3.1 μM, but SI = 8. Overall, six compounds (8, 11–15) 147 showed a low micromolar EC₅₀ and SI > 20. Compound 13, 148 the 3-pivaloyl derivative of 1, was the most selective among the 149 novel synthesized molecules. 150

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 μM . 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%.

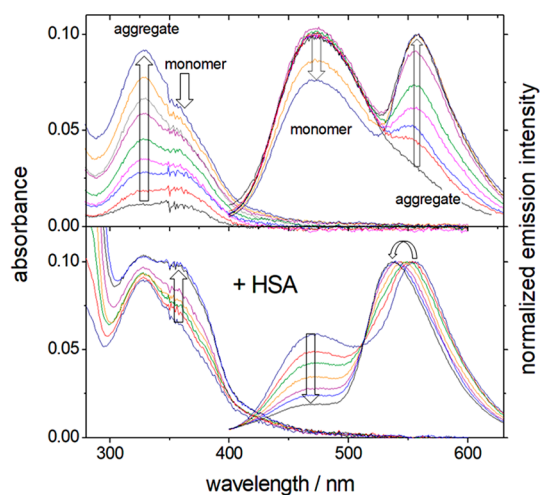


Figure 3. Absorption (left) and fluorescence emission spectra of compound 1 in phosphate buffer at pH 8 in the absence (top) and in the presence of human serum albumin (HSA). Top: effect of increasing concentration of compound 1: 1.25, 2.5, 3.75, 5, 6.25, 7.5, 8.75, 10, 11.25 μM . Bottom: the arrows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

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 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 by 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, $2.5 (\pm 1) \times 10^5 \text{ M}^{-1}$. 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 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 that govern BBB penetration. Compound 1 fulfills the requirements

Table 4. Pharmacokinetic Parameters of Compound 1

Comp.	Dose (mg) and route	Cmax (ng/mL)	Cmax (μM)	Tmax (h)	AUCtot (ng/mL h)	AUCtot (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

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) 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. 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/acsmedchemlett.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)

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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 Trypanosomatidic 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-tox; 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

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