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Development of novel 1,4-benzodiazepine-based Michael acceptors as antitrypanosomal agents

Roberta Ettari [a], Santo Previti [a], Sandro Cosconati [b], Santina Maiorana [a], Tanja Schirmeister [c], Silvana Grasso [a], Maria Zappalà [a,*]

[a] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Annunziata, 98168 Messina, Italy
[b] DiSTABiF, Second University of Naples, Via Vivaldi 43, 81100 Caserta, Italy
[c] Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, 55099 Mainz, Germany

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ABSTRACT

Novel 1,4-benzodiazepines, endowed with a Michael acceptor moiety, were designed taking advantage of a computational prediction of their pharmacokinetic parameters. Among all the synthesized derivatives, we identified a new lead compound (i.e., **4a**), bearing a vinyl ketone warhead and endowed with a promising antitrypanosomal activity against *Trypanosoma brucei brucei* (IC_{50} = 5.29 μM), coupled with a lack of cytotoxicity towards mammalian cells (TC_{50} >100 μM).

Human African Trypanosomiasis (HAT or sleeping sickness) is a parasitic disease of sub-Saharan Africa, caused by two subspecies of protozoa of *Trypanosoma* genus: *T. brucei gambiense* and *T. brucei rhodesiense*.[1] The disease is transmitted by the bite of tsetse fly (*Glossina* genus), with an estimated number of actual cases of 30,000 each year.[2] *T. b. gambiense*, responsible for the 95% of HAT cases, is widespread in central and western Africa and induces a chronic form of the disease. On the contrary, *T. b. rhodesiense* causes a rapid-onset, higher mortality rate, acute form of trypanosomiasis and it is very common in southern and eastern Africa.[3] Both parasitic subspecies produce two main stages of HAT. Stage 1 (also named hemolymphatic stage) initiates after the tsetse fly bite and can persist several weeks. During this stage, the parasite initially lives within the bloodstream and then migrates to the lymph nodes, spleen, and spinal fluid, causing symptoms like rash, fever, muscle aches, and fatigue. If left untreated, stage 1 HAT evolves into the neurological stage (stage 2 HAT), during which the parasite crosses the blood brain barrier (BBB), leading to serious mental deterioration, sleep disturbances, coma and death.[4] Unfortunately, an effective vaccine has not been developed yet, because of the high degree of antigenic variation expressed by the glycoprotein forming their surface coat.[5,6]

Currently, chemotherapy is the sole approach to treat and control the infection. Current HAT therapy is based only on four drugs: suramin and pentamidine are active only on the stage 1 HAT. On the contrary, melarsoprol and eflornithine, which are able to cross BBB, show different drawbacks: melarsoprol, a trivalent arsenical, is very toxic and causes encephalopathy in 5–10% of treated patients,[7] on the contrary, eflornithine, which is safe to administer, lacks of broad-spectrum activity[8,9] being inactive against *T. b. rhodesiense* and its mode of action is trypanostatic rather than trypanocidal.[10] Nifurtimox a 5-nitrofuran derivative with antifungal properties, licensed for use only in Argentina and Germany, is another effective compound which was first used for the treatment of Chagas disease, caused by *T. cruzi*,[11] and is orally bioavailable, unlike other trypanocidal drugs that are parenterally administered. The combination therapy of nifurtimox with eflornithine, is actually the first-line treatment for stage 2 HAT caused by *T. b. gambiense*[12] or an option for melarsoprol-refractory stage 2 HAT caused by *T. b. rhodesiense*.

In this context, in the last decade our research group has been actively involved in the development of several non-peptidic[13] or peptidomimetic[14–16] parasitic cysteine protease inhibitors as antimalarial and antitrypanosomal agents. Among all the synthesized inhibitors, one of the most promising antitrypanosomal agent is represented by the Michael acceptor **1**,[14e] (Fig. 1) characterized by the presence of a 1,4-benzodiazepine (BDZ) nucleus as

* Corresponding author. Tel.: +39 090 6766411; fax: +39 090 6766402.
E-mail address: mzappala@unime.it (M. Zappalà).

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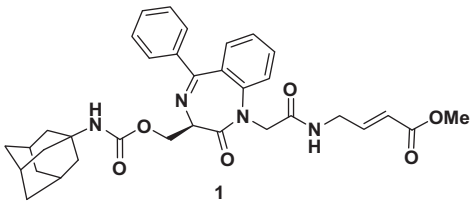

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Figure 1. Structure of lead compound **1**.

recognition moiety and by a hydroxymethyl group at C3 of the scaffold, used to tie an adamantyl nucleus able to create additional interactions with the S3 pocket of the target enzyme. Ester **1** is a potent rhodesian inhibitor (K_i = 2.6 nM), endowed with a good antitrypanosomal activity (IC_{50} = 4.8 μM).[17] While previous lead optimization efforts were instrumental to optimize the activity profile of this inhibitor, however, they were not helpful in enhancing its putative pharmacokinetic properties. Most probably, its high molecular weight is one of the most evident structural feature that should hamper a possible development of ester **1** as chemotherapeutic agent, together with its high *c*log*P* that is a bigger challenge to address.

Starting from these considerations, and with the aim to improve its physicochemical properties, thus obtaining new drug-like antiparasitic agents, better matching the Lipinski's Rule of 5 (RO5), we designed novel simplified 1,4-benzodiazepines, endowed with a Michael acceptor portion (i.e., **2**–**4**). The idea to develop novel 1,4-benzodiazepin-2-ones as antitrypanosomal agents is strongly supported also by literature evidences, that highlighted their good activity against *T. b. brucei*.[18]

The present Letter reports the design, synthesis and the biological evaluation against *T. b. brucei* of the new synthesized compounds **2**–**4**.

To design new analogues of compound **1** we first decided to take advantage of the computational prediction of its pharmacokinetic (PK) parameters. To this end, we employed the QikProp software (QikProp, version 3.4 (2011); Schrödinger, LLC, New York, NY). In addition to predicting molecular properties, QikProp provides ranges for comparing each compound's property with those of 95% of known drugs. Results of this analysis demonstrated that, among all the predicted physicochemical parameters, the one that is falling outside the prescribed limits of drug-likeness is the aqueous solubility. In particular, compound **1** is predicted to have a log*S* of −9.6, indicating an almost null solubility in water solution and consequently no absorption in vivo.

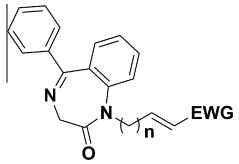
Therefore, we decided to start from our lead compound **1** and to attain a set of structural simplification aimed at optimizing its PK parameters, while maintaining its antiparasitic activity. In this context, we decided to eliminate the methyl carbamoyl side chain, appended to C3 position of the benzodiazepine nucleus, in order to increase the water solubility. Then, we replaced the amide bond with aliphatic chains of different lengths (2–4 carbon atoms) between the BDZ scaffold and the Michael acceptor, in order to stabilize new compounds to proteolysis. Moreover, we also decided to explore different reactive species by substituting the Michael acceptor with α,β-unsaturated esters (**2**), nitriles (**3**) and ketones (**4**) (Table 1).

The design step led to a small library of nine compounds for which preliminary QikProp calculations were attained and compared with the parent lead **1**. Table 1 reports structures of the designed compounds along with the most relevant parameters calculated by QikProp. Indeed, analysis of these data outlines that, apart from compound **3c** and the lead compound **1**, all the other ligands should be active at the central nervous system (CNS), more water soluble and have a high oral absorption. Thus, encouraged by these preliminary predictions we embarked into the synthesis of all the designed derivatives.

The synthesis of Michael acceptors **2a**–**c**, **3a**–**c** and **4a**–**c** has been carried out starting from compounds **5**–**7**, which have been obtained in agreement to our previously reported procedure.[16a]

To introduce the Michael acceptor portion, a dry CH_2Cl_2 solution of **5**–**7** was treated with the required cross-metathesis (CM) partners **8**–**10**, in the presence of the second generation Hoveyda–Grubbs catalyst (Scheme 1). The resulting mixture was heated in a microwave reactor at 100 °C for 2 h to afford the CM products in high yields (76–92%).

Table 1
Structures of the designed compounds along with their calculated QikProp parameters



Compounds	*n*	EWG	Calculated QikProp parameters							
			CNS[a]	Log Po/w[b]	Log*S*[c]	Log HERG[d]	PCaco[e]	Log BB[f]	pMDCK[g]	HOA[h]
1			−2	5.9	−9.2	−7.3	427.8	−1.6	197.6	1
2a	2	COOMe	−1	3.6	−5.1	−6.3	763.3	−0.9	369.5	3
2b	3	COOMe	−1	4.0	−5.6	−6.5	762.9	−1.0	369.3	3
2c	4	COOMe	−2	4.4	−6.0	−6.6	762.9	−1.1	369.2	3
3a	2	CN	−1	3.2	−5.3	−6.0	665.0	−0.9	318.3	3
3b	3	CN	−1	3.6	−5.7	−6.2	664.7	−1.0	318.2	3
3c	4	CN	−2	4.0	−6.2	−6.4	664.6	−1.1	318.1	1
4a	2	COMe	−1	3.5	−4.7	−6.1	1013.5	−0.7	501.9	3
4b	3	COMe	−1	3.9	−5.1	−6.3	1012.3	−0.8	501.3	3
4c	4	COMe	−1	4.3	−5.6	−6.5	1012.6	−0.9	501.4	3

[a] Predicted central nervous system activity on a −2 (inactive) to +2 (active) scale.
[b] Predicted octanol/water partition coefficient.
[c] Predicted aqueous solubility, Log*S*. *S* in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.
[d] Predicted IC_{50} value for blockage of HERG K^+ channels.
[e] Predicted apparent Caco-2 cell permeability in nm/s. Caco2 cells are a model for the gut–blood barrier.
[f] Predicted brain/blood partition coefficient.
[g] Predicted brain/blood partition coefficient. MDCK cells are considered to be a good mimic for the blood–brain barrier.
[h] Predicted qualitative human oral absorption: 1, 2, or 3 for low, medium or high.