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Synthesis and SAR studies of a new trypanosome alternative oxidase inhibitors: imidazoline and benzamidine derivatives

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IQM Instituto de Química Médica

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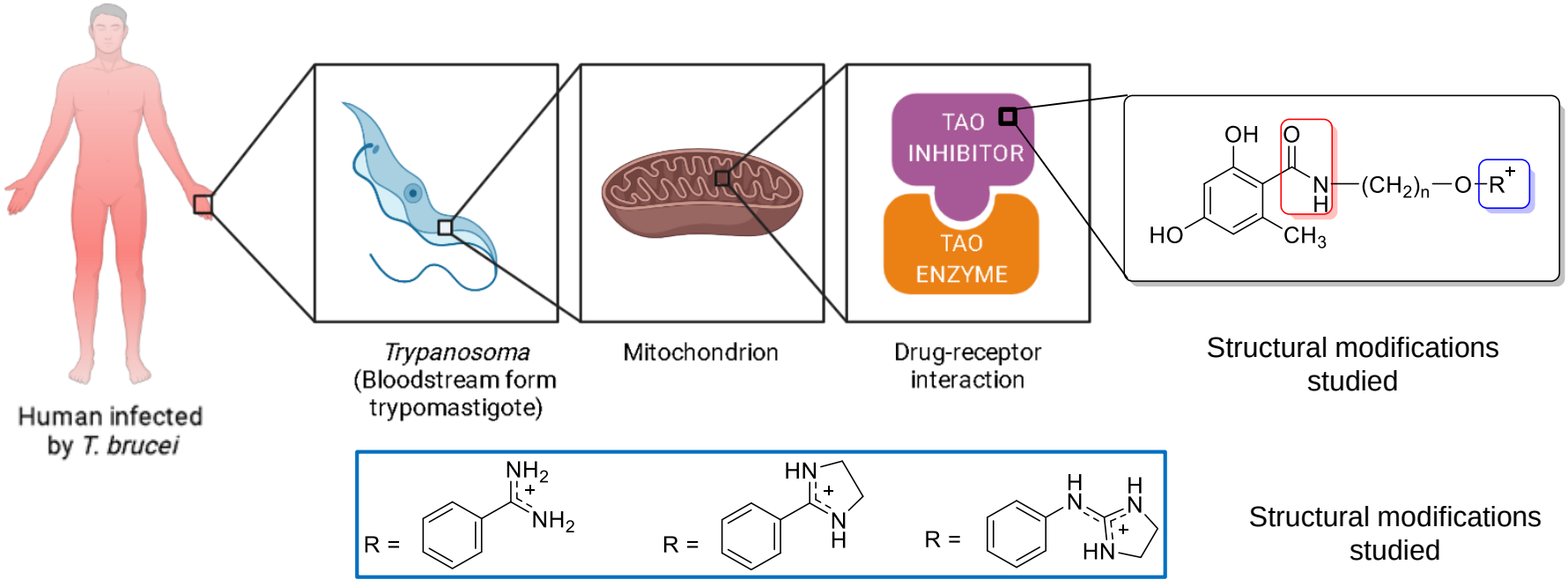


Abstract:

Sleeping sickness or human African trypanosomiasis (HAT), is a vector-borne parasitic disease. It is caused by infection with protozoan parasites belonging to the genus *Trypanosoma* (*T. brucei* sp.). Without treatment, the disease is usually fatal.

Our research of a novel and effective chemotherapy of HAT is based on the inhibition of the Trypanosome Alternative Oxidase (TAO). TAO is essential for the respiration of bloodstream form trypomastigotes because it is the only enzyme available to re-oxidize the NADH produced during glycolysis. This enzyme, which is conserved among trypanosome subspecies and has no counterpart in mammalian cells, is a validated drug target against trypanosomes.

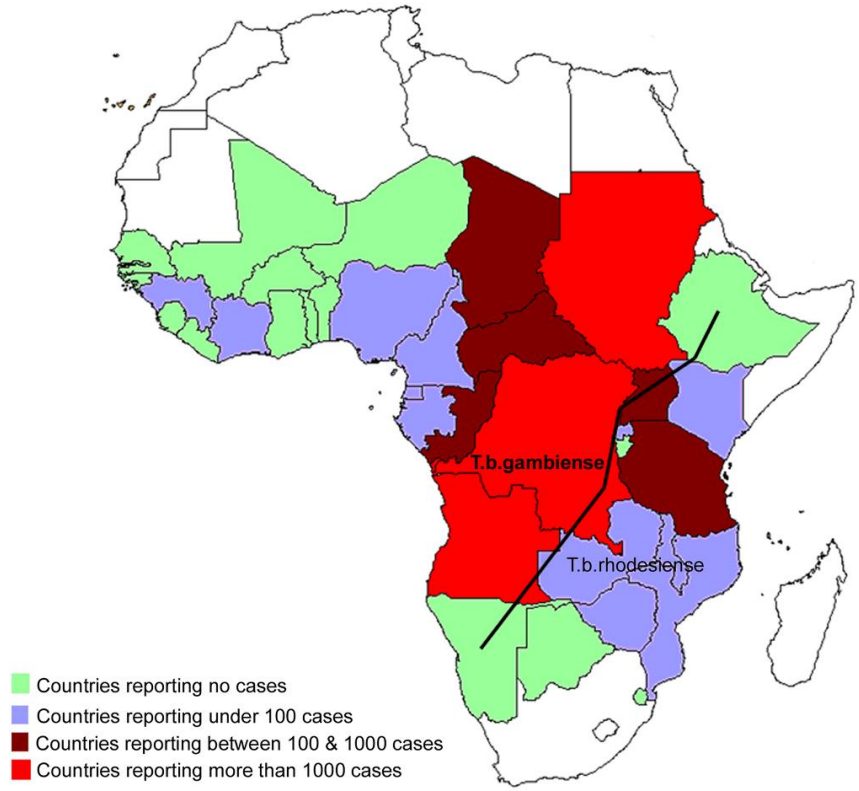
In previous studies, the structure – activity relationships (SAR) of different TAO inhibitors derived from 4-hydroxybenzoate and 4-alkoxybenzaldehyde was investigated. These compounds were shown to exhibit TAO inhibitory activity at the nanomolar level, showing trypanocidal activity in in vitro and in vivo assays.

In the current study, new analogs have been synthesized with the aim of extending the SAR studies of TAO inhibitors. In this case, the ester bond has been replaced by an amide bond, which is more metabolically stable. In addition, new cationic groups such as benzamidinium, 2-phenylimidazolin-3-ium and 2-(phenylamino)imidazolin-3-ium cations have been incorporated.

Keywords: Trypanosome alternative oxidase (TAO) inhibitor; Trypanosoma brucei; benzamidine; imidazoline; structure – activity relationships (SAR).

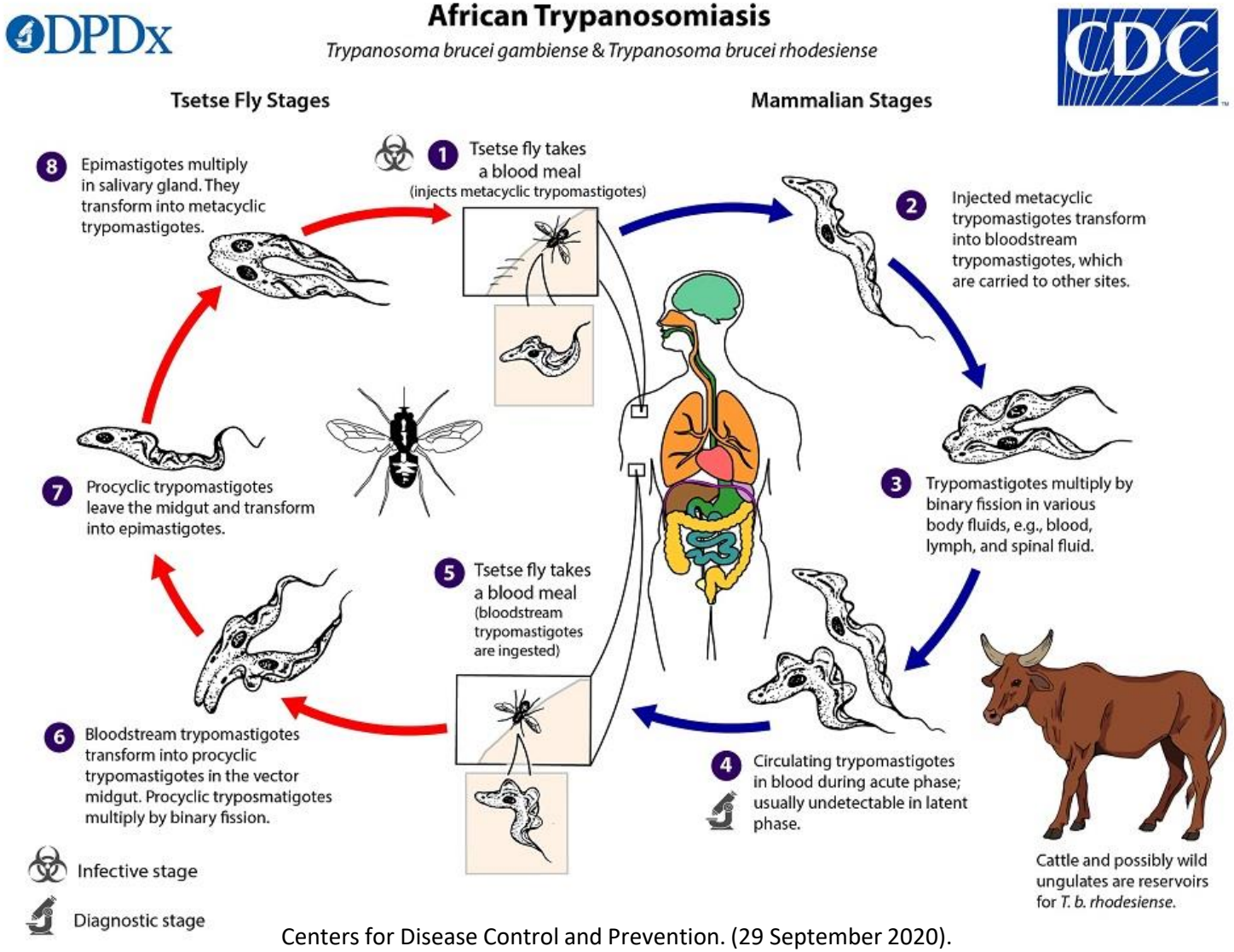
Introduction. Human African trypanosomiasis (HAT)

- Better known as **sleeping sickness**
- Parasitic disease
  - *T. brucei gambiense* (95% of cases)
  - *T. brucei rhodesiense* (<5% of cases)
- Transmitted by infected **Tsetse flies**
- 36 sub-Saharan African countries at risk



Fèvre et al. *PLOS Neglected Tropical Diseases. Rev.* **2008,**2,2.

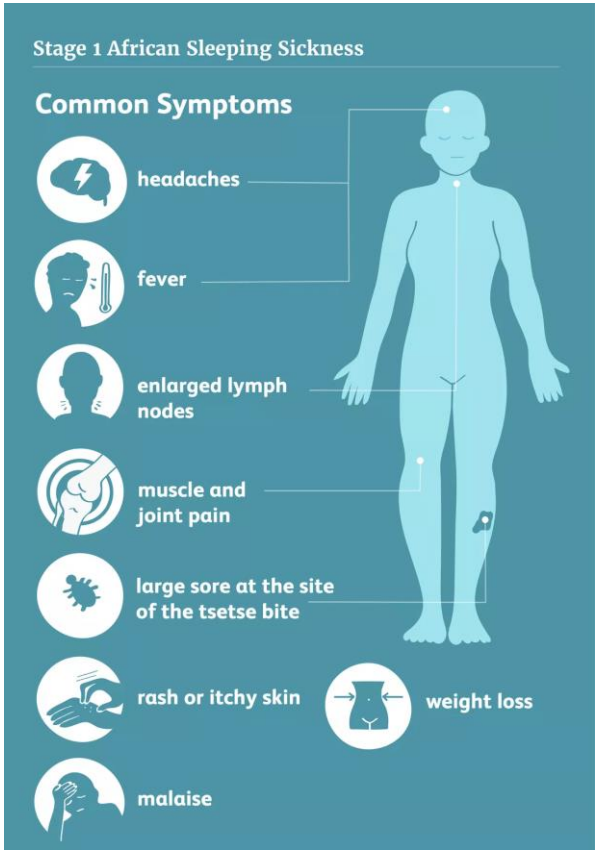
World Health Organisation. (18 May 2021). Trypanosomiasis, human African (sleeping sickness). https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)



Centers for Disease Control and Prevention. (29 September 2020). https://www.cdc.gov/parasites/sleepingsickness/

Diagnosis and stages of sleeping sickness

- Diagnosis by **lymph node aspirate**. Early diagnosis is **difficult** due to the non-specific signs and symptoms.
- 2 stages:
  - **Stage 1** = Haemolymphatic stage. Nonspecific symptoms.
  - **Stage 2** = Meningoencephalic stage (300–500 days after infection). Invasion of de CNS → neuropsychiatric manifestations, coma, death.



Correll, R. An Overview of African Sleeping Sickness. Verywellhealth, **2020** https://www.verywellhealth.com/african-sleeping-sickness-overview-4590129

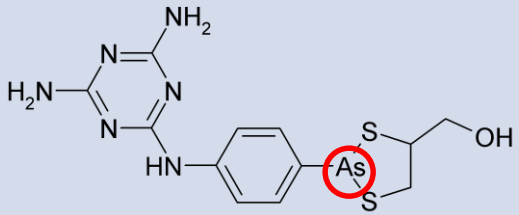
Current treatments

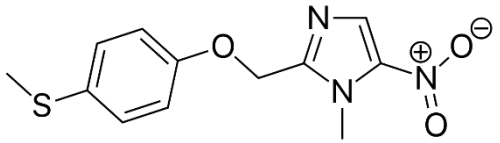
T. b. gambiense

- 1st stage: Pentamidine
- 2nd stage: nifurtimox-eflornithine combination therapy (NECT)
- 1st and 2nd stages: **fexinidazole**

T. b. rhodesiense

- 1st stage: Suramine
- 2nd stage: melarsoprol (highly toxic arsenical drug)





The 2019 WHO interim guidelines for the treatment of human West African trypanosomiasis recommends **FEXINIDAZOLE** (ORAL) for the treatment of gambiense trypanosomiasis

Trypanosome alternative oxidase (TAO) a validated drug target

African trypanosomes adapt their energy metabolism depending on substrate availability

Procyclic form	**Bloodstream** form (BSF)

• Present in the tsetse fly
• **Fully functional cytochrome-dependent respiratory chain**
	• Present in mammals (i.e. human)
	• Use the **glycolysis as main source of ATP**
	  • No cytochrome respiratory pathway
	  • No oxidative phosphorylation

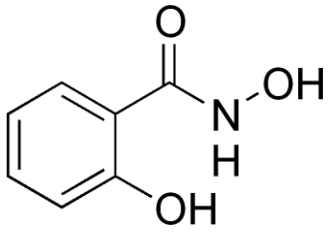
Menzies et al. *Parasitology* **2018**, *145*, 175-183.

Clarkson et al. *J. Biol. Chem.* **1989**, *264*, 17770-17776.

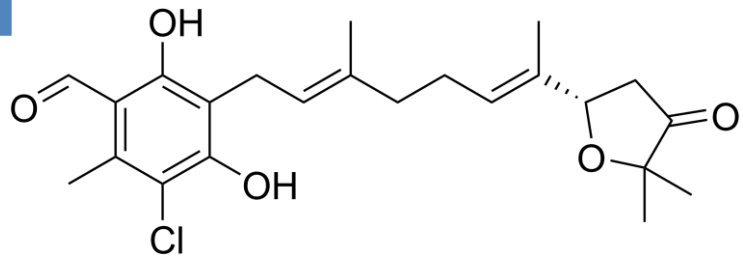
TAO is a validated target of trypanosomes:

Essential for viability os BSF trypanosomes	**Expressed** in all subespecies
Absent in mammals (selectivity)	Sensitive to **specific** inhibitors

TAO inhibitors are active in **mouse models**



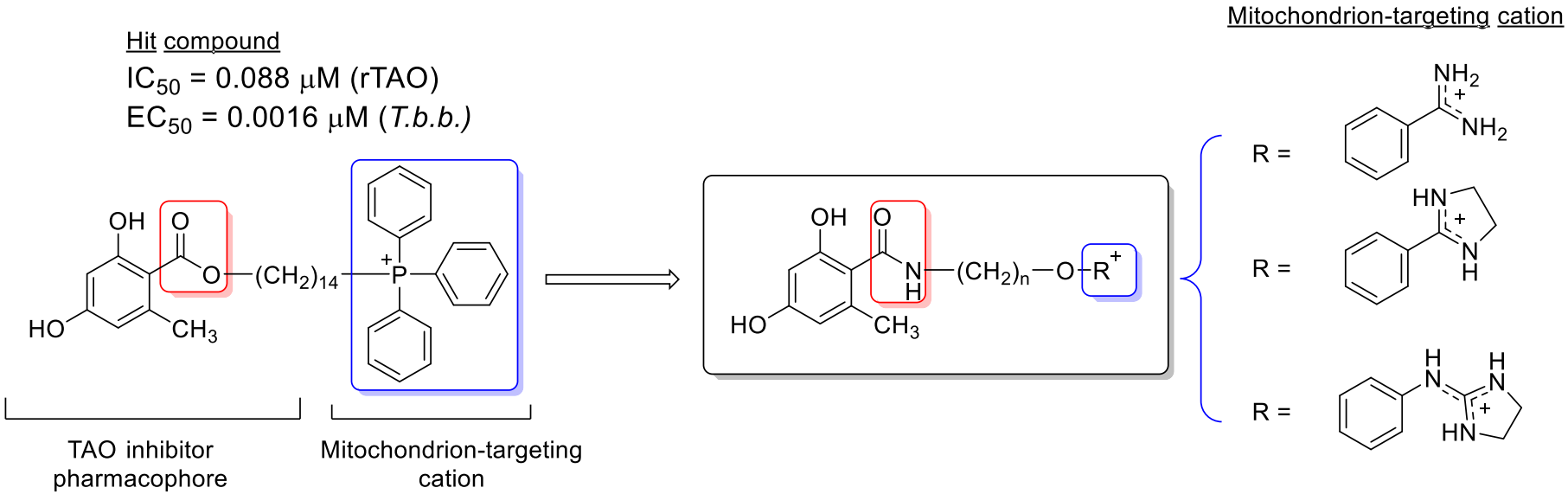
Salicylhydroxamic acid (SHAM)



Ascofuranone

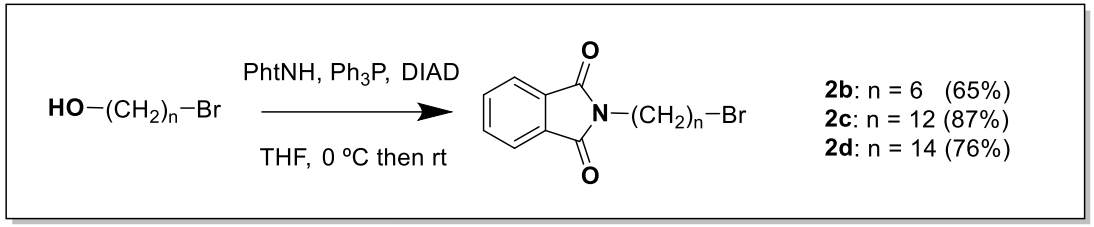
Minu et al. *Trends Parasitol.* **2006**, *22*, 484-491.

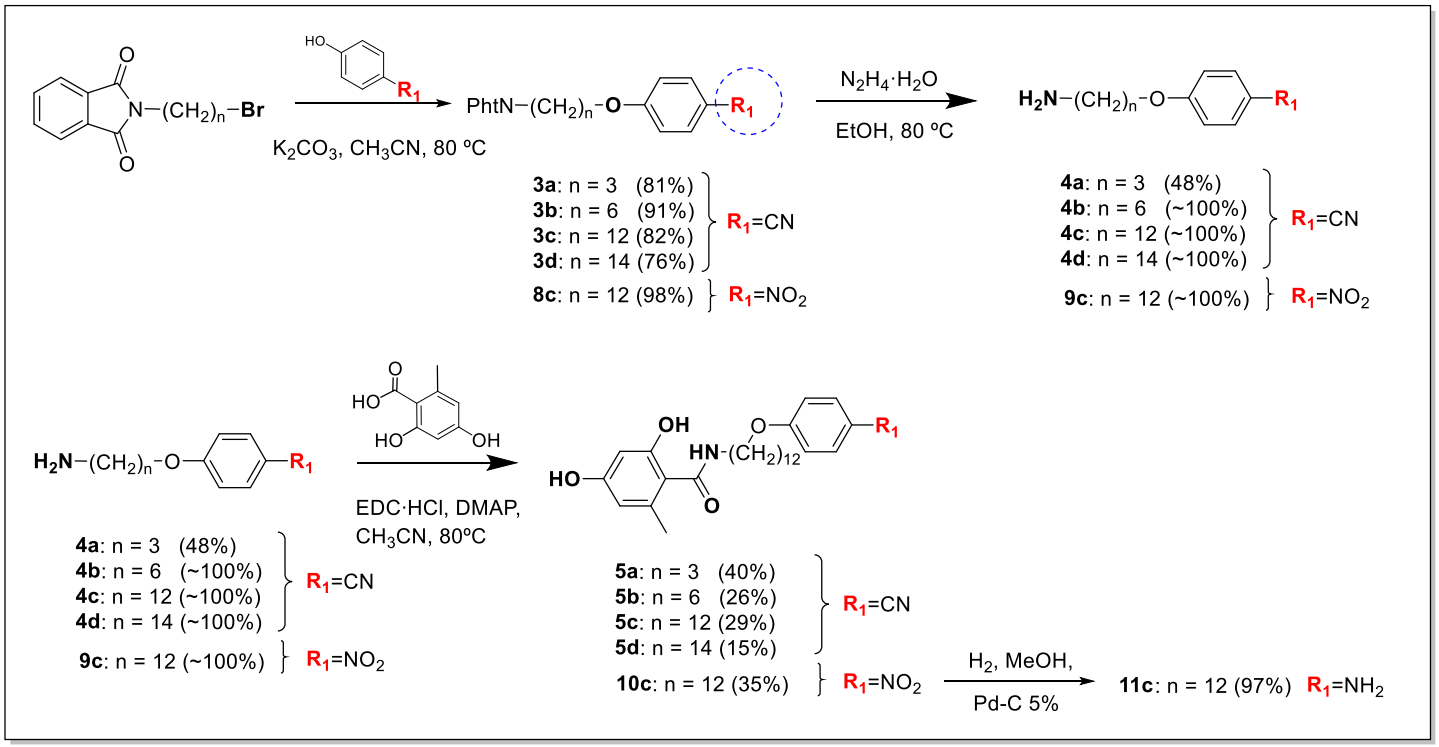
Objective: synthesis of benzamidine and imidazoline TAO inhibitors



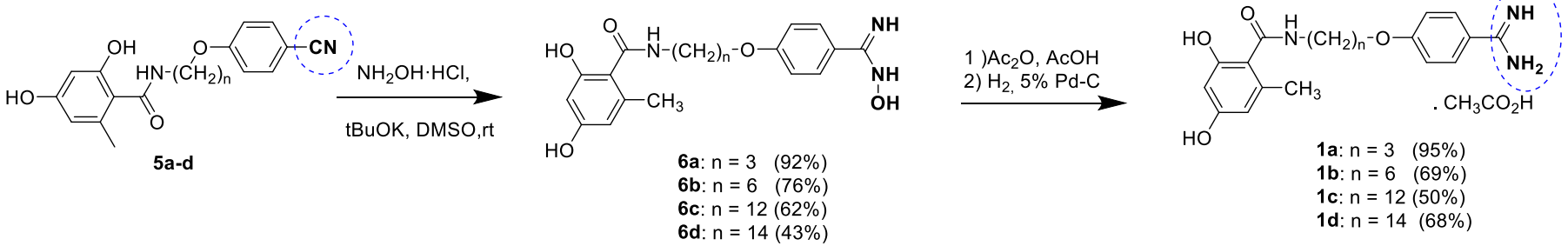
Ebiloma et al. *Eur. J. Med. Chem.* **2018**, *150*, 385-402.

Synthesis of precursors





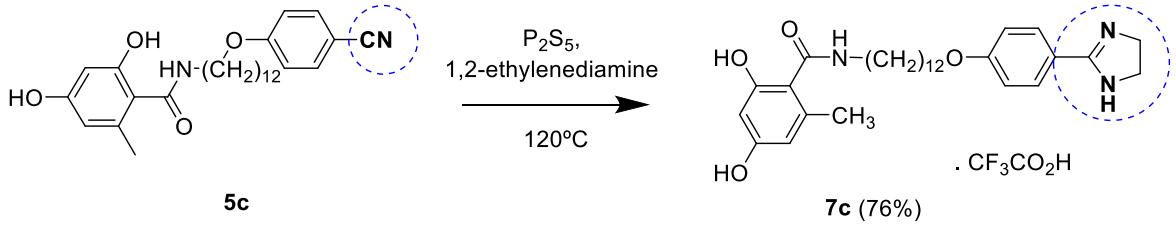
Target compound: Benzamidine (1a-1d)



6a: n = 3 (92%)
6b: n = 6 (76%)
6c: n = 12 (62%)
6d: n = 14 (43%)

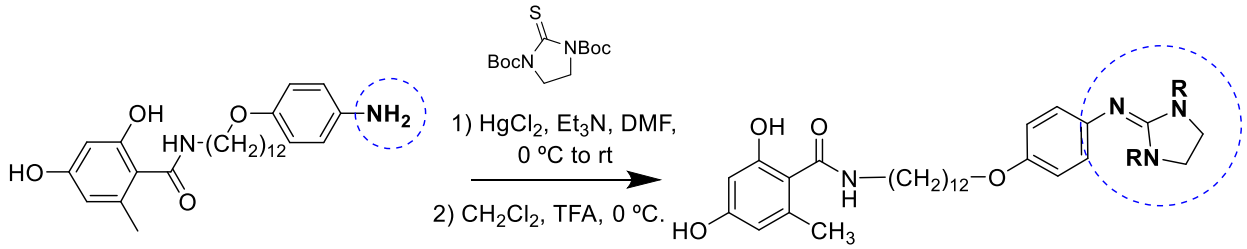
1a: n = 3 (95%)
1b: n = 6 (69%)
1c: n = 12 (50%)
1d: n = 14 (68%)

Target compound: Imidazoline (7c)



7c (76%)

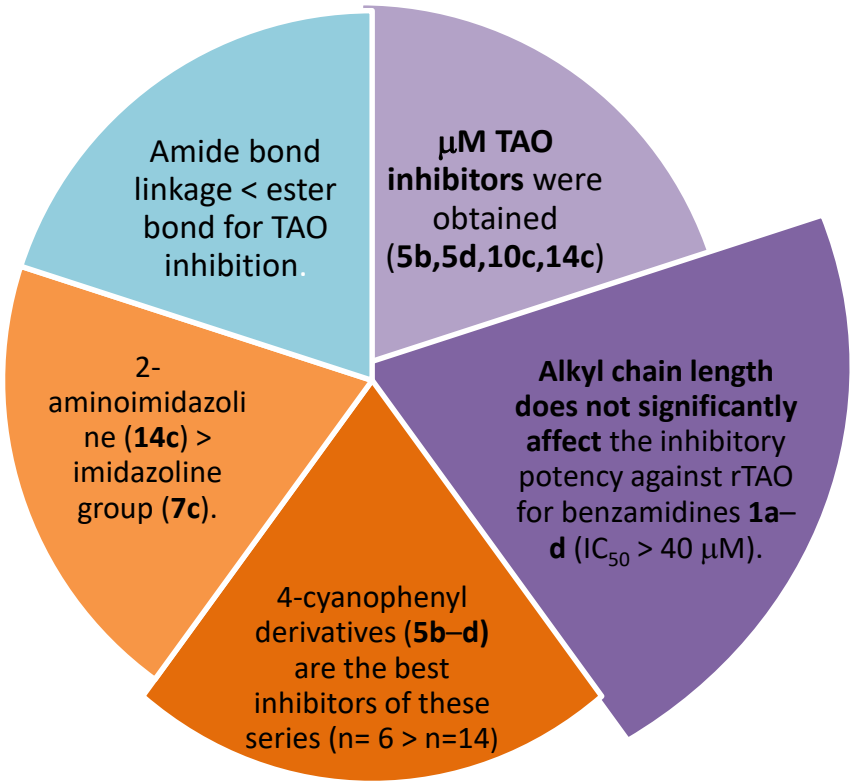
Target compound: Aminoimidazoline (14c)



13c: R = Boc (88%)
14c: R = H (CF_3CO_2H salt) (88%)

Inhibition of recombinant TAO enzyme (rTAO)

Compound	n	rTAO %Inhibition	rTAO IC_{50} (μM)
1a	3	48% (40 μM)	-
1b	6	12,5% (40 μM)	-
1c	12	39,3% (40 μM)	-
1d	14	31,4% (40 μM)	-
5a	3	-	>5
5b	6	-	1,52 ± 0.09
5c	12	27,4% (40 μM)	-
5d	14	89,7% (40 μM)	16,4 ± 0.7
6a	3	14,1% (40 μM)	-
6b	6	-14,9% (40 μM)	-
6c	12	44,8% (40 μM)	-
6d	14	-3,4% (40 μM)	-
7c		10,0% (10 μM)	
10c		53,8% (10 μM)	30,0 ± 1.5
14c		46,3% (10 μM)	22,5 ± 0.3
Ascofuranone		100%	0,002



In vitro activity against *T. b. brucei*

Compound series	n	*T. b. brucei* s427 (WT) EC_{50} (μM)	Cytotoxicity Human cells CC_{50} (μM)
1a	3	>100	>200
1b	6	15.5 ± 0.6	>200
1c	12	3.3 ± 0.2	43.5 ± 5.4
1d	14	18.6 ± 1.1	>200
5a	3	37.2 ± 3.4	>200
5b	6	30.4±0.4	>200
5c	12	15.6 ± 0.7	57.1 ± 0.1
5d	14	14.8 ± 0.5	56.8 ± 0.2
6a	3	>100	>200
6b	6	19.5 ± 1.0	76.8 ± 7.2
6c	12	8.4 ± 1.1	108.9 ± 1.6
6d	14	29.0 ± 2.2	>200
Pentamidine	-	0.0038 ± 0.0004	-
Diminazene	-	0.095 ± 0.011	-
Phenylarsine oxide	-	0.001	0.29

Conclusions

- We have synthesized and characterized a series of amido compounds derived from 2,4-dihydroxy-6-methylbenzoic scaffold with different methylene linker lengths (n = 3, 6, 12, 14) between the TAO pharmacophore and the cationic group.

- Structure – activity relationships (SAR) showed that the substitution of the ester bond defined in previous studies[4,6,7] by an amide bond **does not improve** TAO inhibition nor in vitro activity against *T. brucei.*

Acknowledgments

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