In vitro Anti-Trypanosomal Activities of Indanone-Based Chalcones

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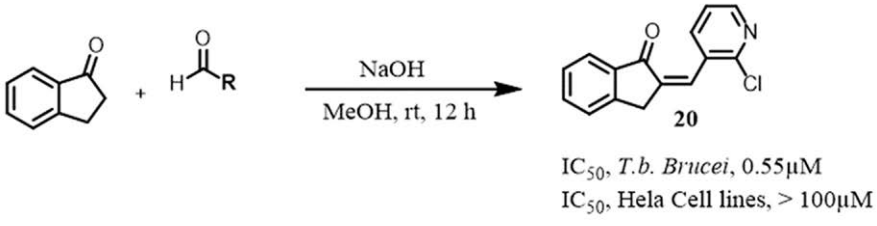
ABSTRACT

Human African trypanosomiasis is a neglected infectious disease that affects mostly people living in the rural areas of Africa. Current treatment options are limited to just four drugs that have been in use of four to nine decades. The life-threatening toxic side-effects associated with the use of these drugs are disconcerting. Poor efficacy, low oral bioavailability, and high cost are other shortcomings of current HAT treatments. Evaluating the potentials of known hits for other therapeutic areas may be a fast and convenient method to discover new hit compounds against alternative targets. A library of 34 known indanone based chalcones was screened against T.b. brucei and nine potent hits, having IC_{50} values between 0.5–8.9 µM, were found. The SAR studies of this series could provide useful information in guiding future exploration of this class of compounds in search of more potent, safe, and low cost anti-trypanosomal agents. ▶ **Graphical Abstract**.



▶ **Graphical Abstract**

Introduction

Human African trypanosomiasis (HAT), a neglected infectious disease that is endemic to sub-Saharan Africa [1], arises from the bite of an infected tsetse fly [2]. It is caused by two sub-species of Trypanosoma brucei, namely T.b. gambiense and T.b rhodesiense[3]. The foregoing sub-species differ in their geographical preference and pathogenicity. T.b. gambiense is solely common in west Africa and causes a chronic form of HAT [4, 5], while T.b rhodesiense is endemic to east Africa and causes an acute form of HAT [6, 7]. Both forms of the disease are fatal if not treated [8].

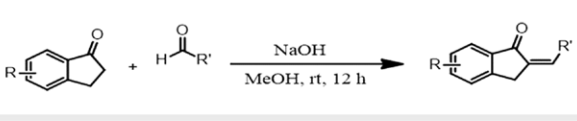
Although tremendous success against HAT has been achieved so far, with fewer deaths attributed to it [1], it's still a public health concern with 2184 new cases reported in 2016 [9] and 60 million

people currently at risk of contracting the disease [10] if effective control measures are lacking. It is important to note that being a neglected disease consigned to the rural areas of sub-Saharan Africa where disease surveillance is challenged by several factors [11], there exists a possibility that the burden of HAT is under-reported.

Control and treatment of HAT relies solely on four chemotherapeutic agents discovered 40 or more years ago: pentamidine, suramin, malarsorprol, and eflornithine [12]. The foregoing drugs are expensive and all suffer from poor oral bioavailability and hence must be administered intravenously [13]. This is not an ideal route of administration for a neglected disease, considering the correlate that it requires special facilities and expertise not common in disease stricken areas. Moreover, these drugs cause life threatening side effects such as hyperglycaemia, bone marrow toxicity, hypoglycaemia, and in some cases death [14]. There have also been issues of treatment failure due the emergence of parasites resistant to current treatment [15].

The aforementioned drawbacks associated with current treatment underscore the need for alternative chemotherapeutic agents against HAT.

In search of cheap anti-trypanosomal agents, we decided to subject our library of synthetic cyclic chalcone analogues to screening against T.b. brucei.



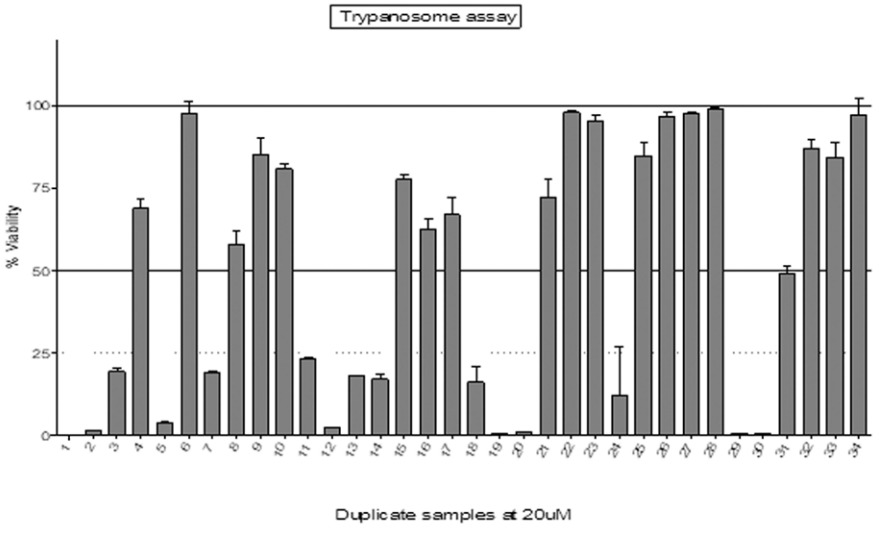
▶ **Fig. 1** Synthesis of target compounds [a]. [a]Reagents and conditions: NaOH, MeOH, rt, 12 h.

Chalcones are notable in that they are easy to synthesise [16]. Moreover, the therapeutic potentials of chalcones cannot be over emphasised as compounds containing this scaffold have been published for their anti-bacterial, anti-viral and anti-malarial potentials [17–19]. These observations suggest that the chalcone class of compounds is a potential source for the discovery of cheap and potent anti-infectives.

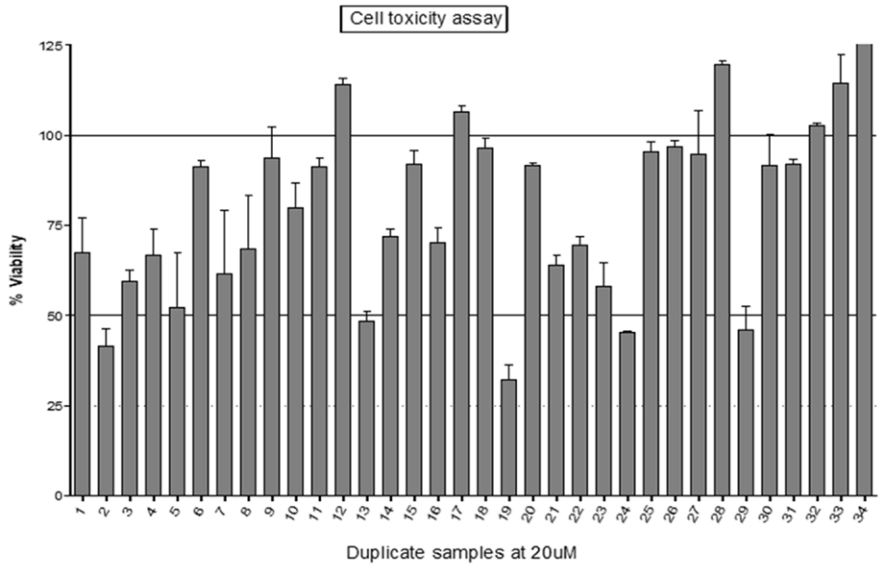
We previously synthesised a series of indanone based chalcone analogues and evaluated their inhibitory activities against recombinant human monoamine oxidase [20]. We were further interested in evaluating the anti-trypanosomal activities of this series. We herein report potent trypanocidal compounds of the indanone-based chalcone class.

The synthesis and characterisation of compounds employed in this study have been previously reported [19, 21] and is depicted in ▶ **Fig. 1** below. Briefly, 6/5-hydroxyl-1-indanone, 5-methoxyl-1-indanone, and 1-indanone were reacted with appropriate aromatic aldehydes using acidic or basic methanol as solvent. The compounds were precipitated out of water and recrystallised from ethanol.

Compounds (1–34) were subjected to in vitro screening against the 427 strain of T.b. brucei to evaluate their anti-trypanosomal activities. After an incubation period of 48 h, the numbers of parasites surviving drug exposure were determined by adding resazurin. Conversion of resazurin to resorufin by living cells was quantified in a multiwell fluorescence plate reader (Exc_{560}/Em_{590}). Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. Results are expressed as percentage (%) viability – the resorufin fluorescence in compound-treated wells relative to untreated controls. Screening was performed at 20 µM final concentration of synthesized compounds and pentamidine as the reference drug. At 20 µM, almost half of the series inhibited parasite viability to below 25 % (▶ **Fig. 2**).



▶ **Fig. 2** Growth inhibitory effect of cyclic chalcones analogues on trypanosome parasites at 20 µM. At this concentration, 15 compounds inhibited parasite viability to below 25 %, which translates to a hit rate of almost 50 %.



▶ **Fig. 3** Cytotoxicity potential of compounds evaluated against HeLa cell lines at 20 µM.

Compounds were further screened in vitro against a human cervix adenocarcinoma (HeLa) cell line to assess their potential cytotoxicities. Screening was performed in duplicate using 20 µM stock solutions of synthesised compounds and emetine as the reference drug, employing resazurin as a cell viability indicator as was done in the trypanosome assay. Compounds resulting in less than 50 % Hela cell viability at 20 µM were assumed to have potential cytotoxicities and hence were not taken further for IC_{50} determination (▶ **Fig. 3**).

Based on Hela cell viability result obtained, 9 out of the 15 compounds inhibiting parasite viability to below 25 % had little effect on HeLa cell viability (> 50 % cell viability at 20 µM). The observation posits that these compounds possess intrinsic anti-trypanosomal activities and pose reduced cytotoxicity risk. These promising compounds were subjected to IC_{50} determination against T.b. brucei and the results are summarised in ▶ **Table 1**.

In comparison with compound **1** (IC_{50}, 3.0 µM), structure activities relationship (SAR) analysis suggest that the presence of an –OH group at position -5 of ring **A** with concurrent attachment of a halogen to ring **B** promotes anti-trypanosomal activities. This is evident in compound **3** for example, which has a lower IC_{50} value of 1.9 µM. We also observed that the substitution pattern on ring **B** greatly influences activities. For instance, the substitution of a bromine atom in compound **7** (IC_{50}, 2.5 µM) with a nitrile unit as is the case in compound **11** leads to almost a three-fold loss in activity (IC_{50}, 8.9 µM). This suggests that electron donating groups attached to ring **B** tend to favour activity over electron withdrawing groups. Also, SAR comparison between compound **5** (IC_{50}, 2.6 µM) and compound **12** (IC_{50}, 0.93 µM) shows clearly that anti-trypanosomal activities increase with increased halogenation of ring **B**. This remark is also evident when comparing compounds **18** (IC_{50}, 3.2 µM), which lacks a chlorine atom on ring **B** and its analogue compound **20** (IC_{50}, 0.55 µM) having a chlorine atom on ring **B.**

The nature of ring **B** also influences anti-trypanosomal activities. Compounds wherein ring **B** is a pyridyl unit elicited potent anti-trypanosomal activities (e. g.**20;** IC_{50}, 0.55 µM), which in most cases were superior to those of phenyl analogues (e. g.**5;** IC_{50}, 2.6 µM). Comparing compound **18** (IC_{50}, 3.2 µM) and **21** ($IC_{50} \geq 200$ µM) suggests that substituting a pyridyl, or phenyl unit with a 5 membered heteroaromatic ring leads to compounds devoid of anti-trypanosomal activities.

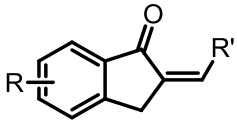
Conclusion

Human African trypanosomiasis is a neglected infectious disease that affects mostly people living in the rural areas of Africa. Current treatment options are limited to just four drugs that have been in use for more than four decades. The life-threatening toxic side-effects associated with the use of these drugs are alarming. Other shortcomings of current HAT treatments include poor efficacy, low oral bioavailability and high cost. All of these factors necessitate the search for new trypanocidal agents.

Being a neglected disease that affects mostly the poorest communities in sub-Saharan Africa, an ideal anti-trypanosomal agent should be relatively cheap. To this end, we decided to investigate the anti-trypanosomal potential of indanone based chalcone analogues. This compound class is cheap to obtain as it is synthesised from readily available starting materials in a simple single synthetic transformation. The anti-trypanosomal activities of this series were established by screening against T.b. brucei. Nine potent hits, all having no cytotoxic effect on a HeLa cell line (>50 % cell viability at 20 µM), were discovered.

It is noteworthy to highlight that the physicohemical properties of a hit compound such as molecular weight, lipophilicity, total polar surface area increase during hit optimization and often lead to compounds with improved activities against the target in question, but poor lead-like properties. In an attempt to avoid poor lead-like properties at a later

▶ **Table 1** Structures, percent viability of parasite cells and IC_{50} values of the most active and non-toxic compounds in vitro.



	Compounds					%viability (IC_{50} µM)
Entry	**R**	**R′(B)**	**MW**	**ClogP**	**tPSA**	***T.b. brucei***
1	-	phenyl	220	3.5	17.1	−0.25(**3.0**)
2	6-OH	phenyl	236	3.4	37.3	1.5 (ND)
3	5-OH	4-F-phenyl	254	3.6	37.3	19.2(**1.9**)
4	5-OH	4-Cl-phenyl	270	3.6	37.3	68.6(ND)
5	5-OH	3-Cl-phenyl	270	4.1	37.3	3.6(**2.6**)
6	5-OH	4-Br-phenyl	315	4.3	37.3	97.6(ND)
7	5-OH	3-Br-phenyl	315	4.3	37.3	18.9(**2.5**)
8	5-OH	4-CH_3-phenyl	250	3.9	37.3	57.6(ND)
9	5-OH	3-CH_3-phenyl	250	3.9	37.3	84.9(ND)
10	5-OH	4-CN-phenyl	261	2.9	61.1	80.6(ND)
11	5-OH	3-CN-phenyl	261	2.9	61.1	23.0(**8.9**)
12	5-OH	3,4-di-Cl-phenyl	305	4.7	37.3	2.0(**0.93**)
13	5-OH	4-OH-phenyl	252	2.8	57.5	18.0(**2.9**)
14	5-OH	3-OH-phenyl	252	2.8	57.5	16.9(ND)
15	5-OH	4-OCH_3-phenyl	266	3.3	46.5	77.4(ND)
16	5-OH	4-$N(CH_3)_2$-phenyl	279	3.6	40.5	62.4(ND)
17	5-OH	4-$CH(CH_3)_2$-phenyl	278	4.8	37.3	66.8(ND)
18	-	3-pyridyl	221	1.9	29.4	16.0(**3.2**)
19	-	2-pyridyl	221	1.9	29.4	0.35(ND)
20	-	2-Cl-3-pyridyl	255	2.7	29.4	0.69(**0.55**)
21	-	2-furanyl	210	2.7	26.3	72.2(ND)
22	-	2-thiophenyl	226	3.2	17.1	97.8(ND)
23	-	2-pyrroyl	209	2.2	29.1	95.3(ND)
24	-	3-thiophenyl	226	3.2	17.1	12.1(ND)
25	-	4-$N(CH_3)_2$-phenyl	263	3.7	20.3	84.5(ND)
26	5-OCH_3	phenyl	250	3.8	26.3	96.6(ND)
27	5-OCH_3	4-$N(CH_3)_2$-phenyl	293	3.9	29.5	97.3(ND)
28	5-OCH_3	cyclohexyl	256	5.2	26.3	99.1(ND)
29	5-OCH_3	2-pyridyl	251	2.3	38.6	0.31(ND)
30	5-OCH_3	3-pyridyl	251	22.3	38.6	0.48(ND)
31	5-OCH_3	2-furanyl	240	2.9	35.5	49.1(ND)
32	5-OCH_3	2-thiophenyl	256	3.4	26.3	86.9(ND)
33	5-OCH_3	5-CH_3-2-furanyl	254	3.4	35.5	84.2(ND)
34	5-OCH_3	5-Br-2-furanyl	319	3.8	35.5	97.0(ND)
PE	-	-	–	–	–	(**0.0095**)

ND, not determined; PE, pentamidine

stage of drug discovery, it has been widely recommended that lead-like hits be prioritised as starting point for hit optimization. Lead-like hits are compounds having molecular weight ≤350, ClogP≤4.2 and tPSA≤90 Å^2. Property profiling of our hit series (▶ **Table 1**) shows that they meet the criteria set out for lead-like hits, making them worth considering for further hit optimization studies.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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