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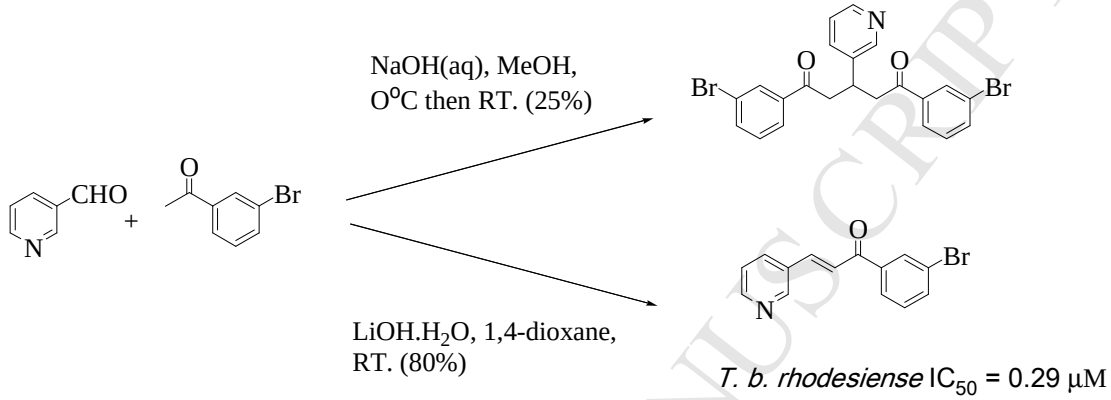

T. b. rhodesiense IC_{50} = 0.29 μM

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ABSTRACT

A library of novel pyridylchalcones were synthesised and screened against *Trypanosoma brucei rhodesiense*. Eight were shown to have good activity with the most potent **8** having an IC_{50} value of 0.29 μM. Cytotoxicity testing with human KB cells showed a good selectivity profile for this compound with a selectivity index of 47. Little activity was seen when the library was tested against *Leishmania donovani*. In conclusion, pyridylchalcones are promising leads in the development of novel compounds for the treatment of human African trypanosomiasis (HAT).

Keywords: Neglected tropical disease; *Trypanosoma brucei rhodesiense; Leishmania donovani;* pyridylchalcone; Claisen-Schmidt.

1. Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a parasitic disease transmitted by the bite of tsetse flies. It occurs in 36 countries in sub-Saharan Africa, where millions of people are at risk of infection [1-3]. HAT is caused by 2 sub-species of *Trypanosoma brucei* and exists as a chronic infection with *T. b. gambiense* or as an acute infection with *T. b. rhodesiense*. Untreated, both forms are usually fatal [1-3]. There are two clinical stages in the progression of HAT. The first corresponds to the multiplication of the parasites in the blood and lymphatic system. The second occurs when these cross the blood-brain barrier to infect the central nervous system causing neurological symptoms to appear. Stage 1 can be treated with pentamidine and suramin whereas melarsoprol, eflornithine, and their combinations with nifurtimox are the only treatments for stage 2. All of these drugs suffer from serious drawbacks including low efficacy, severe toxic side effects and the need for parenteral administration [1-3]. With no vaccine available and limited therapeutic alternatives the continuing emergence of drug resistance is seen as a major public health problem [4]. An urgent need exists for the development of more effective, less toxic and orally available treatments for this neglected tropical disease.

Chalcones (1,3-diphenyl-2-propen-1-ones) are a major class of natural products often found in edible plants. They have received considerable attention due to their wide range of biological actions and have been used as a scaffold in the development of anticancer, antioxidant, antiangiogenic, and anti-inflammatory agents [5]. A number of natural and synthetic chalcones have been shown to possess anti-parasitic activities including licochalcone A **1**, a natural prenylated chalcone isolated from liquorice root (*Glycyrrhiza glabra*), and a potent anti-leishmanial agent [6]. However, the therapeutic use of chalcones has been limited due to their poor bioavailability and rapid metabolic clearance from biological systems [7]. Nevertheless, hybrid molecules incorporating the chalcone moiety are emerging as promising leads for targeting *Leishmania* and *Trypanosoma* species [8-11]. For example, the chalcone-benzoxaborole hybrid **2** gave 100% mouse survival rate and complete elimination of parasites in a *T. b. brucei* murine model [9].

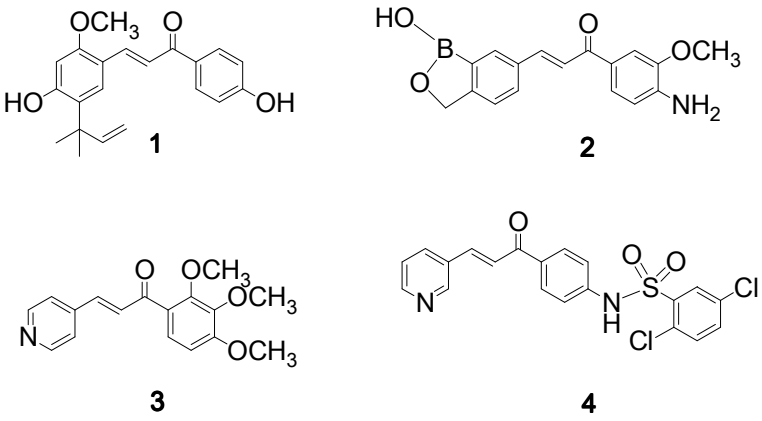


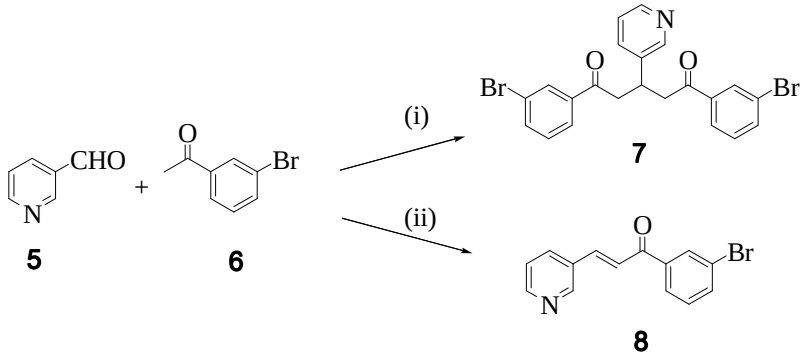
Fig. 1. Anti-parasitic chalcones

Pyridylchalcones, such as **3** and **4**, have been found to exhibit both antimalarial and anti-leishmanial activity [6, 11, 12, 13]. However, there are no examples of them being tested against *Trypanosoma brucei*. With the antitrypanosomal activity of chalcone hybrids in mind [8-11], we were intrigued to determine whether pyridylchalcones would also be active against this target. It was anticipated that pyridyl analogues would exhibit the same low toxicity shown by compounds containing the chalcone nucleus but with improved bioavailability [14]. The introduction of a pyridine group will reduce the lipophilicity of the molecule and increase water solubility, both of which should ease formulation. In addition, lowering the lipophilicty of a chalcone and the inclusion of a heterocyclic A-ring has been associated with an increase in anti-parasitic activity [15].

We report here the synthesis and *in vitro* evaluation of a series of novel pyridylchalcones against *T. b. rhodesiense* and *L. donovani*. Their cytotoxicity against human KB cells was also determined to establish their selectivity profile.

2. Chemistry

Attempts to prepare the 3-(3-pyridyl)chalcone **8** using standard Claisen-Schmidt conditions with 2 equivalents of aqueous sodium hydroxide in methanol yielded an unexpected result [16]. Instead of the desired chalcone, recrystallization from ethanol afforded the diketone **7** in 25% yield (Scheme 1). This product presumably results from conjugate addition of the enolate of 3-bromoacetophenone to the previously formed chalcone. Similar results were obtained when using 3-chloroacetophenone and 4-chloroacetophenone. To our knowledge this unusual reactivity has not previously been reported for 3-pyridinecarboxaldehyde **5**,



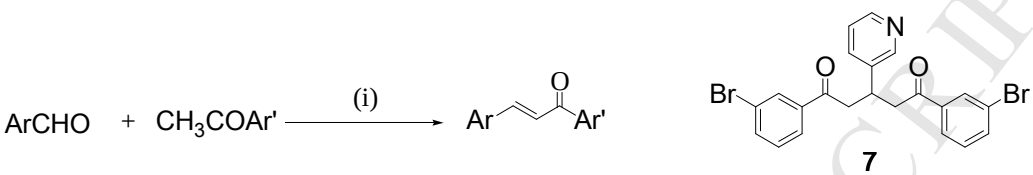
Scheme 1. Reagents and conditions: (i) NaOH(aq), MeOH, 0°C then RT (25%). (ii) LiOH.H_2O, 1,4-dioxane, RT (80%).

although it has been observed with both 2-pyridinecarboxaldehyde and 2-quinolinecarboxaldehyde when reacted with acetophenone under similar conditions [17]. The addition of one equivalent of pyridine to these reactions was found to prevent this conjugate addition. This was thought to be due to the pyridine competing with the aldolate nitrogen for chelation of the metal ion in the intermediate. Unfortunately, this result was not repeated when one equivalent of pyridine was added to our reaction.

Bhagat *et al* report good yields of chalcone with all three isomers of pyridinecarboxaldehyde when reacted with 4-methoxyacetophenone and 10% lithium hydroxide monohydrate in ethanol [18]. Using these conditions, we obtained **8** in a disappointing yield of 39%. It was clear from TLC that **7** was still being formed during this reaction. This proved difficult to remove by recrystallization from ethanol, and column chromatography was required to obtain pure **8**. In a bid to improve yields and simplify purification we investigated the use of potassium hydroxide as a catalyst in 1,4-dioxane. This couple has been found to give good yields of quinoline-2-one based chalcones which had proved difficult to prepare using other conditions [19]. However, with 1.6 equivalents of potassium hydroxide we again obtained a mixture of **8** and **7**. Maintaining 1, 4-dioxane as the solvent but changing the base to lithium hydroxide monohydarate proved the solution to our problems with **8** being obtained in 80% yield after recrystallization from ethanol. TLC showed little or no diketone formation. This method proved suitable for the synthesis of a range of pyridylchalcones (Table 1) in high yield.

3. Results and discussion

The activity of our chalcones against *T. b. rhodesiense* (STIB900) was determined using the Alamar blue growth inhibition assay [20]. Their cytotoxicity against human KB cells was also obtained *in vitro* (Table 1) allowing the calculation of a selectivity index (SI) for each compound where: SI = IC_{50} (KB cells)/ IC_{50} (T. b. r). Of the 17 compounds synthesised, 8 showed good activity against *T. b. rhodesiense* (IC_{50} <0.2 mg/ mL), 8 moderate activity (IC_{50} 0.2-3 mg/ mL) and 1 was inactive (IC_{50} > 3 mg/ mL). The most active compound, with an IC_{50} value of 0.29 µM, was **8** which contained a 3-pyridyl A-ring and a 3-bromophenyl B-



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Scheme 2. Reagents and conditions: (i) $LiOH.H_2O$, 1,4-dioxane, RT.

Table 1. Antitrypanosomal activity and cytotoxicity of synthesized chalcones

N°	*Ar (A-ring)*	*Ar' (B-ring)*	*IC_{50} / µM*		*SI*[b]
			T. b. rhodesiense STIB900[a]	*Cytotoxicity KB Cells*	
7	–	–	12.2	181	15
8	3-Py-	3-Br-Ph-	0.29	13.5	47
9	Ph-	3-Br-Ph-	0.96	18.4	19
10	3-Py-	Ph-	0.72	7.7	11
11	3-Py-	3-MeO-Ph-	0.41	2.3	6
12	3-Py-	4-Br-Ph-	0.34	7.9	23
13	3-Py-	2-Br-Ph-	37.8	>300	>8
14	3-Py-	2-MeO-Ph-	1.14	22.7	20
15	3-Py-	4-MeO-Ph-	0.94	21.7	23
16	3-Py-	3,4-$(MeO)_2$-Ph-	0.98	22.8	24
17	4-Py-	3-Br-Ph-	0.65	23.7	36
18	2-Py-	3-Br-Ph-	0.40	14.7	37
19	3-Br-Ph-	3-Py-	0.69	43.7	63
20	3-Py-	3-Cl-Ph-	0.62	12.7	21
21	3-Py-	3,4-(OCH_2O)-Ph-	4.83	59.4	12
22	3-Py-	3,4-F_2-Ph-	0.72	20.7	29
23	3-Py-	3-HO-Ph-	1.15	21.7	19
24	3-Py-	3-Py-	1.02	17.0	17
	Pentamidine		0.011	–	–
	Melarsoprol		0.005	–	–
	Podophyllotoxin		–	0.058	–

[a] Values are the mean of two independent experiments in triplicate.
[b] Selectivity index (SI) = IC_{50} KB cells/ IC_{50} *T. b. rhodesiense* STIB900.

ring. This compound also showed good selectivity with an SI of 47. Although it was difficult to establish a clear structure activity relationship, certain trends were apparent. The presence of a pyridyl A-ring appears to be important for high activity. When this was replaced by a phenyl, as in **9**, the activity dropped to 0.96 μM. The position of the nitrogen in the pyridine ring does not seem to be as crucial with the 2-pyridyl derivative **18** still having reasonable activity (IC_{50} = 0.40 μM). The 4-pyridyl derivative **17** had a lower IC_{50} value of 0.65 μM but was still considered active. Modifying the B-ring while keeping a 3-pyridyl A-ring can have a significant effect on activity. This was particularly apparent in **13** and **14** which both have substituents at the 2-position in the B-ring. The latter with a methoxy group shows only moderate activity with an IC_{50} value of 1.14 μM, while the former with a bromo is our only inactive compound. Moving the bromo group in **8** to the para position produced a slight reduction in activity with **12** having an IC_{50} of 0.34 μM. A more significant change was evident in **20** where the bromo group in **8** was replaced by a chloro resulting in a doubling of the IC_{50} value to 0.62 μM. With a methoxy group at the meta position in ring B, **11** showed reasonable activity (IC_{50} 0.41 μM) but had a relatively low SI of 6. Moving the methoxy group to the para position significantly reduced activity with **15** having an IC_{50} of 0.94 μM. The only other derivatives with an electron releasing group at this position, **16** (3,4-dimethoxy) and **21** (3,4-methylenedioxy), both exhibited only moderate activity suggesting that electron releasing groups in the B-ring are not conducive to high activity. The phenyl derivative **10** and its 3, 4-difluoro analogue **22** were both active with the same IC_{50} value of 0.72 μM. The phenolic chalcone **23** had moderate activity with an IC_{50} value of 1.15 μM. Taken in conjunction with our other results this could suggest that high activity is favoured by a hydrophobic group at the 3-position in the B-ring. A 3-pyridyl group in the B-ring is not optimum for activity with **19** and **24** giving only moderate IC_{50} values of 0.69 and 1.02 μM respectively. The diketone **7** was found to be inactive.

In view of the literature precedent [11, 12] and our above results with *T. b. rhodesiense* we decided to screen our compound library against *Leishmania donovani* [21]. Using HU3 intracellular amastigotes at 30 μM only **8**, **9**, **12** and **20** showed any activity, with 39.7, 13.8, 11.6 and 2.7% inhibition respectively. At 10 μM no inhibition was observed. Thus, although a number of our derivatives showed promise as potential antitrypanosomal agents they appear to have little activity against *Leishmania*.

4. Conclusion

Initial attempts to prepare 3-pyridylchalcones using standard Claisen-Schmidt conditions with sodium hydroxide were unsuccessful resulting in the formation of a diketone. This problem was overcome by the use of lithium hydroxide monohydrate as base in 1, 4-dioxane. Of the 17 derivatives synthesised eight were shown to have good activity against *T. b. rhodesiense* with the most active **8** having an IC_{50} value of 0.29 μM. This compound showed good selectivity with an SI of 47. Little activity was observed when the library was evaluated against *L. donovani*. In conclusion, pyridylchalcones are promising leads in the development of novel compounds for the treatment of HAT.

5. Experimental section

5.1 Synthetic chemistry

5.1.1 General

All solvents and chemicals were used as purchased without further purification. The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance AV400 NMR spectrometer at 30°C. Chemical shifts are reported in δ units (ppm) relative to either TMS or the residual solvent signal. IR spectra were recorded as KBr discs on a Perkin-Elmer 298 spectrophotometer. HRMS was performed using a Thermo Scientific LTQ Orbitrap XL at the EPSRC UK National Mass Spectrometry Facility at Swansea University. Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus in open glass capillary tubes. TLC was performed on Merck Silica Gel $60F_{254}$ coated plates. Plates were visualised under UV light (254/366nm) and stained with either 2, 4-dinitrophenylhydrazine, iodine or phosphomolybdic acid. Fluka silica gel 60 (30-45μ) was used for flash chromatography. Elemental analyses (C, H, N) was carried out by Warwick Analytical Services using a CE440 elemental analyser. Results were within ±0.4% of the theoretical values.

5.1.2 1, 5-Di(3-bromophenyl)-3-(3-pyridyl)pentane-1,5-dione (**7**)

Sodium hydroxide solution (0.8 mL, 50% w/v, 10.00 mmol) was added to a stirred and cooled (ice bath) solution of 3-bromoacetophenone (0.40 mL, 5.00 mmol) in ethanol (2.5 mL). 3-Pyridinecarboxylate (0.47 mL, 5.00 mmol) was added and stirring continued for 2 h

before allowing the reaction to warm to RT. On the formation of a cream precipitate the reaction was quenched with water (30 mL) and the solid removed by filtration. After washing with water the solid was recrystallized from ethanol affording white crystals (0.305 g, 25%). mp 152-154^{o}C; ^{1}H NMR ($CDCl_3$): δ 3.32 (2H, dd, *J* = 6.6, 16.7 Hz), 3.54 (2H, dd, *J* = 6.6, 16.7 Hz), 4.08 (1H, quin, *J* = 6.6 Hz), 7.15-7.26 (2H, m), 7.36 (2H, t, *J* = 8.3, Hz), 7.60-7.70 (2H, m), 7.86 (2H, d, *J* = 8.3 Hz), 8.07 (2H, s), 8.48 (1H, d, *J* = 1.2 Hz), 8.59 (1H, s); ^{13}C NMR ($CDCl_3$) δ 34.2, 44.2, 123.1, 123.5, 126.6, 130.3, 131.2, 135.4 136.3, 138.3, 138.9, 148.4, 149.2, 196.4; IR (KBr) /cm^{-1} 1678 (C=O); HRMS found $[M+1]^+$ 485.9686, $C_{22}H_{17}Br_2NO_2$ requires $[M+1]^+$ 485.9699; Anal. Calcd $C_{22}H_{17}Br_2NO_2$: C, 54.24; H, 3.52, N, 2.88; Found C, 54.08; H, 3.54; N, 2.91.

5.1.3 General method for chalcone synthesis

Lithium hydroxide monohydrate (0.63 g, 14.95 mmol) was added to a stirred solution of the appropriate aldehyde (9.35 mmol) and ketone (9.35 mmol) in 1,4-dioxane (2.5 mL) at RT. Upon completion, as indicated by the formation of a precipitate and confirmed by TLC the reaction was quenched with water (30 mL). The resulting mixture was extracted with ethyl acetate (3x30 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$) and the solvent removed under vacuum. The crude product was purified by either recrystallization from ethanol or flash chromatography on silica gel.

*5.1.4 (E)-1-(3-Bromophenyl)-3-(3-pyridinyl)prop-2-en-1-one (**8**)*

Recrystallization from ethanol. Pale yellow crystals (80%). mp 126-128^{o}C; ^{1}H NMR (DMSO-d_6): δ 7.51 (1H, dd, *J* = 7.4, 3.9 Hz), 7.56 (1H, t, *J* = 7.7 Hz), 7.81 (1H, d, *J* = 15.8 Hz), 7.87-7.92 (1H, m), 8.11 (1H, d, *J* = 15.8 Hz), 8.16-8.19 (1H, m), 8.35-8.42 (2H, m), 8.64 (1H, dd, *J* = 2.1, 7.4 Hz), 9.06 (1H, d, *J* = 2.8 Hz); ^{13}C NMR (DMSO-d_6) δ 122.4, 123.4, 123.9, 127.6, 130.37, 131.04, 135.31, 135.96, 139.27, 141.4, 150.5, 151.2, 187.7; IR (KBr) /cm^{-1} 1665 (C=O); HRMS found $[M]^+$ 288.0025, $C_{14}H_{10}BrNO$ requires $[M]^+$ 288.0019; Anal. Calcd $C_{14}H_{10}BrNO$: C, 58.36; H, 3.50, N, 4.86; Found C, 58.26; H, 3.44; N, 4.83.

*5.1.5 (E)-1-(3-Bromophenyl)-3-(phenyl)prop-2-en-1-one (**9**)*

Recrystallization from ethanol. Pale yellow crystals (67%). mp 82-84^{o}C; ^{1}H NMR (DMSO-d_6): δ 7.44-7.47 (3H, m), 7.53 (1H, t, *J* = 9.2 Hz), 7.76 (1H, d, *J* = 15.3 Hz), 7.84-7.90 (3H, m), 7.93 (1H, d, *J* = 15.3 Hz), 8.14 (1H, dd, *J* = 1.2, 7.5 Hz), 8.30-8.34 (1H, m); ^{13}C NMR

(DMSO-d_6) δ 121.6, 122.1, 127.6, 128.1, 128.9, 130.5, 130.9, 139.5, 187.8; IR (KBr) /cm^{-1} 1661 (C=O); HRMS found $[M+1]^+$ 288.0048, $C_{15}H_{11}BrO$ requires $[M+1]^+$ 288.0046; Anal. Calcd $C_{15}H_{11}BrO$: C, 62.74; H, 3.86; Found C, 62.38; H, 3.87.

*5.1.6 (E)-1-(Phenyl)-3-(3-pyridinyl)prop-2-en-1-one (**10**)*

Flash chromatography, 40% ethyl acetate: hexane. Lemon yellow solid (67%). mp 93-95°C; ^{1}H NMR ($CDCl_3$): δ 7.36 (1H, dd, *J* = 4.9, 9.9 Hz), 7.49-7.56 (2H, m), 7.58-7.64 (2H, m), 7.79 (1H, d, *J* = 14.8 Hz), 7.91-7.97 (1H, m), 8.00-8.07 (2H, m), 8.63 (1H, dd, J = 1.0, 4.9 Hz), 8.62 (1H, d, J = 1.0 Hz); ^{13}C NMR ($CDCl_3$) δ 123.8, 128.6, 130.7, 132.9, 133.2, 134.6, 137.7, 140.9, 150.0, 151.1, 189.8; IR (KBr) /cm^{-1} 1660 (C=O); HRMS found $[M+1]^+$ 210.0913, $C_{14}H_{11}NO$ requires $[M+1]^+$ 210.0913; Anal. Calcd $C_{14}H_{11}NO$: C, 80.36; H, 5.30, N, 6.69; Found C, 80.36; H, 5.26; N, 6.60.

*5.1.7 (E)-1-(3-Methoxyphenyl)-3-(3-pyridinyl) prop-2-en-1-one (**11**)*

Flash chromatography, 50% ethyl acetate: hexane. Light yellow solid (70%). mp 83-84°C; ^{1}H NMR ($CDCl_3$): δ 3.90 (3H, s), 7.15 (1H, dd, *J* = 3.7, 8.8 Hz), 7.36 (1H, dd, *J* = 4.7, 8.8 Hz), 7.43 (1H, t, *J* = 8.4 Hz), 7.54-7.63 (3H, m), 7.79 (1H, d, *J* = 15.8 Hz), 7.93-7.98 (1H, m), 8.64 (1H, dd, *J* = 1.9, 4.7 Hz), 8.87 (1H, d, *J* = 2.7 Hz). ^{13}C NMR ($CDCl_3$): δ 55.5, 112.9, 119.6, 120.8, 121.3, 123.8, 129.7, 130.7, 139.1, 140.9, 150.3, 151.1, 160.0, 189.5; IR (KBr) /cm^{-1} 1663 (C=O); HRMS found $[M+1]^+$ 240.1019, $C_{15}H_{13}NO_2$ requires $[M+1]^+$ 240.1019; Anal. Calcd $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48, N, 5.85; Found C, 75.53; H, 5.42; N, 5.67.

*5.1.8 (E)-1-(4-Bromophenyl)-3-(3-pyridinyl)prop-2-en-1-one (**12**)*

Recrystallization from ethanol. Fine off white crystals (80%). mp 126-128°C; ^{1}H NMR ($CDCl_3$): δ 7.36 (1H, dd, *J* = 5.3, 9.5 Hz), 7.54 (1H, d, *J* = 15.9 Hz), 7.65 (2H, d, *J* = 8.4 Hz), 7.80 (1H, d, *J* = 15.8 Hz), 7.89 (2H, d, *J* = 8.4 Hz), 7.93-7.98 (1H, m), 8.65 (1H, d, *J* = 3.1 Hz), 8.86 (1H, s); ^{13}C NMR ($CDCl_3$): δ 123.2, 123.8, 128.3, 130.1, 130.5, 131.8, 134.6, 141.5, 150.1, 151.3, 188.7; IR (KBr) /cm^{-1} 1663 (C=O); HRMS found $[M+1]^+$ 289.9997, $C_{14}H_{10}BrNO$ requires $[M+1]^+$ 289.9998; Anal. Calcd $C_{14}H_{10}BrNO$: C, 58.36; H, 3.50, N, 4.86; Found C, 58.34; H, 3.46; N, 4.81.

*5.1.9 (E)-1-(2-Bromophenyl)-3-(3-pyridinyl)prop-2-en-1-one (**13**)*

Recrystallization from ethanol. Pale yellow crystals (37%). mp 248-250^{o}C (decomposed); ^{1}H NMR ($CDCl_3$): δ 7.38-7.42 (2H, m), 7.52 (1H, d, *J* = 15.6 Hz), 7.67 (1H, dd, *J* = 2.2, 5.4 Hz), 7.79 (1H, d, *J* = 15.6 Hz), 7.93-8.14 (2H, m), 8.15 (1H, t, J = 7.2 Hz), 8.65 (1H, d, *J* = 4.7 Hz), 8.87 (1H, s); ^{13}C NMR ($CDCl_3$): δ 123.1, 127.0, 130.3, 131.6, 134.6, 136.0, 139.5, 141.8, 150.2, 151.4, 186.1; IR (KBr) /cm^{-1} 1666 (C=O); HRMS found $[M+1]^+$ 289.9999, $C_{14}H_{10}BrNO$ requires $[M+1]^+$ 289.9998; Anal. Calcd $C_{14}H_{10}BrNO$: C, 58.36; H, 3.50, N, 4.86; Found C, 58.30; H, 3.42; N, 4.88.

*5.1.10 (E)-1-(2-Methoxyphenyl)-3-(3-pyridinyl)prop-2-en-1-one (**14**)*

Recrystallization from ethanol. Pale yellow crystals (44%). mp 91-93^{o}C; ^{1}H NMR (DMSO-d_6): δ 3.91 (3H, s), 7.02 (1H, d, *J* = 8.3 Hz), 7.07 (1H, t, *J* = 7.5 Hz), 7.35 (1H, dd, *J* = 4.2, 7.9 Hz), 7.48-7.54 (1H, m), 7.50 (1H, d, *J* = 15.8 Hz), 7.64 (1H, d, *J* = 15.8 Hz), 7.67 (1H, dd, *J* = 1.7, 7.5 Hz), 7.89 (1H, d, *J* = 7.9 Hz), 8.61 (1H, d, *J* = 4.2 Hz), 8.82 (1H, s); ^{13}C NMR ($CDCl_3$): δ 55.5, 11.4, 120.7, 123.5, 128.7 130.4, 130.6, 133.5, 134.7, 138.9, 149.9, 150.8, 158.3, 192.1; IR (KBr) /cm^{-1} 1656 (C=O); HRMS Found $[M+1]^+$ 240.1017, $C_{15}H_{13}NO_2$ requires $[M+1]^+$ 240.1019; Anal. Calcd $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48, N, 5.85; Found C, 75.14; H, 5.58; N, 5.64.

*5.1.11 (E)-1-(4-Methoxyphenyl)-3-(3-pyridinyl)prop-2-en-1-one (**15**)* [12]

Recrystallization from ethanol. Yellow solid (60%). mp 105-106°C; ^{1}H NMR ($CDCl_3$): δ 3.90 (3H, s), 7.00 (2H, d, *J* = 8.8 Hz), 7.35 (1H, dd, *J* = 4.6, 8.3 Hz), 7.61 (1H, d, *J* = 16.2 Hz), 7.78 (1H, d, *J* = 16.2 Hz), 7.92-7.96 (1H, m), 8.04 (2H, d, *J* = 8.8 Hz), 8.62 (1H, dd, *J* = 4.2, 1.3 Hz), 8.87 (1H, d, *J* = 2.5 Hz); ^{13}C NMR ($CDCl_3$): δ 55.6, 114.0, 123.8, 130.7, 130.9, 134.6, 137.4, 140.1, 149.9, 150.9, 163.7, 188.0; IR (KBr) /cm^{-1} 1662 (C=O); HRMS found $[M+1]^+$ 240.1017, $C_{15}H_{13}NO_2$ requires $[M+1]^+$ 240.1019; Anal. Calcd $C_{15}H_{13}NO_2.0.15H_2O$: C, 74.46; H, 5.54, N, 5.79; Found C, 74.41; H, 5.43; N, 5.77.

*5.1.12 (E)-1-(3, 4-Dimethoxyphenyl)-3-(3-pyridyl)prop-2-en-l-one (**16**)*

Flash chromatography, 60-80% ethyl acetate: hexane. Light yellow solid (67%). mp 91-93°C; ^{1}H NMR ($CDCl_3$): δ 3.99 (6H, s), 6.94 (1H, d, *J* = 7.5 Hz), 7.36 (1H, dd, *J* = 5.0, 8.0 Hz), 7.63 (1H, d, *J* = 15.9 Hz), 7.64 (1H, d, *J* = 2.0 Hz), 7.68 (1H, dd, *J* = 2.0, 7.5 Hz), 7.78 (1H, d, *J* = 15.9 Hz), 7.93-7.96 (1H, m), 8.63 (1H, dd, *J* = 1.5, 5.0 Hz), 8.87 (1H, d, *J* = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 56.2, 110.0, 110.8, 122.7, 123.7, 124.5, 130.9, 134.6, 140.1,

149.40 149.9, 150.9, 153.7, 187.9; IR (KBr) /cm^{-1} 1655 (C=O); HRMS found $[M+1]^+$ 270.1125, $C_{16}H_{15}NO_3$ requires $[M+1]^+$ 270.1125; Anal. Calcd $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61, N, 5.20; Found C, 71.03; H, 5.65; N, 5.14.

*5.1.13 (E)-1-(3-Bromophenyl)-3-(4-pyridinyl)prop-2-en-1-one (**17**)*

Recrystallization from ethanol. Pale yellow crystals (34%). mp 104-106^oC; ^1H NMR ($CDCl_3$): δ 7.34 (1H, t, *J* = 7.1 Hz), 7.41 (2H, d, *J* = 6.1 Hz), 7.53 (1H, d, *J* = 15.6 Hz), 7.64 (1H, d, *J* = 15.6 Hz), 7.66-7.69 (1H, m), 7.87 (1H, dt, *J* = 1.0, 7.1 Hz), 8.07 (1H, t, *J* = 1.0 Hz), 8.63 (2H, d, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 122.1, 123.2, 125.4, 127.1, 130.4, 131.6, 136.2, 139.2, 141.8, 142.4, 150.7, 188.4; IR (KBr) /cm^{-1} 1666 (C=O); HRMS found $[M+1]^+$ 289.9998, $C_{14}H_{10}BrNO$ requires $[M+1]^+$ 289.9998; Anal. Calcd $C_{14}H_{10}BrNO$: C, 58.36; H, 3.50, N, 4.86; Found C, 58.20; H, 3.46; N, 4.81.

*5.1.14 (E)-1-(3-Bromophenyl)-3-(2-pyridinyl)prop-2-en-1-one (**18**)*

Recrystallization from ethanol. Pale yellow crystals (47%). mp 110-112^oC; ^1H NMR ($CDCl_3$): δ 7.40-7.49 (2H, m), 7.55 (1H, t, *J* = 8.8 Hz), 7.72 (1H, d, *J* = 16.0 Hz), 7.85-8.00 (2H, m), 8.10-8.15 (2H, m), 8.20 (1H, s), 8.70 (1H, d, *J* = 4.5 Hz); ^{13}C NMR ($CDCl_3$): δ 125.0, 126.9, 127.5, 130.9, 135.7, 137.2, 143.8, 150.1, 152.6, 188.3; IR (KBr) /cm^{-1} 1664 (C=O); HRMS found $[M+1]^+$ 289.0056, $C_{14}H_{10}BrNO$ requires $[M+1]^+$ 289.0052; Anal. Calcd $C_{14}H_{10}BrNO$: C, 58.36; H, 3.50, N, 4.86; Found C, 58.30; H, 3.45; N, 4.86.

*5.1.15 (E)-3-(3-Bromophenyl)-1-(3-pyridinyl)prop-2-en-1-one (**19**)*

Recrystallization from ethanol. Pale yellow crystals (40%). mp 114-116^oC; ^1H NMR ($CDCl_3$): δ 7.32-7.44 (2H, m), 7.52 (1H, d, *J* = 16.5 Hz), 7.72 (1H, d, *J* = 6.5 Hz), 7.81 (1H, d, *J* = 16.5 Hz), 7.93-7.98 (2H, m), 8.14 (1H, t, *J* = 1.5Hz), 8.64 (1H, d, *J* = 2.7 Hz), 8.87 (1H, s); ^{13}C NMR ($CDCl_3$): δ 123.1, 123.2, 126.6, 130.4, 131.6, 134.6, 136.0, 142.3, 150.2, 151.4, 188.4; IR (KBr) /cm^{-1} 1658 (C=O); HRMS found $[M+1]^+$ 289.0055, $C_{14}H_{10}BrNO$ requires $[M+1]^+$ 289.0052; Anal. Calcd $C_{14}H_{10}BrNO$: C, 58.36; H, 3.50, N, 4.86; Found C, 58.30; H, 3.45; N, 4.81.

*5.1.16 (E)-1-(3-Chlorophenyl)-3-(3-pyridinyl)prop-2-en-1-one (**20**)*

Recrystallization from ethanol. Off white crystals (65%). mp 128-130^oC; ^1H NMR ($CDCl_3$): δ 7.37 (1H, dd, *J* = 5.2, 7.7 Hz), 7.45 (1H, t, *J* = 6.6 Hz), 7.51-7.60 (2H, *m*), 7.80 (1H, d, *J* =

16.0 Hz), 7.89 (1H, d, *J* = 7.6 Hz), 7.94-8.02 (2H, m), 8.65 (1H, d, *J* = 5.2 Hz), 8.86 (1H, s); ^{13}C NMR ($CDCl_3$): δ 123.1, 123.8, 126.6, 128.6, 130.1, 130.4, 133.0, 135.1, 139.3, 141.8, 150.2, 151.4, 188.4; IR (KBr) /cm^{-1} 1665 (C=O); HRMS found $[M+1]^+$ 244.0524, $C_{14}H_{10}NOCl$ requires $[M+1]^+$ 244.0524; Anal. Calcd $C_{14}H_{10}NOCl$: C, 69.00; H, 4.14, N, 5.75; Found C, 68.86; H, 4.12; N, 5.66.

*5.1.17 (E)-1-(3,4-Methylenedioxyphenyl)-3-(3-pyridinyl)prop-2-en-1-one (**21**)*

Recrystallization from ethanol. Off white crystals (55%). mp 138-140°C; ^{1}H NMR ($CDCl_3$): δ 6.10 (2H, s), 6.92 (1H, d, *J* = 7.8 Hz), 7.37 (1H, dd, *J* = 4.3, 7.1 Hz) 7.54 (1H, d, *J* = 2.1 Hz), 7.57 (1H, d, *J* = 14.9 Hz) 7.67 (1H, dd, *J* = 2.1, 7.8 Hz), 7.78 (1H, d, *J* = 14.9 Hz), 7.93-7.98 (1H, m), 8.63 (1H, dd, *J* = 1.1, 4.3 Hz), 8.86 (1H, d, *J* = 1.2 Hz); ^{13}C NMR ($CDCl_3$) δ 102.0, 108.0, 109.4, 123.5, 123.8, 124.9, 130.8, 132.5, 134.6, 140.4, 148.5, 149.9, 151.0, 152.0, 187.6; IR (KBr) /cm^{-1} 1666 (C=O); HRMS found $[M+1]^+$ 254.0811, $C_{15}H_{11}NO_3$ requires $[M+1]^+$ 254.0812; Anal. Calcd $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38, N, 5.53; Found C, 70.91; H, 4.36; N, 5.48.

*5.1.18 (E)-1-(3,4-Difluorophenyl)-3-(3-pyridinyl)prop-2-en-1-one (**22**)*

Recrystallization from ethanol. Pale green crystals (47%). mp 120-121°C; ^{1}H NMR (DMSO-d_6): δ 7.50-7.53 (1H, m), 7.63-7.70 (1H, m), 7.78 (1H, d, *J* = 15.0 Hz), 8.10 (2H, d, *J* = 15.0 Hz), 8.24-8.29 (1H, m), 8.37-8.39 (1H, m), 8.64 (1H, dd, *J* = 1.5, 4.3 Hz), 9.05 (1H, d, *J* = 1.2, 4.3 Hz); ^{13}C NMR (DMSO-d_6) δ 117.9, 118.1, 123.1, 126.5, 130.3, 134.7, 141.4, 150.5, 150.9, 186.5; IR (KBr) /cm^{-1} 1661 (C=O); HRMS found $[M]^+$ 246.0724, $C_{14}H_9NOF_2$ requires $[M]^+$ 246.0725; Anal. Calcd $C_{14}H_9F_2NO$: C, 68.57; H, 3.70, N, 5.71; Found C, 68.48; H, 3.70; N, 5.66.

*5.1.19 (E)-1-(3-Hydroxyphenyl)-3-(3-pyridinyl)prop-2-en-1-one (**23**)*

After quenching with water the mixture was acidified with 1 M HCl before extraction with ethyl acetate. Recrystallization from ethanol. Light yellow crystals (55%). mp 182-184°C; ^{1}H NMR (DMSO-d_6): δ 7.09 (1H, dd, *J* = 2.1, 8.5 Hz), 7.39 (1H, t, *J* = 7.7 Hz)), 7.48-7.51 (2H, m), 7.65 (1H, d, *J* = 6.8 Hz), 7.75 (1H, d, *J* = 14.9 Hz), 8.01 (1H, d, *J* = 14.9 Hz), 8.33 (1H, d, *J* = 7.7 Hz)), 8.62 (1H, s), 9.02 (1H, s), 9.85 (1H, brs, OH); ^{13}C NMR (DMSO-d_6) δ 114.7, 119.7, 120.5, 123.9, 124.1, 129.9, 135.1, 138.7, 140.4, 150.3, 157.8, 188.9; IR (KBr)

/cm^{-1} 1660 (C=O); HRMS found $[M+1]^+$ 226.0861, $C_{14}H_{11}NO_2$ requires $[M+1]^+$ 226.0863; Anal. Calcd $C_{14}H_{11}NO_2$: C, 74.65; H, 4.92, N, 6.22; Found C, 74.51; H, 4.92; N, 6.03.

*5.1.20 (E)-1-(3-pyridinyl)-3-(3-pyridinyl)prop-2-en-1-one (**24**)*

Recrystallization from ethanol. Pale yellow crystals (41%). mp 148-150^{o}C; ^{1}H NMR (DMSO-d_6): δ 7.51 (1H, dd, J = 4.8, 9.0 Hz), 7.62 (1H, dd, *J* = 4.8, 9.0 Hz), 7.83 (1H, d, *J* = 13.7 Hz), 8.12 (1H, d, *J* = 13.7 Hz), 8.38 (1H, d, *J* = 7.2 Hz), 8.48 (1H, d, *J* = 7.2 Hz), 8.63 (1H, d, *J* = 4.8 Hz), 8.85 (1H, d, *J* = 4.8 Hz), 9.06 (1H, s), 9.37 (1H, s); ^{13}C NMR (DMSO-d_6) δ 123.6, 123.9, 130.3, 132.5, 135.3, 141.3, 149.8, 151.3, 153.5, 188.3; IR (KBr) /cm^{-1} 1666 (C=O); HRMS found $[M+1]^+$ 211.0864, $C_{13}H_{10}N_2O$ requires $[M+1]^+$ 211.0866; Anal. Calcd $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79, N, 13.33; Found C, 73.90; H, 4.79; N, 13.36.

5.2 Biological assays

5.2.1 Trypanocidal activity

Trypanosoma brucei rhodesiense STIB 900, a clone of a population isolated in 1982 from a patient in Tanzania. Stock drug solutions were prepared in DMSO at 20 mM and further diluted to the appropriate concentration using medium. Assays were performed in 96-well microtiter plates with each well containing 100 ml of parasite culture (1 x 10^3 bloodstream forms) with serial drug dilutions at 37°C for 72 h in 5% CO_2. Each compound was tested in triplicate with 30 mg/ml the highest concentration of compound used and a 3-fold serial dilution was performed down to a suitable concentration to obtain an IC_{50} value. Control wells were without drug and blanks were medium only. After 72 h of incubation the plates were inspected to assure growth in control wells and to determine the minimum inhibitory concentration (MIC). Subsequently, 20 µL of Alamar Blue™ was added to each well and the plates incubated for another 2-4 h. Plates were read on a Gemini Plate Reader (Molecular Devices) using an excitation wave length of 530 nm and an emission wave length of 580 nm (cut off 550 nm). IC_{50} values were calculated using Prism © software. Pentamidine and melarsoprol were used as positive controls.

5.2.2 Toxicity on KB cells

KB cells, derived from a human carcinoma of the nasopharynx and typically used in assays for antineoplastic agents, were maintained in RPMI 1640 medium (Sigma, UK), 10% heat-

inactivated foetal calf serum, 37°C, 5% CO_2. KB cell monolayers, prepared in 96-well plates, were exposed to the test compounds for 72 h. Podophyllotoxin was used as a positive control. 20 µL of Alamar Blue™ was added to each well. After a further 2–4 h incubation the plates were read (Molecular Devices Gemini™) at EX/EX 530/580, cut-off 550 nm. The IC_{50} values were calculated by sigmoidal regression analysis (Prism ©).

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Highlights

- Improved Claisen-Schmidt condensation using lithium hydroxide monohydrate in 1,4-dioxane.
- Pyridylchalcones show good activity and selectivity against *Trypanosoma brucei.*
- Pyridylchalcones show little activity against *Leishmania donovani*.
- Promising leads in the development of novel compounds for the treatment of sleeping sickness.