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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiprotozoal activity of 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs of combretastatin A-4

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Abstract Eighteen 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs (**5a–j**, **6a–e**, and **7a–c**) of combretastatin A-4 were synthesized with the objective of discovering compounds capable of controlling the growth of *Trypanosoma lewisii*, *Leishmania tarantole*, *Plasmodium falciparum*, and *Giardia lamblia*. Even though the target compounds demonstrated differential cytotoxicity against mammalian cancer cells, with IC_{50} values ranging from 0.5 to >100 μM, the range of activity against *Trypanosoma*, *Leishmania*, and *Plasmodium*, and to a good extent for *Giardia*, was narrow. The IC_{50} values of "active" compounds against the parasites ranged from about 10 μM to slightly greater than 50 μM. Specifically, compounds **5a**, **5g**, **5h**, **6c**, **7a**, and **7c** were not cytotoxic against mammalian cancer cells ($IC_{50} > 100$ μM) but showed good activity against the parasites, except *Giardia* (e.g., compounds **6c** and **7a**), suggesting that these compounds may act in a similar mechanism in parasites. Compounds **5f** and **6b** were previously shown to promote microtubule depolymerization in mammalian cells.

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Introduction

Infections due to protozoan parasites are a major public health concern for billions of people worldwide (Morris *et al.*, 2001). *Trypanosoma*, *Leishmania*, *Plasmodium*, and *Giardia* are protozoa responsible for various tropical diseases such as African sleeping sickness and Chagas disease, leishmaniasis (Zhai *et al.*, 1995), malaria (Olliaro and Wells, 2009), and giardiasis (Gardner and Hill, 2001; Faubert, 2000), which cause considerable medical and veterinary mortality and morbidity (De Souza, 2002).

Tubulin polymerization and microtubule depolymerization are important targets for the discovery and design of medicinal agents. Compounds that perturb microtubule dynamics are currently among the most effective drugs to treat medical conditions including cancer, gout, and helminth infection (Belotti *et al.*, 1996; Taraboletti *et al.*, 2002; Miller *et al.*, 2001; Jordan *et al.*, 1998). The anticancer compound combretastatin-A4 (CA-4 **1**, Fig. 1) is such an example. CA-4 inhibits tubulin polymerization by binding the colchicine binding site of tubulin (Pettit *et al.*, 1987; Hsieh *et al.*, 2003; Pettit *et al.*, 1995; Gaukoger *et al.*, 2001). Unfortunately, CA-4 suffers from low bioavailability and poor solubility in biological media (Galbraith *et al.*, 2003). These limitations have led to the development of structural analogs including a water-soluble phosphate prodrug (CA-4P, **2**), an amino-containing analog (AC-7739, **3**) (Ohsumi *et al.*, 1998) and its amino acid derivative (AVE-8062, **4**) (Tozer *et al.*, 2002) (Fig. 1).

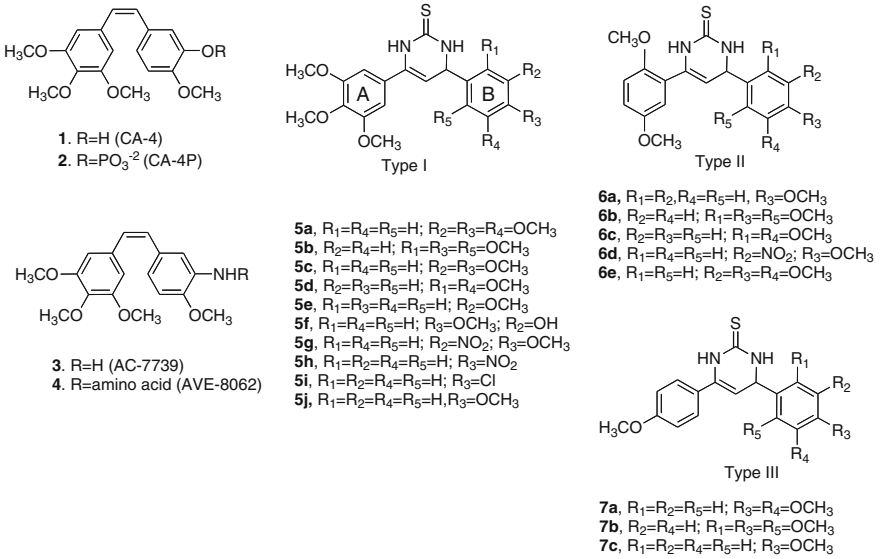


Fig. 1 Structures of combretastatin-A4 (C-A4, **1**) and its water-soluble derivates **2–4**. The 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs are **5a–j**, **6a–e**, and **7a–c**

As part of a program to develop heterocyclic analogs of CA-4 with improved water solubility, a series of novel 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs were designed with the purpose of examining their cytotoxic properties (Lee *et al.*, 2008). To date, the ability of thioxopyrimidine analogs to inhibit the growth of protozoan parasites has not been examined and is the focus of this study. This investigation stems from recent reports that chalcone analogs of CA-4 are endowed with significant antiparasitic activity (Awasthi *et al.*, 2009; Liu *et al.*, 2001; Go *et al.*, 2004; Liu *et al.*, 2003; Dominguez *et al.*, 2001; Narender *et al.*, 2005). Eighteen thioxopyrimidine analogs were selected and divided into three categories, types I–III, and tested for their antiparasitic activity. Type I contains a 3,4,5-trimethoxyphenyl moiety in the A-ring (**5a–j**). Type II contains a 2,5-dimethoxyphenyl group in the A-ring (**6a–e**), and type III contains a 4-methoxyphenyl group in the A-ring **7** (**a–c**). The conformation of analogs **5f** and **6b** were previously assessed by molecular modeling studies using MacSpartan (Johnson *et al.*, 2007; Ruprich *et al.*, 2007). As reported earlier, compounds **5f** and **6b** adopt a twisted geometry similar to that of CA-4 (Kong *et al.*, 2005; Ducki *et al.*, 1998).

Results and discussion

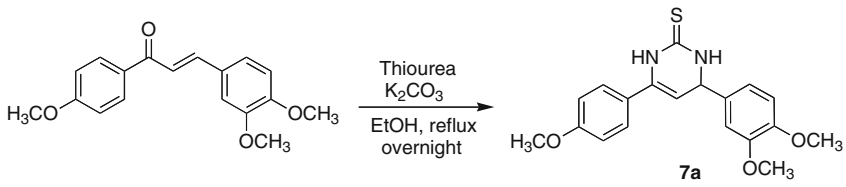
Chemistry

The synthesis of 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs **5a–i**, and **6b**, **d** and their cytotoxic activity against B16 and L1210 cell lines have been reported (Lee *et al.*, 2008). Compound **7c** has also been reported, but its biological activity has not been examined (Al-Hajjar *et al.*, 1979). Compounds **5j**, **6a**, **c**, **e**, and **7a**, **b** were newly synthesized by reaction of the appropriate chalcone (Dickson *et al.*, 2006; Pati *et al.*, 2005) with 2.5 mol equivalents of thiourea and potassium carbonate in refluxing ethanol (overnight). A similar reaction was recently reported in the preparation of curcumin-based thioxopyrimidine analogs (Fadda *et al.*, 2009). On completion, the cooled reaction mixture was poured into ice water, and the precipitate was collected. When needed, the precipitate was recrystallized from methanol. The chemical yields of the new compounds ranged from 52% to quantitative yield. The structures of the new compounds were ascertained by 400 MHz ^{1}H-NMR, infrared plus nominal and high resolution mass spectrometry measurements (Scheme 1).

Biology

The cytotoxicity of the newly synthesized compounds against the growth of murine cancer cells (murine L1210 lymphoma and B16 melanoma) were tested using an in vitro 72-h continuous exposure MTT assay (Carmichael *et al.*, 1987). These experiments were conducted according to a previously published procedure (Lee *et al.*, 2008). The concentration (μM) of each target compound required to inhibit cell growth by 50% relative to an untreated control (IC_{50} value) was determined. The cytotoxicity of the newly synthesized thioxopyrimidine compounds are reported in Table 1 along with previously disclosed results (Lee *et al.*, 2008). It is noteworthy that, in general, the compounds are slightly more active against L1210 leukemia cells than B16 melanoma cells. Except for compound **6e**, the other new compounds (**5j**, **6a**, **6c**, **7a**, and **7b**) are significantly less active (Table 1). Interestingly, only three compounds (**5f**, **6b**, **6e**) show significant cytotoxicity against mammalian

Scheme 1 A representative synthesis of 1,2,3,4-tetrahydro-2-thioxopyrimidine **7a**



cancer cells ($IC_{50} < 1$ μM). However, more of the compounds show very weak activity ($IC_{50} > 100$ μM), e.g., **5a**, **5e**, **5g**, **5h**, **6e**, **7a**, and **7c**.

Since our objective is to discover compounds with poor cytotoxicity against mammalian cells but exhibit high activity against parasitic cells, the compounds were subjected to testing against parasitic cells grown in culture. The 18 targeted compounds were tested for their ability to inhibit the growth of *Trypanosoma lewisii*, *Leishmania tarantole*, *Plasmodium falciparum*, and *Giardia lamblia* in culture. Studies on *Plasmodium* were conducted according to a published procedure (Yanow *et al.*, 2008). *Trypanosoma*, *Leishmania*, and *Giardia* were cultured in RPMI, BHI, and TYI-S-33, respectively, and the cytotoxicity results obtained after 72 h of continuous exposure of the compounds. The activity was assayed using an MTT assay. For the biological studies, the compounds were dissolved in media containing 0.03–0.5% DMSO. This level of DMSO has no effect on the growth of the parasites in culture (data not shown).

Results for the cytotoxicity studies against the growth of four parasites are given in Table 1. Foremost, the compounds are generally active against the parasites. Interestingly, with a few exceptions for *Giardia*, the range of IC_{50} values fall within a narrow range from about 10 μM to slightly greater than 50 μM. In order to confirm the validity of the MTT-based cytotoxicity results, the effects of compound **5f** against the growth of *Giardia* and *Trypanosoma* cells were examined microscopically. The micrographs of *Giardia* and *Trypanosoma* cells treated with compound **5f** (21.5 and 12.1 μM, accordingly) for 72 h are shown in Figs. 2b and 3b, respectively. For comparison, the respective pictures of the untreated parasites are given in Figs. 2a and 3a. Visually, it is clearly evident that the

Table 1 Cytotoxicity and antiprotozoal activity of 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs **5–7**

Agents	Cytotoxicity					
	B16 IC_{50} (μM)	L1210 IC_{50} (μM)	*Leishmania* IC_{50} (μM)[c]	*Trypanosoma* IC_{50} (μM)[c]	*Plasmodium* IC_{50} (μM)[c]	*Giardia* IC_{50} (μM)[c]
5a[a]	>100	>100	46.6	29.3	Nd[b]	24.8
5b[a]	6.3	5.7	46.6	29.6	Nd	20.3
5c[a]	>100	56.3	36.4	52.1	Nd	22.7
5d[a]	36	32	30.3	48.4	Nd	23.5
5e[a]	59.5	>100	19.4	24.3	Nd	40.4
5f[a]	6.0	0.5	12.1	49.2	Nd	21.5
5g[a]	>100	>100	12.5	39.5	Nd	24.3
5h[a]	>100	>100	13.7	50.0	Nd	21.0
5i[a]	55.5	48.3	10.2	27.0	Nd	46.7
5j	41.0	36	12.5	17.9	14.5	22.3
6a	87	80	11.4	16.5	69.4	>100
6b[a]	6.1	0.4	10.4	25.0	17.3	33.8
6c	>100	>100	18.0	27.3	27.9	>100
6d[a]	42	2.2	21.1	33.3	Nd	57.8
6e	6.0	0.5	16.8	15.7	13.3	>100
7a	>100	>100	27.2	25.8	31.0	>100
7b	85	25	25.1	13.4	14.6	>100
7c[d]	>100	>100	17.8	15.2	25.0	Nd

[a] Cytotoxicity data against L1210 and B16 are taken from Lee *et al.* (2008)

[b] Nd is not determined

[c] The error for the antiparasitic studies is ±10%

[d] Taken from Al-Hajjar *et al.* (1979)

number of viable parasites in wells of compound-treated parasites is significantly lower than those in untreated control wells.

Perhaps the most significant discovery from this study is as follows. A number of the thioxopyrimidine compounds (**5c**, **5g**, **5h**, **6c**, **7a**, and **7c**) that show good activity against the parasites exhibit low cytotoxicity against L1210 and B16 cells (IC_{50} values > 100 μM). Of particular interest are the nitro-containing analogs (on the B-ring) **5g** and **5h**. Both compounds have approximately eightfold enhanced activity against *Leishmania*, approximately fourfold increase in activity against *Giardia*, and two-times more activity for *Trypanosoma*, when compared to L1210 and B16 cells. Also of special interest are compounds **7a** and **7c**, the former agent being one of the newly synthesized compounds. These two compounds were inactive against mammalian cells but gave IC_{50} values in the range of 13–25 μM against *L. tarantole*, *T. lewisii*, and *P. falciparum*. Interestingly, the substitution pattern of functional groups on these compounds is different from CA-4. Instead of containing the 3,4,5-trimethoxyphenyl A-ring, which makes CA-4 highly cytotoxic against mammalian cancer cells, compounds **7a** and **7c** contain a *para*-substituted methoxy on the phenyl moiety (A-ring). This difference may explain why compounds **7a** and **7c** are not cytotoxic ($IC_{50} > 100$ μM) against L1210 and B16 cells. The results also show that compounds **7a** and **7c** are not cytotoxic against *G. lamblia*.

Along with the cytotoxicity of compounds **5f** and **6b** against L1210 and B16 cells, the ability of these agents to cause microtubule depolymerization has been reported earlier (Lee *et al.*, 2008). The effects of these compounds on interphase cellular microtubules were evaluated in A-10 aortic smooth muscle cells. With EC_{50} values of 4.4 and 2.9 μM, compounds **5f** and **6b** were concluded to be effective in causing the loss of cellular microtubules, suggesting that microtubule depolymerization might contribute to the observed cytotoxicity against L1210 and B16 cells. Even though these studies have not been conducted on any of the parasites, the authors speculate that it is possible that the target compounds also derive their antiparasitic activity by binding tubulin and causing microtubule depolymerization.

In summary, even though only three compounds, **5f**, **6b**, and **6e**, show significant cytotoxicity against B16 and L1210 cells, almost all 18 1,2,3,4-tetrahydro-2-thioxopyrimidine compounds, **5a–j**, **6a**, **e**, and **7a–c**, show good antiprotozoal activity. Six of the compounds, **5c**, **5g**, **5h**, **6c**, **7a**, and **7c**, are worthy of further studies because of their low cytotoxicity against mammalian cells and good potency against the parasites. Additional benefits of the target compounds **5–7** include their enhanced solubility in aqueous biological media and ease of synthesis.

Characterization of the newly synthesized compounds

5j, Off white solid, quantitative yield; m.p. 222–224°C; TLC (5% MeOH/$CHCl_3$) R_f 0.22; IR (KBr) 3166, 2361, 1663, 1601, 1552, 1452, 1248, 1179, 1131, 1017, 816, 799; ^{1}H-NMR ($CDCl_3$) 7.49 (br s, 1H), 7.30 (d, $J = 8$ Hz, 2H), 6.93 (d, $J = 8$ Hz, 2H), 6.59 (s, 2H), 6.56 (br s, 1H), 5.24 (d, $J = 8$ Hz, 1H), 5.10 (d, $J = 8$ Hz, 1H), 3.88 (s, 6H), 3.86 (s, 3H), 3.83 (s, 3H); EI: M^+ 386 (100%); HRMS: obsd: 386.1304; calcd: 386.1300.

6a, Off white solid, 88% yield; m.p. 152–154°C; TLC (5% MeOH/$CHCl_3$) R_f 0.62, IR (KBr) 3490, 3170, 3070, 2998, 2946, 2832, 1670, 1605, 1553, 1501, 1462, 1297, 1242, 1221, 1176, 1030, 810, 747; ^{1}H-NMR: DMSO-d_6 9.80 (br s, 1H), 8.58 (br s, 1H), 7.28 (d, $J = 8$ Hz, 2H), 6.95 (d, $J = 8$ Hz, 2H), 6.92 (d, $J = 8$ Hz, 2H), 6.81 (s, 1H), 5.13 (d, $J = 8$ Hz, 1H), 5.05 (d, $J = 8$ Hz, 1H), 3.75 (s, 6H), 3.71 (s, 3H); EI: M^+ 356 (100%).

6c Off white solid, 65% yield; m.p. 210–212°C; TLC (5% MeOH/$CHCl_3$) R_f 0.59; IR (KBr) 3177, 2942, 1542, 1535, 1494, 1452, 1262, 1207, 1176, 1083, 1041, 799, 744; ^{1}H-NMR: $CDCl_3$: 8.20 (br s, 1H), 7.30 (d, $J = 8$ Hz, 2H), 6.92 (d, $J = 8$ Hz, 2H), 6.87 (s, 2H), 6.59 (br s, 1H), 5.23

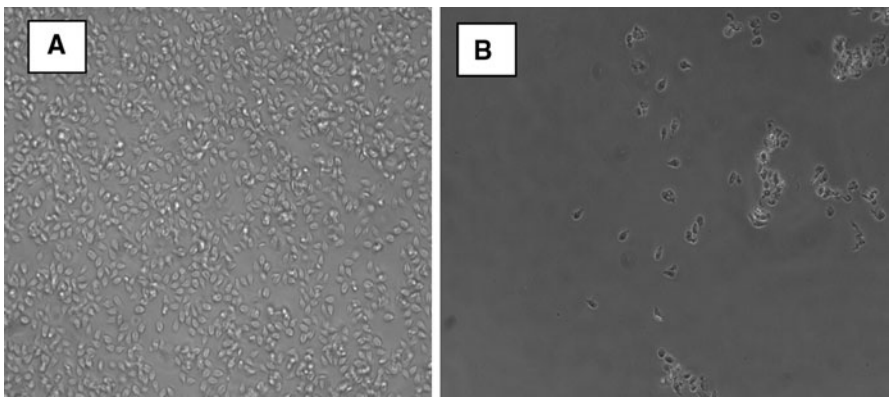


Fig. 2 *Giardia lamblia* control (**a**) and treated (**b**) with 21.5 μM of **5f** for 72 h (×20)

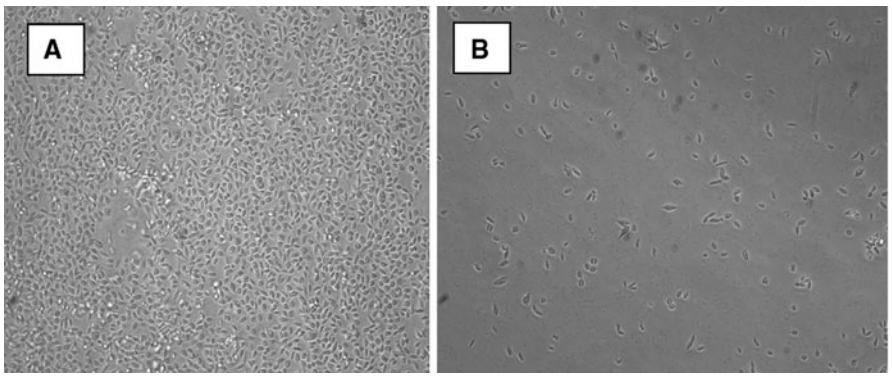


Fig. 3 *Leishmania tarantole* control (**a**) and treated (**b**) with 12.1 μM of **5f** for 72 h (×40)

(d, $J = 4$ Hz, 1H), 5.13 (d, $J = 4$ Hz, 1H), 3.86 (s, 6H), 3.82 (2, 3H), 3.76 (s, 3H); EI: M^+ 386 (100%).

6e Off white solid, 52% yield; m.p. 150–152°C; TLC (5% MeOH/$CHCl_3$) R_f 0.10; IR (KBr) 3180, 2945, 2831, 1673, 1590, 1556, 1494, 1442, 1328, 1276, 1224, 1120, 1037, 816, 730; ^{1}H-NMR: $CDCl_3$: 8.45 (br s, 1H), 6.89 (s, 3H), 6.59 (s, 2H), 6.50 (br s, 1H), 4.95 (d, $J = 8$ Hz, 1H), 4.80 (d, $J = 8$ Hz, 1H), 3.88 (s, 6H), 3.86 (s, 3H), 3.85 (s, 3H), 3.77 (s, 3H); EI: M^+ 416 (100%); HRMS: obsd: 416.1409 calcd: 416.1406.

7a Off white solid, 96% yield; m.p. 198–200°C; TLC (5% MeOH/$CHCl_3$) R_f 0.27; IR (KBr) 3190, 2900, 2850, 1663, 1601, 1552, 1452, 1248, 1179, 1131, 1017, 799; ^{1}H-NMR: $CDCl_3$ 7.66 (br s, 1H), 7.36 (d, $J = 8$ Hz, 2H), 6.92 (d, $J = 8$ Hz, 2H), 6.87 (d, $J = 8$ Hz, 2H), 6.85 (s, 1H), 5.23 (d, $J = 2.8$ Hz, 1H), 5.09 (d, $J = 2.8$ Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H); EI: M^+ 356 (100%).

7b Off white solid, quantitative yield; m.p. 180–182°C; TLC (5% MeOH/$CHCl_3$) R_f 0.37; IR (KBr) 3208, 2994, 2942, 2838, 1670, 1601, 1552, 1511, 1463, 1324, 1297, 1181, 1151, 1120, 1031, 947, 817, 752; ^{1}H-NMR: $CDCl_3$ 7.45 (br s, 1H), 7.35 (d, $J = 8$ Hz, 2H), 6.80 (d, $J = 8$ Hz, 2H), 6.25 (br s, 1H), 6.13 (s, 2H), 5.65 (d, $J = 8$ Hz, 1H), 4.90 (d, $J = 8$ Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 6H); EI: M^+ 386 (100%).

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