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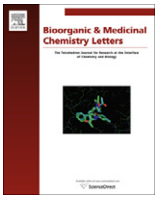

Synthesis and evaluation of oryzalin analogs against *Toxoplasma gondii*

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ABSTRACT

The synthesis and evaluation of 20 dinitroanilines and related compounds against the obligate intracellular parasite *Toxoplasma gondii* is reported. Using in vitro cultures of parasites in human fibroblasts, we determined that most of these compounds selectively disrupted *Toxoplasma* microtubules, and several displayed sub-micromolar potency against the parasite. The most potent compound was N^1,N^1-dipropyl-2,6-dinitro-4-(trifluoromethyl)-1,3-benzenediamine (**18b**), which displayed an IC_{50} value of 36 nM against intracellular *T. gondii*. Based on these data and another recent report [Ma, C.; Tran, J.; Gu, F.; Ochoa, R.; Li, C.; Sept, D.; Werbovetz, K.; Morrissette, N. *Antimicrob. Agents Chemother.* **2010**, *54*, 1453], an antimitotic structure–activity relationship for dinitroanilines versus *Toxoplasma* is presented.

Infection by the apicomplexan parasite *Toxoplasma gondii* can result in miscarriage, birth defects in infants, vision disturbances in immunocompetent hosts, and toxoplasmic encephalitis in the immunocompromised.[1] Acute toxoplasmosis in otherwise healthy individuals is typically treated with the antifolate combination of pyrimethamine and sulfadiazine together with folinic acid.[1] Pyrimethamine has potential side effects, including bone marrow suppression[2] and teratogenicity.[3,4] Resistance to pyrimethamine in malaria chemotherapy can occur through mutations in the dihydrofolate reductase target protein,[5] although no evidence for clinical resistance to pyrimethamine in *Toxoplasma* exists to date.[6] Sulfadiazine use is also complicated by the occurrence of kidney stones,[7] allergic reactions,[8] and the development of resistance.[6] Other alternatives to pyrimethamine–sulfadiazine treatment of toxoplasmosis include clindamycin, atovaquone, and spiramycin,[1] but these drugs each possess their own limitations.[9–11] An ideal drug against human toxoplasmosis would kill the different stages of *T. gondii*, be well distributed in the main sites of infection, including the brain and eyes, and lack the potential for teratogenicity.

Tubulin, which assembles into microtubules, is an essential protein for formation of the mitotic spindle. It continues to be a prime target for established and investigational anticancer agents[12,13] and is also believed to be the molecular target of anthelmintic benzimidazoles.[14] Dinitroanilines are tubulin-binding herbicides which display microtubule selective toxicity for many different classes of protozoan parasites, including *Leishmania*, *Trypanosoma*, *Plasmodium*, and *Toxoplasma*. We have previously characterized the sensitivity of *Toxoplasma* to dinitroanilines and have found that IC_{50} values for commercially available dinitroanilines range from 45 nM to 6.7 μM.[15–17] *Toxoplasma* parasites only replicate inside of host cells, and extracellular parasites do not have dynamic microtubules. Intracellular *Toxoplasma* parasites are sensitive to disruption by dinitroanilines: dinitroaniline-treated parasites lack all microtubules and cannot carry out microtubule-dependent functions including mitosis and cytokinesis.[15,18,19] Following lysis of the original host cell, round, non-polar dinitroaniline-treated parasites are unable to invade new host cells and rapidly die. Thus, dinitroanilines could serve as excellent leads for the discovery of new drugs against toxoplasmosis since they disrupt protozoan parasite microtubules at nanomolar concentrations while showing little or no effect on host cell microtubules.[16,20–23] Computational methods have been used to identify a binding site for the dinitroanilines on parasite tubulin.[16,24] These studies predict that protofilament contacts in the microtubule lattice are disrupted when dinitroanilines selectively bind to protozoan α-tubulin beneath the H1-S2 loop. Analysis of vertebrate α-tubulin through computational methods indicates that oryzalin has non-specific, low affinity interactions with this protein and no consensus binding site, consistent with in vivo and in vitro observations that dinitroanilines do not bind to vertebrate tubulin or disrupt vertebrate microtubules.[20,25–28]

Previous work in our laboratories has shown that structural modification of the commercial dinitroaniline oryzalin leads to increased potency against kinetoplastid parasites.[23,29,30] However, a detailed structure–activity study of dinitroanilines against

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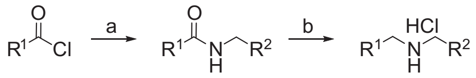
Toxoplasma has not been reported. We were therefore motivated to synthesize analogs of oryzalin (**6i**) and other dinitroaniline herbicides such as trifluralin (**14a**) and dinitramine (**18a**) for testing against *T. gondii*. In the present manuscript, we describe the synthesis and biological activity of such derivatives.

To synthesize several of the desired target compounds, unsymmetrical secondary amines were needed. The required secondary amine salts were synthesized using the method reported previously.[29,31] Hence, the standard coupling of the acid chlorides **1a**–**d** with amines gave the respective amides **2a**–**e**, followed by reduction with borane–THF solution, treatment with methanol, protection with Boc, and deprotection using HCl in dioxane to afford the secondary amine salts **3a**–**e** in good yields (see Scheme 1).



1a-d **2a-e** **3a-e**

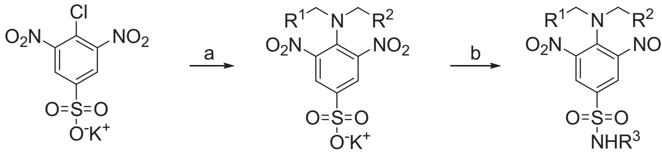
1a: R^1 = Ph
1b: R^1 = 4-Tolyl
1c: R^1 = *c*Hexane
1d: R^1 = CH_2Ph

2a, **3a**: R^1 = Ph, R^2 = CH_2CH_2OH
2b, **3b**: R^1 = Ph, R^2 = *c*Pr
2c, **3c**: R^1 = R^2 = 4-Tolyl
2d, **3d**: R^1 = R^2 = *c*Hexane
2e, **3e**: R^1 = R^2 = CH_2Ph

Scheme 1. Reagents and conditions: (a) RNH_2, Et_3N, CH_2Cl_2, 0 °C to rt, overnight; (b) (i) BH_3–THF (3 equiv), reflux, overnight; (ii) MeOH, reflux, 5 h; (iii) Boc_2O (1.5 equiv) in CH_2Cl_2, rt, overnight; (iv) 4 M HCl in 1,4-dioxane (1.3 equiv), CH_2Cl_2, rt, overnight.

Dinitroaniline sulfonamide target compounds were prepared as shown in Scheme 2. The desired amines reacted with the potassium salt of 4-chloro-3,5-dinitrobenzenesulfonate **4** in the presence of triethylamine to give N^4,N^4-disubstituted-3,5-dinitrobenzenesulfonates **5a**–**e**, **5f**–**h**,[31] and **5i**–**j**[30,31] in good yields. These sulfonates contained traces of the secondary amine salts as determined from their ^{1}H NMR spectra, but these salts did not interfere with further reactions. Sulfonyl chlorides were synthesized from benzenesulfonates **5a**–**j** using PCl_5 in dichloromethane. After brief work up, the resulting sulfonyl chlorides were treated with a methanolic solution of NH_3 or the desired amines in dry THF to afford the target compounds **6a**–**h**, **6i**–**j**,[29] **6k**–**l**, and **6m**–**n**[30] in good to excellent yields. Mono-N^4-alkylated and free N^4-amino sulfonamides were isolated as bi-products, especially for bulky N^4,N^4-disubstituted analogs such as **6a–g**.

The synthesis of **12**, where the nitro moieties present in **6i** were replaced with nitrile groups, was accomplished according to Scheme 3. The sulfonyl chloride corresponding to sulfonate **7**[32] was prepared in situ from **7** using $PCl_5/POCl_3$ in dry chlorobenzene; treatment of this sulfonyl chloride with methanolic NH_3 afforded **8**. An Ullmann reaction[33] between sulfonamide **8** and dipropylamine in the presence of potassium carbonate and catalytic copper gave

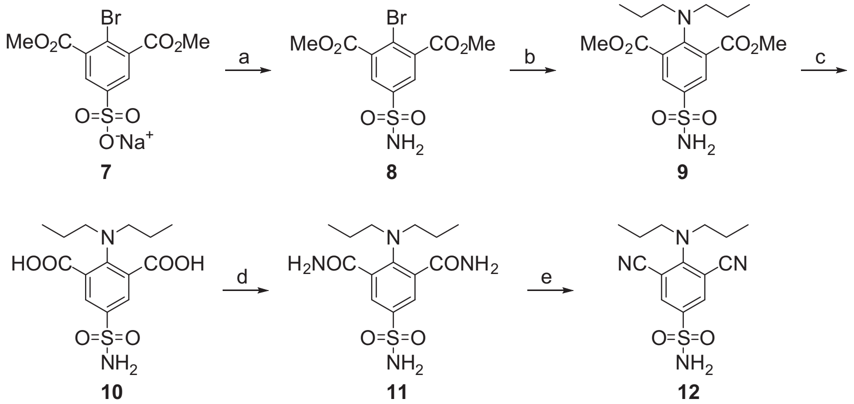


4 **5a-j** **6a-n**

5a: R^1 = Ph, R^2 = CH_2OH
5b: R^1 = Ph, R^2 = $(CH_2)_2OH$
5c: R^1 = R^2 = 4-Tolyl
5d: R^1 = R^2 = *c*Hexane
5e: R^1 = R^2 = CH_2Ph
5f: R^1 = R^2 = Ph
5g: R^1 = Ph, R^2 = *c*Pr
5h: R^1 = R^2 = $CH(CH_3)_2$
5i: R^1 = R^2 = CH_2CH_3
5j: R^1 = R^2 = $(CH_2)_2CH_3$

6a: R^1 = Ph, R^2 = CH_2Cl, R^3 = H
6b: R^1 = Ph, R^2 = $(CH_2)_2Cl$, R^3 = H
6c: R^1 = R^2 = 4-Tolyl, R^3 = H
6d: R^1 = R^2 = *c*Hexane, R^3 = H
6e: R^1 = R^2 = CH_2Ph, R^3 = H
6f: R^1 = R^2 = Ph, R^3 = H
6g: R^1 = Ph, R^2 = *c*Pr, R^3 = H
6h: R^1 = R^2 = $CH(CH_3)_2$, R^3 = H
6i: R^1 = R^2 = CH_2CH_3, R^3 = H
6j: R^1 = R^2 = $(CH_2)_2CH_3$, R^3 = H
6k: R^1 = R^2 = CH_2CH_3, R^3 = $(CH_2)_2OH$
6l: R^1 = R^2 = CH_2CH_3, R^3 = 4-phenol
6m: R^1 = R^2 = CH_2CH_3, R^3 = Ph
6n: R^1 = R^2 = CH_2CH_3, NHR^3 = $N(CH_3)_2$

Scheme 2. Reagents and conditions: (a) secondary amine, reflux, or secondary amine hydrochloride, Et_3N, MeOH, reflux, 4 h; (b) (i) PCl_5, CH_2Cl_2, rt, 3 h; (ii) NH_3 (7 N in MeOH), or amine, Et_3N, THF, 0 °C to rt, 2 h.



Scheme 3. Reagents and conditions: (a) (i) $PCl_5/POCl_3$, chlorobenzene, reflux, 24; (ii) NH_3 (0.5 M in 1,4-dioxane), 0 °C, 1 h, rt, 1 h; (b) dipropylamine, K_2CO_3, Cu, dioxane, reflux, 5 h; (c) 3 N NaOH, MeOH, reflux, 2 days; (d) (i) CCl_3COCCl_3, PPh_3, THF, 0 °C, 1 h; (ii) NH_3/dioxane, rt, 1 h; (e) $PdCl_2$, CH_3CN/H_2O, 24 h.