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Synthesis, Structural Determination, and Pharmacology of Putative Dinitroaniline Antimalarials

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Abstract: A series of novel, homologous compounds possessing the general formula N^1-N^2 -bis(2,6-dinitro-4-trifluoromethylphenyl)-1, n -diamino alkanes (where $n = 4, 6, 10$ or 12), were designed to probe inter- and intra- binding site dimensions in malarial parasite (*Plasmodium*) tubulin. Various crystal structures, including chloralin and trifluralin, an isopropyl dimer, and 2,6-dinitro-4-trifluoromethylphenylamine, were determined. Dinitroanilines, when soluble, were evaluated both in culture and in vivo. Trifluralin was up to 2-fold more active than chloralin against cultured parasites. The isopropyl dimer was water soluble (>5 mM) and revealed activity superior to that of chloralin in culture. The effects of selected dinitroanilines upon the mitotic microtubular structures of *Plasmodium*, the putative target of these dinitroanilines, were also determined. Electronic properties of the molecules were calculated using DFT (HF/6-31+G* level) to ascertain whether incorporation of such a pharmacophore could allow both QSAR and rational development of more selectively toxic antiparasitic agents.

Introduction

Malaria is a parasitic infection transmitted to humans (and other animals) by female *Anopheles* mosquitoes when taking a blood meal.^[1] The increase in drug resistant forms of *Plasmodium*,

especially of the *P. falciparum* species,^[2] continues to threaten tropical and sub-tropical regions of the world and with climate change, infections could spread to other, currently malaria free zones.

The most recent guidelines for the treatment of uncomplicated malarial infections suggest various antimalarial combination therapies which include: artemether and lumefantrine, artesunate and (amodiaquine or mefloquine); dihydroartemisinin + piperazine or a triple combination therapy of artesunate + sulfadoxine-pyrimethamine.^[3] However, adverse side effects of certain antimalarial drugs in current use,^[4] and the appearance and propagation of drug resistant strains of *Plasmodium*^[2b] necessitate the discovery and development of new and less toxic chemotherapeutic agents with novel modes of action.^[5]

A wide variety of endogenous compounds and xenobiotics produce directly (or indirectly) reactive oxygen species (ROS) that can induce oxidative stress in both the infected host and parasite. Often ROS are generated by electron transfer (ET) or other routes mediated by free radicals. Principal ET functionalities are quinones (or their precursors), conjugated imines, metal complexes and aromatic nitro compounds (ArNO₂). Certain dinitroaniline herbicides,^[6] (Figure 1) and their analogues^[7] which are photo-labile^[8] also exhibit activity against Leishmaniasis, a disease endemic to tropical and sub-tropical regions,^[6] both in culture and *in vivo*.^[7, 9] These compounds have also exhibited activity against a range of parasites commonly infecting humans and domesticated animals, including *Plasmodium*,^[10] *Cryptosporidium*,^[11] *Leishmania*,^[9a, 12] *Entamoeba*^[13], *Babesia*^[14] and *Toxoplasma*.^[15] One such nitroaromatic compound, trifluralin **1** (Figure 1),^[16] a widely used commercial pre-emergent herbicide, was initially considered the active agent against leishmaniasis.^[6] However, subsequent investigations implicated its industrial precursor, chloralin **5** as the true active molecule.^[17] **5** was found to be a contaminate of commercial samples of trifluralin, confounding ascription of bioactivity within the dinitroaniline class of compounds. Since **5** is one hundred times more potent than **1** against *Leishmania* promastigotes and targets parasite tubulin,^[18] it has attracted significant attention from parasitologists. Reversible polymerisation of tubulin facilitates organelle transport, chromosome segregation, and maintains cell structure integrity.^[10a, 19] Although, tubulins are well-conserved protein families, slight sequence differences between parasite and human tubulins can drastically affect the activity of certain microtubule inhibitors making them candidate drug targets.^[18c, 20] In the case of benzimidazole anthelmintics,^[21] this differential toxicity has been exploited in chemotherapy.^[22] The inhibitory activity of **5** has been associated with displacement of its reactive C-4 chloro moiety by cysteine residues present in *Leishmania* tubulin, thereby inhibiting microtubule assembly.^[17a]

Dinitroanilines appear to inhibit distinct parts of the developmental cycles of protozoal parasites. For example oryzalin **3** irreversibly arrested trophozoite mitosis in *E. histolytica*.^[13] In *Toxoplasma gondii*, dinitroanilines appear to block nuclear division by inhibition of intra-nuclear spindle formation, but other cytoskeletal components were also differentially affected by the drugs tested.^[15] Isolation of oryzalin-resistant *Toxoplasma gondii*

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clones shows that resistance can be induced by point mutations, in α -tubulin.^[23]

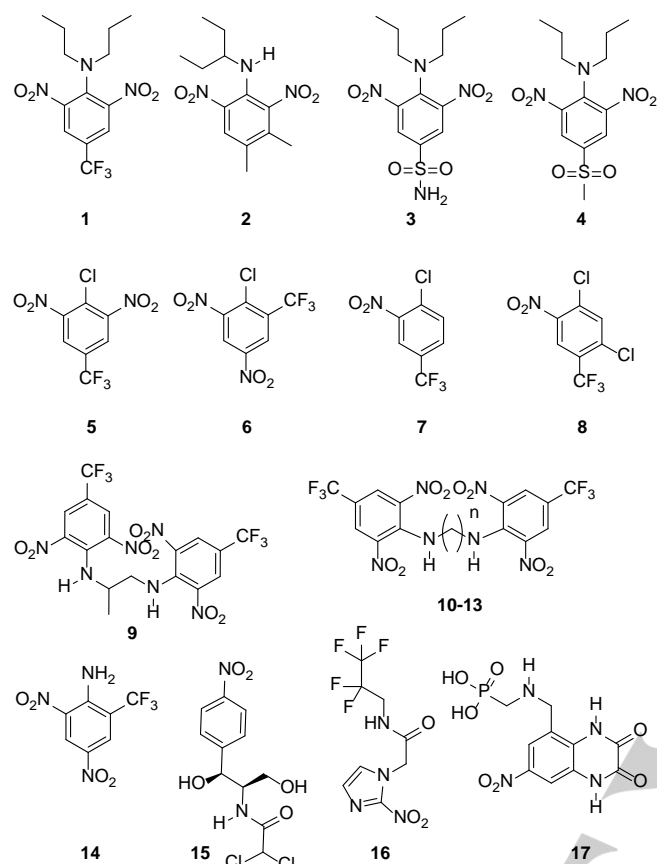


Figure 1. Chemical structures and numbering. Trifluralin **1**, pendimethalin **2**, oryzalin **3**, desaminomethyloryzalin **4**, chloralrin **5**, 2-chloro-3, 5-dinitrobenzotrifluoride (isochloralin) **6**, 4-chloro-3-nitrobenzotrifluoride **7**, 2,4-dinitro-5-nitrobenzotrifluoride **8**, N^1, N^3 -bis-(2,6-dinitro-4-trifluoromethyl-phenyl)-propane-1,2-diamine, where $n = 2$, **9**; where $n = 4$, **10**; where $n = 6$, **11**; where $n = 10$, **12**; where $n = 12$, **13**, 2,6-dinitro-4-trifluoromethyl-phenylamine **14**, chloramphenicol **15**; 2-2-nitro-1H-imidazol-1-yl-N-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5) **16**, [(7-nitro-2,3-dioxo-1,2,3,4-tetrahydro-quinoxalin-5-ylmethyl)-amino]-methyl)-phosphonic acid (AMP397) **17**.

Studies of the effects of dinitroaniline compounds against malarial parasites have shown that they are selectively toxic to parasite rather than host cells but lack the potency required for further development as antimalarial agents.^[10a, 24] The study of Kaidoh *et al.*^[24c] showed by electron microscopy that micromolar (μM) concentrations of **1** caused fragmentation and increased diameter of microtubules and dissolution of the sub-pellicular microtubule complex in *P. falciparum* gametocytes. Fennell *et al.*^[25] using asexual blood-staged parasites demonstrated that **1** and **3** inhibited progression through schizogony, blocked mitotic division, and caused accumulation of abnormal microtubular structures. Moreover, radiolabelled **1** interacted with purified, recombinant parasite tubulins but to a much lesser extent with bovine tubulins. **3** was also an inhibitor of liver-stage schizogony.^[26]

Identification of parasite tubulin as a novel target in *Leishmania*^[17a] suggested to us that low cost dinitroaniline herbicides, such as **1**, merited further evaluation and development as potential antimalarial agents. This study consisted of the syntheses, purification and quantum mechanical study and pharmacological evaluation of selected dinitroaniline analogues against *Plasmodium* in culture and *in vivo*. The presence of fluorine in these compounds imparts desirable

pharmacological properties and delays premature metabolism.^[27] Since both structural and electronic features dictate pharmacodynamic (drug-receptor interactions), this information was sought through X-ray diffraction of selected dinitroanilines. We also report the preparation and activity of a novel trifluralin dimer active against *P. falciparum* in culture.

Results and Discussion

Synthetic studies and compound selection procedure

Several compounds were chosen from our drug bank for initial, concurrent evaluation against a lethal strain of *P. berghei* in mice and chloroquine-sensitive and -resistant strains of *P. falciparum* in culture. Selected substances were not compromised by poor physical properties^[28] in order to facilitate testing in culture. Experiments *in vivo* were not restricted by aqueous solubility, because the subcutaneous (s.c.) route was used for injections formulated in dimethyl sulfoxide (DMSO) and olive oil.

Mindful that the industrial precursor of **1** has been proposed to be the active antiparasitic agent, we included in this study an isomer of **5**, isochloralin **6** and the structurally related **8**. We hypothesised that evaluation of the antimalarial activity of this potentially *iso*-lipophilic isomer of **5** would indicate if steric congestion, around the labile halide group, depresses the previously postulated attack by thiols presumed present within the target receptor. Structure-activity relationship analyses of **5** implicate the displaceable chloride moiety activated by a single nitro group as the pharmacophore; these contributed to selecting compound **7** for antimalarial tests. Hence, a judicious selection and evaluation of compounds depicted in Figure 1 could reveal a functional antimalarial pharmacophore for dinitroanilines.

The initial aim was to estimate the average *inter-* or *intra*-binding site dimensions in tubulin polymers using a homologation strategy. Consequently, a series of dimers (compounds **9** - **13**, Figure 1) were designed for preliminary evaluation in culture.

Compounds **1** and **2** (Figure 1) were synthesised using a commercial robot (Anachem SK233) capable of automated synthesis coupled to a HPLC-MS system,^[29] which screened a wide variety of bases and solvents (data not shown) to establish optimal reaction conditions and reagents that effectively promoted $\text{S}_{\text{N}}\text{Ar}$ reactions between a variety of amines and various *mono*-, *di*- and *tri*-nitro substituted haloarenes. Inclusion of an organic weak base, triethylamine to scavenge HCl generated during the $\text{S}_{\text{N}}\text{Ar}$ reaction, and toluene at reflux as solvent, under a protective blanket of argon, proved superior to other base/solvent conditions examined. High boiling solvents were especially problematic as dinitroanilines tend to readily sublime under moderate vacuum. A transient red colouration noted during drop-wise addition of reagents during reactions suggested participation of the nitro group in stabilising the labile Meisenheimer intermediate complex formed during the dechloro-amination reaction.^[30]

The construction of **9** proved interesting since refluxing **5** with 1,3-diaminopropane provided a compound that proved insoluble in chloroform so a comparative NMR spectrum could not be obtained. The NMR spectrum in DMSO was unsymmetrical suggesting that the product was not the postulated compound. A crystal suitable for a diffraction study revealed that a rare rearrangement to the isopropyl dimer, a situation described in the literature during the base catalysed preparation of (2-diethylamino-propyl)-carbamic acid (2-methyl-cyclohexylester) from *N,N*-diethyl-propane-1,3-diamine.^[31]

X-ray Crystallography

The structures of **1**, **5**, **8**, **9** and **14** are given in Figure 2 and structural details have been deposited with the Cambridge Crystallographic Data Centre (reference numbers CCDC 219352 - 219356). Crystal data and refinement details are shown in Table S1. In the structure of **1**, the nitrogen atom has a trigonal environment with the three subtended angles at nitrogen adding up to $359.6(2)^\circ$. The plane of C(4), N(7), C(71), C(81) intersects

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that of the phenyl ring at an angle of $36.4(2)^\circ$. The torsion angles of the ethyl groups are somewhat surprising being C4-N7-C71-C72 $126.1(4)$, N7-C71-C72-C73 $-63.2(7)$ and C4-N7-C81-C82 $118.9(5)$, N7-C81-C82-C83 $-172.3(4)^\circ$. Hence, one propyl group is in a *-gauche* conformation and the other is in the expected *trans* environment. The two nitro groups make angles of $47.5(2)$ and $54.6(2)^\circ$ with the phenyl ring.

Clearly, the rotation of the two-alkyl groups out of the plane is a steric effect concomitant with an opposite rotation from the nitro groups. Thus, with reference to Figure 2, O(32) is below the plane while C(71) is above and O(52) is above the plane while C(81) is below. The O...C distances in the two cases are $2.869(6)$ and $2.942(5)$ Å.

In the structure of chloralin **5** the two molecules in the asymmetric unit show different types of disorder. Molecule A contains rotational disorder of the $-CF_3$ group, whereas molecule B contains rotational disorder of one nitro group with the oxygen atoms alternatively above and below the ring plane as well as rotational disorder of the $-CF_3$ group. In A, the two nitro groups

intersect the ring plane at angles of $61.6(3)$, $34.9(5)^\circ$ and in B $63.0(3)$ and $53.9(3)$ or $56.9(10)^\circ$. So it is clear that the presence of the chloride substituent intermediate between the two nitro groups has had a similar effect on the orientation of the nitro groups to the $-N(\text{propyl})_2$ group in **1**.

In the structure of **8** the nitro group is twisted out of the plane of the aromatic ring by an angle of $38.3(2)^\circ$. This is despite the fact that there is a substituent only on one side of the nitrate. In this structure the $-CF_3$ group is ordered unlike the situation in **1** and **5**, presumably this is due to the presence of the adjacent Cl(6) atom. It is noteworthy that the $-CF_3$ group takes up a conformation in which two of the fluorine atoms are almost equidistant from the adjacent Cl(6) atom at $3.114(3)$, $3.128(4)$ Å.

The structure of the trifluralin dimer *N',N''-di-(2,6-dinitro-4-trifluoromethylphenyl)-1,3-propylenediamine* **9** was obtained as a racemic crystal and is somewhat surprising because the two amine nitrogen atoms are only $2.908(9)$ Å apart because the linkage between them contains several torsion angles that are *gauche* rather than *trans* as might be expected;

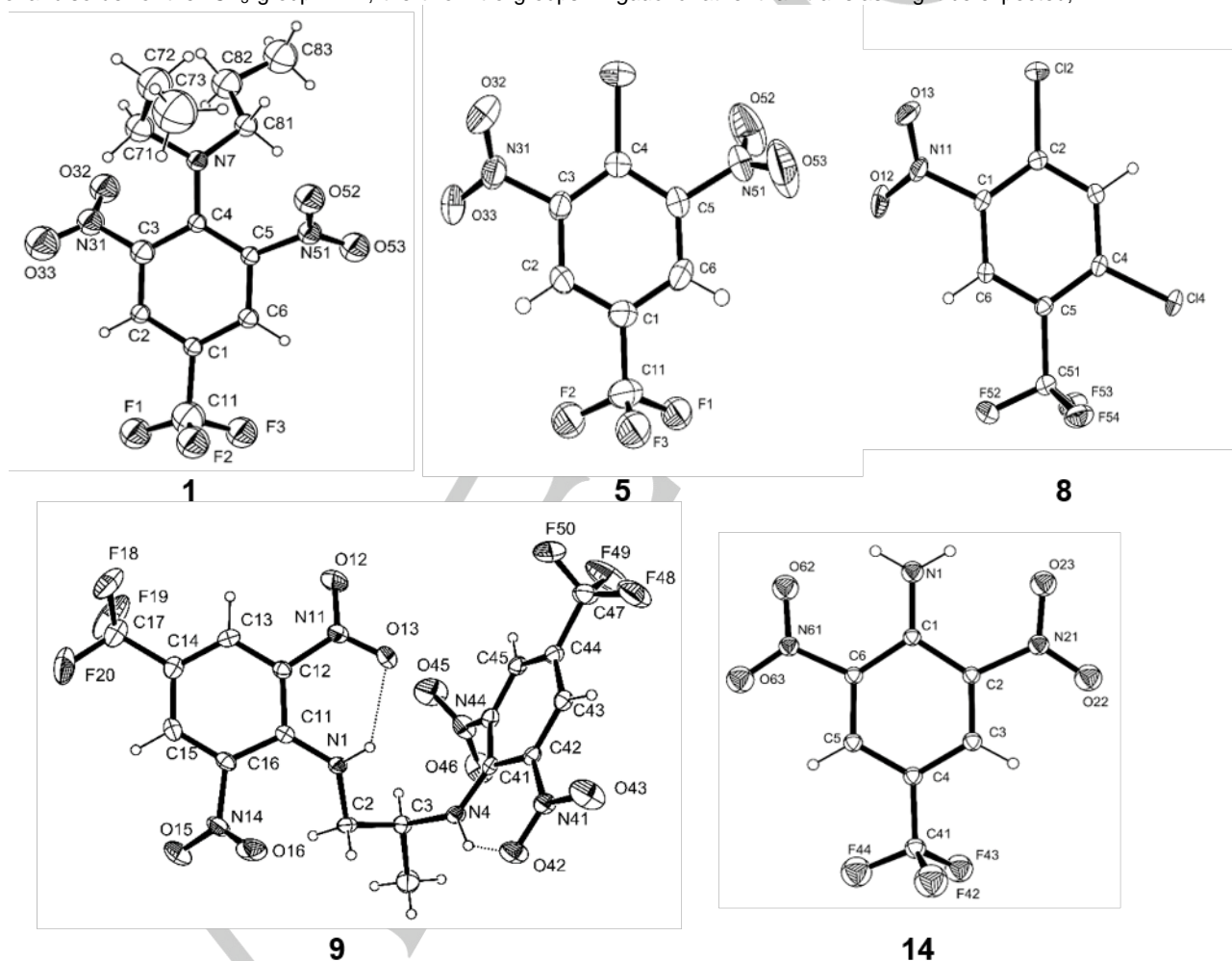


Figure 2. The structures of **1**, **5**, **8**, **9** and **14** with ellipsoids at 25% probability, and intramolecular hydrogen bonds are shown as dotted lines. **1** has one propyl group in a *-gauche* and the other in a *-trans* conformation. The $-CF_3$ group is disordered over two orientations, only the major component is shown. Molecule A is shown for **5** with one set of positions for the disordered $-CF_3$ group. Molecule B has a similar basic geometry but rotational disorder in one nitrate and a $-CF_3$ group. The conformation of the $-CF_3$ group in **8** ensures that fluorine atoms are staggered with respect to the adjacent Cl(6). **9** has N...N distances of $2.642(5)$ and $2.639(5)$ Å.

being for C(5)-C(4)-N(41)-C(42) $-152.3(4)^\circ$, C(4)-N(41)-C(42)-C(43) $136.7(4)^\circ$, N(41)-C(42)-C(43)-N(44) $65.4(4)^\circ$, C(42)-C(43)-N(44)-C(51) $-103.8(5)^\circ$, C(43)-N(44)-C(51)-C(56) $159.3(4)^\circ$. The two amine nitrogen atoms N(41) and N(44) both form

intramolecular hydrogen bonds with their adjacent oxygen atom O(72), O(52), respectively, with dimensions N...O, N-H...O and H...O $2.642(5)$ Å, 130.6° , 2.00 Å and $2.639(5)$ Å, 127.6° , 2.02 Å respectively Å). These two nitro groups intersect the plane of the

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benzene ring at angles of 9.5(11), 7.9(7)°, respectively, while the other nitro groups which are sterically hindered by the carbon atoms C(1) and C(3) are twisted by angles of 40.2(6), 53.8(5)° out of the plane of the benzene rings. The two aromatic rings intersect at an angle of 60.7(2)° though there are no intramolecular hydrogen bonds between them, nor indeed any close contacts between the two aromatic rings and their substituents.

As further confirmation of the correlation between the rotation of the nitro group relative to the phenyl ring and the substituent(s) on the amine nitrogen, the structure of 2,6-dinitro-4-trifluoromethyl-phenylamine **14** was also determined.

Here, the two-nitro groups are adjacent to an amine group and formed hydrogen bonds via O32 and O52. Dimensions were O...H 2.01, 2.01 Å, O...H-N 128, 130° and O...N 2.630(9), 2.642(9) Å, respectively. As a consequence the angles of rotation of the two groups relative to the ring were 9.8(12) and 8.6(10)° thus showing that steric effects are paramount in considering the angle of rotation of the nitro groups in the molecules reported here.

Theoretical calculations

Quantum mechanics calculations using the Gaussian03 program [32] were carried out to establish whether packing effects had any serious effects on the molecular geometry of these complexes. Calculations on trifluralin **1** using the crystal structure as an input model, converged at the B3LYP/6-31+G* level with the N(7) having a trigonal environment. The angle between the plane of C(4), N(7), C(71), C(81) and the phenyl ring was 38.6° while the nitro groups intersected the phenyl ring at values of 42.1, 42.0° confirming that the conformation found in the crystal structure is not affected significantly by crystal packing, although it might be expected that both N-propyl groups would have the *trans* conformation though this would not be feasible due to clashes between the chains.

Equivalent calculations have been carried out on this molecule with the NPr₂ groups replaced with (a) NH₂ and (b) NHMe. In molecule (a), the calculation resulted in a planar conformation in which both nitro groups and the NH₂ group were coplanar with the phenyl ring, angles of intersection 0.4° presumably forming some weak interaction between the amine hydrogen atoms and the nitro groups. This can be compared to the crystal structure of **14** where the nitro groups are slightly twisted out of the plane of the phenyl ring by angles of 9.8(12), 8.6(10)°. In the case of molecule (b), the nitro group adjacent to the N-H group remained closely planar with the phenyl ring making an intersection of 8.1° while the nitro group adjacent to the methyl group twisted by 49.8°. This result can be compared to that found in the crystal structure of the trifluralin dimer **9** where there are two independent -NHR groups and the angles found are 9.5(11), 7.9(7)° for the nitro group adjacent to -NH and 40.2(6), 53.8(5)° for the nitro group adjacent to -NR.

These experimental and theoretical results show that the bulk of the NPr₂ group in **1** has a similar effect to that of the chlorine in chloralin **5** on the twist in the adjacent nitro groups. However, with a primary amine, both adjacent nitro groups will be coplanar with the nitro group while only one nitro group in a secondary amine is twisted significantly from the plane. This difference in structure of the adjacent nitro groups may indicate that only the tertiary amine will be active and that the presence of a hydrogen atom bonded to the nitrogen and forming a weak hydrogen bond to the adjacent nitro group may affect activity.

We next investigated the electronic properties of molecules **5**, **6**, **7** and **8** (Figure 1) to establish whether there were any obvious correlations with activity, the first two being active and latter two inactive. Selected parameters are shown in Table 1. Clearly all but the charge on the chlorine can be correlated with biological activity but with only four test compounds, no decisive conclusions can be drawn here but may be helpful in drawing

conclusions as to which substituents enhance electron transfer in future studies (see EPR section).

Table 1. Selected physicochemical and electronic properties of compounds tested in culture and in vivo.

	charge	HOMO(a.u.)	LUMO(a.u.)	C-Cl(Å)	μ(D)
	on Cl				
5	0.361	-0.319	0.136	1.720	1.601
6	0.430	-0.323	-0.147	1.722	1.826
7	0.360	-0.310	0.136	1.760	4.301
8	0.357,	-0.302	0.126	1.741,	2.978
	0.327			1.735	

Pharmacological evaluations

Six potential antimalarials were evaluated against the cloned *P. falciparum* subline FCH5.C2 [33] and the chloroquine- and pyrimethamine-resistant strain K1/Thailand (Table 2), and *in vivo* against lethal *P. berghei* (Table 3), previously utilised to construct our functional *in vivo* receptor for bisquinolines. [34] Both evaluations used commercially (Sigma-Aldrich) available chloroquine diphosphate and artemisinin as standard antimalarial drugs.

Table 2. Antimalarial activity of dinitroanilines against two cultured strains of *P. falciparum*.

Compound	IC ₅₀ FCH5.C2		IC ₅₀ K1/Thailand	
	48 h [μM]	72 h [μM]	48 h [μM]	72 h [μM]
1	5.9 ^[a]	2.7 ^[c]	6.5 ^[c]	3.2 ^[c]
5	7.1	5.9	10.0	6.4
6	7.3	7.8	11.0 ^[a]	5.5
7	>64 ^[c]	>64 ^[c]	>64 ^[c]	>64 ^[c]
8	>64 ^[c]	>64 ^[c]	>64 ^[c]	>64 ^[c]
9	4.8 ^[b]	4.9	3.8 ^[c]	3.8 ^[a]

[a] Different from chloralin at p<0.05, Student's t-test. [b] Different from chloralin at p<0.01, Student's t-test. [c] Different from chloralin at p<0.001, Student's t-test.

Table 3. Antimalarial activity of dinitroaniline compounds against *P. berghei* N in mice.

Compound	Dose/ μmol kg ⁻¹	<i>P. berghei</i> parasitaemia % control
1	75	89 ± 11
	149	84 ± 16
	298	87 ± 10
5	597	87 ± 8
	46 ^T	71 ± 14
	92 ^T	79 ± 7
	185 ^T	49 ± 9 ^[b]
6	46 ^T	66 (54 – 78) ^[a]
	92 ^T	
7	228	86 ± 8
	443	98 ± 10
8	85 ± 16	
	96	78 ± 11
	192	98 ± 4
	385	95 ± 13

Compounds were injected s.c. twice daily (total number of doses = 5) in mice (initial group sizes 5-10) inoculated with *P. berghei* N. Parasitaemias were

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determined 72 hr after inoculation and expressed as percentages (mean \pm SEM for 4 – 7 animals on day 3, except [a] average and range for 2 mice) of control values in concurrent vehicle-treated (control) malarial mice. [b] $P < 0.05$; ^T denotes toxicity (hypothermia, weight loss, reduced motor activity) associated with therapy.

Antimalarial activity *in vivo*

Results show that **1** was not antimalarial in the *in vivo* three day-suppression test, against *P. berghei* N in mice, used in this study with doses in the range 25 - 200 mg kg⁻¹ (75 – 597 μ mol kg⁻¹) s.c. twice daily (Table 3). All animals in this study survived treatment with **1**. In contrast, **5** was found to be toxic; a single injection of chloralrin 200 mg (739 μ mol) or 100 mg (370 μ mol) kg⁻¹ s.c. caused death or necessitated humane killing, therefore, doses of 12.5, 25 and 50 mg (46, 92 and 185 μ mol respectively) kg⁻¹ s.c. twice daily were used in the three day malaria suppression test. Antimalarial activity was evident with **5** at 25 (92 μ mol; $P < 0.1$) and 50 mg (185 μ mol; $P < 0.05$) kg⁻¹ s.c.; ID₅₀ 50 mg (185 μ mol) kg⁻¹, but toxicity was evident as hypothermia, weight loss and decreased motor activity in some animals. A readily available, theoretically isolipophilic isomer of **5**, 2-chloro-3,5-dinitrobenzotrifluoride **6**, was also toxic following a single injection s.c. of 100 mg (370 μ mol) kg⁻¹. Reduced doses of 25 mg (92 μ mol) and 12.5 mg (46 μ mol) kg⁻¹ twice daily also caused toxic effects similar to **5** in mice used in the antimalarial screen. Although, **6** at 12.5 mg (46 μ mol) kg⁻¹ reduced *P. berghei* parasitaemia to 66 % of that seen in vehicle-treated mice, the low number of survivors on day 3 (2 from initial group of five mice) makes this result statistically insignificant). Compounds 4-chloro-3-nitrobenzotrifluoride **7** (50 and 100 mg kg⁻¹; 228 and 443 μ mol kg⁻¹, respectively) and 2,4-dichloro-5-nitrobenzotrifluoride **8** (25, 50 and 100 mg kg⁻¹; 96, 192 and 385 μ mol kg⁻¹, respectively) were neither antimalarial (Table 1) nor toxic in mice during the period of the three day suppression test.

It is relevant to note that **1** is also inactive *in vivo* against *Cryptosporidium parvum* (dosed at 100 mg kg⁻¹)^[11] and is neither carcinogenic or tumorigenic in male B6C3F1 mice or in Osborne-Mendel rats of either sex.^[35] **1** is rapidly cleared in rats by faecal and urinary excretion with only 10 % remaining unmetabolised.^[36] Rapid metabolism and clearance in rodents^[37] may account for the inactivity of **1** in rodent models of *Plasmodium* and *Cryptosporidium in vivo*. Another possibility is that the high log P value of **1** (4.81) constrains this substance at or around the site of injection.^[11]

We were unable to separate the antimalarial effects of **5** or its analogue, **6** from their toxicity in mice. The compounds 4-chloro-3-nitrobenzotrifluoride **7** and 2,4-dinitro-5-nitrobenzotrifluoride **8** had no antimalarial activity or toxicity and thus appear to lack the pharmacophore associated with **5** and **6**.

Antimalarial activity in culture

Compounds **9** – **13** were not fully soluble in DMSO at suitable concentrations (>5 mM), limiting acquisition of a more complete QSAR profile. Although, **5** may well be the active toxic and antileishmanial component of **1** in other investigations^[17a], it was not a contaminant in our rigorously purified samples.

Antimalarial activity was evaluated after 48 and 72 h incubations using asynchronous cultures of chloroquine-sensitive and -resistant strains of *P. falciparum* (Table 2). **1** was slightly but significantly more active than **5** against both strains. The observed activity of **1** is consistent with previously published data.^[24-26, 38] The potency of the compound was unaffected by the resistances of the K1/Thailand strain to unrelated agents. The theoretically isolipophilic isomer of **5**, **6**, displayed similar activity to **5**. Activity was abolished by the removal of one of the nitro- groups of **5**, **7**, and was not restored by the extra Cl in **8**. The trifluralin dimer **9** was slightly more active than **1** so is possible that only one half of this dimer interacts at the receptor site, particularly as the

relatively folded conformation makes it unlikely that both aromatic rings can simultaneously interact at a putative target site. However, interestingly, the activity of **9** was either superior or equal to the monomeric **5** and **6**. Disappointingly, other members of the homologous series proved insoluble at the levels required for testing on cultured parasites. When strategies for improving the solubility of these putative antimalarials are discovered, it will be interesting to see whether a dimer can be found that is superior to **1** as a result of an optimised linker length.

Examination of mitotic microtubular structures of **1**- or **5**-treated parasites by immunofluorescence revealed a breakdown of the normal hemispheres and microtubule-organising centres and their replacement by fragmented tubulin labelling (Figure 3).

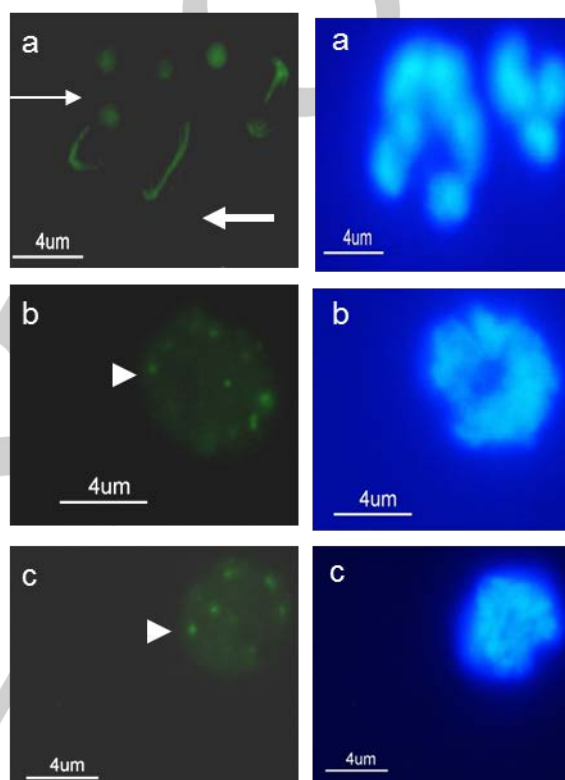


Figure 3. Mitotic microtubular structures of cultured *P. falciparum* parasites viewed by immunofluorescence using antibodies to *P. falciparum* β -tubulin (left) and DAPI nuclear stain (right). (a) Untreated parasites: note microtubule-organizing centres (small arrow) and hemispheres (large arrow) and distinct nuclear bodies (right). (b) treated with **5**. Note in both (b) and (c) tubulin fluorescence in small dots with slight, diffuse background and loss of normal microtubular structures (arrowheads), and disorganisation of nuclear DNA (right). Exposure to inhibitors was 20 μ M for 6 h.

This may be the result of net depolymerisation of these microtubules, but it is not clear why the same diffuse labelling seen with vinblastine, dolastatin and auristatin PE^[39] is not apparent. This effect is likely to be more relevant to growth inhibition than the previously reported effects on invasion and the f-MAST^[38] because of the lower concentrations used in the present study.

Dow *et al.*^[10b] reported the activities of **1**, **5**, pendimethalin, benfluralin and **3** against *P. berghei* in culture and in a rat model of malaria. They reported that while moderately active against cultured parasites (IC₅₀, 1.3 μ M), **1** did not reach sufficient plasma levels in rats to obtain an antimalarial effect *in vivo*. Their results differ slightly from ours in the lower activity of **5** against cultured

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parasites (IC_{50} , $16 \mu\text{M}$) but this could be a species difference. **5** was not tested *in vivo* by these investigators.

Mechanism of antimalarial action

Support for an *ipso*-de-chloro-thiolation mechanism (compare to the aforementioned synthesis of **9**, where the amine is replaced by a cysteine residue) is provided by evidence that **5** interferes with *Leishmania* tubulin^[19] and microtubule assembly in culture ($IC_{50} = 22 \mu\text{M}$), whereas **1**, which possesses the much poorer amino-*bis*-propyl leaving group, does not.^[40] The mechanism of inhibition of thiol dependent enzymes has been reviewed.^[41] However, the proposed mechanism of antitubulin action involving certain mono-nitroanilines has been questioned.^[18a] Analogues tested against purified tubulin failed to inhibit microtubule assembly, but had antileishmanial activity in culture, which suggests that further analogue development may reveal non-toxic agents. **1** may bind to its receptor by non-covalent electrostatic and van der Waals interactions, whereas **5** can form a covalent adduct with nucleophilic side chains of parasite protein amino acids. Computational modelling has suggested binding sites within both dinitroanilines in kinetoplastid and apicomplexan α -tubulin. However, there are four amino acid differences between the kinetoplastid and apicomplexan binding site residues, which might contribute to the differential efficacy of specific dinitroanilines.^[42]

Although, one electron reduction of the nitro group is the basis of the antiparasitic action of nitroimidazole drugs,^[43] this does not appear to be the mode of action in intact cells.^[44] For instance, 5-nitrofurfural *N*-butyl semicarbazone^[45] has been shown to have antitrypanosomal activities, targeting trypanothione reductase through a nitro anion radical mechanism. Nitric oxide donors have been reported to inhibit *Leishmania* cyteine proteinase^[46] and a similar mode of action could apply to *Plasmodium*. Although, nitrofurantoin radical anion and GSH cannot be detected under physiological conditions within parasites,^[43a] such experiments need to be performed for dinitroanilines against both drug-resistant and -sensitive *Plasmodium*.

EPR Studies of trifluralin-heme interactions

Previous investigations have indicated that **3** has an effect on the liver stage of the *Plasmodium* life cycle.^[26] As this organ is a major site of drug deactivation and occasionally inactivation during xenobiotic metabolism especially with heme containing enzymes such as cytochrome P450's, the interaction of reduced **1** with heme was of interest. In order to elucidate the interaction of **1** with heme, continuous-wave (CW) electron paramagnetic resonance (EPR) spectra (Figure 4) were recorded in order to investigate the spin state of the heme iron. The spectrum of hemin is typical of high spin ferric heme ($S = 5/2$) with turning points at $g_{\perp}^{\text{eff}} = 6.0$ and $g_{\parallel}^{\text{eff}} = 1.99$. Upon addition of 4-fold molar excess of **1** under aerobic conditions a dramatic decrease in the intensity of the ferric resonance was seen with no subsequent new signals as has previously been observed with compounds related to mefloquine.^[47] These observations suggest a change of iron spin state to an EPR silent state. The mechanism of action will need further investigation.

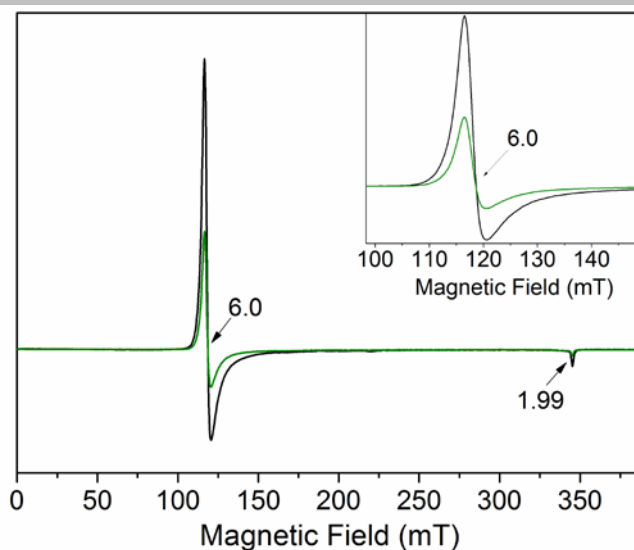


Figure 4. EPR spectra of hemin and hemin mixed with trifluralin **1**. 9 GHz CW EPR spectra of a solution of $200 \mu\text{M}$ hemin (black), hemin on manual 5 sec mixing of 4-fold molar excess of **1** (green). Hemin concentration was $200 \mu\text{M}$ in all experiments. Inset shows magnification of the g_{\perp}^{eff} region. Spectra were recorded at 5 K, 100 kHz, 0.4 mT modulation amplitude, 1 mW, 1 scan.

Mechanism of toxicity

The presence of halogen in natural products, previously considered a rare occurrence, has been identified in over 2600 compounds.^[48] Non-toxic, low molecular weight drugs containing both nitro and trifluoromethyl groups have been successfully designed including EF5 **16**^[49] and antiepileptic AMP397 **17**,^[50] although possessing a "structural alert" (the nitro group),^[51] sufficient safety data have initiated clinical development.^[52] Similar effects on mammalian tubulin have been described with small aromatic electrophiles such as 1-fluoro-2, 4-dinitrobenzene and 2,4-dichlorobenzyl thiocyanate^[53] suggesting that the antimalarial activity may involve, in part, formation of a covalent adduct within parasite tubulin^[54] but may also result in toxicity to the host.

The mechanism of action of several clinically useful drugs including vasodilators such as nitro-glycerine, radiosensitisers such as etanidazole (SR2508), which targets brainstem glioma,^[55] and antibiotics such as metronidazole,^[56] certain antileishmanials^[57] and putative anticancer agents^[44] require the presence of a nitro group. Chloramphenicol **15**, a halogenated nitrobenzene antibiotic produced by *Streptomyces venezuelae*^[48] and found in the moon snail *Lunatia heros*,^[58] lacks systemic toxicity following topical application that prevents opportunistic infections following eye surgery^[59]. However, it has been noted that compounds containing a nitro group are over-represented in the Ames test whether the compounds are metabolically activated or not.^[51b] In the case of **5** the results of this study indicate toxicity may preclude further development of this compound.^[60] **5** was found in this study to be antimalarial both in culture and *in vivo*, but its antimalarial activity was associated with host toxicity. This toxicity indicates a lack of selectivity by **5** for *Plasmodium* and suggests the compound is not preferentially sequestered by the parasite to an adequate degree for antimalarial drug use. **1** displayed antimalarial activity in culture, but not *in vivo* in mice. **1** also failed to display significant toxicity in this study. As indicated above, Dow *et al.*^[10b] reported a similar profile; **1** inhibited *P. berghei* in culture but not *in vivo* in rats following oral administration. These researchers suggested **1** was inactive *in vivo* because plasma concentrations attained after oral dosing were inadequate for antimalarial activity, perhaps due to the high

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solubility of **1** in host body fat,^[10b] or reduction by a first pass effect in the liver. Measurements of the uptake of radiolabelled **1** into *P. falciparum* suggested that accumulation in membranes may also limit the activity of **1** in cultured parasites.^[61] However, since the trifluralin class of compounds undergo extensive metabolism, at least within the rat,^[62] secondary xenobiotic metabolites could also be responsible for antiparasitic activity.^[26] Since dinitrophenols, putative metabolites of chloralrin type compounds, have significant toxicity, development of this class of compound will require extensive pre-clinical toxicity screening.

Conclusions

The long-term aims of our research on dinitroanilines as potential antiparasitic drugs are to: i) reduce the toxicity of the nitro moieties; ii) decrease the possibility of idiosyncratic drug reactions by structural modification;^[63] iii) ensure selective uptake into parasites; iv) measure intra and inter-subunit tubulin binding site dimensions and v) identify the trifluralin pharmacophore. These aims require compounds with improved physical properties compared to trifluralin and the novel dimers reported here.

5 can be considered a target for further development, because it is active against *Plasmodium*, however, it does display toxicity in the host. The discrepancy between the antimalarial activities of **1** in culture and *in vivo* cannot be readily explained. Low bioavailability due to uptake into host body fat and/or rapid metabolism and excretion may explain the lack of activity in laboratory rodents *in vivo*. The number of compounds examined so far is insufficient for establishing robust QSAR. In addition, compounds with better drug formulation profiles could be generated by molecular modifications that enhance aqueous solubility. Unlike in *Leishmania*, **1** [and also **9**] was found in this study to have activity against parasites in culture that could not be ascribed to contaminants in the test substances. Identification of a compound of similar potency to **1** against drug-resistant and -sensitive strains of *Plasmodium* in culture **9** indicates that the presence of the halide is not an absolute requirement for antimalarial activity. This observation suggests the nitro group may be undergoing bioactivation and may act by production of ROS as evidenced by interaction with reduction of Fe(III) within heme.^[46]

Both QSAR studies^[64] and drugs co-crystallised with putative target receptors have enabled rational drug evolution towards more selectively toxic antiparasitic agents.^[52, 65] A computational docking study of **3**, **1** and **5** to tubulin, found in *Toxoplasma gondii*, suggests that in this parasite, they exert their action by disrupting M-N loop contacts.^[23] Studies have shown that antimetabolic herbicides can bind to an unidentified site on malarial parasite tubulin and, notably, block development of liver-stage *Plasmodium* parasites.^[26] Consequently, emphasis must now shift to understanding which of these are responsible for activity and must in turn serve as further lead compounds in the developing the next cycle in eventually developing clinically useful drugs.

Further studies are needed to develop selective accumulation of such compounds into parasites whilst sparing host mitochondrial apparatus; the original concept of selective toxicity as promoted by A. Albert^[66] has indicated that it is not that a compound is not toxic but it should be selectively toxic. If we develop compounds known to selectively accumulate in mitochondria, incorporation of dinitroaniline groups into frameworks^[67] may provide more effective compounds. In conclusion, until the target site of dinitroanilines in *Plasmodium* has been characterised, and details of the receptor site clarified, it will be difficult to identify selectively toxic compounds of this structural class that have the high safety margins required for clinical use.

Supporting Information Summary

Full experimental details and spectroscopic characterizations are given in the supporting information.

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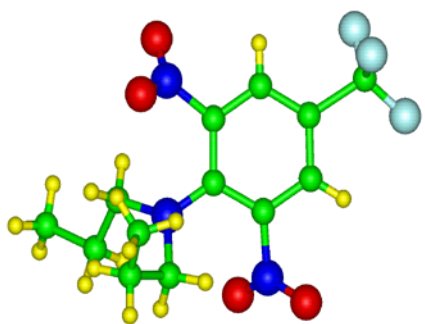
Keywords: antimalarial • chloralrin • trifluralin • DFT • X-ray

- [1] L. H. Miller, D. I. Baruch, K. Marsh, O. K. Doumbo, *Nature* **2002**, *415*, 673-679.
- [2] a) X. Z. Su, L. A. Kirkman, H. Fujioka, T. E. Wellems, *Cell* **1997**, *91*, 593-603; b) T. E. Wellems, C. V. Plowe, *J Infect Dis* **2001**, *184*, 770-776.
- [3] *Guidelines for the treatment of malaria*, 3rd Edition ed., World Health Organization, **2015**.
- [4] a) F. Nosten, R. N. Price, *Drug Safety* **1995**, *12*, 264-273; b) B. Rouveix, *Med Maladies Infect* **1999**, *29*, 326s-332s; c) M. M. van Riemsdijk, J. M. Ditters, M. C. J. M. Sturkenboom, J. H. M. Tulen, R. J. Ligthelm, D. Overbosch, B. H. C. Stricker, *Eur J Clin Pharmacol* **2002**, *58*, 441-445.
- [5] C. Le Manach, A. T. Nchinda, T. Paquet, D. G. Cabrera, Y. Younis, Z. Han, S. Bashyam, M. Zabiulla, D. Taylor, N. Lawrence, K. L. White, S. A. Charman, D. Waterson, M. J. Witty, S. Wittlin, M. E. Botha, S. H. Nondaba, J. Reader, L. M. Birkholtz, M. B. Jimenez-Diaz, M. S. Martinez, S. Ferrer, I. Angulo-Barturen, S. Meister, Y. Antonova-Koch, E. A. Winzeler, L. J. Street, K. Chibale, *J Med Chem* **2016**, *59*, 9890-9905.
- [6] M. M. Y. Chan, D. Fong, *Science* **1990**, *249*, 924-926.
- [7] J. D. Berman, *Antimicrob Agents Ch* **1994**, *38*, 1692-1692.
- [8] M. G. S. Tagle, M. L. Salum, E. I. Bujan, G. A. Arguello, *Photoch Photobio Sci* **2005**, *4*, 869-875.
- [9] a) J. W. Benbow, E. L. Bernberg, A. Korda, J. R. Mead, *Antimicrob Agents Ch* **1998**, *42*, 339-343; b) J. D. Berman, *Clin Infect Dis* **1997**, *24*, 684-703; c) P. C. Melby, *Curr Opin Infect Dis* **2002**, *15*, 485-490.
- [10] a) A. Bell, *Parasitol Today* **1998**, *14*, 292-292; b) G. S. Dow, A. Armson, M. R. Boddy, T. Itenge, D. McCarthy, J. E. Parkin, R. C. A. Thompson, J. A. Reynoldson, *Exp Parasitol* **2002**, *100*, 155-160.
- [11] A. Armson, S. W. Kamau, F. Grimm, J. A. Reynoldson, W. M. Best, L. M. MacDonald, R. C. A. Thompson, *Acta Trop* **1999**, *73*, 303-311.
- [12] G. Bhattacharya, J. Herman, D. Delfin, M. M. Salem, T. Barszcz, M. Mollet, G. Riccio, R. Brun, K. A. Werbovetz, *J Med Chem* **2004**, *47*, 1823-1832.
- [13] A. Makioka, M. Kumagai, S. Kobayashi, T. Takeuchi, *J Parasitol* **2002**, *88*, 994-999.
- [14] M. G. Silva, A. Domingos, M. A. Esteves, M. E. M. Cruz, C. E. Suarez, *Int J Parasitol-Drug* **2013**, *3*, 59-68.
- [15] T. J. W. Stokkermans, J. D. Schwartzman, K. Keenan, N. S. Morrisette, L. G. Tilney, D. S. Roos, *Exp Parasitol* **1996**, *84*, 355-370.
- [16] Q. F. Soper, Eli Lilly and Co Ltd (GB), U S, Patent US3257190A, **1966**.
- [17] a) H. L. Callahan, C. Kelley, T. Pereira, M. Grogl, *Antimicrob Agents Ch* **1996**, *40*, 947-952; b) M. M. Y. Chan, J. S. Tzeng, T. J. Emge, C. T. Ho, D. N. Fong, *Antimicrob Agents Ch* **1993**, *37*, 1909-1913; c) M. M. Y. Chan, M. Grogl, C. C. Chen, E. J. Bienen, D. Fong, *P Natl Acad Sci USA* **1993**, *90*, 5657-5661; d) M. M. Y. Chan, M. Grogl, H. Callahan, D. Fong, *Antimicrob Agents Ch* **1995**, *39*, 1609-1611.
- [18] a) K. K. Pitzer, K. A. Werbovetz, J. J. Brendle, J. P. Scovill, *J Med Chem* **1998**, *41*, 4885-4889; b) B. P. Chatterji, B. Jindal, S. Srivastava, D. Panda, *Expert Opin Ther Pat* **2011**, *21*, 167-186; c) *Tubulin-Binding Agents*, Springer, **2009**.

- [19] K. G. Jayanarayan, C. S. Dey, *J Clin Pharm Ther* **2002**, *27*, 313-320.
- [20] K. A. Werbovetz, *Tubulin as an Antiprotozoal Drug Target, Vol. 2*, Benthan Science, **2002**.
- [21] Q. A. Mckellar, E. W. Scott, *J Vet Pharmacol Ther* **1990**, *13*, 223-247.
- [22] E. Lacey, *Int J Parasitol* **1988**, *18*, 885-936.
- [23] N. S. Morrisette, A. Mitra, D. Sept, L. D. Sibley, *Mol Biol Cell* **2004**, *15*, 1960-1968.
- [24] a) J. Nath, I. Schneider, *Clin Res* **1992**, *40*, A331-A331; b) R. E. Fowler, A. M. C. Smith, J. Whitehorn, I. T. Williams, L. H. Bannister, G. H. Mitchell, *Mol Biochem Parasit* **2001**, *117*, 187-200; c) T. Kaidoh, J. Nath, H. Fujioka, V. Okoye, M. Aikawa, *J Eukaryot Microbiol* **1995**, *42*, 61-64.
- [25] B. J. Fennell, J. A. Naughton, E. Dempsey, A. Bell, *Mol Biochem Parasit* **2006**, *145*, 226-238.
- [26] E. Dempsey, M. Prudencio, B. J. Fennell, C. S. Gomes-Santos, J. W. Barlow, A. Bell, *Mol Biochem Parasit* **2013**, *188*, 116-127.
- [27] F. M. D. Ismail, *J Fluorine Chem* **2002**, *118*, 27-33.
- [28] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv Drug Deliv Rev* **2001**, *46*, 3-26.
- [29] D. F. Emiabata-Smith, D. L. Crookes, M. R. Owen, *Org Process Res Dev* **1999**, *3*, 281-288.
- [30] a) E. Buncel, J. M. Dust, F. Terrier, *Chem Rev* **1995**, *95*, 2261-2280; b) F. Terrier, *Nucleophilic Aromatic Displacement. The Influence of the Nitro Group*, VCH Publishers, Weinheim, **1991**; c) G. A. Artamkina, M. P. Egorov, I. P. Beletskaya, *Chem Rev* **1982**, *82*, 427-459; d) F. Terrier, *Chem Rev* **1982**, *82*, 77-152.
- [31] J. G. Du, G. Xu, H. K. Lin, G. W. Wang, M. L. Tao, W. Q. Zhang, *Green Chem* **2016**, *18*, 2726-2735.
- [32] M. J. T. Frisch, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V.G.; Montgomery, J. A.; Stratman, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Latham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M.W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; and Pople, A. , Gaussian, Inc., Gaussian, Inc., **1998**.
- [33] A. Bell, B. Wernli, R. M. Franklin, *Parasitol Res* **1993**, *79*, 146-152.
- [34] a) F. M. D. Ismail, M. J. Dascombe, P. Carr, S. E. North, *J Pharm Pharmacol* **1996**, *48*, 841-850; b) F. M. D. Ismail, M. J. Dascombe, P. Carr, S. A. M. Merette, P. Rouault, *J Pharm Pharmacol* **1998**, *50*, 483-492.
- [35] N. T. Program, *Natl. Toxicol. Program Tech. Rep. Ser.* **1978**, *34*, 1-96.
- [36] F. U. Erkog, R. E. Menzer, *J Agr Food Chem* **1985**, *33*, 1061-1070.
- [37] A. R. Jacobson, J. T. Gerig, *Chem Res Toxicol* **1988**, *1*, 304-311.
- [38] R. E. Fowler, R. E. Fookes, F. Lavin, L. H. Bannister, G. H. Mitchell, *Parasitology* **1998**, *117*, 425-433.
- [39] B. J. Fennell, S. Carolan, G. R. Pettit, A. Bell, *J Antimicrob Chemoth* **2003**, *51*, 833-841.
- [40] K. A. Werbovetz, J. J. Brendle, D. L. Sackett, *Mol Biochem Parasit* **1999**, *98*, 53-65.
- [41] R. Leung-Toung, W. R. Li, T. F. Tam, K. Karimian, *Curr Med Chem* **2002**, *9*, 979-1002.
- [42] C. Ma, J. Tran, F. Gu, R. Ochoa, C. Li, D. Sept, K. Werbovetz, N. Morrisette, *Antimicrob Agents Ch* **2010**, *54*, 1453-1460.
- [43] a) C. Miller, L. K. Folkes, C. Mottley, P. Wardman, R. P. Mason, *Arch Biochem Biophys* **2002**, *397*, 113-118; b) C. W. Jefford, P. A. Cadby, L. C. Smith, D. F. Pipe, *Pharmazie* **1982**, *37*, 395-402; c) J. D. Maya, S. Bollo, L. J. Nunez-Vergara, J. A. Squella, Y. Repetto, A. Morello, J. Perie, G. Chauviere, *Biochem Pharmacol* **2003**, *65*, 999-1006.
- [44] B. C. Giovannella, J. S. Stehlin, H. R. Hinz, A. J. Kozielski, N. J. Harris, D. M. Vardeman, *Int J Oncol* **2002**, *20*, 81-88.
- [45] a) H. Cerecetto, R. Di Maio, G. Ibarruri, G. Seoane, A. Denicola, G. Peluffo, C. Quijano, M. Paulino, *Farmaco* **1998**, *53*, 89-94; b) H. Cerecetto, R. Di Maio, M. Gonzalez, M. Risso, P. Saenz, G. Seoane, A. Denicola, G. Peluffo, C. Quijano, C. Olea-Azar, *J Med Chem* **1999**, *42*, 1941-1950; c) H. Cerecetto, R. Di Maio, M. Gonzalez, M. Risso, G. Sagrera, G. Seoane, A. Denicola, G. Peluffo, C. Quijano, A. O. M. Stoppiani, M. Paulino, C. Olea-Azar, M. A. Basombrio, *Eur J Med Chem* **2000**, *35*, 343-350.
- [46] L. Salvati, M. Mattu, M. Colasanti, A. Scalone, G. Venturini, L. Gradoni, P. Ascenzi, *Bba-Protein Struct M* **2001**, *1545*, 357-366.
- [47] A. J. Fielding, V. Lukinovic, P. G. Evans, S. Alizadeh-Shekalgourabi, R. H. Bisby, M. G. B. Drew, V. Male, A. Del Casino, J. F. Dunn, L. E. Randle, N. M. Dempster, L. Nahar, S. D. Sarker, F. G. C. Reinhard, S. P. de Visser, M. J. Dascombe, F. M. D. Ismail, *Chem-Eur J* **2017**, *23*, 6811-6828.
- [48] G. W. Gribble, *Pure Appl Chem* **1996**, *68*, 1699-1712.
- [49] C. J. Koch, S. M. Hahn, K. Rockwell, J. M. Covey, W. G. McKenna, S. M. Evans, *Cancer Chemoth Pharm* **2001**, *48*, 177-187.
- [50] W. Suter, A. Hartmann, F. Poetter, P. Sagelsdorff, P. Hoffmann, H. J. Martus, *Mutat Res-Gen Tox En* **2002**, *518*, 181-194.
- [51] a) M. J. Strauss, *Ind Eng Chem Prod Rd* **1979**, *18*, 158-166; b) O. Llorens, J. J. Perez, H. O. Villar, *Int J Quantum Chem* **2002**, *88*, 107-117.
- [52] J. L. Burgaud, E. Ongini, P. Del Soldato, *Ann Ny Acad Sci* **2002**, *962*, 360-371.
- [53] Y. C. Lee, R. A. Yapple, R. Baldrige, M. Kirsch, R. H. Himes, *Biochim Biophys Acta* **1981**, *671*, 71-77.
- [54] R. L. Bai, C. Duanmu, E. Hamel, *Biochim Biophys Acta* **1989**, *994*, 12-20.
- [55] K. J. Marcus, S. C. Dutton, P. Barnes, C. N. Coleman, S. L. Pomeroy, L. Goumnerova, A. L. Billett, M. Kieran, N. J. Tarbell, *Int J Radiat Oncol* **2003**, *55*, 1182-1185.
- [56] P. Poli, M. A. de Mello, A. Buschini, R. A. Mortara, C. N. de Albuquerque, S. da Silva, C. Rossi, T. M. A. D. Zucchi, *Biochem Pharmacol* **2002**, *64*, 1617-1627.
- [57] G. Chauviere, B. Bouteille, B. Enanga, C. de Albuquerque, S. L. Croft, M. Dumas, J. Perie, *J Med Chem* **2003**, *46*, 427-440.
- [58] C. A. Price, E. M. Lynch, B. A. Bowie, D. J. Newman, *J Antibiot* **1981**, *34*, 118-119.
- [59] S. Walker, C. J. M. Diaper, R. Bowman, G. Sweeney, D. V. Seal, C. M. Kirkness, *Eye* **1998**, *12*, 875-879.
- [60] I. T. Reeve, M. G. Miller, *Chem Res Toxicol* **2002**, *15*, 352-360.
- [61] J. A. Naughton, R. Hughes, P. Bray, A. Bell, *Biochem Pharmacol* **2008**, *75*, 1580-1587.
- [62] J. O. Nelson, P. C. Kearney, J. R. Plimmer, R. E. Menzer, *Pestic Biochem Phys* **1977**, *7*, 73-82.
- [63] K. Samuel, W. J. Yin, R. A. Stearns, Y. S. Tang, A. G. Chaudhary, J. P. Jewell, T. Lanza, L. S. Lin, W. K. Hagmann, D. C. Evans, S. Kumar, *J Mass Spectrom* **2003**, *38*, 211-221.
- [64] X. H. Du, C. Guo, E. Hansell, P. S. Doyle, C. R. Caffrey, T. P. Holler, J. H. Mckerrow, F. E. Cohen, *J Med Chem* **2002**, *45*, 2695-2707.
- [65] a) P. Kovacic, L. E. Becvar, *Curr Pharm Design* **2000**, *6*, 143-167; b) C. C. Wang, *J Med Chem* **1984**, *27*, 1-9.
- [66] A. Albert, *Selective Toxicity. The physico-chemical basis of therapy*, Springer, **1985**.
- [67] A. M. Thompson, P. D. O'Connor, A. J. Marshall, A. Blaser, V. Yardley, L. Maes, S. Gupta, D. Launay, S. Braillard, E. Chatelain, B. Wan, S. G. Franzblau, Z. Ma, C. B. Cooper, W. A. Denny, *J. Med. Chem.* **2018**, *61*, 2329-2352.

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A series of novel dinitroaniline compounds were designed to probe inter- and intra- binding site dimensions in malarial parasite (*Plasmodium*) tubulin. Various crystal structures, including chloralinalin and trifluralin, an isopropyl dimer, and 2,6-dinitro-4-trifluoromethyl-phenylamine, were determined. Dinitroanilines, when soluble, were evaluated both in culture and *in vivo*. A new dimeric homologue of trifluralin showed antimalarial activity *in vitro*. Rational development of more selectively toxic antiparasitic agents is discussed.



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