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Synthesis and evaluation of phosphoramidate and phosphorothioamidate analogues of amiprophos methyl as potential antimalarial agents

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ABSTRACT

A series of phosphoramidate and phosphorothioamidate compounds based on the lead antitubulin herbicidal agents amiprophos methyl (APM) and butamifos were synthesised and evaluated for antimalarial activity. Of these compounds, phosphorothioamidates were more active than their oxo congeners and the nature of both aryl and amido substituents influenced the desired activity. The most active compound was **46**, *O*-ethyl-*O*-(2-methyl-4-nitrophenyl)-*N*-cyclopentyl phosphorothioamidate, which was more effective than the lead compound.

Malaria is a devastating parasitic disease caused by apicomplexan species of the genus *Plasmodium*, notably the most virulent species *Plasmodium falciparum*, and is responsible for about 800,000 deaths per annum, many of these in children and pregnant women, and most of these in Africa.[1] Most current therapies for malaria target the blood stage in the life cycle of the *Plasmodium* parasite.[2] The prototypic agent quinine and its derivatives, the 4-aminoquinolines such as chloroquine, target primarily the asexual, erythrocytic stages of the disease, while the 8-aminoquinolines such as primaquine target hepatic stages. Other agents include anti-folates (pyrimethamine–sulfadoxine), inhibitors of mitochondrial electron transport (atovaquone) and artemisinins, whose mechanism of action is controversial. While this last class of drugs has undoubtedly revolutionised the treatment of malaria and are the agents of choice in malarial treatment protocols as artemisinin based combination therapies, the ever present spectre of resistance is a constant concern, especially in areas where sub-optimal dose regimes are employed. Current best-practice favours the use of more than one agent to reduce the chance of spontaneous mutation and to prevent resistance. Despite these measures, worryingly, reports of artemisinin resistance have emerged.[3] The need for novel therapeutics to combat this insidious pathogen is thus evident, including agents targeting novel parasitic targets.[4] One potential class of antimalarial agents may be inhibitors of the protein tubulin, and the activity of anti-tubulin compounds as antiparasitic agents has been recently reviewed.[5,6] Although a ubiquitous protein, there is significant amino acid sequence heterogeneity between mammalian and parasite tubulins, prompting the hope that selective antitubulin agents may prove useful.[7] Typical antitubulin agents such as taxanes and *Vinca* alkaloids, while potent against *Plasmodium*, are non-selective. In contrast, herbicidal agents of the dinitroaniline class such as trifluralin **1** have shown activity against *P. falciparum* with minimal cytotoxicity.[8] The site of action of dinitroaniline herbicides in plant and protozoal species is controversial and as yet unproven, but is likely to be located on α-tubulin.[9] Electron microscopic autoradiography showed radioactive trifluralin associated with microtubule fragments.[10] In an in vivo model of mouse malaria, trifluralin was inactive against *Plasmodium berghei* despite submicromolar activity in vitro, an effect ascribed to poor absorption.[11] Trifluralin has poor aqueous solubility, with a reported *c* log *P* of 5.322,[12] and as well as affecting its pharmacokinetics this may cause it to accumulate in membranous compartments of the parasite and its host erythrocyte, away from its site of action in the cell.[13] A further disadvantage regarding the use of dinitroanilines such as trifluralin as lead compounds is their potential carcinogenicity.[14] Despite these drawbacks, many dinitroanilines have been synthesised and tested against both apicomplexan and kinetoplastid parasites. Of the many derivatives evaluated, some of the more interesting compounds with activity against apicomplexans are shown in Figure 1.

Armson has reported IC_{50} values for trifluralin and the sulfonamide dinitroaniline oryzalin **2** against *Cryptosporidium* of 750 and 800 nM, respectively.[15] Oryzalin and its analogues have also been investigated for activity against *Toxoplasma gondii*. While oryzalin itself had an IC_{50} value of 0.25 μM, benzenediamine **3**

Abbreviations: APM, amiprophos methyl.

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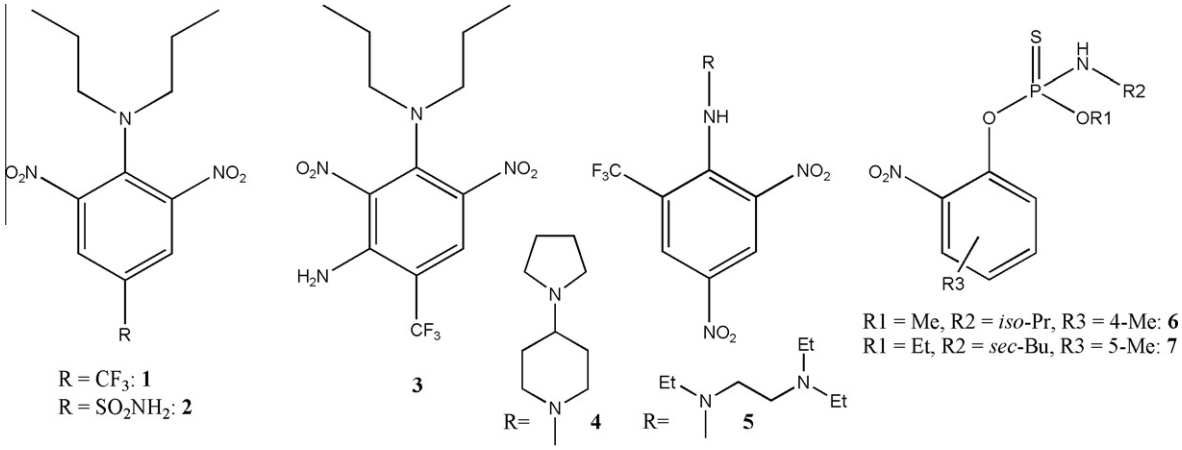


Figure 1. Antitubulin herbicidal compounds and derivatives.

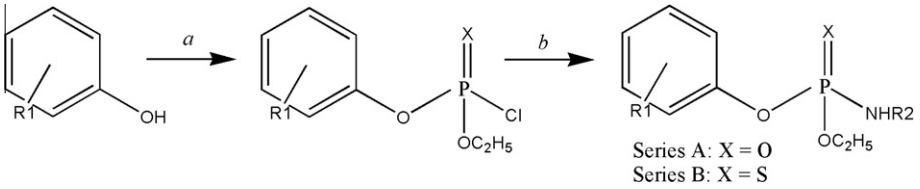
had an IC_{50} value of 36 nM.[16] Regioisomeric dinitroanilines exhibited interesting activity against several parasites, with **4** and **5** the most potent, both with an IC_{50} value of 0.44 μM against *P. falciparum*.[17]

Phosphorothioamidate herbicides, typified by APM **6** and butamifos **7** (Fig. 1), are considered to bind tubulin in the same way as the dinitroanilines. APM was shown competitively to inhibit the binding of ^{14}C oryzalin to tobacco tubulin, indicating the formation of a moderate affinity tubulin/APM complex that may interact with the ends of microtubules. APM concentrations that inhibited plant cell growth were within the threshold range of APM concentrations that depolymerised cellular microtubules, suggesting that growth inhibition is caused by microtubule depolymerisation.[18] Molecular modelling studies have shown that the electrostatic surfaces of the phosphorothioamidates are very similar to those of the dinitroanilines, with the electronegative domains of the *ortho* nitro (-phenyl) group and the phosphorothio group matching both the shape and spacing of the equivalent regions of the dinitroanilines.[19] In addition, plant species with resistance to dinitroanilines also show cross resistance to phosphorothioamidates.[20,21] The observation that APM and the dinitroanilines **1** and **2** prevented *P. falciparum* erythrocytic shizogony, blocked mitosis and resulted in accumulation of ab normal microtubular structures[22] suggested that the phosphorothioamidate class of herbicides were also worthy of investigation as potential antimalarial lead compounds. In that study, the IC_{50} value of APM against *P. falciparum* was 3.5 μM, comparable to that for trifluralin of 2.9 μM, while the *N*-phenylcarbamate herbicide chloropropham lacked any inhibitory effect up to 128 μM. In addition, neither trifluralin nor APM showed any inhibitory effect on mammalian (Vero monkey kidney) cells at concentrations up to 64 μM. Phosphorothioamidates also lack the potentially toxophoric dinitroaniline motif. Cognisant of these facts, we decided to synthesise and test a range of phosphoramidate and phosphorothioamidate analogues of APM and butamifos and test these compounds for activity against *P. falciparum*.

Original syntheses for pesticidal phosphorothioamidates involve sequential substitution of a pentavalent phosphorus species. One available approach is the reaction of an anhydrous alkali metal phenolate and an alkali metal monohydric alkoxide with an *N*-substituted dichlorothiophosphoramide in an excess of the alcohol used to prepare the alkoxide.[23] The order of substitution is not critical, and the order of addition of the phenolate and alkoxide may be reversed. Indeed, it is also possible to react either as starting material an *O*-alkyl dichlorothiophosphate or an *O*-phenyl dichlorothiophosphate sequentially with the remaining two synthons. As we first wished to generate a series of phosphoramidate compounds, our synthetic strategy employed the commercially available *O*-alkylated reagent ethyl phosphorodichloridate. Sequential displacement of the chlorine atoms in this compound was accomplished easily with equimolar quantities of phenol and amine synthons, as shown in Scheme 1, and the desired phosphoramidates **8**–**23** were obtained in 17–73% yields (Table 1). Analogous phosphorothioamidates, including **6**, were prepared by the sequential reaction of ethyl phosphorothiodichloridate with phenolic and amine synthons.

Having varied the phenolic substituents in phosphoramidates **8**–**23**, we then wished to investigate the effect of varying the nature of the amide functionality, by introduction of primary, secondary and tertiary nitrogen species, and also branched and cyclic systems. These compounds, **25**–**49**, were synthesised using analogous methodology to that used in Scheme 1, and the resultant phosphoro(thio)amidates are shown in Table 2. Structures for the proposed target compounds were confirmed with the aid of ^{1}H, ^{13}C and ^{31}P NMR spectroscopy and mass spectrometry analysis, while the purity of the compounds was assessed by elemental analysis. Synthetic procedures, spectral data and testing methodology are provided in the Supplementary section.

Tables 1 and 2 show the anti-malarial activity of the phosphoroamidate and phosphorothioamidate compounds tested. In our experiments, APM had an IC_{50} value of 4 μM. All compounds in Table 1 reflected the intention of preserving the isopropylamido group of **6** intact while varying the nature of the aromatic moiety. Phosphoramidate regioisomers **9**–**14** were devoid of activity or almost so, and while replacement of the 2-nitro group by cyano- or methoxy- substituents did not improve activity, replacement by a halogen, as in compounds **16** and **18**, reduced the IC_{50} value to 39 μM. The mono-substituted trifluoromethylphenol derivatives **19**–**21** demonstrated potential, with the *meta* and *para* compounds **20** and **21** the better of those tested, both with IC_{50} values of 50 μM. Bulkier naphthalene groups as in **22** and **23** failed to improve activity. The compounds listed in Table 2 show the effect of varying the amido substituent within both APM-like and *p*-trifluoro or halogen series. Within APM/butamifos-like compounds **25**–**31**, the most potent was the *n*-butyl **26**. Within the trifluoro series **32**–**44**, elongation of the amido alkyl moiety to C5 enhanced activity whether acyclic or cyclic; this is exemplified by *N*-pentyl **36**, at 4.5 μM and its *N*-cyclopentyl analogue **38**, at 8.6 μM. Replacement of the oxygen atom by sulfur raised activity threefold, as shown by a comparison of **37** and **38** (26 vs 8.6 μM). Interestingly, compounds **45** and **46**, both 2-methyl-4-nitro substituted phosphorothioamidates, had activities of 6.9 and 1.6 μM, respectively. To compare the effect of the X group on phosphorus further,



Scheme 1. Synthesis of substituted phosphoramidates and phosphorothioamidates (**8**–**49**). Reagents and conditions: (a) $C_2H_5XPCl_2$; Et_3N; THF (series A) or $PhCH_3$ (series B); 0 °C; N_2; (b) $R2NH_2$; Et_3N; THF (series A) or $PhCH_3$ (series B); 0 °C; N_2.

Table 1
Anti-malarial activity of phosphoroamidates **8**–**23** as assessed by inhibition of pLDH at 72 h

Compound	R^1	R^2	X	IC_{50} (μM)	*c* log *P*[a]
6 (APM)	4-CH_3-2-NO_2	*i*-Propyl	S	4	3.50
8	5(H)	*i*-Propyl	O	>128	2.68
9	4-CH_3-2-NO_2	*i*-Propyl	O	126	3.11
10	2-CH_3-4-NO_2	*i*-Propyl	O	128	3.11
11	5-CH_3-2-NO_2	*i*-Propyl	O	>128	3.11
12	2-CH_3-5-NO_2	*i*-Propyl	O	128	3.11
13	3-CH_3-4-NO_2	*i*-Propyl	O	79	3.11
14	2-CH_3-3-NO_2	*i*-Propyl	O	128	3.11
15	2-CN-4-CH_3	*i*-Propyl	O	128	2.97
16	2-Br-4-CH_3	*i*-Propyl	O	39	3.94
17	2-CH_3O-4-CH_3	*i*-Propyl	O	128	2.90
18	2-Cl-4-CH_3	*i*-Propyl	O	39	3.67
18b	2-Cl-4-CH_3	—	O	102	6.23
19	2-CF_3	*i*-Propyl	O	87	3.57
20	3-CF_3	*i*-Propyl	O	50	3.57
21	4-CF_3	*i*-Propyl	O	50	3.57
22	2-Naphthol	*i*-Propyl	O	72	3.69
23	1-NO_2-2-Naphthol	*i*-Propyl	O	87	3.64
24	4-CH_3-2-NO_2	*i*-Propyl	S	—	3.84

[a] *c* log *P* values calculated using MarvinSketch 5.1.4 from ChemAxon.

Table 2
Anti-malarial activity of compounds **25**–**49** as assessed by inhibition of pLDH at 72 h

Compound	R^1	R^2	X	IC_{50} (μM)	*c* log *P*[a]
6 (APM)	4-CH_3-2-NO_2	*i*-Propyl	S	4	3.50
25	4-CH_3-2-NO_2	*n*-Propyl	O	>128	3.16
26	4-CH_3-2-NO_2	*n*-Butyl	O	28	3.56
27	4-CH_3-2-NO_2	*i*-Butyl	O	75	3.56
28	4-CH_3-2-NO_2	*n*-Pentyl	O	51	3.95
29	4-CH_3-2-NO_2	Cyclopentyl	O	47	3.54
30	5-CH_3-2-NO_2	*n*-Propyl	O	102	3.16
31	5-CH_3-2-NO_2	*i*-Butyl	O	>128	3.56
32	4-CF_3	NH_2	O	79	2.56
33	4-CF_3	*n*-Butyl	O	32	4.02
34	4-CF_3	*sec*-Butyl	O	40	4.04
35	4-CF_3	Cyclobutyl	O	45	3.60
36	4-CF_3	*n*-Pentyl	S	4.5	5.15
37	4-CF_3	Cyclopentyl	O	26	4.00
38	4-CF_3	Cyclopentyl	S	8.6	4.73
39	4-CF_3	Cyclohexyl	O	43	4.40
40	4-CF_3	*n*-Heptyl	O	44	5.21
41	4-CF_3	*N*,*N*-Diethyl	O	48	3.74
42	4-CF_3	Piperidino	O	84	3.78
43	4-CF_3	Pyrrolidino	O	56	3.38
44	4-CF_3	Morpholino	O	98	2.72
45	2-CH_3-4-NO_2	*n*-Pentyl	S	6.9	4.69
46	2-CH_3-4-NO_2	Cyclopentyl	S	1.6	4.27
47	4-Br	Cyclopentyl	O	17	3.91
48	4-Br	Cyclopentyl	S	23	4.64
49	4-Br	Cyclopentyl	—	128	4.63

[a] *c* log *P* values calculated using MarvinSketch 5.1.4 from ChemAxon.

we prepared **47**–**49**. However, unusually, oxo and thio congeners were similarly active at 17 and 23 μM, respectively, while the tricoordinate species was inactive.

Known SAR for herbicidal activity[24] predicts that deactivating aromatic substituents such as a nitro group may enhance activity. With regard to antimalarial activity, Table 1 shows that while this may be a shared effect, as suggested by the activity of trifluoromethyl derivative **21**, it is difficult to ascertain within the phosphoramidate series, due to the fact that many compounds (e.g., nitro-substituted compounds **10**–**15**) were devoid or almost devoid of activity. This may suggest an underlying requirement for the phosphorothio rather than the phosphoro moiety. Compared to APM, its *O*-ethyl phosphoramidate analogue has a 20-fold higher IC_{50} value. This suggests the sulfur analogues may be a great deal more potent than their oxygen congeners. In vivo, however, oxidation of thiophosphate compounds to their phosphate analogues occurs via cytochrome-P450-dependent desulfuration in the liver.[25] Interestingly, trifluoro compound **21** has been previously reported; a German patent describes the use of 4-trifluoromethylphenyl(thio)phosphoric acid amides as pesticides.[26]

Variation of the amido functionality on the nitrogen however had an obvious effect on activity. Within the amido homologues **25**–**30**, this effect was not as dramatic as within the *para*-trifluoro series **32**–**44**, as increasing the chain length and branching did improve activity up to C5, as evidenced by the IC_{50} values of compounds **36** and **38**, at 4.5 and 8.6 μM, respectively. This indicated that pentyl or cyclopentylamino substituents were optimal, as further enhancing the lipophilic nature of the side chain resulted in reduced activity, as shown by the *n*-heptyl compound **40**. Analysis of the predicted *c* log *P* values as shown in Tables 1 and 2 revealed a modest correlation with activity for all compounds ($R^2 = 0.504$). Interestingly, the degree of substitution on nitrogen also appeared to affect activity within the *para*-trifluoro phosphoramidate series. The primary amido **32** was not very potent at 79 μM, yet tertiary amides **41**–**43** displayed some activity, comparable to the secondary phosphoramides among **33**–**40**.

The present study has allowed us to conclude that an electron-withdrawing group on the phenolic ring may enhance activity within the phosphoroamidate series, particularly in the *para*-position. Additional aromatic alkyl substitution may enhance activity, although it does not appear to be crucial. For most compounds tested, phosphorothioamidates were more active than their oxo congeners. Within these, an alkylamido C5 residue seems to be optimal. Of all compounds prepared, **36** and **46** are most worthy of further investigation.

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Supplementary data

Supplementary data (experimental procedures and spectral data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.088.

References and notes

1. WHO. World Malaria Report, 2010.
2. Schlitzer, M. *Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 149.
3. Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P.; Lindegardh, N.; Socheat, D.; White, N. J. *N. Engl. J. Med.* **2009**, *361*, 455. Erratum in: *N. Engl. J. Med*. **2009**, 361, 1714..
4. Sahu, N. K.; Sahu, S.; Kohli, D. V. *Chem. Biol. Drug Des.* **2008**, *71*, 287.
5. Kappes, B.; Rohrbach, P. *Future Microbiol.* **2007**, *2*, 409.
6. Fennell, B. J.; Naughton, J. A.; Barlow, J.; Brennan, G.; Fairweather, I.; Hoey, E.; McFerran, N.; Trudgett, A.; Bell, A. *Expert Opin. Drug Discov.* **2008**, *3*, 501.
7. Bell, A. *Parasitol. Today* **1998**, *14*, 234.
8. Nath, J.; Schneider, J. *Clin. Res.* **1992**, *40*, 331A.
9. Nyporko, Y.; Yemets, A.; Brytsun, V.; Lozinsky, M.; Blume, Y. *Tsitol. Genet+* **2009**, *43*, 56.
10. Kaidoh, T.; Nath, J.; Fujioka, H.; Okoye, V.; Aikawa, M. *J. Eukaryot. Microbiol.* **1995**, *42*, 61.
11. Dow, G. S.; Armson, A.; Boddy, M. R.; Itenge, T.; McCarthy, D.; Parkin, J. E.; Thompson, J. C. A.; Reynoldson, J. A. *Exp. Parasitol.* **2002**, *100*, 155.
12. Mitra, A.; Sept, D. *J. Med. Chem.* **2006**, *49*, 5226.
13. Naughton, J. A.; Hughes, R.; Bray, P.; Bell, A. *Biochem. Pharmacol.*, 2008. 1580.
14. Saghir, S. A.; Charles, G. D.; Bartels, M. J.; Kan, L. H. L.; Dryzga, M. D.; Brzak, K. A.; Clark, A. J. *Toxicol. Lett.* **2008**, *180*, 38.
15. Armson, A.; Sargent, K.; MacDonald, L. M.; Finn, M. P.; Thompson, R. C.; Reynoldson, J. A. *FEMS Immunol. Med. Mic.* **1999**, *26*, 109.
16. Endeshaw, M. M.; Li, C.; de Leon, J.; Yao, N.; Latibeaudiere, K.; Premalatha, K.; Morrissette, N.; Werbovetz, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5179.
17. Best, W. M.; Sims, C. G.; Thompson, R. C. A.; Armson, A.; Reynoldson, J. A. Antiparasitic compounds. WO/2006/108224.
18. Murthy, J. V.; Kim, H. H.; Hanesworth, V. R.; Hugdahl, J. D.; Morejohn, L. C. *Plant Physiol.* **1994**, *105*, 309.
19. Ellis, J. R.; Taylor, R.; Hussey, P. J. *Plant Physiol.* **1994**, *105*, 15–18.
20. Vaughn, K. C.; Marks, M. D.; Weeks, D. P. *Plant Physiol.* **1987**, *83*, 956.
21. James, S. W.; Silflow, C. D.; Stroom, P.; Lefebvre, P. A. *J. Cell Sci.* **1993**, *106*, 209.
22. Fennell, B.; Naughton, J.; Dempsey, E.; Bell, A. *Mol. Biochem. Parasitol.* **2006**, *145*, 226.
23. Moyle, C. L.; Kenaga, E. E. U.S. Patent 2,252,574; Moyle, C. L. U.S. Patent 2,252,576.
24. Blume, Ya.; Nyporko, A.; Yemets, A.; Baird, W. *Cell Biol. Int.* **2003**, *27*, 171.
25. Tang, J.; Chambers, J. *J. Biochem. Mol. Toxic.* **1999**, *13*, 261.
26. Buerstinghaus, R.; Varwig, J.; Kiehs, K.; Adolphi, H. Ger. Offen. 1984, DE 3,231,191.