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# Antimalarial 3-arylamino-6-benzylamino-1,2,4,5-tetrazines

Duong Nhu [a], Sandra Duffy [b], Vicky M. Avery [b], Andrew Hughes [c], Jonathan B. Baell [a,*]

[a] Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia
[b] Discovery Biology, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, Queensland, Australia
[c] La Trobe University, Bundoora, Victoria, Australia

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ABSTRACT

We report on novel 3-arylamino-6-benzylamino-1,2,4,5-tetrazines with potent activity against *Plasmodium falciparum*.



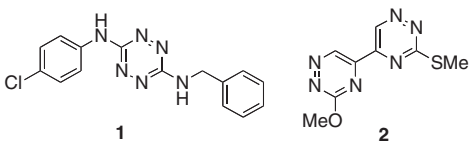
Malaria is one of the most detrimental infectious parasitic diseases which poses a threat to more than two billion lives worldwide as estimated in 2002.[1] Although eradication has been successful in certain areas,[2] malaria is still prevalent in developing countries, especially in Africa, which has the highest mortality in children under the age of five.[3,4]

In humans, malaria is caused by four strains of single-celled eukaryotic protozoa of the genus *Plasmodium*. These strains include *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium falciparum*, among which *P. falciparum* is associated largely with lethal cases.[3,5] Individuals with malaria generally experience fevers and chills as a result of the body's inflammatory response. In addition, more severe symptoms, involving multiple organs and tissues, result from the destruction of red blood cells (RBCs) and are manifested by lactic acidosis, malarial anaemia and cerebral malaria.[3,6] Genetic conditions such as sickle cell anaemia, however, confer some protection against the disease and the trait is consequently preserved in regions where malaria becomes endemic.[3]

Intensive studies have focused on vaccine and drug development targeting different stages of the parasite life cycle to combat the disease.[7–9] The search for effective vaccines, either with subunit or whole parasite approaches, is challenging due to the polymorphic nature of the parasites.[7–10] In addition, cost is also a concern as malaria is problematic in third world countries.[7] Drug treatments have been adopted and a majority of antimalarial agents in the clinic act on the intra-erythrocytic stages.[5] In the review by Frederich et al.,[5] these drugs have been divided into four major groups based on the biochemical pathways underlying their modes of action. These include nucleic acid synthesis, protein synthesis, oxidative stress and heme metabolism.

Chloroquine has a long history in treatment of malaria; however, emergence of resistant strains of *Plasmodium* raises the need for ongoing research on alternative efficacious drug candidates.[11] Although artemisinin analogues have shown improved performance against chloroquine and multi drug-resistant strains of *Plasmodium*,[5,12] besides the aforementioned financial constraint, resistance to these drugs has been reported in some areas of South-East Asia.[9] Therefore, development of novel antimalarial agents, satisfying the criteria of being both potent and synthetically economical, is of interest.

Through screening a set of in-house compounds at the Swiss Tropical Research Institute against a panel of parasites,[13] we found that compounds **1** and **2** in Figure 1 were potently toxic to *P. falciparum*. As shown in Table 1, compound **1** exhibited submicromolar activity towards *P. falciparum* with an $EC_{50}$ of 440 nM and was more than 30 times less toxic towards other parasites and mammalian cells. The triazine dimer **2** was even more potent towards




1 WEHI-0033352 2 WEHI-0059743

**Figure 1.** Two in-house heterocycles screened against a panel of parasites and found to have potent activity against *P. falciparum*.

* Corresponding author.
*E-mail address:* jbaell@wehi.edu.au (J.B. Baell).

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**Table 1**
Antiparasitic activity profile of **1** and **2**

| Assay | $EC_{50}$ (μM)[a] | |
|---|---|---|
| | **1** | **2** |
| *P. falciparum*[b] | 0.44 | 0.18 |
| *L. infantosum*[c] | 16 | 8.6 |
| *T. cruzi*[d] | 15 | 16 |
| *T. brucei*[e] | >32 | 5.7 |
| Cytotoxicity[f] | >32 | 33 |

[a] Values are means of three experiments, ±50%.
[b] *Plasmodium falciparum* 3D7 strain, erythrocytic stage, chloroquine was used as a control, $EC_{50}$ 30 μg/ml.
[c] *Leishmania infantosum*, amastigote stage, miltefosine was used as a control, $EC_{50}$ 2.4 μg/ml.
[d] *Trypanosoma cruzi* Tulahaen C2C4 strain, amastigote stage, nifurtimox was used as a control, $EC_{50}$ 0.24 μg/ml.
[e] *Trypanosoma brucei rhodesiense* strain STIB 900, bloodstream form. Suramin ($EC_{50}$ 0.13 μg/ml) was used as a control.
[f] Rat skeletal myoblast cell L-6 strain, Tamoxifen was used as a control, $EC_{50}$ 4.9 μg/ml.

*P. falciparum* with an $EC_{50}$ of 180 nM and was also highly selective, with the next most potent activity being against *Trypanosoma brucei* with an $EC_{50}$ of 5.7 μM.

However, routine quality control indicated that triazine **2** was comprised of mostly a compound with a molecular weight of 252 instead of the expected 236. While this matter was being investigated (to be reported in due course), tetrazine **1** was selected for a structure–activity relationship (SAR) investigation and these results are now reported.

A small group of 3,6-disubstituted 1,2,4,5-tetrazines with ambiguous activity in a mouse model of malaria (*P. berghei*) have been reported by Werbel et al.[14] These compounds were restricted to 3-phenyl-6-methylamino-1,2,4,5-tetrazines and so are quite distinct to the tetrazines reported here, that are also more drug-like by virtue of being deactivated through 3,6-diamino substitution. There is very little literature on the synthesis of asymmetrically-substituted 3,6-diaminotetrazines. Rusinov et al. reported a convenient synthesis that utilized a bis pyrazole derivative[15] (**5**) as shown in Figure 2. Hence reaction of guanidine hydrochloride with hydrazine gives triaminoguanidine **3** and this in turn is reacted with acetylacetone to give **4**, the oxidation of which gives **5**. We anticipated that bis pyrazole **5** would be more conveniently handled than the highly activated bis chloro counterpart and so utilized this in the synthesis of **1** as shown in Figure 2. Here, the least nucleophilic amine is reacted first (4-chloroaniline for **1**) with **5** so that the second substitution can proceed without forcing conditions. This protocol worked well for all cases where, like **1**, $R^1NH$ represented an aniline and so the system remained relatively activated towards the second incoming nucleophile $R^2NH2$, this also being a more reactive alkylamine. In this way, **1**, **6**, **7**, **10**, **15** and **16** were readily made. The reader is referred to Table 2 for elaboration of the R groups.

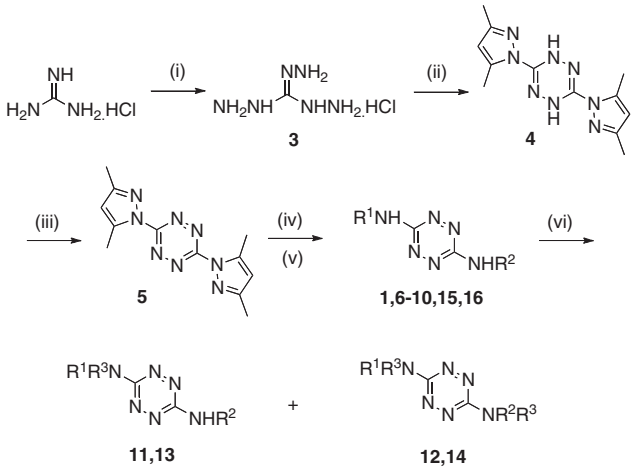


**Figure 2.** Synthesis of asymmetrically-substituted tetrazines. (i) hydrazine hydrate, 1,4-dioxane, reflux 2 h (80%); (ii) acetylacetone (2 equiv), water, rt to 70 °C (60–90%); (iii) $MnO_2$ (10 equiv)/silica, dichloroethane, rt 2 h (57–69%); (iv) $R^1NH_2$ (1.5 equiv), acetonitrile, 40–50 °C; (v) $R^2NH_2$ (1.5 equiv), acetonitrile, reflux; (vi) $R^3X$ (10 equiv), $K_2CO_3$ or $Cs_2CO_3$, KI (if X not I), *N*,*N*-dimethylacetamide, 80–90 °C.

Compounds **15** and **16** involved the use of exotic amine nucleophiles which were made as shown in Figures 3 and 4, respectively. For **15**, the required aniline was made by alkylating 3-hydroxyaniline with mesylated *N*,*N*-dimethylaminoethanolamine, giving **17** as shown in Figure 3. This could then be used in step (iv) in Figure 2 en route to the synthesis of **15**.

Compound **16** required the synthesis of benzylamine **18** as shown in Figure 4. This involved alkylation of BOC-protected 3-hydroxybenzylamine followed by deprotection. The resulting amine, **18**, could then be used in step (v) in Figure 2 en route to the synthesis of **16**.

In some cases, where $R^1NH$ was an alkylamine and not an aniline, the second incoming amine nucleophile in addition displaced the first amine nucleophile. Hence, **9** was produced in a 1:1 mixture with the 3,6-bis(*n*-butylamino) counterpart resulting from the additional displacement of the *N*-benzyl group with butylamine (Table 2).

This mixture was inseparable and the single peak in the HPLC trace was suggestive of a tightly bound complex. In the case of methylamine, only 5% of the product mixture comprised **8**, the remaining 95% being the 3,6-bis(methylamino) by-product. Nevertheless enough of **8** could be separated and purified for testing. This unusual propensity for the benzylamino group to be displaced in this system remains unexplained.

Synthesis of N-alkylated derivatives of **1** was readily undertaken. As also shown in Figure 2, alkylation of the exocyclic nitrogen atoms with $R^3X$ proceeded smoothly under standard conditions to give the monoalkylated compounds **11** ($R^3$ = Me), using MeI, or **13** ($R^3 = CH_2CH_2NMe_2$), using $ClCH_2CH_2NMe_2$, with the alkyl group preferentially reacting at the most acidic NH, this being the aniline $R^1NH$. Small amounts of the respective dialkylated by-products **12** and **14** were also obtained.

Compounds were tested for toxicity to *P. falciparum* and to mammalian cells. The biological results are summarized in Table 1. Here it can be seen that the levels of inhibition of *P. falciparum* by compound **1** is similar to that obtained previously. Compound **1** is potently anti-malarial with an $EC_{50}$ of 1.1 μM and is significantly less toxic to mammalian cells, with 58% cell death only at 29 μM.

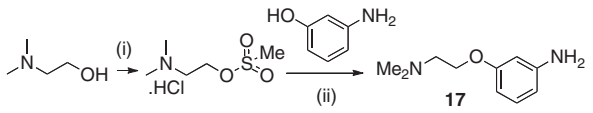


**Figure 3.** (i) Methanesulfonyl chloride, dry ether, 0 °C to rt; (ii) NaH, *N*,*N*-dimethylformamide, 0 °C to rt.

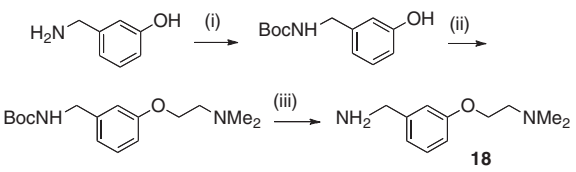


**Figure 4.** (i) $Boc_2O$, tetrahydrofuran, 0 °C to rt (96%); (ii) NaH, $ClCH_2CH_2NMe_2$, *N*,*N*-dimethylformamide, 0 °C to rt (73%); (iii) 1.25 M HCl in methanol, 1 h (22%).

Compounds **6** and **7** are suggestive of very sharp SAR, as extension of the benzyl group by one methylene or incorporation of a branched carbon, respectively appears to largely destroy activity. Keeping the *N*-benzyl group constant, variations in the left hand side were then investigated. Replacement of the *N*-phenyl group with an *N*-methyl group (compound **8**) led to a loss of activity and the similarly poor activity of *N*-butyl compound **9** indicated that hydrophobicity of the substituent alone was insufficient to restore activity. Remarkably, even the des-chloro *N*-phenyl compound **10** was completely inactive, suggesting very sharp negative SAR and that a chloro in the 4-position was vital for good activity.

One way of improving solubility and permeability and hence drug-likeness is through decreasing crystallinity and this can often be achieved with N-alkylation. Hence we made and tested the *N*-methyl compound **11** and showed that such modification was well tolerated with activity being similar to that of **1**. However, cytotoxicity to mammalian cells was significant with an $EC_{50}$ of 12 μM. Solubility can be increased further by installation of a solubilizing group and in this regard we targeted tertiary amines as they can be membrane permeable and have been shown to improve activity in other antimalarial compounds through accumulation in the acidic food vacuole of the parasite.[16]

To this end we made and tested amine **13**, but it exhibited weaker activity suggesting that while the *N*-methyl group in **11** was acceptable, further elongation of the *N*-alkyl group was not. In order to explore other positions that may not interfere, we made two compounds where the tertiary amine was attached via a tether to the *meta* position of either the aniline ring or the benzyl ring. It was envisaged that by being on the *meta* position, there would be the maximum topographical space in which to move to either locate a favorable interaction or avoid an unfavorable interaction.

Where this group was on the aniline ring, activity was weaker and compound **15** exhibited an $EC_{50}$ of 15 μM. In contrast, this group was better tolerated on the benzyl ring, and compound **16** returned an $EC_{50}$ of 5 μM although it was also reasonably cytotoxic with an $EC_{50}$ of 21 μM.

Also shown in parentheses in Table 2 for compounds **1**, **6**, **11**, **13**, **15** and **16** are the biological activities against the chloroquine-resistant Dd2 strain of *P. falciparum*. It can be seen that these compounds are essentially equipotent towards this strain, suggesting that their mode of activity is independent to that of chloroquine.

In summary, we report novel heterocycles based on 3-(4-chlorophenylamino-6-(benzylamino)-1,2,4,5-tetrazines that are active against *P. falciparum*. SAR was probed and revealed both the 4-chloroaniline and benzylamino substituents were essential for activity. However, the aniline nitrogen atom could be methylated (**11**) and a solubilizing group installed on the benzyl ring (**16**) with maintenance of reasonable potency in the low micromolar range. We recommend caution in progressing this class of compounds because they have a tendency to be toxic towards mammalian cells and it currently cannot be ruled out that this may be linked to their observed antimalarial activity.

**Table 2**
Biological data for tetrazines

| Compd | $R^1$ | $R^2$ | $R^3$ | $EC_{50}$ (μM)[a] | |
|---|---|---|---|---|---|
| | | | | *Pf*[b] | Cytotox[c] |
| **1** | 4-ClPh | $CH_2Ph$ | — | 1.1 (1.1)[d] | 58%@ 29 μM |
| **6** | 4-ClPh | $CH_2CH_2$-Ph | — | 85% @115 μM (88%@115 μM) | NA@ 115 μM |
| **7** | 4-ClPh | CH(Me)-Ph | — | NA@38 μM | NA@38 μM |
| **8** | Me | $CH_2Ph$ | — | NA@115 μM | NA@115 μM |
| **9**[e] | *n*-Bu | $CH_2Ph$ | — | NA@115 μM | NA@115 μM |
| **10** | Ph | $CH_2Ph$ | — | NA@115 μM | NA@115 μM |
| **11** | 4-ClPh | $CH_2Ph$ | Me | 2.4 (3.5)[d] | 12 |
| **12** | 4-ClPh | $CH_2Ph$ | Me | NT[f] | NT[f] |
| **13** | 4-ClPh | $CH_2Ph$ | $CH_2CH_2$-$NMe_2$ | 13 (20)[d] | 38 |
| **14** | 4-ClPh | $CH_2Ph$ | $CH_2CH_2$-$NMe_2$ | NA@115 μM | NA@115 μM |
| **15** | 3-($Me_2N$-$CH_2CH_2$-O)Ph | $CH_2Ph$ | — | 15 (13)[d] | 52%@38 μM |
| **16** | 4-ClPh | $CH_2Ph$(3-O $CH_2CH_2$-$NMe_2$) | — | 5[g] (6)[d] | 21 |

[a] Values are means of two experiments, ±50%.
[b] *Plasmodium falciparum* 3D7 strain, erythrocytic stage, chloroquine was used as a control, $EC_{50}$ 4 nM.
[c] HEK, puromycin was used as a control, $EC_{50}$ 411 nM.
[d] *Plasmodium falciparum* Dd2 strain (chloroquine-resistant), erythrocytic stage, chloroquine was used as a control, $EC_{50}$ 173 nM.
[e] Tested as a 1:1 mixture with inseparable 3,6-bis(*n*-butyl) by-product.
[f] Not tested.
[g] Approximate: poorly fitting curve.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.036.

## References and notes

1. Snow, R. W.; Guerra, C. A.; Noor, A. M.; Myint, H. Y.; Hay, S. I. *Nature* **2005**, *434*, 214.
2. Gallup, J. L.; Sachs, J. D. *Am. J. Trop. Med. Hyg.* **2001**, *64*, 85.
3. Haldar, K.; Milner, D. A.; Murphy, S. C., Jr.; Taylor, T. E. *Annu. Rev. Pathol.: Mech. Dis.* **2007**, *2*, 217.
4. Greenwood, B. M.; Bojang, K.; Whitty, C. J. M.; Targett, G. A. T. *Lancet* **2005**, *365*, 1487.
5. Frederich, M.; Dogne, J.-M.; Angenot, L.; Mol, P. D. *Curr. Med. Chem.* **2002**, *9*, 1435.
6. Miller, L. H.; Baruch, D. I.; Marsh, K.; Doumbo, O. K. *Nature* **2002**, *415*, 673.
7. Good, M. F. *Trends Parasitol.* **2005**, *21*, 29.
8. Brady, R. L.; Cameon, A. *Curr. Drug Target* **2004**, *5*, 137.
9. D'Alessandro, U. *Expert Opin. Pharmacother.* **2009**, *10*, 1291.
10. Hisaeda, H.; Yasutomo, K.; Himeno, K. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 700.
11. Cosledan, F.; Fraisse, L.; Pellet, A.; Guillou, F.; Mordmuller, B.; Kremsner, P. G.; Moreno, A.; Mazier, D.; Maffrand, J.-P.; Meunier, B. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17579.
12. Crespo, M. D. P.; Avery, T. D.; Hassen, E.; Fox, E.; Robinson, T. V.; Valente, P.; Taylor, D. K.; Tilley, L. *Antimicrob. Agents Chemother.* **2008**, *52*, 98.
13. This was undertaken at the Swiss Tropical Research Institute. Compounds **1** and **2** belonged to primary screening hit sets derived from screening our high throughput screening library against *Trypanosoma brucei*-derived farnesyl pyrophosphate synthase (FPPS) and *Trypanosoma cruzi*-derived trypanothione reductase, respectively. The potent and selective antimalarial activity of compounds **1** and **2** was therefore serendipitous and may have arisen via mechanisms unrelated to the enzyme targets for the initial screen. We have recently disclosed the results of the trypanothione reductase high throughput screen (Ref. 17).
14. Werbel, L. M.; McNamara, D. J.; Colbry, N. L.; Johnson, J. L.; Degnan, M. J.; Whitney, B. *J. Heterocycl. Chem.* **1979**, *16*, 881.
15. Rusinov, G. L.; Latosh, N. I.; Ganebnykh, I. I.; Ishmetova, R. I.; Ignatenko, N. K.; Chupakhin, O. N. *Russ. J. Org. Chem.* **2006**, *42*, 757.
16. Kelly, J. X.; Smilkstein, M. J.; Brun, R.; Wittlin, S.; Cooper, R. A.; Lane, K. D.; Janowsky, A.; Johnson, R. A.; Dodean, R. A.; Winter, R.; Hinrichs, D. J.; Riscoe, M. K. *Nature* **2009**, *459*, 270.
17. (a) Holloway, G. A.; Baell, J. B.; Fairlamb, A. H.; Novello, P. M.; Parisot, J. P.; Richardson, J.; Watson, K. G.; Street, I. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1422; (b) Holloway, G. A.; Charman, W. N.; Fairlamb, A. H.; Brun, R.; Kaiser, M.; Kostewicz, E.; Novello, P. M.; Parisot, J. P.; Richardson, J.; Street, I. P.; Watson, K. G.; Baell, J. B. *Antimicrob. Agents Chemother.* **2009**, *53*, 2824.