Aminobenzimidazoles as Antimalarials with an Unknown Mechanism of Action

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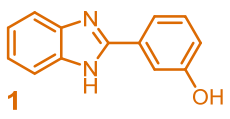
1. Introduction

- Malaria poses a significant global health challenge and is prevalent in tropical regions of the world, particularly sub-Saharan Africa.
- Resistance is emerging to many current antimalarial treatments, exemplified by high-failure rates to artemisinin combination therapies in South-East Asia.[1]
- Antimalarials acting via novel mechanisms of action are urgently required to combat this rising problem.

2. Lead Compound Discovery

- Apical membrane antigen 1 (AMA1) is a potential therapeutic target as it has been shown to have an essential role in erythrocytic invasion.[2]
- Our group undertook a fragment screen to identify small molecule inhibitors of AMA1, with hits containing a benzimidazole core (**1**).[3]
- Structural elaboration led to 2-aminobenzimidazoles (ABIs) which were not active against AMA1.
- A phenol derivative (**2**) was found to be potent against *P. falciparum* 3D7 with an IC_{50} of 77 nM against *Pf* 3D7 over 72 h despite no apparent binding to AMA1.
- AstraZeneca identified a range of benzimidazoles active against *Pf* through a phenotypic HTS where ABI **2** was the sole phenol identified and retained potency against a range of resistant *Pf* strains.[4]



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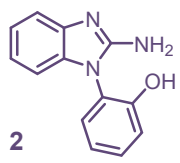

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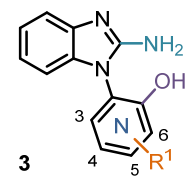
3. Structural Development

- Our group has expanded on the structure-activity relationship (SAR) and found the 1,4-amino alcohol relationship is crucial and positional alteration or replacement of the NH_2 or OH results in a loss of activity.
- Replacing the phenol ring with aliphatic rings or substitution of N in the ring results in reduced activity.
- Substitution is well tolerated around the phenol with two 5-substituted derivatives (Me and OMe) twice as potent.
- Methyl substitution at positions 4-, 5- and 6- improved antimalarial potency.
- Exploring the SAR effects of substitution around the benzimidazole requires a new synthetic route.



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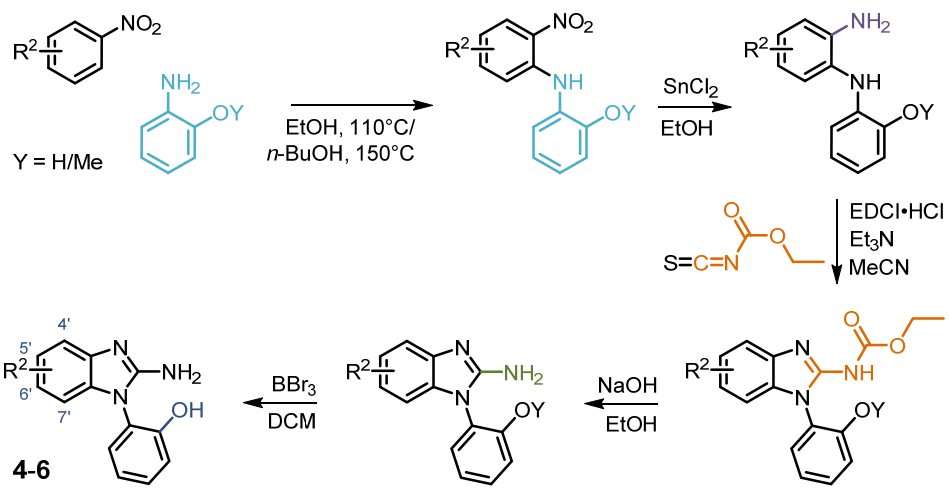
#	R^1	IC_{50} (nM) ± S.E.M
2	-	77 ± 4
3a	3-Me	135 ± 9
3b	4-Me	56 ± 6
3c	5-Me	42 ± 4
3d	6-Me	61 ± 7

4. Benzimidazole Substitution



4-6

- Initial attempts at benzimidazole substitution were not chemoselective and resulted in inseparable mixtures.
- Multistep synthetic route was required to selectively synthesise substituted benzimidazoles at each position.
- Ring closure was low yielding due to by-products of the reactive phenol so it was protected as an anisole.
- Anisoles required 150 °C in the first step so were reacted in *n*-butanol rather than at 110 °C in ethanol for phenols.

#	R^2	IC_{50} (nM) ± S.E.M
4a	4'-F	532 ± 49
4b	5'-F	184 ± 25
4c	6'-F	127 ± 42
4d	7'-F	386 ± 32
5a	4'-Br	491 ± 51
5b	5'-Br	211 ± 32
5c	6'-Br	118 ± 13
5d	7'-Br	411 ± 38
6a	4'-Me	15 ± 2
6b	5'-Me	19 ± 2
6c	6'-Me	17 ± 2

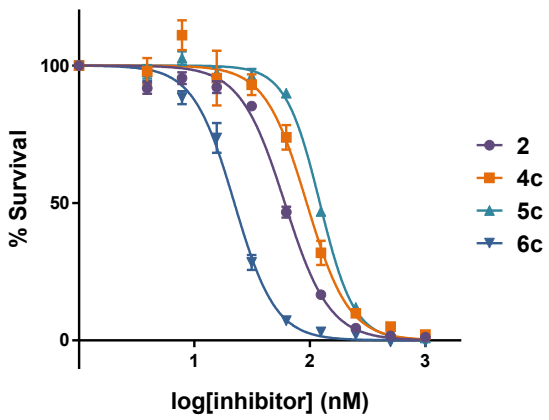


Figure 1: 72 h IC_{50} against *Pf* 3D7 of **2** compared to 6'-substituted benzimidazoles (**4c**, **5c** and **6c**)

- Benzimidazole substitution is tolerated at the 6'-position with minor losses in activity for 6'-fluoro (**4c**) and bromo (**5c**) derivatives (**Figure 2**).
- Methyl substituents (**6a-c**) were much more potent than **2** regardless of ring position.
- Brominated derivatives allow synthetic access to more diverse substituents.

5. Disubstitution

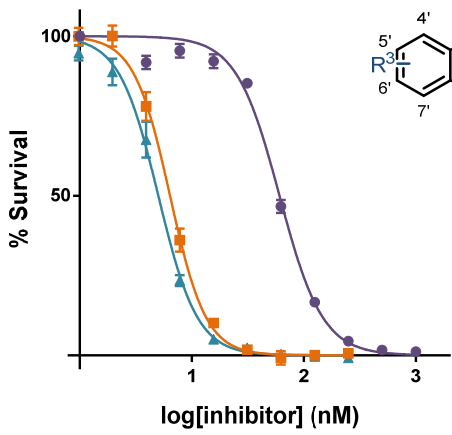
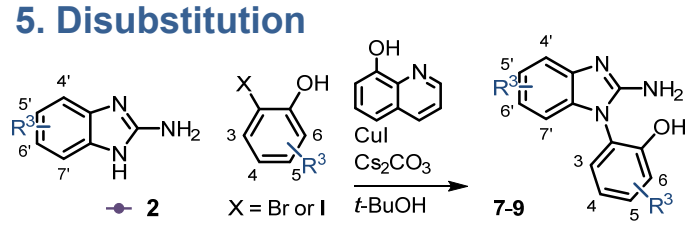


Figure 2: 72 h IC_{50} against *Pf* 3D7 of **2** compared to disubstituted benzimidazoles (**7** and **9**)

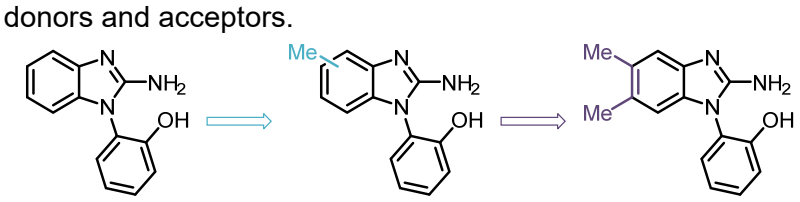


#	R^3		IC_{50} (nM) ± S.E.M
7	4-Me	5-Me	6.4 ± 0.5
8	4-Me	6-Me	23 ± 2
9	5'-Me	6'-Me	5.4 ± 0.3
10	5-Me	6'-Me	16 ± 2

- Disubstituted derivatives **7-9** were synthesised through a Cu(I) selective *N*-arylation.[5]
- Compound **10** was synthesised through the chemoselective multistep scheme of other substituted benzimidazoles using a methylated anisole.
- Disubstitution resulted in enhanced antiplasmodial activity.
- Compounds **7** and **9** are the only two ABIs with sub 10 nM potency.
- Substituted benzimidazoles are low molecular weight so there is chemical space to improve the pharmacokinetic properties.

6. Conclusions & Future Directions

- Benzimidazole substitution is well tolerated at the 6'-position.
- Methyl substituents on the benzimidazole ring resulted in the most potent ABI derivatives to date. Disubstitution of the benzimidazole or phenol rings resulted in potency of less than 10 nM.
- Larger benzimidazole substituents will be explored along with hydrogen-bond donors and acceptors.



IC_{50} = 77 ± 4 nM IC_{50} = 15-19 nM IC_{50} = 5.4 ± 0.3 nM

- The pharmacokinetic properties of substituted benzimidazoles will be assessed for viability as drug compounds.
- The mechanism of action is under investigation using various methods including resistance generation, click chemistry and multiple omics techniques.

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