

A Concise Silylamine Approach to 2-Amino-3-hydroxy-indoles with Potent *in vivo* Antimalaria Activity

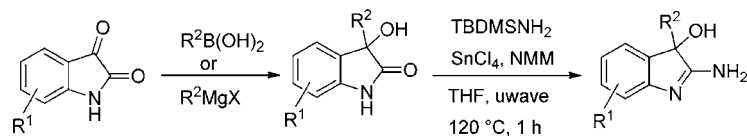
Sameer Urgaonkar,[†] Joseph F. Cortese,[†] Robert H. Barker,[‡] Mandy Cromwell,[‡] Adelfa E. Serrano,[§] Dyann F. Wirth,^{†,||} Jon Clardy,^{†,⊥} and Ralph Mazitschek^{*,†,♯}

Infectious Disease Initiative Program, The Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, Massachusetts 02142, Drug Discovery and Development, Genzyme Corporation, Waltham, Massachusetts 02451, University of Puerto Rico School of Medicine, San Juan, P.R. 00936-5067, Department of Immunology and Infectious Disease, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, and Center for Systems Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

ralph@broad.harvard.edu

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ABSTRACT



R¹ = 5-Cl, R² = 1-naphthyl

P. falciparum EC₅₀ (3D7) = 30 nM

P. falciparum EC₅₀ (Dd2) = 37 nM

The development of a concise strategy to access 2-amino-3-hydroxy-indoles, which are disclosed as novel antimalarials with potent *in vivo* activity, is reported. Starting from isatins the target compounds are synthesized in 2 steps and in good yields via oxindole intermediates by employing *tert*-butyldimethylsilyl amine (TBDMSNH₂) as previously unexplored ammonia equivalent.

Malaria is the most deadly parasitic infectious disease with an estimated 300–500 million cases and a death toll of 0.8–1.2 million in 2008 alone.¹ Of the four *Plasmodium* species that are relevant for humans, *P. falciparum* accounts for most of the fatalities.¹ These already grim statistics are likely to become even grimmer as *P. falciparum* strains that are resistant to

commonly used antimalaria chemotherapeutic agents emerge and spread throughout Africa and parts of Asia.

The most recent antimalarial drug class was introduced in 1996.² In an effort to address the urgent need for new drugs, especially ones with new cellular targets or chemotypes that could delay the emergence of resistance, we have screened small-molecule libraries for novel, drug-like compounds with whole-cell antimalarial activity and limited susceptibility to established mechanisms of drug resistance.³

[†] The Broad Institute of Harvard and MIT.

[‡] Genzyme Corporation.

[§] University of Puerto Rico School of Medicine.

^{||} Harvard School of Public Health.

[⊥] Harvard Medical School.

[♯] Massachusetts General Hospital.

(1) Aregawi, M. Cibulskis, R. Otten, M. Williams, R. Dye, C. *World Malaria Report 2008*; World Health Organization: Geneva, 2008.

(2) Ekland, E. H.; Fidock, D. A. *Int. J. Parasitol.* **2008**, *38*, 743.

(3) Baniecki, M. L.; Wirth, D. F.; Clardy, J. *Antimicrob. Agents Chemother.* **2007**, *51*, 716.

Very recently, two similar efforts have also been reported.^{4,5} Out of ~79,000 compounds,⁶ 104 inhibitors with nanomolar activity against drug-sensitive (3D7) and multidrug-resistant (Dd2, HB3) *P. falciparum* strains were identified.³ One of the hits, the 2-amino-5-chloro-3-hydroxy-3-phenylindole **1a** (see Figure 1) was particularly attractive because of its

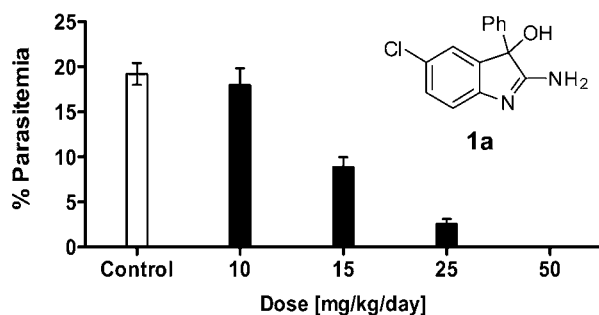


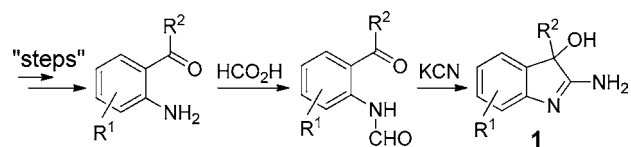
Figure 1. *In vivo* antimalaria activity of 2-aminoindole **1a**. Following inoculation of Swiss Albino mice with *P. berghei* parasites, **1a** was administered i.p. once daily for 4 days, and parasitemia was determined on day 5.

unusual and compact 2-amino-3-hydroxy-indole core structure. In addition, compound **1a** achieved excellent exposure in mouse pharmacokinetic studies, and more importantly, when tested in a 4-day suppressive *P. berghei* mouse model **1a** demonstrated very good *in vivo* efficacy,⁷ causing a dose-dependent decrease in parasitemia with undetectable levels of parasites at the highest dose tested and no signs of adverse side effects (Figure 1).

Encouraged by this promising *in vivo* activity we began to explore and optimize this compound class. A thorough literature survey revealed that 2-amino-3-hydroxy-indoles are virtually unexplored for biological activity, which we suspect results at least in part from the limited synthetic methodology to access this class. To date, four distinct approaches have been reported.^{8–11} We found the methods reported by Bell et al. to prepare the 2-amino-3-hydroxy-indoles *via* reaction of KCN with formylated or dichloroa-

cyclated 2-aminobenzophenones to be the most appropriate (Scheme 1).⁹

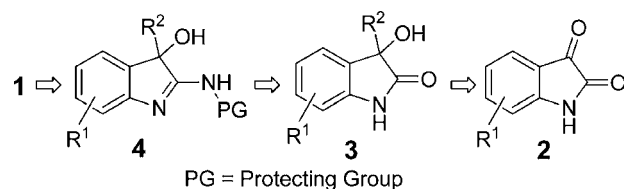
Scheme 1. Traditional Synthetic Strategy to Access 2-Amino-3-hydroxy-indoles via Cyanohydrins



Unfortunately, this strategy, although readily scalable and attractive for process scale syntheses, is limited for early stage exploratory medicinal chemistry due to the lack of commercially available 2-aminobenzophenones and the inherent lack of enantioselectivity. As a result, we developed a general, short and efficient method that would (a) provide analogues in quantities that satisfy early stage drug discovery requirements, (b) tolerate a wide variety of functional groups, (c) begin with commercially available diversely functionalized building blocks, and (d) avoid the use of cyanide while (e) offering the potential to enantioselectively access 2-amino-3-hydroxy-indoles.

We envisioned a three-step reaction sequence starting from isatins, which are widely available and inexpensive starting materials as shown in Scheme 2.

Scheme 2. Retrosynthetic Analysis Starting from Isatin (2)



Grignard addition or Rh-catalyzed addition¹² of boronic acids to isatin **2** would give 3-hydroxy-3-aryl-oxindole **3**. Oxindole **3** could then react with a protected ammonia equivalent (e.g., allylamine) to yield the corresponding protected 2-amino-3-hydroxy-indole **4**, which on deprotection would result in the desired 2-amino-3-hydroxy-indole **1**. Furthermore, boronic acids¹³ and electron-rich arenes¹⁴ can be added to isatins with high enantioselectivity. This method would also allow access to 2-amino-3-hydroxy-indoles enantioselectively.

On the basis of the well-precedented nature of the first step, we directed our efforts toward the conversion of 3-hydroxyoxindoles **3** to allyl-protected 2-amino-3-hydroxy-indoles **4**. We were somewhat encouraged by variable

(4) Gamo, F.-J.; Sanz, L. M.; Vidal, J.; Cozar, C. d.; Alvarez, E.; Lavandera, J.-L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. *Nature* **2010**, *465*, 305.

(5) Guiguemde, W. A.; Shelat, A. A.; Bouck, D.; Duffy, S.; Crowther, G. J.; Davis, P. H.; Smithson, D. C.; Connelly, M.; Clark, J.; Zhu, F.; Jiménez-Díaz, M. B.; Martínez, M. S.; Wilson, E. B.; Tripathi, A. K.; Gut, J.; Sharlow, E. R.; Bathurst, I.; El Mazouni, F.; Fowble, J. W.; Forquer, I.; McGinley, P. L.; Castro, S.; Angulo-Barturen, I.; Ferrer, S.; Rosenthal, P. J.; Derisi, J. L.; Sullivan, D. J.; Lazo, J. S.; Roos, D. S.; Riscoe, M. K.; Phillips, M. A.; Rathod, P. K.; Van Voorhis, W. C.; M Avery, V.; Guy, R. K. *Nature* **2010**, *465*, 311.

(6) Analysis of screening libraries and assay results was supported by *Instant JChem*, *JKlustor*, and *Screen*; ChemAxon: Budapest, Hungary; www.chemaxon.com/products.html.

(7) Peters, W.; Robinson, B. L. In *Parasitic Infection Models*; Zak, O., Sande, M. A., Eds.; Academic Press: London, 1999; pp 757.

(8) Wittig, G.; Kleiner, H.; Conrad, J. *Liebigs Ann. Chem.* **1929**, 469, 1.

(9) Bell, S. C.; Wei, P. H. L. *J. Heterocycl. Chem.* **1969**, *6*, 599.

(10) Ishizumi, K.; Inaba, S.; Yamamoto, H. *J. Org. Chem.* **1974**, *39*, 2581.

(11) Benincori, T.; Sannicola, F. *J. Org. Chem.* **1988**, *53*, 1309.

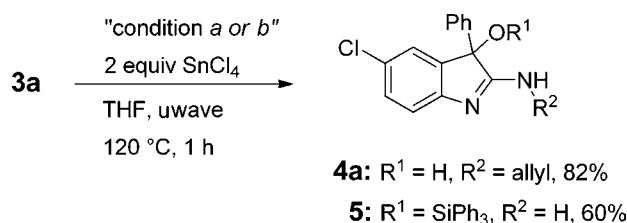
(12) Toullec, P.; Jagt, R.; de Vries, J.; Feringa, B.; Minnaard, A. *Org. Lett.* **2006**, *8*, 2715.

(13) Shintani, R.; Inoue, M.; Hayashi, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 3353.

(14) Hanhan, N. V.; Sahin, A. H.; Chang, T. W.; Fettingner, J. C.; Franz, A. K. *Angew. Chem., Int. Ed.* **2010**, *49*, 744.

conversions (~30–50%) obtained upon heating 5-chloro-3-hydroxyoxindole **3a** with an excess of allylamine in the presence of 5 mol % of PTSA and 4 Å molecular sieves.¹⁵ Attempts to increase the yields by employing other drying agents such as Na₂SO₄, MgSO₄ or the use of Dean–Stark apparatus were not successful. We anticipated that better conversions could be achieved with the use of a Lewis acid, which not only would catalyze the transformation but would also efficiently entrap the water formed. Exploring a set of Lewis acids confirmed our hypothesis, with both SnCl₄ and Ti(O*i*Pr)₄ efficiently and more importantly, reproducibly promoting the conversion of **3a** to **4a** (Scheme 3). Further-

Scheme 3. Amidations with Allylamine and Triphenylsilylamine Conditions^a



^a Conditions: (a) excess allylamine for **4a**, (b) 2 equiv of Ph₃SiNH₂ and 4 equiv NMM for **5**.

more, while microwave conditions reduced the reaction times from 12–18 h to 40–60 min, the addition of NMM (*N*-methyl morpholine) as an acid scavenger allowed the amine coupling partner to be used in nominal amount.

Disappointingly, the deprotection of the allyl group in **4a** proved challenging despite the plethora of deallylation protocols for amines and amides.^{16,17}

Next, we turned our attention to identifying an ammonia equivalent that would allow for more facile deprotection. On the basis of literature precedents of employing a silylated ammonia equivalent in unrelated transformations,¹⁸ we decided to explore commercially available Ph₃SiNH₂. Unexpectedly, as shown in Scheme 3, the reaction of **3a** with Ph₃SiNH₂ under the optimized conditions produced *O*-silylated 2-amino-3-hydroxy-indole **5**, in 60% yield while only trace amounts of the desired desilylated product **1a** were observed. Aminoindole **5** is probably formed by the intramolecular migration of the silyl group. Attempts to desilylate **5** using TBAF or other fluoride sources resulted in hydrolysis of **5** and in recovery of the original hydroxyoxindole starting material **3a**.

Encouraged by the finding that a silylamine appears well-suited to install the desired amine functionality, we suspected that less sterically demanding analogues of Ph₃SiNH₂ such as *tert*-butyldimethylsilyl amine (TBDMSNH₂) might allow

deprotection under condition that would prevent hydrolysis of the amine. Pleasingly, we were able to directly isolate the desired 2-aminoindole product **1a** in 60% isolated yield. The structure of **1a** was unambiguously established by X-ray analysis revealing that the newly installed substituent appears to favor the tautomer with an exoamine rather than an exoimine geometry (see Supporting Information).

Although TBDMSNH₂ has been utilized in the context of its ligand attributes for metal complexes,^{19–21} this is, to our knowledge, the first report on the application of TBDMSNH₂ in organic synthesis.

The synthetic utility of this novel SnCl₄-promoted amidation reaction with TBDMSNH₂ as an ammonia surrogate was then explored using a series of substituted 3-hydroxyoxindoles. A variety of functional groups in the indole ring, including halogens, nitro, and alkyl ether are well tolerated in the amidation process (Table 1). The amidation reaction worked equally well under Ti(O*i*Pr)₄ promoted conditions.

The substrate scope with respect to the substituent at the 3-position of oxindoles was also found to be broad. Thus, the aryl group at the 3-position possessing functional groups such as ester (entry h) and alkyne (entry d) could be employed in this process. In the case of an aryl group substituted with an enolizable ketone, the use of Ti(O*i*Pr)₄ in lieu of SnCl₄ was critical for the success of the reaction (entry i, Table 1), as is the indole with free nitrogen as R² (entry p).

Interestingly, an aryl group substituted with the sulfonamide group yielded the aminoindole with TBDMS group on sulfonamide nitrogen with this method. However, deprotection with HF/pyridine gave the aminoindole **1q** (entry q). It is worth noting that several of these 2-amino-3-hydroxyindoles would be difficult to access or would require functional group manipulations via a cyanohydrin route significantly lengthening the synthesis. In addition, our methodology allowed for direct access of *N*(1)-alkyl iminoindole using the corresponding oxindole as starting material (entry r).

To ascertain whether an enantioenriched 3-hydroxyoxindole would transform into 2-amino-3-hydroxy-indole under the reaction conditions without any loss in enantiomeric excess, we prepared chiral 3-hydroxyoxindole (*S*)-**3a**²² (>95% ee) and subjected it to SnCl₄ promoted amidation reaction with TBDMSNH₂. Gratifyingly, the corresponding 2-amino-3-hydroxy-indole (*S*)-**1a** was obtained in good yield without any measurable loss of enantiomeric excess (>95% ee) indicating no racemization occurred during the reaction (Scheme 4).

The initial exploratory SAR against drug-sensitive (3D7) and drug-resistant (Dd2) parasite strains indicates that 2-amino-3-hydroxy-indoles are in general more active when R² is either an *ortho*-substituted electron-rich aromatic ring (entries c and m, Table 1) or a naphthyl moiety (entry n,

(15) Meyer, R.; Zwiesler, M. *J. Org. Chem.* **1968**, *33*, 4274.

(16) Escoubet, S.; Gastaldi, S.; Bertrand, M. *Eur. J. Org. Chem.* **2005**, *2005*, 3855.

(17) Unsatisfactory yields were obtained when a RhCl₃-catalyzed allyl deprotection strategy was applied. Zacuto, M.; Xu, F. *J. Org. Chem.* **2007**, *72*, 6298.

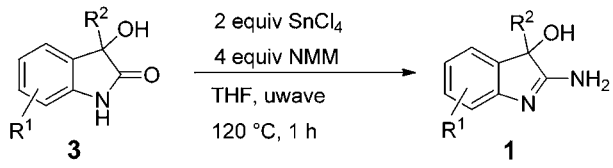
(18) Huang, X.; Buchwald, S. *Org. Lett.* **2001**, *3*, 3417.

(19) West, R.; Boudjouk, P. *J. Am. Chem. Soc.* **1973**, *95*, 3983.

(20) Bowser, J.; Neilson, R.; Wells, R. *Inorg. Chem.* **1978**, *17*, 1882.

(21) Quallich, G.; Makowski, T.; Sanders, A.; Urban, F.; Vazquez, E. *J. Org. Chem.* **1998**, *63*, 4116.

(22) Hewawasam, P.; Erway, M.; Moon, S.; Knipe, J.; Weiner, H.; Boissard, C.; Post-Munson, D.; Gao, Q.; Huang, S.; Gribkoff, V.; Meanwell, N. *J. Med. Chem.* **2002**, *45*, 1487.

Table 1. Direct Conversion of **3** to **1** with TBDMSNH₂


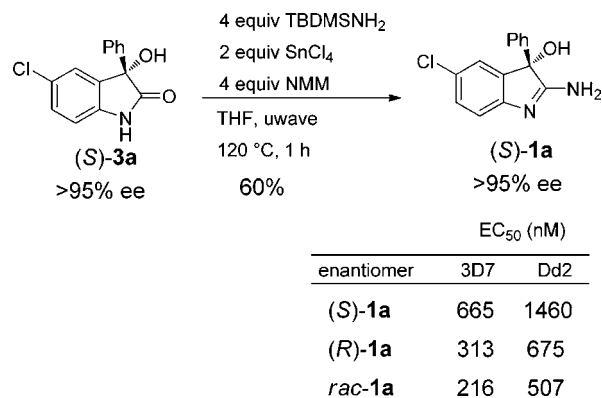
entry	R ¹	R ²	yield (%) ^a	EC ₅₀ (nM)	
				3D7	Dd2
a	5-Cl	Ph	60 (56) ^b	216	507
b	5-I	Ph	64	289	338
c	5-Cl	<i>o</i> -OMePh	43	24	57
d	5-Cl	$\frac{1}{2}$ ≡-Ph	45	1160	1127
e	5-Cl	$\frac{1}{2}$ △	58	104	465
f	5-F	<i>p</i> -OMePh	52	85	359
g	5-Cl	<i>iso</i> -butyl	50	232	270
h	5-Cl	<i>m</i> -CO ₂ MePh	38	783	1578
i	5-Cl	<i>m</i> -AcPh	30 ^b	529	1525
j	5-Cl	CH ₂ -(<i>o</i> -tolyl)	53	63	139
k	4-Br	CH ₂ Ph	51	>5000	>5000
l	5-OMe	Ph	49 (48) ^b	24	385
m	H	<i>o</i> -tolyl	53	112	152
n	5-Cl	1-naphthyl	67	30	37
o	5-NO ₂	Ph	30 ^b	>5000	>5000
p	5-Cl	3'-(5'-OMe-indolyl)	40 ^b	>5000	>5000
q	5-Cl	<i>p</i> -SO ₂ NH ₂ Ph	45 ^{b,c}	>5000	>5000
r ^d	H	Ph	39	>5000	>5000
		Chloroquine		16	216

^a Isolated yields. ^b Ti(OiPr)₄ was used instead of SnCl₄ and NMM. ^c TBS-protected aminoindole was formed, which on treatment with HF/pyridine gave **1q**; yield reported is over two steps. ^d The indole nitrogen is methylated (see Supporting Information).

Table 1), which is possibly a result of the increased dihedral angle of the biaryl system and thereby generating a more favorable binding conformation for its target(s). 2-Amino-3-hydroxy-indoles **1** with benzylic and alkyl groups at R² also displayed potent antimalarial activity for R¹ as 5-Cl. Furthermore, protection of the 3-hydroxyl (Scheme 3 compound **5**; EC₅₀ > 5 μM) or 2-amino groups (Scheme 3 compound **4a**; EC₅₀ > 5 μM) was found to be detrimental.

The 3-hydroxy-oxindoles (**3**) which potentially can be regenerated by hydrolysis of the 2-amino group did not exhibit any significant antimalarial activity.

A 2-fold difference in *in vitro* activity between the two enantiomers of **1a** was noted and interestingly, racemate was found to be more active than either of the two enantiomers (Scheme 4).

Scheme 4. Synthesis of Enantioenriched 2-Amino-3-hydroxy-indole **1a**

In summary, we have identified 2-amino-3-hydroxy-indoles as a novel chemical class with potent *in vitro* and *in vivo* antimalaria activity. We have developed a concise synthetic strategy to efficiently synthesize analogues in quantities sufficient for medicinal chemistry exploration. This method establishes the unprecedented use of TBDMSNH₂ as an ammonia surrogate and allows for the first enantioselective synthesis of 2-amino-3-hydroxy-indoles. It is likely that TBDMSNH₂ will find use in other transformations requiring protected ammonia equivalents.

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Supporting Information Available: Experimental procedures and characterization; ¹H and ¹³C NMR, chiral SFC chromatogram, and X-ray structure of **1a**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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