Novel 4-Aminoquinolines Active against Chloroquine-Resistant and Sensitive *P. falciparum* Strains that also Inhibit Botulinum Serotype A

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Received June 17, 2008

Abstract: We report on the initial result of the coupling of 4-amino-7-chloroquinoline with steroidal and adamantane constituents to provide small molecules with excellent in vitro antimalarial activities (IC_{90} (W2) down to 6.74 nM). The same entities also inhibit the botulinum neurotoxin serotype A light chain metalloprotease at low micromolar levels (7−31 μM). Interestingly, structural features imparting increased antimalarial activity also provide increased metalloprotease inhibition, thus allowing for simultaneous compound optimizations against distinct targets.

Malaria parasites contain acidic food vacuoles (FV)[a] that are responsible for hemoglobin hydrolysis, and it is these vacuoles that appear to be the site of action for a number of existing antimalarials. The heme, obtained via hemoglobin degradation in the FV, is transformed into insoluble hemozoin pigment, while the globin is hydrolyzed into individual amino acids. Antimalarial drugs appear to kill the parasite either by producing toxic free radicals in the FV or by blocking the formation of hemozoin from heme molecules once they are liberated from hemoglobin, as in the case of 4-amino-7-chloroquinolines (4,7-ACQs)[1] The antiparasitic effects of compounds possessing the 4,7-ACQ are presumably caused by the toxicity of nonpolimerized heme, possibly through membrane disruption. The development of widespread drug-resistance to chloroquine (CQ), the standard antimalarial drug, has resulted in severe health issues for countries in malaria endemic regions.[2] Therefore, significant focus has been placed on the syntheses of peroxide antimalarials[3] as well as the development other molecules that attack heme polymerization.[4] Currently, those of greatest interest, due to their low toxicity, are new 4,7-ACQs,[5] 4-pyridones,[6] combinations of 4,7-ACQs with drug-reversals,[7] ferrocenes,[8] and bisquinolines.[9] A promising drug candidate possessing the 4,7-ACQ moiety appears to be AQ-13, a des-methyl chloroquine derivative.[10]

Antimalarial agents possessing the 4,7-ACQ have previously been shown to inhibit the metalloprotease activity of the botulinum neurotoxin serotype a light chain (BoNT/A LC),[11,12] a potential biothreat agent. This serotype, along with botulinum neurotoxin serotypes B−G, are responsible for the characteristic neuroparalysis associated with the disease state botulism.[13] Structurally, BoNTs consist of a heavy chain (HC) and a light chain (LC), which are connected by a disulfide bridge. The HC binds to neurons and transports the LC into the cell cytosol. The LC is a zinc metalloprotease that cleaves components of the soluble NSF-ethylmaleimide-sensitive factor attachment protein receptor complex (which mediates the exocytosis of acetylcholine into neuromuscular junctions). Of the seven known serotypes (i.e., A−G), A and E cleave SNAP-25 [synaptosomal-associated protein (25 KDa)] (19), B, D, F, and G cleave vesicle-associated membrane protein (20−23), and serotype C cleaves both SNAP-25 and syntaxin 1. BoNT/A possesses a lethal dose of 1−5 ng kg^{-1} for humans.[13] For perspective, this lethality is 10^6 times greater than cobra toxin and ca. 10^{11} times greater than cyanide. Currently, the only available treatment for botulism (i.e., once BoNTs have innervated) is critical care mechanical ventilation. However, as BoNTs remain active for several weeks,[13] this avenue of treatment is not practical for widespread application, hence the need to identify and develop small molecule inhibitors of the enzyme's metalloprotease.

Recently, we reported the synthesis of 4,7-ACQ's possessing a steroidal, cholic acid carrier, and their inhibitory activity against the BoNT serotype A light chain (BoNT/A LC) metalloprotease.[12] Here, we show that the same compounds are also potent antimalarial agents. Moreover, we report the syntheses and the antimalarial and BoNT/A LC inhibitory potencies of novel 4,7-ACQ-adamantane derivatives and describe how the structure activity data for both sets of compounds follow very similar trends for both targets.

The syntheses of the compounds represented in Charts 1 and 2 are described in detail in the Supporting Information and in ref 12 The quintessential feature of our synthetic approach is the simple and efficient preparation of the target compounds using readily available and inexpensive starting materials. The chiral centers in the steroidal components are not prone to racemization, so products were obtained as single enantiomers. In an alternative approach, the achiral aminoquinolines were prepared.[14]

In vitro antimalarial activity is given in Table 1. General observations regarding antimalarial activity include:[15] (1) compounds with an amide functionality linking the 4,7-ACQ moiety to the steroid, or to adamantane, are inactive (the exception is **5**, which is less active than MFQ against W2 but has comparable activity to MFQ against D6 and the multidrug

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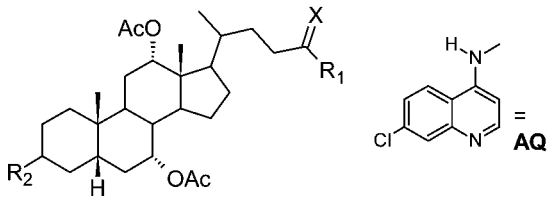
[a] Abbreviations: FV, food vacuole; CQ, chloroquine; MFQ, mefloquine; ART, artemisinin; SI, the selectivity index (SI) is defined as the ratio of the IC_{90} for the resistant versus sensitive strain; BoNT, botulinum neurotoxin; BoNT/A, BoNT serotype A; LC, light chain; 4,7-ACQ, 4-amino-7-chloroquinoline.

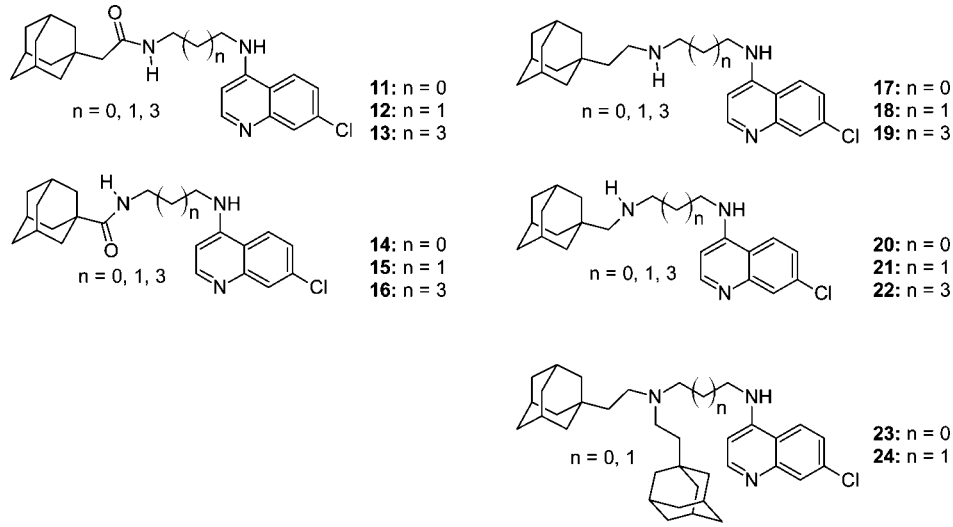
10.1021/jm800737y CCC: $40.75
Published on Web 07/19/2008

Chart 1



1: $R_1 = OCH_3$, $R_2 = \alpha$-AQ, X = O
2: $R_1 = NHPr^n$, $R_2 = \alpha$-AQ, X = O
3: R_1 = AQ, $R_2 = \alpha$-OAc, X = O
4: R_1 = AQ, $R_2 = \alpha$-OAc, $X = H_2$
5: $R_1 = NHCH_2CH_2AQ$, $R_2 = \alpha$-OAc, X = O
6: R1 = $NHCH_2CH_2AQ$, R2 = α-OAc, $X = H_2$
7: $R_1 = OCH_3$, $R_2 = \beta$-$NHCH_2CH_2AQ$, X = O
8: $R_1 = OCH_3$, $R_2 = \alpha$-$NHCH_2CH_2AQ$, X = O
9: $R_1 = OCH_3$, $R_2 = \alpha$-$OCH_2CH_2NHCH_2CH_2AQ$, X = O
10: $R_1 = OCH_3$, $R_2 = \alpha$-OCH_2CH_2AQ, X = O

Chart 2



resistant TM91C235 strain), (2) 4,7-ACQs with two ionizable nitrogens, i.e., **6**, **8**, and **9**, are more potent against all three strains than monoamino analogues **4**, **1** (**2**), and **10**, respectively (Table 1), (3) the most active compounds are the epimeric steroidal 4,7-ACQs **7** and **8** and adamantane derivatives **20**, **21**. In particular, steroid containing **7** and **8** are more active than ART against all tested strains, while ethanediamino derivative **20** is as active as ART against the highly resistant W2 strain and more active than ART against the CQ susceptible D6 and the multidrug resistant TM91C235 strains. The propanediamino congener **21** is 2−3 times more active than CQ against the D6 strain, as active as ART against the TM91C235 strain, and slightly less active against W2 strain.

Analyzing the selectivity index (SI), it is evident that most of compounds are more active against the D6 strain than against the CQ resistant strain. However, several features merit further elaboration. SIs for the two adamantane derivatives, **20** and its homologue **21**, are almost identical in each instance, revealing that the two compounds themselves are equally potent against both drug resistant strains. In addition, both compounds have very similar activity to ART against both strains. Another group of compounds that merits additional analysis are steroidal derivatives **6**, **7**, and **8**. **6** is two times less active against the D6 and W2 strains than CQ and ART, respectively, but is 1.5−4 times more active than CQ and MFQ against the TM91C235 strain. A significant feature is that this compound has an $SI_{W2/D6}$ of 0.95.[16] In addition to their superior activity against all three strains, compared to CQ, MFQ, and ART, epimeric aminoquinolines **7** and **8** are also more active against the W2 strain than against the D6 strain ($SI_{W2/D6}$ 0.65 and 0.91, respectively). The observed in vitro antiprotozoal behavior of **7** and **8** is very similar to ART, an unusual characteristic for an aminoquinoline that does not possess additional functionalities, such as hydroxybenzene or ferrocene. The side products of the reductive amination (Supporting Information), compounds **23** and **24**, were several times less active against all strains, presumably due to their two voluminous adamantyl components.

All compounds shown in Charts 1 and 2 were examined for BoNT/A LC metalloprotease inhibitory activity using a previously described HPLC-based assay.[17] Compounds in Chart 1 were examined previously.[12] For Chart 2 compounds, diaminoethyl derivatives **20** and **17** and diaminopropyl derivatives **21** and **18** were found to inhibit the enzyme. IC_{50} values for these compounds, as well as those examined previously,[12] are shown in Table 2. Interesting structural features resulting in increase/decrease antimalarial activity also follow similar trends for BoNT/A LC inhibition, specifically: (1) compounds incorporating an amide in the linker between the 4,7-ACQ and either the steroidal or adamantane components were inactive, (2) 4,7-ACQs with two ionizable nitrogens, i.e., **6**, **7**, **8**, **17**, etc., are the most potent inhibitors of the BoNT/A LC, while those possessing a single ionizable nitrogen demonstrate poor[11] to no activity, (3) inhibitors possessing the cholic acid moiety, i.e., **6**, **7**, and **8**, are more potent than inhibitors possessing the adamantine motif, most likely due to greater steric occupancy of the enzyme's substrate binding cleft, and (4) **23** and **24**, which each possess a second adamantane group, were also inactive against the BoNT/A LC, most likely due to their increased steric bulk.

Table 1. In Vitro Antimalarial Activities of Aminoquinoline Compounds against *P. falciparum* Strains

	D6[a] (nM)		TM91C235[b] (nM)		W2[c] (nM)		
compd	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	SI[d]
1	10.65	20.71	30.05	68.60	107.72	563.15	3.31/27.20
2	68.18	98.17	99.46	366.71	208.98	1851.51	3.74/18.86
3	3022.31	6915.40	3506.06	6058.39	7191.35		
4	15.75	30.71	37.04	70.49	139.61	1176.50	2.30/38.31
5	19.01	21.13	28.79	34.78	48.85	70.57	1.65/3.34
6	16.87	34.21	27.74	77.92	11.38	32.50	2.28/0.95
7	6.17	10.43	11.01	24.97	3.38	6.74	2.39/0.65
8	9.47	12.35	12.83	24.39	5.74	11.18	1.97/0.91
9	14.07	22.78	32.18	75.95	17.83	24.60	3.33/1.08
10	33.05	99.26	50.52	72.84	111.03	147.21	0.73/1.48
11	78.50	157.33	238.10	382.92	253.81	396.84	
12	105.87	293.60	201.00	464.34	337.19	852.69	
13	267.28	347.38	602.18	776.84	517.72	660.39	
14	201.68	464.76	438.69	685.39	484.51	724.44	
15	213.69	370.46	524.68	787.15	877.99	1358.80	
16	511.34	631.71	765.01	984.39	884.29	1408.66	
17	8.59	13.65	17.76	24.43	15.66	30.96	1.79/2.27
18	11.43	23.27	42.11	171.09	33.66	56.09	7.35/2.41
19	21.92	24.75	67.23	168.39	159.71	310.82	6.80/12.55
20	4.26	8.23	8.73	14.93	8.40	15.22	1.81/1.85
21	3.83	6.41	7.39	20.20	12.10	20.05	3.15/3.13
22	15.19	18.94	75.34	161.25	130.95	167.69	8.51/8.85
23	51.85	69.97	100.71	225.20	78.15	113.35	3.22/1.62
24	52.22	78.31	109.43	171.29	83.21	132.30	2.19/1.69
CQ[e]	12.44	16.38	124.24	243.15	392.09	529.86	14.84/32.35
MFQ[e]	11.25	28.06	33.46	106.04	4.96	14.70	3.78/0.52
ART[f]	9.0	12.80	13.04	17.40	6.7	11.5	1.36/0.90

[a] *P. falciparum* African D6 clone. [b] *P. falciparum* multidrug resistant TM91C235 strain (Thailand). [c] *P. falciparum* Indochina W2 clone. [d] The selectivity index (SI) is defined as the ratio of the IC_{90} for the resistant versus sensitive strain, TM91C235/D6 and W2/D6, respectively. [e] Control compounds. Average of seven replicates. [f] Average of greater than eight replicates.

Table 2. In Vitro Inhibitory Activity of Aminoquinolines against BoNT/A LC

compd	inhibition IC_{50} (μM)
6[a]	10.00 (±0.80)
7[a]	17.00 (±1.7)
8[a]	7.00 (±1.0)
21[b]	31.98 (±3.2)
20[b]	18.92 (±2.8)
18[b]	50.10 (±2.8)
17[b]	11.81 (±1.6)

[a] Results taken from ref 12. [b] IC_{50} values were calculated from plots of concentration versus inhibition (see Experimental Section, Supporting Information, for details), and are the averages of five determinations.

Interestingly, inhibitors **20** and **17**, possessing ionizable nitrogen positions separated by an ethenyl spacer, are 1.7 and 4.2 fold more potent than their respective analogues, **21** and **18**, which both possess a corresponding propenyl spacer. Moreover, the longer corresponding pentenyl spacer found in **19** and **22** completely obviates activity. This agrees with results from our previous study,[12] which showed that N^1-(7-chloroquinolin-4-yl)-ethane-1,2-diamine inhibited the BoNT/A LC with 1.5 fold more potency than N^1-(7-chloroquinolin-4-yl)-propane-1,3-diamine. Finally, **23** and **24**, which each possess a second adamantane group, were also inactive against the BoNT/A LC, most likely due to their increased steric bulk.

In conclusion, we have synthesized novel 4,7-ACQ derivatives that target both heme polymerization in malaria and BoNT/A LC zinc metalloprotease activity. The screening of these compounds against three *P. falciparum* strains indicated that our N^1-[(7-chloro-4-quinolinyl)-1,2-ethanediamine and 1,3-propanediamines coupled with steroid and adamantyl carriers are good-to-excellent inhibitors of CQ-susceptible and CQ-resistant *P. falciparum* strains. Out of 14 ionizable diamines, five compounds (**7**, **8**, **17**, **20**, **21**) are more active than CQ (IC_{90} = 12.8−6.4 nM). In addition, 12 compounds are more active against highly CQ-resistant W2, and 13 are more active against multidrug resistant TM91C235, than chloroquine. Of particular importance is the finding that steroidal aminoquinolines **7** and **8** are more active against the W2 strain than artemisinin, with SIs (W2/D6) of 0.65 and 0.91, respectively. A compelling aspect of this study is that the most active 4,7-ACQ derivatives examined in the antimalarial screens are also the most potent inhibitors of BoNT/A LC metalloprotease activity and follow similar structure activity trends for both. This is extraordinary given that fact that the two distinct targets share completely different mechanisms of action. Hence, 4,7-ACQ derivatives provide an efficient synthetic paradigm for simultaneously developing novel small molecules that can be used to treat either malaria infection or BoNT/A poisoning.

Acknowledgment. This work has been partially supported by the Ministry of Science of Serbia (grant no. 142022) and the Serbian Academy of Sciences and Arts. Botulinum studies were funded by the U.S. Army Medical Research and Material Command Research plan no. 02-4-3U-057 and IAA no. Y3-CM-100505 (MRMC and NCI). The contents have been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation or publication. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting true views of the Department of the Army, the Department of Defense, or the National Institutes of Health. For J.C.B. and R.G., this project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. For J.C.B. and R.G., this research was supported (in part) by the Developmental Thera-

peutics Program in the Division of Cancer Treatment and Diagnosis of the National Cancer Institute.

Supporting Information Available: Full experimental section. This material is free of charge via the Internet at http://pubs.acs.org.

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(16) To the best of our knowledge, the selectivity index <1 involving an 3-chloro-4-aminoquinoline and W2 clone was found only on few occasions: bis(aminoquinolines) (ref 7), ferroquine (ref 6b), and AQ-40 (ref 8b).

(17) (a) Schmidt, J. J.; Stafford, R. G. A high-affinity competitive inhibitor of type A botulinum neurotoxin protease activity. *FEBS Lett.* **2002**, *532*, 423–426. (b) Schmidt, J. J.; Bostian, K. A. Proteolysis of synthetic peptides by type A botulinum neurotoxin. *J. Protein Chem.* **1995**, *14*, 703–708. (c) Schmidt, J. J.; Bostian, K. A. Endoproteinase activity of type A botulinum neurotoxin: substrate requirements and activation by serum albumin. *J. Protein Chem.* **1997**, *16*, 19–26. (d) Schmidt, J. J.; Stafford, R. G.; Bostian, K. A. Type A botulinum neurotoxin proteolytic activity: development of competitive inhibitors and implications for substrate specificity at the S1¢ binding subsite. *FEBS Lett.* **1998**, *435*, 61–64.

JM800737Y