In Vitro Antiplasmodial, Antiamoebic, and Cytotoxic Activities of Some Monomeric Isoquinoline Alkaloids

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Twenty-one alkaloids have been assessed for activities against *Plasmodium falciparum* (multidrug-resistant strain K1) in vitro; 18 of these are reported for the first time. Two protoberberine alkaloids, dehydrodiscretine and berberine, were found to have antiplasmodial IC_{50} values less than 1 μM, while seven alkaloids—allocrytopine, columbamine, dehydroocoteine, jatrorrhizine, norcorydine, thalifendine, and ushinsunine—had values between 1 and 10 μM. These results are discussed in the context of structure−activity relationships. Compounds were also assessed for antiamoebic and cytotoxic activities, but none was significantly active except for berberine, which was moderately cytotoxic.

Diseases caused by protozoa are responsible for considerable morbidity and mortality throughout the developing world. Chief among these is malaria, which is estimated to kill between 1.5 and 2.7 million people each year.[1] Amoebiasis is another important protozoal disease which is less fatal than malaria but causes considerable morbidity.[2] In a previous study,[3] we assessed the in vitro antiplasmodial, antiamoebic, and cytotoxic activities of a series of bisbenzylisoquinoline alkaloids with the result that several alkaloids were identified as having potent antiplasmodial and/or antiamoebic activities. In this paper, we report the in vitro evaluation of a number of monomeric isoquinoline alkaloids in an attempt to discover further alkaloids with antiprotozoal properties and, where possible, to gain information on structure−activity relationships.

Results and Discussion

The in vitro IC_{50} values of the alkaloids against *Plasmodium falciparum*, *Entamoeba histolytica*, and KB cells (human carcinoma of the nasopharynx) are shown in Table 1. The structures of some of the compounds are illustrated in Figure 1.

In the aporphine group, norcorydine possessed the highest antiplasmodial activity (IC_{50} = 3.08 μM), and corydine, its *N*-methyl analogue, was 7-fold less active. Isocorydine, which differs from corydine in the positioning of the hydroxyl/methoxyl substituents (Figure 1), was more than 10-fold less active than norcorydine. Catalpifoline (6-*O*-methylnorcorydine) was 7-fold less active than norcorydine. These results suggest that a secondary amino function and a phenolic substituent enhance the in vitro antiplasmodial activity of aporphines. Norcorydine was found to have only weak activity against *E. histolytica* and to be nontoxic to KB cells (IC_{50} >733 μM), clearly showing selective toxicity against *P. falciparum*. The low cytotoxicity of these alkaloids is consistent with a previous report in which corydine and isocorydine were found to be inactive in vivo against the Walker 256 tumor.[4] Antiplasmodial activity was also shown by ushinsunine (IC_{50} = 5.99 μM) and dehydroocoteine (IC_{50} = 5.78 μM); the latter alkaloid was 6-fold more active than its C-6a−C-7 saturated analogue ocoteine. Recently, the aporphine alkaloid (−)-roemrefidine has been reported to have potent in vitro and in vivo antimalarial activities with relatively low cytotoxicity.[5]

None of the four morphinanone alkaloids tested was significantly active in any of the tests. In contrast, the protoberberine group included the alkaloids with the highest antiplasmodial activities. Dehydrodiscretine (IC_{50} = 0.64 μM) and berberine (IC_{50} = 0.968 μM) were the most active. Variations in the substitution pattern as seen in the other members of this group resulted in some loss of activity. The activities of berberine and jatrorrhizine against *P. falciparum* (strain K1) reported here are similar to those found by Vennerstrom and Klayman[6] (using strains D-6 and W-2). However, in this study canadine (tetrahydroberberine) was found to have very weak antiplasmodial activity against strain K1 (IC_{50} >147μM), whereas the latter authors[6] reported marked in vitro activity against strains D-6 (IC_{50} = 1.64 μM) and W-2 (IC_{50} = 5.13 μM). The low antiplasmodial activity of the nonquaternary alkaloid canadine (tetrahydroberberine) found in this study, in contrast to the high activity of berberine, may indicate that a quaternary nitrogen is required for activity in this series of alkaloids. However, the two alkaloids also differ with respect to the saturation in ring C as a consequence of the quaternization of the ring-B nitrogen.

Of the two protopine alkaloids, allocrytopine was moderately potent against *P. falciparum*, while protopine was 7-fold less active. The presence of the methylenedioxy group in protopine is clearly detrimental to activity.

None of the compounds tested exhibited significant activity against *E. histolytica*. Berberine has been reported to inhibit the growth of *E. histolytica*, *Giardia lamblia*, and *Trichomonas vaginalis* in vitro,[7] but high concentrations (1 mg/mL) were needed. This is consistent with the weak effect seen in this study (IC_{50} = 111 μM). Berberine (as

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Table 1. Antiplasmodial, Antiamoebic, and Cytotoxic Activities of Alkaloids and Standard Drugs

	activity against		
alkaloid	*P. falciparum* IC_{50} μM±SEM (*n*)	*E. histolytica* IC_{50} μM (95% CI)	KB cells IC_{50} μM±SEM (*n*)
aporphine type			
catalpifoline	24.1 ± 2.34 (4)	147	>733
corydine	22.3 ± 1.46 (3)	90.6(47.2−174)	>733
dehydroocoteine	5.78 ± 1.37 (4)	136	>341
isocorydine	37.0 ± 0.22 (4)	>147	>733
magnoflorine iodide	>107	111(64.6−191)	>533
norcorydine	3.08 ± 0.66 (3)	76.4−153	>733
ocoteine	34.8 ± 3.36 (4)	51.2(45.0−58.2)	74.3 ± 12.3 (4)
thalicminine	16.2 ± 3.91 (4)	75.1(57.8−97.0)	>685
ushinsunine	5.99 ± 0.47 (4)	not tested	42.5 ± 4.85 (4)
morphinanone type			
metaphanine hydrobromide	>117	58.6−117	>586
O-methylflavinantine	207 (2)	73.4−147	>734
sinoacutine	>95	76.4−153	>764
sinomenine	>152	56.2(41.2−77.4)	>759
protoberberine type			
berberine chloride	0.968 ± 0.16 (6)	111(77.7−160)	7.32 ± 1.07 (4)
columbamine chloride	1.92 ± 0.48 (4)	156(89.6−273)	77.9 ± 18.4 (4)
dehydrodiscretine chloride	0.639 ± 0.059 (4)	66.9−134	>335
jatrorrhizine chloride	3.15 ± 0.97 (4)	82.7(74.9−111)	>335
thalifendine chloride	7.91 ± 1.83 (4)	116(86.1−156)	>699
protopine type			
allocrytopine	5.08 ± 1.05 (4)	135	>338
protopine	34.0 ± 4.92 (4)	70.7−142	>354
tetrahydroprotoberberine type			
canadine	>147	126(74.0−214)	>734
standard drugs			
chloroquine diphosphate	0.20 ± 0.03 (8)	not tested	not tested
emetine dihydrochloride	not tested	2.23(1.99−2.46)	not tested
podophyllotoxin	not tested	not tested	0.008 ± 0.003 (4)

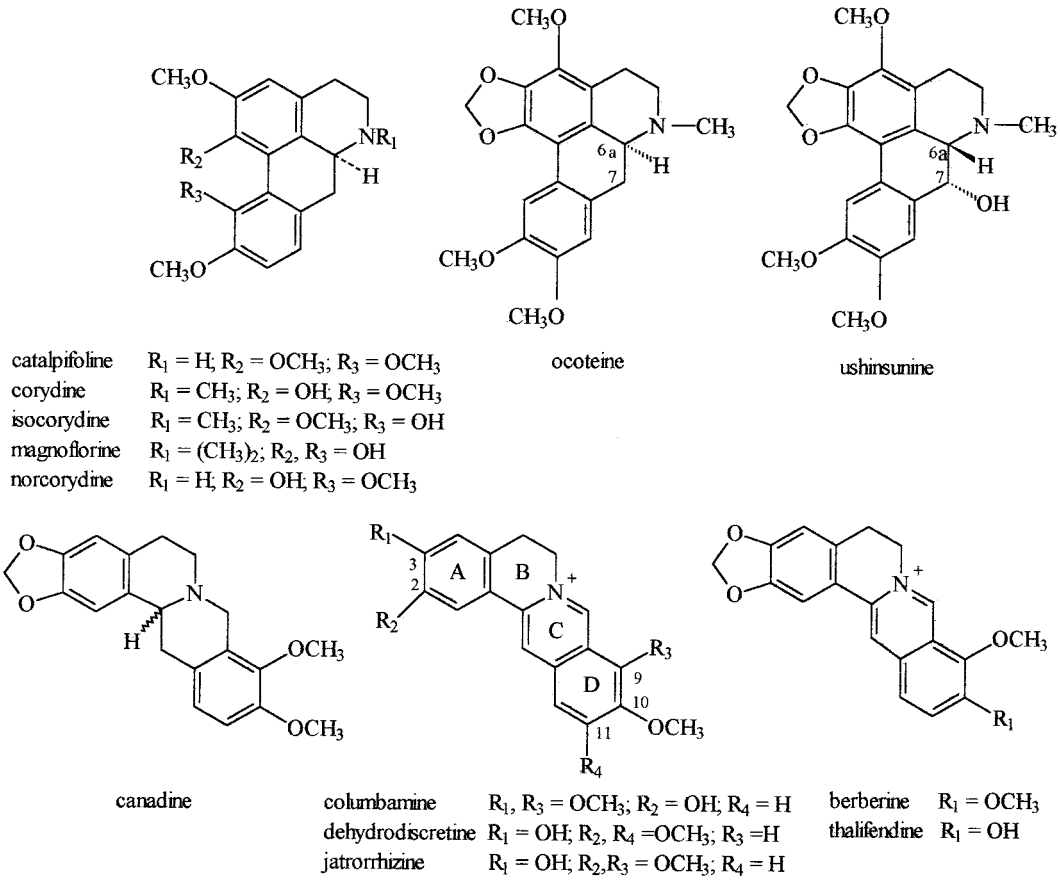

Figure 1. Structures of some of the alkaloids used in this study.

sulfate) in doses of 2 mg/kg by intramuscular injection or 3 mg/kg orally has been reported to be effective in preventing the development of hepatic amoebiasis in guinea pigs inoculated with *E. histolytica*,[8] suggesting that activity in vivo is greater than that seen in vitro. This finding is in contrast to the lack of in vivo activity seen against *Plasmodium berghei* in mice.[6] Further research is needed to explain these observations.

In conclusion, this study has identified a number of monomeric isoquinoline alkaloids that have significant in

vitro antiplasmodial activities and are worthy of further investigation as leads to new antimalarial agents. In particular, dehydrodiscretine is highly active and selectively toxic to malaria parasites. Dehydrodiscretine is the only 2,3,10,11-tetraoxygenated protoberberine alkaloid that was tested; the other, less active compounds were all 2,3,9,-10-tetraoxygenated protoberberines. Structure−activity relationships reveal that antiplasmodial activity is influenced by the position of oxygenation, nature of the substituent, and the oxidation state of the ring.

Experimental Section

Compounds. The alkaloids used in this study were available from a collection in one of our laboratories (PLS). Chloroquine diphosphate, emetine dihydrochloride, and podophyllotoxin were available from Sigma.

Biological tests. In vitro testing against *E. histolytica* (strain NIH 200) was carried out using amoebae grown in microplates as described previously.[9] After 72 h of incubation at 37 °C in the presence of serial dilutions of the compounds under test, inhibition of growth was assessed by fixing and staining the organisms with eosin and measuring the optical density with a microplate reader at 490 nm. Activity against *P. falciparum* (multidrug-resistant strain K1) was determined by measuring the inhibition of incorporation of [^{3}H]-hypoxanthine into red blood cells infected with malaria parasites as previously reported.[10] Cytotoxicity against KB cells was determined using a method similar to that used for assessing antiamoebic activity.[11]

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