for monitoring compounds 1 and 2 and set at 500 mV for monitoring compounds 3 and 4. The peak heights gave the tissue concentrations (nmol/g of tissue) from calibration curves. These were prepared from standard solutions (authentic samples dissolved in 0.02 M formic acid). Corrections were made for recoveries (for compound 1, 79.6%, SEM = 2.3%, n = 10; for compound 2, 84.3%, SEM = 1.7%, n = 12; for compound 3, 89.7%, SEM = 1.5%, n = 14; for compound 4, 86.8%, SEM = 1.2%, n = 21).

In Vitro Aromatic Hydroxylation. Compounds 1 and 2 were incubated with crude rat liver microsomes in 10-mL glass tubes at 37 °C as previously described.[6] Incubation constituents were NADPH (0.1 mM), $MgCl_2$ (2.5 nM), Tris buffer, pH 7.4 (0.1 M), microsomes (0.5 mg of protein/mL), and H_2O to a final volume of 1.0 mL. After incubation for 3 min, the reaction was stopped by adding 50 μL of perchloric acid (60%). Supernatants were assayed for compounds 3 and 4 (as described above) without further purification. In control incubation samples (one without microsomes and one without test compound) compounds 3 and 4 were not detectable. Apparent kinetic constants (K_m and V_{max}) were calculated from Lineweaver-Burk plots with three to four concentrations, each measured in triplicate.

In Vitro O-Methylation. Incubations of compounds 3 (0.025-0.5 mM) and 4 (0.05-1 mM) with rat liver COMT were performed essentially as described earlier.[6] After 15 min, 50 μL of perchloric acid (60%) was added to the incubation mixture, and the O-methylated products were measured in the supernatant by the same method as described above for the determination of compounds 1 and 2, except for the electrode potential, which now was set to 750 mV. Compounds 5 and 8 had retention times of (10% *i*-PrOH) 13 and 7 min, respectively. The only methoxy hydroxy compound detected in the incubation samples of compound 4 had a retention time of 12 min. This suggests that compound 4 is in vivo methylated to compound 7 instead of 8. In incubation samples of compound 3, two methoxy hydroxy compounds were detected, with retention times of 13 min (5) and 9 min (probably 6). Concentrations of compounds 6 and 7 were calculated from the calibration curves of their isomers 5 and 8, respectively, assuming an identical electrochemical sensitivity for both isomers in each case. Apparent kinetic constants were calculated from Lineweaver-Burk plots with three to four concentrations, each measured in triplicate.

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Registry No. 1, 69367-50-6; 2, 80300-08-9; 3, 72189-85-6; 3·HBr, 113925-13-6; 4, 113925-14-7; 4·HBr, 113925-15-8; 5, 113925-16-9; 5·HCl, 113925-17-0; 6, 113925-18-1; 7, 113925-19-2; 8, 113925-20-5; 8·HCl, 113925-21-6; 9·HCl, 104931-31-9; 10, 87411-78-7.

Protoberberine Alkaloids as Antimalarials

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The protoberberine alkaloids berberine (1), palmatine (2), jatrorrhizine (3), and several berberine derivatives (4-10) were tested for antimalarial activity in vitro against *Plasmodium falciparum* and in vivo against *Plasmodium berghei*. The berberine derivatives 4-10 were designed and synthesized to maximize structural diversity within a modest set of compounds. Palmatine (2) and jatrorrhizine (3) were isolated as their chlorides from *Enantia chlorantha*. None of the protoberberine alkaloids was active in vivo, although compounds 1, 2, 3, 5, and 6 exhibited a potency comparable to that of quinine in vitro.

Berberine (1) is probably the most widely distributed of all alkaloids, having been found in plants of the nine botanical families, Berberidaceae, Papaveraceae, Runnunculaceae, Rutaceae, Menispermaceae, Rubiaceae, Rhamnaceae, Magnoliaceae, and Annonaceae. This and allied protoberberine alkaloids such as palmatine (2) have been used extensively in folk medicine.[1] At the present time, the only practical therapeutic application of berberine is in the treatment of cutaneous leishmaniasis,[2-9] although various claims have been made concerning its use in the treatment of malaria.[1,5,10-18] Whereas several investigators found berberine to be inactive in experimental and clinical malaria,[5,14] others claimed it to be valuable alone[16,17] or as an adjunct to quinine.[12,13] Berberine has also been claimed to be beneficial in the treatment of the splenomegaly of malaria.[10,11] In a recent in vitro study,[18] berberine chloride at 50 μM was found to completely block protein synthesis in *Plasmodium falciparum*. Finally, aqueous extracts from the African plant *Enantia chlorantha* Oliver, which contains the protoberberine alkaloids palmatine, columbamine, and jatrorrhizine,[19] have been claimed to be therapeutically useful in the treatment of malaria.[20]

Although antimalarial effects have been ascribed to berberine, little quantitative experimental data exist to substantiate such claims. In this paper we present data on the antimalarial properties of berberine (1) and its

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(1) Thakur, R. S.; Srivastava, S. K. *Cent. Inst. Med. Aromatic Plants* **1982**, *4*, 249.
(2) Varma, R. L. *Indian Med. Gaz.* **1927**, *62*, 84.
(3) DasGupta, B. M.; Dikshit, B. B. *Indian Med. Gaz.* **1929**, *64*, 67.
(4) Devi, A. L. *Indian Med. Gaz.* **1929**, *64*, 139.
(5) Chopra, R. N.; Dikshit, B. B.; Chowan, J. S. *Indian J. Med. Res.* **1932**, *19*, 1193.
(6) Shideman, F. E. *Bull. Natl. Formul. Comm.* **1950**, *18*, 3.
(7) Narian, L.; Dutta, G. P. *Indian J. Parasitol.* **1978**, *2*, 83.
(8) El-On, J.; Greenblatt, C. L. *Curr. Ther. Res.* **1983**, *33*, 660.
(9) Ghosh, A. K.; Bhattacharyya, F. K.; Ghosh, D. K. *Exp. Parasitol.* **1985**, *60*, 404.
(10) Waldropo, C. P. *Biol. Inst. Clin. Quir.* **1926**, *2*, 355.
(11) Quintana, H.; Soto, N. A. *Semana Med.* **1926**, *33*, 943.
(12) Andre, A. P. *Rev. Med. Cubana* **1936**, *38*, 976.
(13) Brahmachari, U. *Indian Med. Gaz.* **1944**, *79*, 259.
(14) Pessoa, S. B. *Acta Med. (Rio de Janeiro)* **1942**, *9*, 191.
(15) Glasby, J. S. *Encyclopedia of the Alkaloids*; Plenum: New York, 1975; Vol. 1, p 203.
(16) Dalton, D. R. *The Alkaloids*; Marcel Dekker: New York, 1979; p 371.
(17) Hahn, F. E.; Ciak, J. *Antibiotics. Vol. III*, Corcoran, J. W., Hahn, F. E., Eds.; Springer-Verlag: New York, 1975; p 578.
(18) Elford, B. C. *Parasitol. Today* **1986**, *2*, 309.
(19) Hamonniere, M.; Leboeuf, M.; Cave, A.; Paris, R. R. *Planta Med. Phytother.* **1975**, *9*, 296.

0022-2623/88/1831-1084$01.50/0

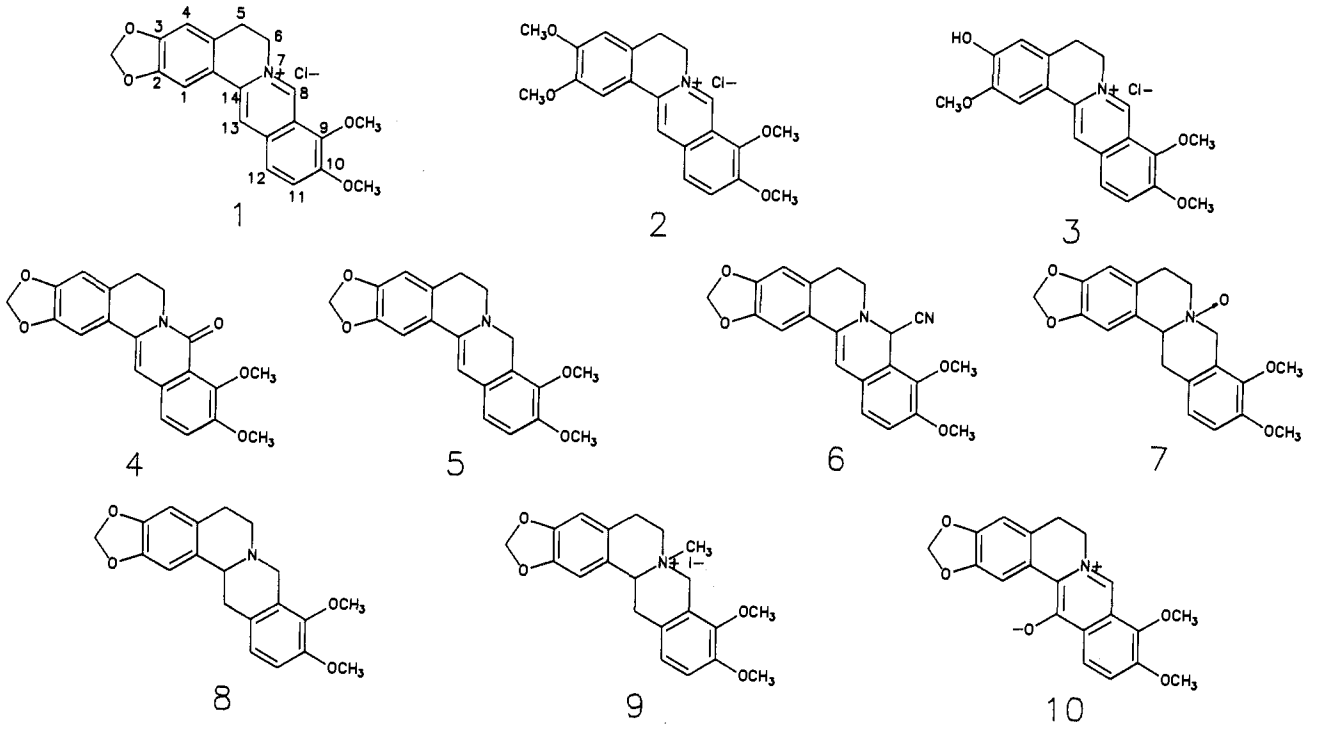

Figure 1. Protoberberine alkaloids.

Table I. Antimalarial Activity of Protoberberines against *P. falciparum* in Vitro and *P. berghei* in Vivo

no.	mp, °C	lit, mp, °C	yield, %	*P. falciparum* D-6 clone: IC_{50}, ng/mL	*P. falciparum* W-2 clone: IC_{50}, ng/mL	*P. berghei*: 40, 160, 640 mg/kg, T - C,[a] days
1				141	148	0.6, 1.0, T[b]
2	203–205	205[c]		281	163	0.5, T, T
3	206–208	206[d]		422	1607	N/A
4	200–201	198–200[e]	39	557	2008	0.1, 0.5, 0.3
5	155–161	157–159[f]	56	120	171	-0.2, T, T
6	178–181	184–186[g]	89	101	143	N/A
7	195–196	188–190[h]	43	565	558	0, 0.2, 0
8	169–171	170–171[i]	72	555	1739	0.1, 0.1, 0.3
9	248–250	248[j]	85	970	1345	N/A
10	258–261	260–262[k]	91	568	701	-0.2, -0.2, 0.2

[a] T - C is the mean survival time of the treated mice beyond that of the control animals. This value must be ≥6.2 days in order for a test compound to be considered active. [b] Toxic. [c] Feist, K.; Sandstede, G. *Arch. Pharm. (Weinheim, Ger.)* **1918**, *256*, 1. [d] Feist, K. *Arch. Pharm. (Weinheim, Ger.)* **1907**, *245*, 586. [e] Reference 24, Haworth, R. D.; Koepfli, J. B.; Perkin, W. H., Jr. *J. Chem. Soc.* **1927**, *548*. [f] Reference 25. [g] Reference 30, upon rapid heating. [h] Reference 29. [i] Bradsher, C. K.; Dutta, N. L. *J. Am. Chem. Soc.* **1960**, *82*, 1145. [j] Reference 27. [k] Reference 28.

derivatives (**4–10**), as well as palmatine (**2**) and jatrorrhizine (**3**) (Figure 1), isolated from the bark of *E. chlorantha*.

Chemistry

Palmatine and jatrorrhizine were isolated as their chlorides from the bark of *E. chlorantha* by using a modified procedure combining several aspects of previous reported[19,21–23] extractions of berberine alkaloids from various plant sources. The synthetic berberine derivatives were chosen to maximize structural diversity in order to establish a range-finding structure–activity relationship with a modest series of compounds. Except for 8-cyanodihydroberberine (**6**) and tetrahydroberberine *N*-oxide (**7**), derivatives of berberine were synthesized by using literature procedures.[24–28] The synthesis of **7** was reported by the hydrogen peroxide treatment of tetrahydroberberine (**8**),[29] but we were unable to duplicate this procedure. Instead, we obtained **7** in a straightforward manner by treating **8** with *m*-chloroperbenzoic acid (MCPBA). 8-Cyanodihydroberberine (**6**) was obtained by treatment of a methanolic solution of berberine chloride (**1**) with aqueous sodium cyanide in high yield. In an early report[30] it had been claimed that **6** was the product resulting from

(20) We have been informed by Dr. A. O. Onabanjo that in present day Nigerian antimalarial folk medicine, several small pieces of the bark of *E. chlorantha* (known as "Awa-opa") are added to $^3/_4$ of a bottle of beer and the balance of the bottle is made up with soda pop to mask the bitter taste. After macerating for 2–3 days, one-half cup of the liquid is given twice daily for 3 consecutive days.

(21) Hamerslag, F. E. *The Technology and Chemistry of Alkaloids*; D. Van Nostrand: New York, 1950; pp 129–151.
(22) Seitz, G. *Naturwissenschaften* **1959**, *46*, 263.
(23) Buzas, A.; Egnell, C. *Ann. Pharm. Fr.* **1965**, *23*, 351.
(24) Perkin, W. H., Jr. *J. Chem. Soc.* **1918**, *113*, 722.
(25) Elliot, I. W. *J. Heterocycl. Chem.* **1967**, *4*, 639.
(26) Russell, P. B. *J. Am. Chem. Soc.* **1956**, *78*, 3115.
(27) Sallay, I.; Ayers, R. H. *Tetrahedron* 1963, *19*, 1397.
(28) Hanaoka, M.; Mukai, C.; Nagami, K.; Okajima, K.; Yasuda S. Chem. *Pharm. Bull.* **1984**, *32*, 2230.
(29) Takemoto, T.; Kondo, Y.; Kondo, K. *Yakagaku Zasshi* **1963**, *83*, 162.
(30) Robinson, G. M.; Robinson, R. *J. Chem. Soc.* **1917**, *111*, 958.

the treatment of berberine sulfate with potassium cyanide, but structural characterization was lacking.

Biological Results and Discussion

The protoberberine derivatives were tested in vitro against two clones of human malaria, *P. falciparum* D-6 (Sierra Leone clone) and W-2 (Indochina clone). The former clone is a strain that is resistant to mefloquine and the latter to chloroquine, pyrimethamine, sulfadoxine, and quinine. In vivo testing was done in *Plasmodium berghei* parasitized mice. In the in vitro screen, compounds **1**, **2**, **3**, **5**, and **6** exhibited a potency comparable to that of quinine. None of the compounds, however, were active in vivo against *P. berghei*. Moreover, higher activity in the in vitro screen seems to correlate with a corresponding toxicity in the in vivo model. (See Table I.)

The more potent compounds **1**, **2**, **3**, **5**, and **6** in the in vitro model possessed either a dihydro or quaternary protoberberine structure. The *trans*-stilbene substructure present in these compounds listed above was a minimum, but not sufficient, requirement for greater potency as oxyberberine (**4**) and berberinephenolbetaine (**10**) were much less active. The alkyl-substitution pattern at the 2,3-catechol function of the protoberberine quaternary alkaloids **1** and **2** had only a minor influence on activity; a free phenolic function at the 3-position in protoberberine quaternary alkaloid **3**, however, lowered activity significantly. Tetrahydroberberine (**8**) was less active as were two tetrahydro derivatives **7** and **9**. Tetrahydroberberine *N*-oxide (**7**) has a formal positive charge on the nitrogen atom, and *N*-methyltetrahydroberberinium iodide (**9**) has a full positive charge on the nitrogen atom. Therefore, potency in these tetrahydroberberines was not improved by incorporation of a positively charged nitrogen. On the basis of results seen with this modest series of protoberberine alkaloids, several structural features are required to maintain potency in vitro against *P. falciparum*. These include an alkyl-substituted 2,3-catechol function as part of either a quaternary protoberberine structure or a dihydroprotoberberine structure with a neutral nitrogen atom.

The activity seen with dihydroberberine (**5**) and 8-cyanodihydroberberine (**6**) may result in part from their oxidation to the corresponding quaternary structures. Bodor et al.[31] have calculated second order rate constants of ca. 4–20 mol^{-1} s^{-1} for the oxidation of dihydroberberine to berberine under a variety of in vitro conditions.

In conclusion, these results confirm those of Elford,[18] who found berberine to be a potent inhibitor in vitro of both nucleic acid and protein biosynthesis in *P. falciparum*. Many studies have in fact demonstrated a strong interaction of berberine with DNA;[32-35] however, the lack of in vivo antimalarial activity seen in mice with all the protoberberine alkaloids tested agrees with those clinical reports[5,14] which had claimed berberine to be inactive as an antimalarial drug.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were run as KBr disks on a Nicolet 20SXB spectrophotometer. NMR spectra were run on a JEOL FX90Q spectrometer using TMS as an internal standard. HPLC chromatograms were run on a Varian Vista 5500/402 chromatograph/data station using a Varian Micropak (MCH-5-N-CAP) C-18 reverse-phase column eluting with H_2O/CH_3CN (6:4) with 0.1% TFA and detection at 345 nm. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI.

Isolation of Palmatine (2) and Jatrorrhizine (3) Chlorides. A modified isolation procedure based on the one described by Hamerslag[21] for the isolation of berberine from golden root-seal was employed. Ground bark from *E. chlorantha* (323 g) was treated with 1500 mL of toluene at reflux for 7 h and twice with 1250 mL of Et_2O at reflux for 14 h. Each extraction was followed by three washings with fresh solvent, and the washes and extracts were discarded. After the bark had dried at room temperature, it was extracted three times with 1250 mL of MeOH at reflux for 9 h, and the extracts were combined. Solvent removal at reduced pressure provided 16 g of a brown residue. Three successive extractions of this residue with 200 mL of 5% HCl with heating on a steam bath followed by filtration afforded a yellow-brown filtrate. Removal of the water in vacuo provided 12.1 g of a brown residue, which was dissolved in a mixture of $CHCl_3$ and MeOH and applied to an acidic alumina chromatography column. Elution with $CHCl_3$/MeOH mixtures as described by Cava et al.[36] provided palmatine (**2**) chloride (4.72 g) and jatrorrhizine (**3**) chloride (0.33 g). The identity of these alkaloids was confirmed by melting point, elemental analysis, and IR and NMR spectra. Synthesis of the known tetrahydropalmatine and tetrahydrojatrorrhizine from palmatine and jatrorrhizine, respectively, using $NaBH_4$ in 10% aqueous MeOH[37] provided confirmation of the identity of the alkaloids.

8-Cyanodihydroberberine (6). Sodium cyanide (50 mmol, 2.45 g) dissolved in 100 mL of water was added to a solution of 2.0 g of 1 chloride dihydrate (5 mmol) dissolved in 100 mL of MeOH with heating on a steam bath. Heating was continued for 15 min following the formation of a homogeneous solution. Cooling afforded 8-cyanodihydroberberine (1.61 g, 89%) as orange-brown cubes: mp 178–181 °C (lit.[30] mp 184–186 °C); IR 2960, 2938, 2906, 2831, 2211 (CN), 1622, 1606, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.88–3.59 (m, 4 H), 3.87 (s, 3 H), 3.97 (s, 3 H), 5.75 (s, 1 H), 5.96 (s, 2 H), 6.14 (s, 1 H), 6.59 (s, 1 H), 6.86 (s, 2 H), 7.16 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 29.54, 47.91, 49.97, 56.04, 60.91, 98.13, 101.16, 104.09, 107.77, 113.84, 116.55, 116.98, 119.74, 124.08, 126.89, 128.41, 138.38, 144.39, 146.94, 147.75, 150.57; MS, *m/z* 362 (M^+, 37), 361 (100), 360 (14), 346 (19), 338 (11), 337 (55), 336 (95), 334 (16), 332 (13), 331 (16), 321 (25), 310 (25), 306 (14), 304 (12), 292 (14), 278 (31), 276 (13), 139 (9), 101 (8). Anal. ($C_{21}H_{18}N_2O_4$) C, H, N.

Tetrahydroberberine *N*-Oxide (7). To a solution of tetrahydroberberine (10 mmol, 3.39 g) in CH_2Cl_2 at 0 °C was added MCPBA (15 mmol, 2.59 g) in one portion. The reaction mixture was stirred for 1.5 h at 0 °C before dilution with 250 mL of CH_2Cl_2 and extraction with 3 × 100 mL of 1 N KOH and 100 mL of brine. Drying the organic phase with K_2CO_3 and solvent removal in vacuo provided a brown foam, which was crystallized from CH_3CN to afford 1.51 g (43%) of tetrahydroberberine *N*-oxide as fine tan crystals: mp 195–196 °C (lit.[29] mp 188–190 °C); IR 2913, 2908, 2900, 1499, 1487, 1461 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.44–2.75 (m, 2 H), 3.09–4.10 (m, 4 H), 3.88 (s, 3 H), 3.89 (s, 3 H), 4.35–4.77 (m, 3 H), 5.93 (s, 2 H), 6.64 (s, 1 H), 6.72 (s, 1 H), 6.82 (d, J = 10 Hz, 1 H), 6.97 (d, J = 10 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.67, 29.49, 55.71, 60.04, 64.54, 67.79, 67.85, 100.89, 105.50, 108.37, 111.73, 123.05, 123.48, 125.00, 125.49, 125.54, 125.81, 145.53, 146.67, 150.41; MS, *m/z* 355 (M^+, 25), 354 (18), 340 (31), 339 (66), 338 (100), 337 (80), 336 (32), 322 (25), 308 (9), 292 (8). Anal. ($C_{20}H_{21}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

Biological Methods. The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al.[38] and Milhous et al.[39] Two *P. falciparum* malaria parasite clones, designated as Indochina (W-2) and Sierra

(31) Bodor, N.; Brewster, M. E. *Eur. J. Med. Chem.* **1983**, *18*, 235.
(32) Yamagishi, H. *J. Cell. Biol.* **1962**, *15*, 589.
(33) Krey, A. K.; Hahn, F. E. *Science (Washington, D.C.)* **1969**, *166*, 755.
(34) Hahn, F. E.; Krey, A. K. *Progr. Mol. Subcell. Biol.* **1971**, *2*, 134.
(35) Maiti, M.; Chaudhuri, K. *Indian J. Biochem. Biophys.* **1981**, *18*, 245.
(36) Cava, M. P.; Reed, T. A.; Beal, J. L. *Lloydia* **1965**, *28*, 73.
(37) Tani, C.; Imanishi, I.; Nishijo, J. *J. Pharm. Soc. Jpn.* **1970**, *90*, 407.
(38) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
(39) Milhous, W. K.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. *Antimicrob. Agents Chemother.* **1985**, *27*, 525.

Leone (D-6), were utilized in susceptibility testing. Test compounds were dissolved in DMSO and serially diluted with culture media. The uptake of tritiated hypoxanthine was used as an index of inhibition of parasite growth. The compounds described herein were tested against a drug-sensitive strain of *P. berghei* (strain KBG 173) in mice according to methods previously described.[40]

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(40) Osdene, T. S.; Russell, P. B.; Rane, L. *J. Med. Chem.* **1967**, *10*, 431.

Registry No. 1, 633-65-8; 2, 10605-02-4; 3, 6681-15-8; 4, 549-21-3; 5, 483-15-8; 6, 113975-46-5; 7, 3906-36-3; 8, 522-97-4; 9, 61774-67-2; 10, 113975-47-6.

Graphics Computer-Aided Receptor Mapping as a Predictive Tool for Drug Design: Development of Potent, Selective, and Stereospecific Ligands for the 5-HT_{1A} Receptor

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A conformational study of four 5-HT_{1A} (serotonin) receptor ligands ((*R*-(−)-methiothepin, spiperone, (*S*)-(−)-propranolol, and buspirone) led to the definition of a pharmacophore and a three-dimensional map of the 5-HT_{1A} antagonist recognition site. These models were used to design new compounds and successfully predict their potency, stereospecificity, and selectivity. For example, 8-[4-[(1,4-benzodioxan-2-ylmethyl)amino]butyl]-8-azaspiro[4.5]decane-7,9-dione (1, MDL 72832) has nanomolar affinity (pIC_{50} = 9.14) for the 5-HT_{1A} binding site in rat frontal cortex. As predicted, the *S*-(−) enantiomer of 1 was more active than its *R*-(+) enantiomer (pIC_{50} = 9.21 and 7.66, respectively) and a naphthalene analogue of 1 displayed the expected improved selectivity.

Graphics computer technology that has been developed during the last decade is an important new tool for drug design. It has proven particularly useful in the determination of crystallographic structures and for the theoretical mechanistic studies of the interaction between a substrate and a receptor of known structure.[1] On the other hand, when receptor structure is totally unknown, the graphics computer has generally been used a posteriori to account for structure–activity relationships.

We report here the rational application of the computer-aided receptor mapping technique to the a priori design of a series of novel molecules with high affinity, stereospecificity, and selectivity for a particular receptor. Our objective was the central 5-HT_{1A} receptor subtype.[2] The program was stimulated by the observation that the novel centrally active 5-HT (5-hydroxytryptamine, serotonin) receptor agonist 8-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (8-OH-DPAT)[3] shows remarkable potency and selectivity for a subtype of the central 5-HT_1 recognition site, designated 5-HT_{1A}.[4] Subsequent work on the behavioral effects of 8-OH-DPAT and 5-MeO-DMT (5-methoxy-*N*,*N*-dimethyltryptamine), as well as the reported clinical properties of buspirone, a compound having a high affinity for the 5-HT_{1A} receptor, strongly suggested that antagonists at this site would have desirable therapeutic potential as novel anxiolytic agents.[5-7] These observations led us to try to design new 5-HT_{1A} receptor antagonists with optimized potency and selectivity.

Table I. Affinity of Compounds Used in Creating Pharmacophore for Central 5-HT recognition Sites in Rat Frontal Cortex

compound	pIC_{50} 5-HT_{1A}	5-HT_{1B}	5-HT_2
8-OH-DPAT	8.52	5.42	5.00
(−)-methiothepin	7.02	6.74	8.20
(+)-methiothepin	6.07	5.49	8.25
spiperone	6.91	6.00	8.67
propranolol	6.77	6.31	5.10
buspirone	7.66	4.90	5.47

Receptor Mapping and Drug Design

The general approach that has been followed was defined by Marshall[8] and can be outlined as follows: (i) critical examination of compounds active or inactive at the target receptor, (ii) graphics computer-aided definition of a pharmacophore, (iii) three-dimensional graphics computer-assisted mapping of the recognition site, and (iv) use of the previously defined pharmacophore and receptor map to design original putative optimized ligands.

In spite of its limitations, this approach proved to be very efficient in the case of the 5-HT_{1A} recognition site, as will be discussed below.

(i) Activity Evaluation. 8-OH-DPAT has been shown to display both high affinity and selectivity for the 5-HT_{1A} recognition site in vitro,[4] and the tritiated analogue has been used to label this site selectively in the brain.[9]

(1) Cohen, C. *Adv. Drug Res.* **1985**, *14*, 41.
(2) Pedigo, N.; Yamamura, H.; Nelson, D. *J. Neurochem.* **1981**, *36*, 222.
(3) Arvidsson, L.-E.; Hacksell, U.; Nilsson, J.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wikstrom, H. *J. Med. Chem.* **1981**, *24*, 921.
(4) Middlemiss, D.; Fozard, J. *Eur. J. Pharmacol.* **1983**, *90*, 151.
(5) *Brain 5-HT_{1A} Receptors*; Dourish, C., Ahlenius, S., Hutson, P., Eds.; Ellis Horwood: Chichester, U.K., 1987.
(6) Glaser, T.; Traber, J. *Eur. J. Pharmacol.* **1983**, *88*, 137.
(7) Peroutka, S. *Biol. Psychiatry* **1985**, *20*, 971.
(8) Humblet, C.; Marshall, G. *Annu. Rep. Med. Chem.* **1980**, *15*, 267.

0022-2623/88/1831-1087$01.50/0