Laboratory Evaluation of the Phototoxic Potency of Quinolinemethanols

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A series of quinolinemethanols which are structurally related to quinine form a large pool of potential antimalarial agents. One of them, 6,8-dichloro-2-phenyl-α-2-piperidyl-4-quinolinemethanol (I), has been reported to cause photosensitization in man.[1] This undesirable reaction is the major reason why the antimalarial potential of this class of compounds has not been realized. The phototoxic response of mice injected with I and other known photosensitizers has been found to parallel human experience.[2] Aside from a single *in vitro* study,[3] quinine has not been implicated in phototoxic reactions of animals. Our pilot studies revealed that quinine is not a photosensitizer for albino mice exposed to uv radiation and suggested that structural modification of I might produce other quinolinemethanols free of phototoxicity. The testing in albino mice of a series of quinolinemethanols for phototoxicity forms the basis of this preliminary report.

Experimental Section

I.C.R. female mice, 20–25 g, 5–7 weeks old, were used in this study. Groups of four control and four treated animals were exposed simultaneously in plastic boxes with woven wire lids to a flat field of uv. The chemical agents were suspended in a vehicle of methylcellulose and Tween 80 and administered by the intraperitoneal route 30 min prior to exposure. Control animals received a like volume of the suspending medium and were otherwise handled and housed jointly with their respective treated partners. The radiation source consisted of four General Electric F40 BLB lamps arranged in a plane 30 cm above the table top. A window-glass filter interposed between the source and the animals reduced the intensity of short-wavelength radiation and effectively confined the spectrum to wavelengths greater than 3200 Å. The measured average intensity of radiation at the top of the animals was 4.9×10^3 ergs/cm²/sec. Experimental groups were exposed for 70 hr. Phototoxic potency was scored at the end of irradiation and daily thereafter for a 10-day period.[4]

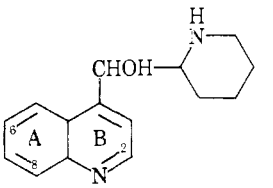
A minimum perceptible erythema (MPE) was noted in control mice after a period of 72 hr. This MPE dose influenced our choice of radiation exposure time, which was standardized at 70 hr. In the routine test, control mice showed minimal changes, and their treated partners expressed photosensitization by gross inflammation in the skin of the ears and tail. These cutaneous lesions vary from erythema to necrosis depending upon the relative degree of phototoxicity. A similar syndrome has been described for albino mice by Hausmann[5] and for guinea pigs by Sams.[6] By testing each compound at several dosages, we have been able to compare their activity in terms of the minimum phototoxic dose and thus circumvent decisions between various degrees of swelling or depth of erythema.

Results and Discussion

Table I shows the effect on phototoxic potency of substitutions on the quinoline nucleus of a series of α-2-piperidylquinolinemethanols. With the exception of II and XII, all the analogs proved to be phototoxic. However, none was more potent than I, the parent compound. It appears that position 2 of the B ring must be substituted to ensure phototoxicity and that substitutions on the A ring lead to increased potency.

TABLE I

THE PHOTOTOXICITY OF QUINOLINEMETHANOLS. α-2-PIPERIDYL DERIVATIVES



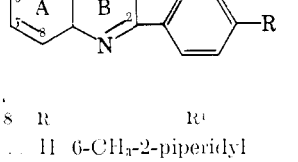
No.	Position 6	Position 8	Position 2	MED[a] (mice), mg/kg ip
I[b]	Cl	Cl	C_6H_5	5
II[b]	...	Cl	...	Negative
III[c]	...	CH_3	C_6H_5	33
IV[c]	...	...	C_6H_5	50
V[c]	Cl	Cl	*p*-ClC_6H_4	6.6
VI[b]	CH_3	CH_3	*p*-FC_6H_4	12.5
VII[b]	...	CF_3	*p*-$CH_3C_6H_4$	25
VIII[b]	...	...	*p*-$CH_3OC_6H_4$	10
IX[c]	...	...	4-Piperidyl	50
X[c]	...	...	Morpholino	100
XI[b]	...	...	Cyclohexyl	40
XII[c]	...	...	*i*-Pr	Negative

[a] F. Y. Wiselogle, Ed., "Survey of Antimalarial Drugs, 1941–1945," Edwards Bros., Ann Arbor, Mich., 1946. [b] From R. E. Lutz, University of Virginia, Charlottesville, Va. [c] Minimum effective phototoxic dose.

The compounds listed in Table II are analogs of I in which the α-2-piperidyl group has been replaced by various heterocyclic moieties. There is obvious side-chain influence in the phototoxic potency of these quinolinemethanols. The results suggest that an aza arene (XIV *vs.* VII) in the side chain markedly reduces photosensitivity.

TABLE II

THE PHOTOTOXICITY OF QUINOLINEMETHANOLS. HETEROCYCLIC MOIETIES

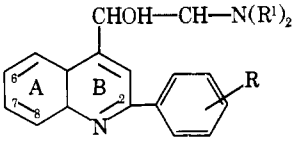


No.	Position 6	Position 8	R	R¹	MED[a] (mice), mg/kg ip
XIII[c]	Cl	...	H	6-CH_3-2-piperidyl	33
XIV[b]	...	CF_3	H	2-Pyridyl	300
XV[c]	Cl	Cl	Cl	1-Adamantylaminomethyl	200
XVI[d]	...	...	H	2-Pyrrolidyl	75
XVII[c]	Cl	Cl	H	Piperidinomethyl	200
XVIII[c]	Cl	Cl	Cl	Morpholinomethyl	50
XIX[c]	Cl	Cl	Cl	4-CH_3-1-piperazinylmethyl	5
XX[c]	F	F	F	4-CH_3-1-piperazinylmethyl	25

[a] From J. Burkhalter, University of Michigan, Ann Arbor, Mich. [b] From R. E. Lutz, University of Virginia, Charlottesville, Va. [c] From E. Atkinson, Arthur D. Little, Inc., Cambridge, Mass. [d] F. Y. Wiselogle, Ed., "Survey of Antimalarial Drugs, 1941–1945," Edwards Bros., Ann Arbor, Mich., 1946. [e] Minimum effective phototoxic dose.

(1) T. N. Pullman, L. Eichelberger, A. H. Alving, R. Jones, Jr., R. Craige, Jr., and C. M. Whorton, *J. Clin. Invest.*, **27**, 15 (1948).
(2) A. E. Ison and H. Blank, *Invest. Dermatol.*, **48**, 288 (1967).
(3) G. Calenoff, *Nature*, **166**, 443 (1950).
(4) The principles of laboratory animal care as promulgated by the National Society for Medical Research, were observed.
(5) W. Hausmann, *Biochem. Z.*, **30**, 276 (1910).
(6) W. Sams, *Arch. Dermatol.* **94**, 773 (1966).

TABLE III
THE PHOTOTOXICITY OF QUINOLINEMETHANOLS. DI-N-ALKYLS



No.	Position 6	7	8	R	R¹	MED[g] (mice), mg/kg ip
XXI[a]	OCH_3			3,4-Cl_2	Et	25
XXII[a]	OCH_3	Cl		4-OCH_3	Et	25
XXIII[a]	Cl			3,4-$(OCH_3)_2$	Et	100
XXIV[a]	OCH_3			3,4-Cl_2	n-C_5H_{11}	400
XXV[b]		Cl	CH_3	4-Cl	Isopentyl	25
XXVI[c]	OCH_3			H	n-Bu	150
XXVII[a]	OCH_3	Cl		4-OCH_3	n-Bu	50
XXVIII[h]	CH_3	Cl		4-Cl	n-Bu	50
XXIX[c]	CH_3		CH_3	4-Cl	n-Bu	33
XXX[c]				4-Cl	n-Bu	66
XXXI[c]	Cl		Cl	4-Cl	n-Bu	25
XXXII[c]		Cl		4-Cl	n-Bu	33
XXXIII[d]		CF_3		4-Cl	n-Bu	25
XXXIV[e]		F		4-F	n-Bu	50
XXXV[f]	Cl		Cl	4-Cl(3-CH_3)[h]	n-Bu	12.5
XXXVI[f]	Cl		Cl	2,4-Cl_2	n-Bu	12.5
XXXVII[f]	Cl		Cl	2,4-Cl_2(3-CH_3)[h]	n-Bu	100
XXXVIII[c]	Cl		Cl	3,4-Cl_2	n-Bu	50
XXXIX[d]	Cl		Cl	3-CF_3	n-Bu	100
XL[c]	Cl		Cl	H	n-C_6H_{13}	260
XLI[c]			Cl	4-Cl	n-C_6H_{13}	100
XLII[c]			Cl	4-Cl	n-C_6H_{13}	100
XLIII[a]	OCH_3			3,4-Cl_2	n-C_6H_{13}	100
XLIV[a]	OCH_3	Cl		4-OCH_3	n-C_6H_{13}	400
XLV[d]	Cl		Cl	3-CF_3	n-C_6H_{13}	300

[a] From R. Rowlett, Virginia Institute for Scientific Research, Richmond, Va. [b] From T. Biel, Aldrich Chemical Co., Milwaukee, Wis. [c] F. Y. Wiselogle, Ed., "Survey of Antimalarial Drugs, 1941–1945," Edwards Bros., Ann Arbor, Mich., 1946. [d] From A. Saggiomo, Research Institute of Temple University, Philadelphia, Pa. [e] From E. Atkinson, Arthur D. Little, Inc., Cambridge, Mass. [f] From Walter Reed Army Institute of Research, Washington, D. C. [g] Minimum effective phototoxic dose. [h] CH_3 on 3 position of B ring.

The structures described in Table III are analogs of I bearing various di-N-alkyls in the side chain. All proved to be more or less phototoxic. Considered as groups, the potency appears to vary inversely with the length of the alkyl group on the nitrogen in the side chain.

Albino mice are convenient and sensitive indicators of photosensitivity. Their small size, sparsely haired ears and tail, and uniform response to uv radiation makes it possible to screen many compounds in a short time and with minimum expense. In our studies, no attempt was made to score the degree of cutaneous response in the irradiated animals. Rather, multiple drug doses were employed to determine the minimum effective dose for potency comparison. Even such a simple comparison is hazardous since the excretion time and tissue level of individual compounds may vary widely. Perhaps a more meaningful comparison could be related to an index derived from the ratio of maximum tolerated dose to minimum effective dose. Of course, the problem of drug concentration during irradiation is present in all tests whether the agent is administered orally, parenterally, or topically.

Certain tentative assumptions have evolved from a study of our preliminary results.

(1) Substitution of the 2 position of the quinoline nucleus with a functional group is necessary to ensure the ability of quinoline methanols to sensitize mice to radiation. The phototoxic potency of 2-substituted compounds varies with different functional groups in a manner that may suggest an association with their relative electronegativities.

(2) Definite side-chain influence is present in this series of quinolinemethanols. Azaarenes and long alkyl substitutions on nitrogen in the side chain tend to reduce the phototoxic potency.

(3) A-Ring substitution usually results in an increased phototoxic response with Cl > CH_3 > OCH_3. Additional testing will be required to substantiate this observation.

It is probable that the combination of a functional group in the 2 position and a methanol side chain is important in photosensitization. The 2-phenyl analogs of such aminoquinolines as chloroquine and primaquine do not elicit a phototoxic response in mice (unpublished data). Likewise, quinine which is a well-known quinolinemethanol lacking 2 substitution is not a photosensitizer. The parent compound of our series (I) might be viewed as a tricyclic aromatic ring system with coplanar features. It has been hypothesized that all such ring systems have potential photosensitizing ability.[7] Various analogs of I were synthesized

(7) W. L. Fowlks, *J. Invest. Dermatol. Suppl.*, **32**, 223 (1959).

for use in studying the effect of a blocked 2-phenyl group on phototoxic potency. Steric hindrance of the phenyl group was attempted by substituting a methyl group in the 3 position of the quinoline nucleus, and by chlorine substitution in the 2 position of the phenyl ring. From the results in Table III (XXXV, XXXVI, XXXVII), it can be seen that steric hindrance of the phenyl group decreased the relative phototoxic potency. Further efforts in this direction are planned.

The Influence of the Carboxyl Group upon the Antibacterial Activity of Rifamycins[1]

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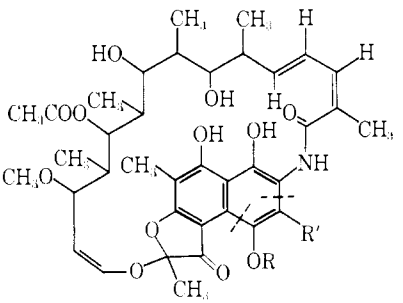
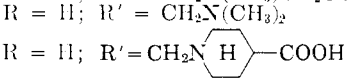
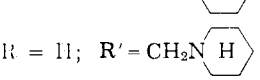
It has been demonstrated[2] that rifamycin B (I), one of the products of the metabolism of *Streptomyces mediterranei*,[3] is inactive *per se*. In aqueous media rifamycin B undergoes an oxidative and hydrolytic transformation to rifamycin S[4] which possesses high antibacterial activity. Rifamycin S is easily reduced to the hydroquinone form, rifamycin SV (II), which is now employed in the therapy of staphylococcal and other gram-positive infections, biliary tract infections, tuberculosis, and leprosy.[5] The structural difference between rifamycin B and rifamycin SV consists in the absence in the latter of the glycolic moiety bearing a free carboxyl group.[6]



I, R = CH_2COOH; R′ = H (rifamycin B)
II, R = H; R′ = H (rifamycin SV)
IIIa, R = H; R′ = $CH_2N(CH_3)CH_2COOH$
IIIb, R = H; R′ = $CH_2N(CH_3)_2$



IVa, R = H; R′ = CH_2N(H)—COOH

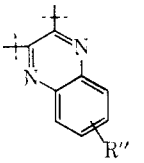


IVb, R = H; R′ = CH_2N(H)

Va, R = H; R′ = $CH{=}NNHC_6H_4COOH$-*p*
Vb, R = H; R′ = $CH{=}NNHC_6H_5$
VIa, R = H; R′ = $CH{=}NN(CH_3)CH_2COOH$
VIb, R = H; R′ = $CH{=}NN(CH_3)_2$
VIIa, R = H; R′ = $CH{=}NN{=}CHC_6H_4COOH$-*p*
VIIb, R = H; R′ = $CH{=}NN{=}CHC_6H_5$

In a previous paper[7] we described the synthesis and properties of a class of derivatives of rifamycin B in which the carboxyl group was blocked (amides and hydrazides). These derivatives possessed *in vitro* antibacterial activity in most instances comparable to that of rifamycin SV.

Moreover, very recently[8] we pointed out the lowering of the antibacterial activity when the aromatic hydrogen in rifamycin SV is replaced by glutathionyl, carboxymethylthio, or β-carboxyethylthio residues. These findings lead us to infer that the presence of the carboxyl function in the rifamycin molecule could be responsible for the weakening of its antibacterial activity, and therefore we undertook the study of a number of semisynthetic rifamycins with a free carboxyl group, among some classes in which antimicrobial activity was demonstrated to be generally maintained. The products selected for this comparison include some Mannich derivatives of rifamycin SV (IIIa and IVa), hydrazones of 3-formylrifamycin SV (Va, VIa, and VIIa), and a carboxyrifazine (VIIIa). These derivatives were tested



VIIIa, R″ = COOH
VIIIb, R″ = H (rifazine)

for the *in vitro* antibacterial activity and compared with the analogous rifamycins without carboxyl group, already described (IIIb and IVb,[9] Vb, VIb, and VIIb,[10] VIIIb[11]).

Biological Results.—Table I summarizes the minimum inhibitory concentration of the carboxyrifamycins against some typical strains and compares it with that of the corresponding rifamycins without a carboxyl group. The resulting data clearly confirm a decrease of activity for the rifamycins investigated, when compared to the corresponding derivatives without the carboxyl group. The ratio of activity between derivatives with and without the carboxyl group presents large variations according to the type of derivatives taken into consideration and to the test microorganism. Particularly, *Staphylococcus aureus* is more sensitive to the structural variation here examined. In fact, the introduction of a carboxyl group induces a decrease of activity from 1:1000 (VIIIa:VIIIb) to 1:10 (IVa:IVb) against this strain.

Streptococcus hemolyticus is less sensitive to these structural modifications; in some instances (IVa *vs.* IVb and VIIa *vs.* VIIb) there is no significant modification of activity. All the carboxyrifamycins are practically inactive against gram-negative bacteria and present only limited activity on *Mycobacterium tuberculosis;* on the contrary, the parent compounds show moderate activity on the former and are highly active on the latter.

(1) Rifamycins. XLIX.
(2) S. Füresz and M. T. Timbal, *Chemotherapia*, **7**, 200 (1963).
(3) P. Sensi, A. M. Greco, and R. Ballotta, *Antibiot. Ann.*, 262 (1959–1960).
(4) P. Sensi, R. Ballotta, A. M. Greco, and G. G. Gallo, *Farmaco, Ed. Sci.*, **16**, 165 (1961).
(5) N. Bergamini and G. Fowst, *Arzneimittel-Forsch.*, **15**, 951 (1965).
(6) W. Oppolzer, V. Prelog, and P. Sensi, *Experientia*, **20**, 336 (1964).
(7) P. Sensi, N. Maggi, R. Ballotta, S. Füresz, R. Pallanza, and V. Arioli, *J. Med. Chem.*, **7**, 596 (1964).
(8) N. Maggi and R. Pallanza, *Farmaco, Ed. Sci.*, **22**, 307 (1967).
(9) N. Maggi, V. Arioli, and P. Sensi, *J. Med. Chem.*, **8**, 790 (1965).
(10) N. Maggi, R. Pallanza, and P. Sensi, *Antimicrobial Agents Chemotherapy*, 770 (1965).
(11) G. G. Gallo, C. R. Pasqualucci, N. Maggi, R. Ballotta, and P. Sensi, *Farmaco, Ed. Sci.*, **21**, 68 (1966).