0026-895X/93/020191-09$03.00/0

MOLECULAR PHARMACOLOGY, 43:191-199



# Quinolone Antibacterial Agents: Relationship between Structure and *In Vitro* Inhibition of the Human Cytochrome P450 Isoform CYP1A2

UWE FUHR, GÜNTER STROBL, FRANCESC MANAUT, EVA-MARIA ANDERS, FRITZ SÖRGEL, ELENA LOPEZ-DE-BRINAS, DANIEL T. W. CHU, ANDRE G. PERNET, GERHARD MAHR, FERRAN SANZ, and A. HORST STAIB

*Department of Clinical Pharmacology, University Hospital Frankfurt, Germany (U.F., E.-M.A., A.H.S.), GSF-Research Center for Environment and Health, 8042 Neuherberg, Germany (G.S.), IMIM (UAB), Passeig Maritim 25, 08003 Barcelona, Spain (F.M., E.L.-D.-B., F.Sa.), IBMP Institute for Biomedical and Pharmaceutical Research, Schleifweg 3, 8501 Nürnberg-Heroldsberg, Germany (F.Sö., G.M.), and Abbott Laboratories, Abbott Park, Chicago, Illinois (D.T.W.C., A.G.P.)*

Received March 24, 1992; Accepted November 6, 1992

**SUMMARY**

The inhibitory effect of 44 quinolone antibacterials and derivatives (common structure, 4-oxoquinoline-3-carboxylic acid) on cytochrome P450 isoform CYP1A2 activity was tested using human liver microsomes and caffeine 3-demethylation as a specific test system for this enzyme. By direct comparison of molecules differing structurally in only one position, the following structure-activity relationships were found. 3′-Oxo derivatives had a reduced or similar activity and M1 metabolites (cleavage of piperazinyl substituent) had a greater inhibitory activity, compared with the parent molecule. Alkylation of the 7-piperazinyl substituent resulted in a reduced inhibitory potency. Naphthyridines with an unsubstituted piperazinyl group at position 7 displayed a greater inhibitory potency than did corresponding quinoline derivatives. Derivatives with a fluorine substitution at position 8 had only a minor effect. Molecular modeling studies with inhibitors and caffeine showed that it is possible to explain the potency of the quinolones to inhibit CYP1A2 on a molecular level. The keto group, the carboxylate group, and the core nitrogen at position 1 are likely to be the most important groups for binding to the active site of CYP1A2, because the molecular electrostatic potential of all inhibitors is very similar to that of caffeine in these regions. The presence of a piperazinyl substituent, however, seems to be no prerequisite for inhibitory potency. Finally, an equation to estimate the potency to inhibit CYP1A2 was developed by quantitative structure-activity relationsip analysis.

Quinolone antibacterial agents, currently being introduced into the therapy of a wide range of infections in humans, share a common basic molecular structure that is attributed to 4-oxoquinoline-3-carboxylic acid (Fig. 1A). This quinoline core may be modified by replacement of the carbon atoms at position 8 (naphthyridine) or positions 6 and 8 (pyridopyrimidine) of this ring system by nitrogen and by other atom or substituent substitutions, especially at positions 1, 6, 7, and/or 8. For simplicity, all these substances here are refered to as "quinolones."

In addition to their antimicrobial effect, mediated by inhibition of bacterial gyrase (1–3), the quinolones exert a second important biological effect as the result of inhibition of an enzyme. This effect decreases the metabolism of xenobiotics in humans and occasionally causes adverse drug reactions (4). Inhibition of the biotransformation of caffeine and theophylline by quinolones has been described *in vivo* (5–7) and *in vitro* (8–10). In investigations with human liver microsomes, we provided evidence that this effect is caused by a competitive inhibition of CYP1A2 activity (8). This has been shown for ofloxacin, lomefloxacin, enoxacin, ciprofloxacin, and pipemidic acid (8) by measuring their effects on caffeine 3-demethylation (Fig. 1B). CYP1A2 is known to be responsible for the high affinity component described for this reaction in human liver (11–13). The extent of inhibition varied between substances, but structural requirements for a marked inhibitory effect on CYP1A2 activity are unknown to date. However, this is of considerable relevance, because a prediction of inhibitory potency may aid in the selection of safer drugs for further development from a series of otherwise similar compounds.

The aim of this investigation was to gain insight into the nature of the quinolone inhibition of CYP1A2 and to assess the relationship between the structure of gyrase inhibitors and their potency to inhibit CYP1A2 activity. To this end we carried out *in vitro* inhibition studies and applied molecular modeling methods and QSAR analysis.

**ABBREVIATIONS:** CYP1A2, cytochrome P450 isoform CYP1A2; QSAR, quantitative structure-activity relationship; 1,7-DMX, 1,7-dimethylxanthine; $k'$, capacity factor for quinolones in reverse phase high performance liquid chromatography; $k_w'$, $k'$ extrapolated to 0% methanol in the liquid phase; $IC_{50}$, inhibitor concentration required to reduce metabolite formation rate by 50%; HPLC, high performance liquid chromatography.

## Materials and Methods

**Quinolones.** The 44 quinolone congeners tested in this study were available as either drugs or corresponding metabolites or were synthesized during development of new quinolones. The substances (for structures, see Table 1) were supplied by the following companies: ofloxacin and desmethylofloxacin, Hoechst AG (Frankfurt, Germany); lomefloxacin, Searle (Dreieichenhain, Germany); pipemidic acid, Madaus (Köln, Germany); piromidic acid, Dainippon Pharmaceutical (Osaka, Japan); ciprofloxacin, M1-ciprofloxacin, oxociprofloxacin, enrofloxacin, and M1-enrofloxacin, Bayer AG (Wuppertal, Germany); enoxacin, M1-enoxacin, and oxoenoxacin, Gödecke (Freiburg, Germany); difloxacin, temafloxacin, and a series of new compounds with code numbers starting with either "A" or "X" (which were all synthesized by D. T. W. Chu, Abbott) (14–16), Abbott (Chicago, IL); pefloxacin and oxopefloxacin, Rhône-Poulenc (Antony, France); norfloxacin and oxonorfloxacin, Merck Sharp & Dohme (Rahway, NJ); nalidixic acid and amifloxacin, Winthrop (Norderstedt, Germany); fleroxacin, desmethylfleroxacin, oxofleroxacin, and AM735, Kyorin (Tokyo, Japan); and cinoxacin, Lilly (Gießen, Germany).

**Other chemicals.** Methylxanthines were obtained from the following companies: caffeine, Serva (Heidelberg, Germany); 1,7-DMX, Aldrich (Milwaukee, WI); and hydroxypropyltheophylline, Fluka (Buchs, Switzerland). NADP (98%), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase (from yeast, purity grade II, 140 units/ml) were purchased from Boehringer (Mannheim, Germany). Acetonitrile, methanol, and tetrahydrofuran were chromatography-grade products from Merck (Darmstadt, Germany). All other chemicals (analytical grade) were purchased from Merck.

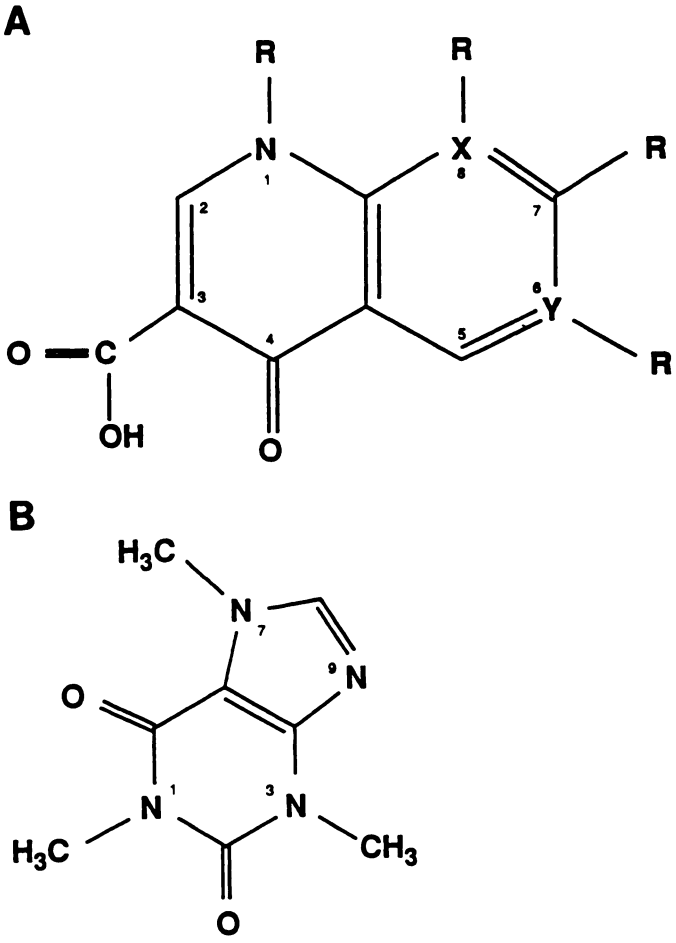


**Fig. 1.** Chemical structures of CYP1A2 substrate and inhibitors used (structures are turned in order to match the orientation in Figs. 2, 4, and 5). A, General structure of quinolone antibacterial agent derivatives. X = H, Y = H, quinoline derivatives; X = N, Y = H, naphthyridine derivatives; X = N, Y = N, pyridopyrimidine derivatives. B, Structure of caffeine (1,3,7-trimethylxanthine).

**Activity of CYP1A2.** The preparation of human liver microsomes, the incubation procedure, and the HPLC assay of 1,7-DMX have been described previously (8, 9). Briefly, incubations were carried out in 0.1 mM potassium phosphate buffer, pH 7.4, containing about 2 mg/ml microsomal protein, an NADPH-generating system, caffeine, and the quinolone to be tested . The incubation was stopped after 15 min by addition of 20% (w/v) trichloroacetic acid. The concentration of both caffeine and the quinolones used was 500 $\mu$M, because this was in the range of concentrations for which transformed caffeine formation rates were linear with respect to the substrate concentrations in the Hanes plots and with respect to the concentrations of quinolone inhibitors in the Dixon plots (8). Additionally, the difference in effect of the quinolones was greatest at this concentration (which for caffeine corresponds to the $K_m$ value for the high affinity component of the test reaction) (8). Also, the amount of caffeine metabolites could be measured easily even at reduced formation rates in the presence of a potent inhibitor. For each substance, incubations with and without inhibitor using human liver microsomes from four donors were separately carried out in duplicate. $IC_{50}$ and $K_i$ values were not measured because of the small amounts of substances available (<1 mg for some compounds) and the low inhibitory potency observed in many cases, which would have necessitated very high concentrations of the poorly soluble inhibitors. Some of the results from the studies mentioned above (8, 9) have been included in the analysis presented here.

**Evaluation of inhibitory effect.** The 3-demethylation of caffeine was used to determine CYP1A2 activity. Means of 1,7-DMX formation rates were calculated for duplicate incubations. The inhibitory effect of a compound in the microsomes from each donor was determined as the activity in the presence of this compound relative to the activity in its absence (eq. 1).

$$\%\ \text{remaining activity} = \frac{\text{1,7-DMX formation rate with inhibitor}}{\text{1,7-DMX formation rate without inhibitor}} \times 100 \quad (1)$$

Incubations with inhibitor and the corresponding controls without inhibitor were carried out within the same experiment. This effect is presented as mean ± standard deviation of four microsomal preparations.

**Lipophilicity assay.** Transformed relative retention times (log $k_w'$) in reverse phase HPLC were determined in order to estimate the lipophilicity of the majority of the compounds ($n = 29$) (17, 18). Briefly, compounds were injected onto a 5-$\mu$m C18 Nucleosil column (Macherey & Nagel, Düren, Germany) and eluted with buffer, pH 7.4, containing varying percentages of methanol. UV absorbance was measured at 270 nm to detect the substances. Capacity factors were calculated from retention times. The graph of log $k'$ versus percentage of methanol, obtained by quadratic regression, was extrapolated to 0% methanol. A linear correlation coefficient between log $k_w'$ values and inhibitory effect on CYP1A2 was calculated to evaluate the influence of lipophilicity on inhibitory potency.

**Assessment of structure-activity relationships.** Primarily, we conducted a descriptive analysis of results by comparing the inhibitory effects of substances differing in structure at only one position. This was completed by correlating inhibitory activity with lipophilicity.

Computer-assisted molecular modeling studies were carried out using the workstation IRIS 4D/5OGT (Silicon Graphics, Munich, Germany). The structures of inhibitors were built up interactively using the SYBYL program (19). Energy minimizations were done by means of the MAXIMIN2 option of SYBYL (method, conjugate gradient; termination option, gradient; root mean square gradient, 0.002 kcal/mol·Å$^2$). All force field calculations were carried out considering electrostatic interactions. For consistency, all energy values were calculated with version 5.3 of SYBYL. Conformational analysis was carried out using the SEARCH option of SYBYL. The procedure applied depended

on the number of rotatable bonds within the molecule. The torsional angles of terminal alkyl groups were only investigated when intramolecular steric interactions were expected. In the case of one or two rotatable bonds, conformational space was searched using 10° increments. In the case of three or more rotatable bonds, 30° increments were applied to limit the number of conformations. In the latter case additional searches were started in the ±30° environment of low energy conformers, using 10° increments. To make sure that all relevant conformers were taken into account, an energy limit as high as 50 kcal/mol was chosen in SYBYL SEARCH. Low energy conformers were then energy minimized (for conditions, see above), and for superpositions only those conformers were chosen whose energy was not more than 5 kcal/mol higher than that of the lowest energy conformer. Almost all of the conformers used for superposition (up to five per molecule) had an energy not more than 3 kcal/mol above the energy of the lowest energy conformer. However, the energies of all conformers used for the development of the pharmacophor model (one per molecule, i.e., the optimal one of the up to five conformers tested) were within 2 kcal/mol. Molecular electrostatic potentials were calculated for optimized structures using the POTENTIALS option of SYBYL.

For QSAR analysis, the three-dimensional structures of the compounds were built with the ALCHEMY II program (20), and their geometries were optimized using the semiempirical AM1 program (21) of AMPAC (22). From these structures, a multiple stepwise regression analysis (23) was carried out using properties and parameters of a subset of molecules ($n$ = 38) that included all substances with a quinoline or a naphthyridine core and with a (substituted) piperazinyl or a methyl group at position 7. The parameters that were chosen on the basis of the descriptive analysis of the inhibitory effect and of electrostatic and steric properties of quinolones were as follows: kind of core (naphthyridine/quinoline with fluorine at position 8/quinoline with hydrogen at position 8); volume, maximum and minimum charges, and hydrophobicity of substituents at positions 1 and 7; and molecular electrostatic potential minima generated by the substituent at position 7, which was computed and analyzed using the program MEPMIN (24).

## Results

Structures of the compounds tested and their effects are listed in Table 1, sorted by structural properties. Almost all the quinolone antibacterial agent derivatives tested had an inhibitory effect on caffeine 3-demethylation and thus on CYP1A2 activity. The most active compound was the M1 metabolite of ciprofloxacin, which is formed by cleavage and deethylation of the piperazinyl substituent. This metabolite reduced the rate of 1,7-DMX formation under chosen *in vitro* conditions to 12.2 ± 2.5% of that without inhibitor. Among the clinically used quinolones, fleroxacin had no effect, whereas enoxacin had the most pronounced effect (25.1 ± 1.7% remaining activity).

Molecular modeling methods were applied to obtain more detailed insight into possible reasons for the different inhibitory potencies of the compounds tested. We compared the structure of the specific CYP1A2 substrate caffeine with the structures of the inhibitors tested. This procedure is justified because the type of inhibition determined for some quinolone antibacterial agents was competitive (8). Competitive inhibitors and substrates bind to the same site and are mutually exclusive.

Comparison of the molecular electrostatic potential of caffeine with those of competitive inhibitors (e.g., ciprofloxacin) showed a strong resemblance. The negative potential caused by the carbonyl oxygen atoms at positions C2 and C6 of caffeine overlapped with the negative potential caused by the carbonyl oxygen atom at position C4 and that of one oxygen atom of the carboxyl group at position C3 of quinolone derivatives, respectively (Fig. 2). The positive potential caused by the hydrogen atoms of the methyl groups at positions N1 and N7 of caffeine overlapped with the positive potential caused by the hydrogen atom of the carboxyl group and the hydrogen atoms of the substituent at position N1 and that bound to position C2 of quinolone derivatives, respectively.

Although there were major structural differences in the group of substances tested, the following effects of structural elements can be summarized. They were defined by direct comparison of activities of molecules differing in structure at only one position and in some cases could be explained using molecular modeling.

**Core.** Naphthyridines with a piperazinyl substituent at position 7 ($n$ = 3) showed a stronger inhibitory effect than corresponding quinoline derivatives. However, the two naphthyridines with further substitution of the piperazinyl group showed reduced or similar inhibitory potency, compared with quinolines (Fig. 3A). The superposition model has the N8 nitrogen atom of naphthyridines exerting a negative potential at the position where the N9 nitrogen atom of caffeine also exerts a negative potential, whereas quinolines have a positive molecular electrostatic potential in this region (Fig. 4). Therefore, the model suggests that the reason for the more pronounced effect of some naphthyridine derivatives, compared with that of quinolone derivatives, is caused by the closer electronic similarity of the former to caffeine.

**Substituents at position 8.** In general derivatives with a fluoro substituent at position 8 were only weak inhibitors. Direct comparison was only possible between fleroxacin and AM735. Fleroxacin was inactive, whereas AM735 had a moderate inhibitory potency.

**Substituents at position 1.** As for the substituent at position N1 of quinolone antibacterial agents, electronic as well as steric properties seem to govern the affinity for the active site. In general, small hydrophobic substituents like ethyl and cyclopropyl groups seem to be favored. However, there are compounds that have substituents like the 1′-pyrrolidyl or the 1′-piperidyl group that also are strong inhibitors. Conformational analyses and comparison of the molecular electrostatic potentials of these molecules suggest that the negative potential caused by the nitrogen atoms of the substituents at position N1 lie in the same region as the negative potential of the N9 nitrogen atom of caffeine mentioned above.

**Substituents at position 7.** (a) M1 metabolites (cleavage and deethylation of the piperazinyl substituent) ($n$ = 3) had more marked inhibitory activity than did the parent molecules (Fig. 3B). (b) Methylation/ethylation of the 7-piperazinyl substituent at position 3′ or 4′ ($n$ = 9) was related to a reduced inhibitory potency (Fig. 3C). (c) Compounds with a 3′-oxopiperazinyl ring ($n$ = 5) had reduced or similar activity, compared with the corresponding parent molecule (Fig. 3D). However, regarding the substituent at position C7 of quinolones, superpositions suggest that a piperazinyl ring is not a prerequisite for inhibitory potency. This finding is in accordance with inhibition studies, because quinolones with fluoro and methyl substituents at position C7 also were strong inhibitors *in vitro*.

Taken together, the pharmacophor for quinolone inhibition of CYP1A2, i.e., the common arrangement of functional groups shown by all quinolone inhibitors, consists of two areas with positive potentials and two domains with negative potentials (Fig. 5). Substituents at positions N1 and C7 may enhance the potency of inhibition (or reduce activity for steric reasons) but

TABLE 1

**Structures of 4-oxoquinoline-3-carboxylic acid (quinolone) derivatives and related compounds and inhibitory effects on human CYP1A2 activity**

Inhibition values are mean ± standard deviation of four microsome donors.

| Substance name or code | Type of core | Substitution of the core at position[a] | | | | Inhibition of human CYP1A2 (remaining activity)[b] |
|---|---|---|---|---|---|---|
| | | 8 | 1 | 6 | 7 | |
| | | | | | | % of control |
| Cinoxacin | Cinnoline | H | Ethyl | -O-C-O- (ring) | | 84.2 ± 7.4 |
| Nalidixic acid | Naphthyridine | | Ethyl | H | Methyl | 33.4 ± 5.6 |
| Enoxacin | Naphthyridine | | Ethyl | F | Piperazinyl | 25.1 ± 1.7 |
| M1-enoxacin | Naphthyridine | | Ethyl | F | $NH_2$-$(CH_2)_2$-NH- | 21.3 ± 4.4 |
| Oxoenoxacin | Naphthyridine | | Ethyl | F | 3′-Oxopiperazinyl | 76.8 ± 5.9 |
| A 60919 | Naphthyridine | | Cyclopropyl | F | Piperazinyl | 18.6 ± 3.2 |
| A 57132 | Naphthyridine | | 4′-Fluorophenyl | F | Piperazinyl | 31.3 ± 3.8 |
| A 57274 | Naphthyridine | | 2′,4′-Difluorophenyl | F | Piperazinyl | 43.3 ± 4.0 |
| X 2 | Naphthyridine | | 2′,4′-Difluorophenyl | F | 3′,3′-Dimethylpiperazinyl | 88.1 ± 5.2 |
| Pipemidic acid | Pyridopyrimidine | | Ethyl | | Piperazinyl | 40.7 ± 2.8 |
| Piromidic acid | Pyridopyrimidine | | Ethyl | | 1′-Pyrrolidyl | 59.0 ± 2.5 |
| Desmethylofloxacin | Pyridobenzoxacine | -O-$CH_2$-$CH(CH_3)$- (ring) | | F | Piperazinyl | 73.3 ± 9.0 |
| Ofloxacin | Pyridobenzoxacine | -O-$CH_2$-$CH(CH_3)$- (ring) | | F | 4′-Methylpiperazinyl | 89.2 ± 9.9 |
| Desmethylfleroxacin | Quinoline | F | 2′-Fluoroethyl | F | Piperazinyl | 80.5 ± 3.9 |
| Fleroxacin | Quinoline | F | 2′-Fluoroethyl | F | 4′-Methylpiperazinyl | 104.4 ± 14.2 |
| Oxofleroxacin | Quinoline | F | 2′-Fluoroethyl | F | 3′-Oxo-4-methylpiperazinyl | 93.9 ± 5.5 |
| Lomefloxacin | Quinoline | F | Ethyl | F | 3′-Methylpiperazinyl | 76.6 ± 7.1 |
| Norfloxacin | Quinoline | H | Ethyl | F | Piperazinyl | 44.3 ± 8.9 |
| Oxonorfloxacin | Quinoline | H | Ethyl | F | 3′-Oxopiperazinyl | 52.1 ± 5.7 |
| Pefloxacin | Quinoline | H | Ethyl | F | 4′-Methylpiperazinyl | 78.0 ± 4.8 |
| Oxopefloxacin | Quinoline | H | Ethyl | F | 3′-Oxo-4′-methylpiperazinyl | 84.5 ± 5.4 |
| AM 735 | Quinoline | H | 2′-Fluoroethyl | F | 4′-Methylpiperazinyl | 75.7 ± 10.7 |
| Ciprofloxacin | Quinoline | H | Cyclopropyl | F | Piperazinyl | 30.6 ± 6.7 |
| M1-ciprofloxacin | Quinoline | H | Cyclopropyl | F | $NH_2$-$(CH_2)_2$-NH- | 12.2 ± 2.5 |
| Oxociprofloxacin | Quinoline | H | Cyclopropyl | F | 3′-Oxopiperazinyl | 43.4 ± 5.0 |
| Enrofloxacin | Quinoline | H | Cyclopropyl | F | 4′-Ethylpiperazinyl | 46.1 ± 5.8 |
| M1-enrofloxacin | Quinoline | H | Cyclopropyl | F | $CH_3$-$CH_2$-NH-$(CH_2)_2$-NH- | 18.6 ± 2.2 |
| X 3 | Quinoline | H | Cyclopropyl | F | F | 29.9 ± 9.6 |
| A 56559 | Quinoline | H | Methylamino | F | Piperazinyl | 47.5 ± 5.3 |
| Amifloxacin | Quinoline | H | Methylamino | F | 4′-Methylpiperazinyl | 78.6 ± 3.8 |
| A 56301 | Quinoline | H | Dimethylamino | F | 4′-Methylpiperazinyl | 84.6 ± 4.5 |
| A 56618 | Quinoline | H | 1′-Pyrrolidyl | F | 4′-Methylpiperazinyl | 44.8 ± 2.1 |
| A 56551 | Quinoline | H | 1′-Piperidyl | F | 4′-Methylpiperazinyl | 76.3 ± 1.8 |
| A 60450 | Quinoline | H | 1′-Pyrryl | F | 4′-Methylpiperazinyl | 61.2 ± 3.2 |
| A 56772 | Quinoline | H | 4′-Methylphenyl | F | Piperazinyl | 31.0 ± 3.2 |
| A 60916 | Quinoline | H | 4′-Aminophenyl | F | Piperazinyl | 52.2 ± 2.9 |
| A 57592 | Quinoline | H | 2′,6′-Dimethylphenyl | F | 4′-Methylpiperazinyl | 78.7 ± 3.6 |
| Difloxacin | Quinoline | H | 4′-Fluorophenyl | F | 4′-Methylpiperazinyl | 78.7 ± 7.3 |
| A 57531 | Quinoline | H | 2′,4′-Difluorophenyl | F | Piperazinyl | 68.4 ± 4.7 |
| Temafloxacin | Quinoline | H | 2′,4′-Difluorophenyl | F | 3′-Methylpiperazinyl | 90.0 ± 4.5 |
| X 1 | Quinoline | H | 2′,4′-Difluorophenyl | F | 3′,3′-Dimethylpiperazinyl | 81.3 ± 3.7 |
| A 56836 | Quinoline | H | 3′-Pyridyl | F | Piperazinyl | 65.7 ± 3.0 |
| A 56608 | Quinoline | H | 4′-Pyridyl | F | Piperazinyl | 84.8 ± 5.6 |
| A 56607 | Quinoline | H | 4′-Pyridyl | F | 4′-Methylpiperazinyl | 86.8 ± 7.2 |

[a] Position relative to that of the quinoline core.
[b] Human CYP1A2 activity was determined as the rate of caffeine 3-demethylation (see Materials and Methods).

are not expected to be necessary for affinity of the compound for the active site of CYP1A2.

**Effect of lipophilicity on inhibitory potency.** Lipophilicity and inhibitory effect of the quinolones (Fig. 6) did not show any significant correlation for the 29 compounds tested ($r = 0.346$), for naphthyridines ($r = 0.401$, $n = 5$), or for the quinolines ($r = 0.204$, $n = 22$).

**QSARs.** Four of the parameters that were tested with respect to a possible influence on the inhibitory effect (see Materials and Methods) showed a statistically significant influence. Inhibitory potency increased when (a) the depth of the molecular electrostatic potential minimum generated by the substituent in position 7 increased ($p < 0.001$), (b) there was no substituent at position 8 (i.e., either in the case of a naphthyridine core or in quinoline congeners with a hydrogen bond to position C8) ($p < 0.01$), (c) the maximum charge of the substituent at position 7 increased ($p < 0.05$), or (d) the volume of the substituent at position 1 decreased ($p < 0.05$).

The equation obtained was

$$(196 \pm 35) + (1.56 \pm 0.41) \cdot MINI7 + (27.9 \pm 9.9) \cdot CORE2 - (189 \pm 71) \cdot CMAX7 + (0.41 \pm 0.15) \cdot VOL1 \quad (2)$$

where *MINI7* is the electrostatic minimum generated by the substituent at position 7 (kcal/mol), *CORE2* describes position 8 of the core (value was 0 for naphthyridines and for quinolines with a hydrogen at this position and was 1 in the presence of a fluoro substituent), *CMAX7* reflects the maximum charge of the substituent at position 7 (kcal/mol), and *VOL1* indicates the volume of the substituent at position 1 (Å$^3$). The correlation

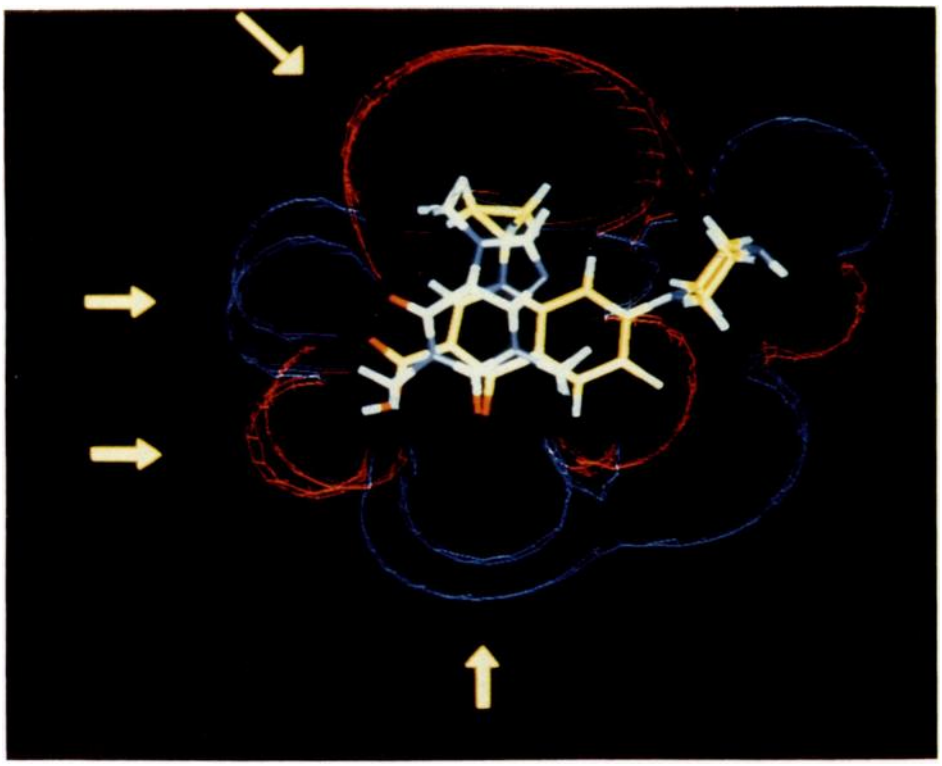

**Fig. 2.** Superposition of caffeine and ciprofloxacin. *Arrows*, regions that are highly similar in the two molecules. *Blue grid*, negative potentials; *red grid*, positive potentials. Carbon atoms of caffeine are *white* and those of ciprofloxacin are *yellow*.

coefficient obtained using eq. 2 was $r^2 = 0.56$ ($p < 0.00005$). A standard error of 16.8% (percentage of remaining activity) was found for the residuals. These QSAR results support those obtained using molecular modeling.

## Discussion

### Suitability of Methods Used to Determine CYP1A2 Activity

By different approaches, it has been shown that the high affinity component of caffeine 3-demethylation is highly specific for human CYP1A2 activity *in vitro* (11–13). Therefore, the use of this metabolic pathway even in the presence of numerous other cytochromes P450, as in the case of human liver microsomes, is expected to almost exclusively (>90%) reflect CYP1A2 activity under chosen incubation conditions with low caffeine concentrations.

The competitive type of inhibition *in vitro* has been shown for only ciprofloxacin and lomefloxacin (both quinolines), enoxacin (a naphthyridine), ofloxacin (a pyridobenzoxacine), and pipemidic acid (a pyridopyrimidine) (8). However, we presume that this mechanism may hold for all substances tested, because the major structural characteristics of the molecule core were unchanged and congeners of all relevant groups showed this competitive type of inhibition. In this case, $K_i$ and $IC_{50}$ values may theoretically be calculated from a single concentration of both substrate and inhibitor tested (once $K_m$ and $V_{max}$ values are known for this reaction and microsome sample). The function that describes the relationship between $K_i$ values and metabolite formation rate (and/or percentage of inhibition) is strictly monotonic. Therefore, the information given by percentage of inhibition data obtained in the present study is qualitatively similar to that provided by the more often used $K_i$ or $IC_{50}$ parameters in this case, although it is less precise. Percentages were not transformed to avoid a multiplication of the experimental error and other pitfalls.

### Quinolone Moieties Involved in Binding to CYP1A2

Our results show that the 4-oxo-3-carboxylic acid moiety of the quinolones, which is essential for antibacterial activity, may also be involved in binding to CYP1A2. Affinity may be modified significantly by substituents at positions 1, 7, and 8, thus influencing the inhibitory potency of these compounds.

Because CYP1A2 binds not only caffeine and quinolone derivatives but also other planar aromatic lipophilic compounds (25), it seems possible that the core of the molecule itself, or a part of it, is also attached directly to the binding site. The size of the substituent at the position 1 nitrogen (which may hinder interaction between this nitrogen and a carboxylate group of the CYP1A2) influenced inhibitory activity of the quinolones. Molecular modeling results show that molecular electrostatic potentials and the three-dimensional structures of caffeine as a substrate and of the quinolones are very similar for certain regions of the molecules (see Figs. 2 and 4). In both structures, core nitrogen atoms are part of these regions, supporting the possible role of nitrogen atoms in substrate and inhibitor binding to CYP1A2.

### Lack of Effect of Inhibitor Lipophilicity

In the heterogeneous system of microsome suspensions, the lipophilicity of a substance is expected to influence its concentration at the cytochrome binding site. A lack of correlation between lipophilicity and inhibitory effect of quinolone derivatives found here indicates that differences in binding affinities, rather than the differences in concentrations, determine the extent of inhibition of caffeine 3-demethylation.

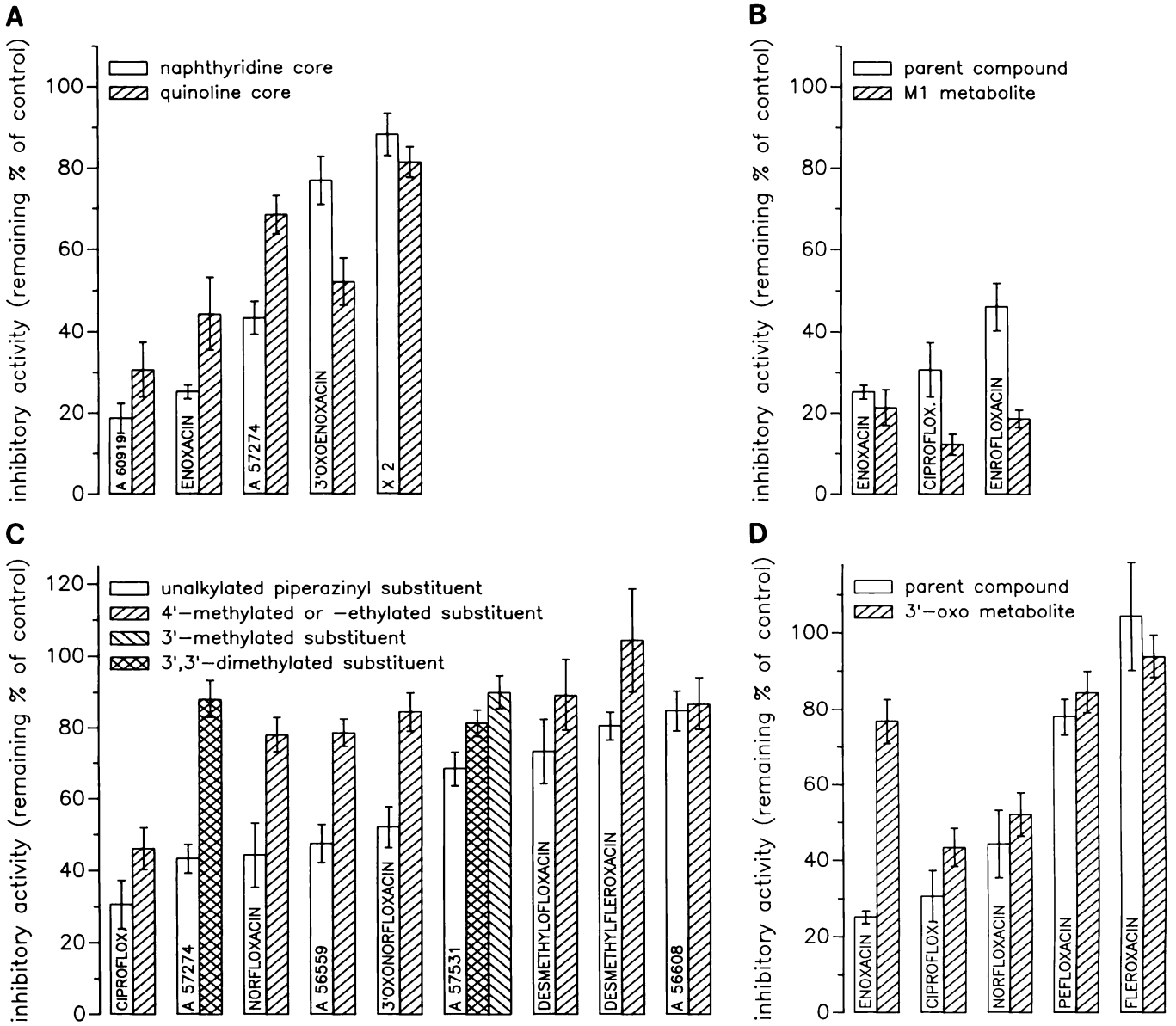


**Fig. 3.** Direct comparison of inhibitory effect on CYP1A2. A, Naphthyridines versus quinolines. B, Parent compounds versus M1 (cleavage and deethylation of the piperazinyl substituent) derivatives. C, Substances with a 3′- or 4′-methylpiperazinyl or 4′-ethylpiperazinyl substituent at position 7 versus those with an unsubstituted piperazinyl substituent at position 7. D, Parent compounds versus 3′-oxo derivatives. CYP1A2 activity is given as remaining activity as a percentage of control (mean ± standard deviation).

### Quinolone Inhibition of CYP1A2 and Antibacterial Activity

Effects of single alterations of quinolone structures were observed to result in consistent changes of inhibitory activity on CYP1A2, thus making it probable that corresponding changes will be found for substances not yet tested. Similar descriptive approaches have been used to assess the relationship between the structures of the quinolones and their antimicrobial activity (26–29).

**Position 1.** In terms of antimicrobial activity, both the steric (groups with dimensions similar to those of an ethyl moiety were considered optimal) and electrostatic properties of substituents were reported to have an important influence, whereas fluorination or chlorination of a substituent present at position 1 was assumed to improve activity by changes in pharmacokinetic properties (28, 29). These findings correspond to our results showing that an increase in the size of the substituent at position 1 correlates with reduced inhibitory activity on CYP1A2.

**Position 7.** Antimicrobial activity of quinolones with a piperazinyl substituent at position 7 did not differ substantially from that of compounds with a 4′-methylpiperazinyl group, but only minor activity was found for smaller substituents, as present in M1 metabolites and in nalidixic acid (26–29). These relationships are very different from those observed for inhibition of CYP1A2 *in vitro*, where methylation or ethylation of the piperazinyl substituent decreased inhibitory potency, whereas cleavage and deethylation of the piperazinyl ring resulted in very active compounds.

**Position 8.** Inhibition of bacterial gyrase was similar for naphthyridines and corresponding quinoline derivatives (28, 29) and slightly less pronounced for congeners with a fluoro substituent at position 8, compared with the unsubstituted

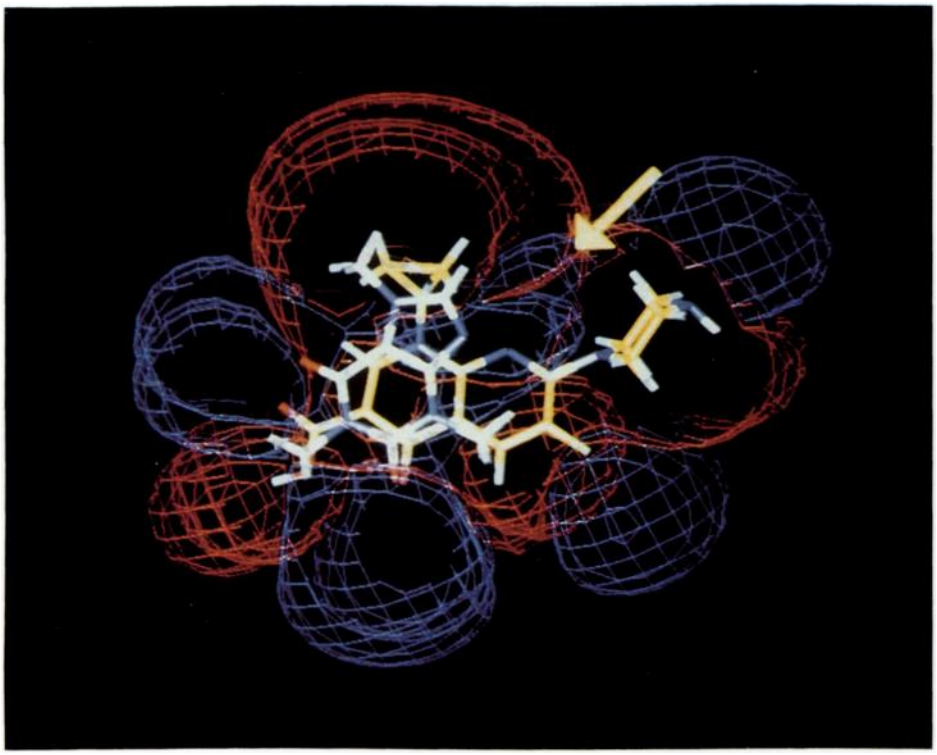

**Fig. 4.** Superposition of caffeine and A 60919 (the naphthyridine analogue of ciprofloxacin). *Arrow*, region where A60919 (N8) as well as caffeine (N9) have a negative molecular electrostatic potential. In contrast, ciprofloxacin has a positive molecular electrostatic potential in this region (see Fig. 2). *Blue grid*, negative potentials; *red grid*, positive potentials. Carbon atoms of caffeine are *white* and those of A60919 are *yellow*.

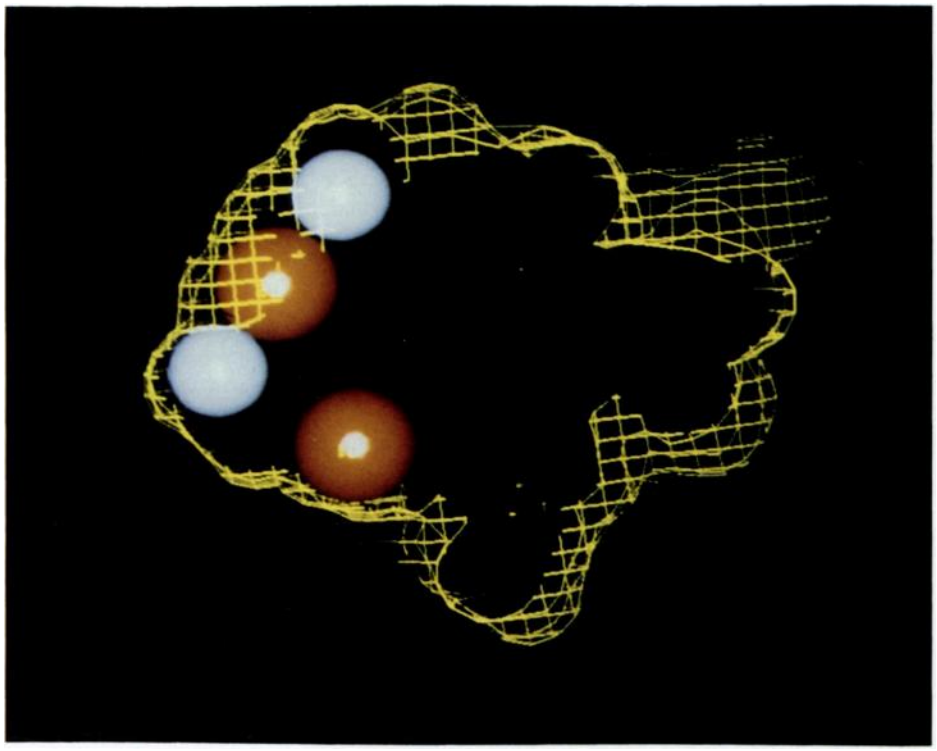

**Fig. 5.** Pharmacophor for inhibitors of CYP1A2. The four groups that may be involved directly in the binding of substrates or competitive inhibitors to the active site of CYP1A2 are represented by the *white* (positive potentials) and *red* (negative) *spheres*. *Yellow grid* (clipped in the direction of the *z*-axis), overall surface of the tested inhibitors. This figure has the same orientation as Figs. 2 and 4.

quinoline (27). An augmented activity of 8-fluorinated compounds *in vivo* was attributed merely to the more favorable pharmacokinetic properties (26, 28, 29). Differences between substances in inhibitory properties on CYP1A2 again do not correspond to antimicrobial activities; reduction of CYP1A2 activity was most pronounced for naphthyridines, less marked for quinolines, and further reduced by fluorination at position 8.

Thus, substituents at position 1 seem to produce similar changes in inhibitory effects on both bacterial gyrase and human CYP1A2, whereas substitutions at positions 7 and 8 do not result in changes that are comparable for the two biological effects.

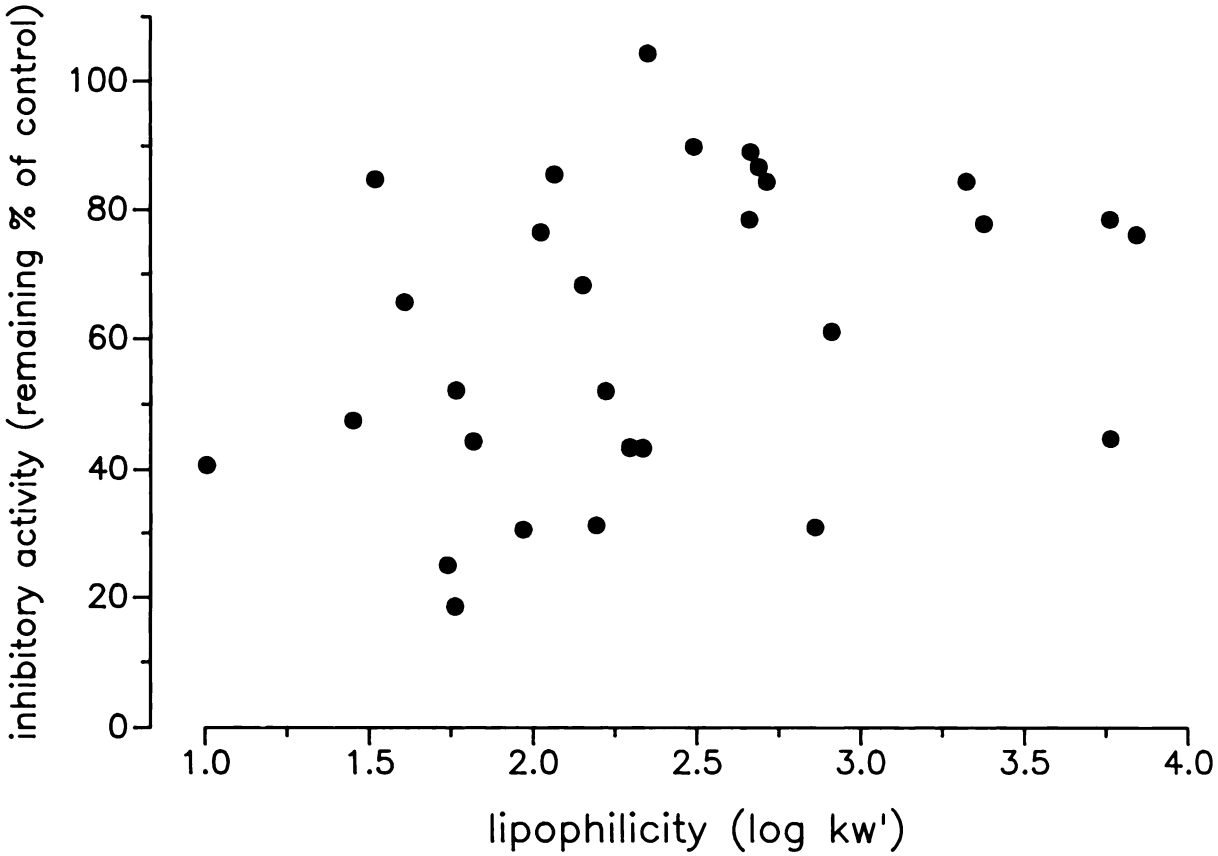


**Fig. 6.** Lack of correlation between lipophilicity and inhibitory effect. Lipophilicity was determined in 29 quinolones using reverse phase HPLC retention times (see Materials and Methods). CYP1A2 activity is given as mean remaining activity, as a percentage of control. The overall correlation coeffecient was $r = 0.346$ ($p > 0.05$, not significant).

Using these differences, it seems possible to find a compound with excellent effects on bacterial growth but minor effects on drug metabolism by human CYP1A2. Substituents at positions 7 and 8 may most likely produce divergence between the effects on bacterial gyrase and human CYP1A2. A molecule expected to be optimal would be a quinoline with a cyclopropyl ring at position 1, a fluorine at position 8, and an alkylpiperazinyl at position 7 of the core. Sparfloxacin, an investigational quinolone that became available after these calculations were finished, shows a structure similar to that described above; it has a quinoline core, a cyclopropyl group at position 1, a fluorine at position 8, a 3′,5′-(*cis*)-dimethylpiperazinyl group at position 7, and additionally an amino group at position 5. This molecule has very good antimicrobial activity *in vitro* (30), did not cause inhibition of theophylline metabolism *in vivo* (31), and reduced activity of CYP1A2 under *in vitro* conditions to not less than 61.2 ± 11.9% of the corresponding control. These additional results provide evidence that data obtained using the methods described above may give important information on a quinolone with considerable practical relevance.

### Prediction of Quinolone Inhibition of CYP1A2?

Nearly all quinolones inhibited the 3-demethylation of caffeine. However, inhibitory potency even of the most effective quinolones was orders of magnitude weaker than that of "classical" CYP1A2 inhibitors like 7,8-benzoflavone (11) or furafylline (12). Quinolones nevertheless have been shown to cause clinically relevant drug interactions *in vivo* (5, 6). As a rough estimate, we previously found that *in vitro* inhibition to 61% of control or less under chosen *in vitro* conditions results in clinically relevant drug interactions *in vivo* in most cases (9), and many of the compounds tested here are at the edge of this limit. An attempt to use the equation obtained in the QSAR study was made in order to identify quinoline and naphthyridine derivatives expected to have a relevant inhibitory effect *in vivo*, although not more than 56% of the variability in inhibitory effect was caused by the variables in this equation. Using inhibition to 61% of control as the cut-off point (9), a sensitivity (potent inhibitors identified by the equation as a percentage of potent inhibitors determined in human liver microsomes) of 82% and a specificity (weak inhibitors identified by the equation as a percentage of weak inhibitors determined in human liver microsomes) of 71% were found. This does not enable a safe distinction between inhibitors with and without clinical effects but, nevertheless, may help to reduce the probability of unexpected adverse drug reactions as a result of quinolone interactions.

#### Acknowledgments

We wish to thank K. Nelson-Kirsten for her critical review of the manuscript.

#### References

1. Andriole, V. T. (ed.). *The Quinolones*. Academic Press, London (1988).
2. Neuman, M. Clinical pharmacokinetics of the newer antibacterial 4-quinolones. *Clin. Pharmacokinet.* **14**:96–121 (1988).
3. Nix, D. E., and J. J. Schentag. The quinolones: an overview and comparative appraisal of their pharmacokinetics and pharmacodynamics. *J. Clin. Pharmacol.* **28**:169–178 (1988).
4. Harder, S., A. H. Staib, C. Beer, A. Papenburg, W. Stille, and P. M. Shah. 4-Quinolones inhibit biotransformation of caffeine. *Eur. J. Clin. Pharmacol.* **35**:651–656 (1988).

5. Harder, S., P. M. Shah, and A. H. Staib. Gyrasehemmstoffe: Zentralnervöse Nebenwirkungen und Hemmung der Methylxanthinelimination. *Dtsch. Med. Wochenschr.* **115**:270–273 (1990).
6. Wijnands, W. J. A., T. B. Vree, and C. L. A. van Herwaarden. The influence of quinolone derivatives on theophylline clearance. *Br. J. Clin. Pharmacol.* **22**:677–683 (1986).
7. Mahr, G., F. Sörgel, G. R. Grannemann, M. Kinzig, P. Muth, K. Patterson, U. Fuhr, P. Nickel, and U. Stephan. Effects of temafloxacin and ciprofloxacin on the pharmacokinetics of caffeine. *Clin. Pharmacokinet.* **22** (Suppl. 1):90–97 (1992).
8. Fuhr, U., T. Wolff, S. Harder, P. Schymanski, and A. H. Staib. Quinolone inhibition of cytochrome P-450-dependent caffeine metabolism in human liver microsomes. *Drug Metab. Dispos.* **18**:1005–1010 (1990).
9. Fuhr, U., E.-M. Anders, G. Mahr, F. Sörgel, and A. H. Staib. Inhibitory potency of quinolone antibacterial agents on cytochrome P450IA2 activity *in vivo* and *in vitro*. *Antimicrob. Agents Chemother.* **36**:942–948 (1992).
10. Sarkar, M., R. E. Polk. P. S. Guzelian, C. Hunt, and H. T. Karnes. *In vitro* effect of fluoroquinolones on theophylline metabolism in human liver microsomes. *Antimicrob. Agents Chemother.* **34**:594–599 (1990).
11. Butler, M. A., M. Iwasaki, F. P. Guengerich, and F. K. Kadlubar. Human cytochrome $P450_{PA}$ (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci. USA* **86**:7696–7700 (1989).
12. Sesardic, D., A. R. Boobis, B. P. Murray, S. Murray, J. Segura, R. de la Torre, and D. S. Davies. Furafylline is a potent and selective inhibitor of cytochrome P450IA2 in man. *Br. J. Clin. Pharmacol.* **29**:651–663 (1990).
13. Fuhr, U., J. Doehmer, N. Battula, C. Wölfel, C. Kudla, Y. Keita, and A. H. Staib. Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. *Biochem. Pharmacol.* **43**:225–235 (1992).
14. Chu, D. T. W., P. B. Fernandes, A. K. Claiborne, E. Pihuleac, C. W. Nordeen, R. E. Maleczka, and A. G. Pernet. Synthesis and structure relationships of novel aryl-fluoroquinolone antibacterial agents. *J. Med. Chem.* **28**:1558–1564 (1985).
15. Chu, D. T. W., P. B. Fernandes, A. K. Claiborne, H. E. Gracey, and A. G. Pernet. Synthesis and biological activity of novel aryl-fluoronaphthyridine antibacterial agents. *J. Med. Chem.* **29**:2363–2369 (1986).
16. Chu, D. T. W., P. B. Fernandes, R. E. Maleczka, C. W. Nordeen, and A. G. Pernet. Synthesis and structure-activity relationship of 1-aryl-6,8-difluoroquinolone antibacterial agents. *J. Med. Chem.* **30**:504–509 (1987).
17. Jaehde, U. Charakterisierung von Transport- und Eliminationsvorgängen neuerer Gyrase-Hemmer im menschlichen Organismus. Ph.D. thesis, Freie Universität, Berlin (1989).
18. Jaehde, U., F. Sörgel, R. Metz, G. Mahr, J. Zürcher, K. G. Naber, and W. Schunack. Quantitative structure-pharmacokinetic relationships of gyrase inhibitors in man. *Eur. J. Pharmacol.* **183**:1857–1858 (1990).
19. N. N. SYBYL Molecular Modeling System. Tripos Associates, Inc., St. Louis, MO (1988).
20. N. N. Alchemy II Program. Tripos Associates, Inc., St. Louis, MO (1988).
21. Dewar, M. J. S., E. G. Zoebish, E. F. Healy, and J. J. P. Stewart. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **107**:3902–3909 (1985).
22. Dewar, M. J. S., and J. J. P. Stewart. AMPAC Program. Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN, 596 (1986).
23. Armitage, P., and G. Berry. *Statistical Methods in Medical Research*. Blackwell Scientific Publications, Oxford, UK, 305–307 (1987).
24. Sanz, F., F. Manaut, J. Jose, J. Segura, M. Carbo, and R. de la Torre. Automatic determination of MEP patterns of molecules and its application to caffeine metabolism inhibitors. *J. Mol. Struct. (Theochem.)* **170**:171–180 (1988).
25. Lewis, D. F., C. Ioannides, and D. V. Parke. Molecular dimensions of substrate binding site of cytochrome P-448. *Biochem. Pharmacol.* **35**:2179–2185 (1986).
26. Chu, D. T. W., and P. W. Fernandez. Structure-activity relationships of the fluoroquinolones. *Antimicrob. Agents Chemother.* **33**:131–135 (1989).
27. Ohta, M., and H. Koga. Three-dimensional structure-activity relationship and receptor mapping of *N*-substituents of quinolone antibacterials. *J. Med. Chem* **34**:131–139 (1991).
28. Radl, S. Structure-activity relationships in DNA gyrase inhibitors. *Pharmacol. Ther.* **48**:1–17 (1990).
29. Rustige, C., D. T. W. Chu, F. Sörgel, and B. Wiedemann. New aspects on structure-activity relationships of quinolones, in *Program and Abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D. C., 153 (1990).
30. Hindler, J. A., F. G. Sandoval, L. S. Young, and C. B. Inderlied. *In vitro* activity of sparfloxacin (AT-4140) and 13 other agents against clinical isolates including ciprofloxacin-resistant strains of staphylococci, enterococci and Gram-negative bacilli, in *Program and Abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington D. C., abstract 1204 (1990).
31. Mahr, G., R. Seelmann, B. Gottschalk, U. Stephan, and F. Sörgel. No effect of sparfloxacin on the metabolism of theophylline in man, in *Program and Abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington D. C., abstract 1259 (1990).

**Send reprint requests to:** Dr. med. Uwe Fuhr, Department of Clinical Pharmacology, University Hospital Frankfurt, Theodor-Stern-Kai 7, 6000 Frankfurt/Main 70, Germany.