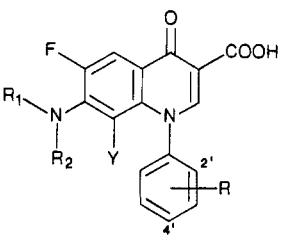
Synthesis and Structure–Activity Relationship of 1-Aryl-6,8-difluoroquinolone Antibacterial Agents

Daniel T. W. Chu,* Prabhavathi B. Fernandes, Robert E. Maleczka, Jr., Carl W. Nordeen, and Andre G. Pernet

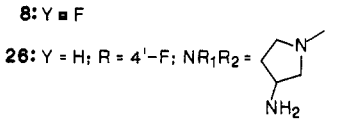
Anti-infective Research Division, Abbott Laboratories, Abbott Park, Illinois 60064. *Received July 30, 1986*

A series of new arylfluoroquinolones has been prepared. These derivatives are characterized by having fluorine atoms at the 6- and 8-positions, substituted amino groups at the 7-position, and substituted phenyl groups at the 1-position. The in vitro antibacterial potency is greatest when the 1-substituent is 2,4-difluorophenyl and the 7-substituent is a 3-amino-1-pyrrolidinyl group. 1-(4-Fluorophenyl)-6,8-difluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**22**) was found to possess excellent in vitro potency and in vivo efficacy.

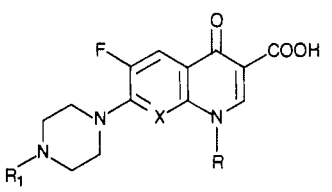
In an earlier paper, we reported the synthesis and antibacterial activities of 7-(substituted amino)-6-fluoro-1-aryl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**1**).[1] These compounds possess a 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety, a common feature found in 4-quinolones. These include norfloxacin (**2**),[2] perfloxacin (**3**),[3] ofloxacin (**4**),[4] ciprofloxacin (**5**),[5] and enoxacin (**6**).[6]

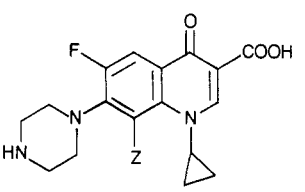


1: Y = H
8: Y = F



26: Y = H; R = 4'-F; NR_1R_2 =





2: R = C_2H_5; R_1 = H; X = CH
3: R = C_2H_5; R_1 = CH_3; X = CH
4: X, R = $COCH_2CH(CH_3)$; R_1 = CH_3
6: R = C_2H_5; R_1 = H; X = N
7: R = C_2H_4F; R_1 = CH_3; X = CF
27: R = *p*-F-C_6H_4; R_1 = H; X = CH
28: R = *p*-F-C_6H_4; R_1 = CH_3; X = CH

5: Z = H
9: Z = F

The optimization of substituents in 4-quinolones has been reported. Quantitative structure–activity relationship (QSAR) analysis of a set of N-1 allyl and alkyl derivatives suggested an optimum STERIMOL length of 4.2 Å, corresponding approximately to an ethyl group.[7] This generalization obviously does not include aryl substituents as shown by the high activity reported for N-1 phenyl analogues.[1] The good biological activities associated with quinolones having N-1 2'-*trans*-phenyl-1'-cyclopropyl substituent[8] provides additional examples that there is a substantial bulk tolerance available at N-1. Hence, the reason for optimization of activity at N-1 ethyl group in quinolones with N-1 aliphatic substitution may lie elsewhere. AM 833 (**7**), a 6,8-difluoro-4-quinolone analogue, was reported to possess good in vivo potency upon oral administration in systemic mouse protection test.[9] An extrapolation of the biological activity from N-1 alkyl to N-1 aryl analogues in 4-quinolones may not be predicted with certainty. We prepared a series of 6,8-difluoro-4-quinolones with the introduction of an aryl substitution at the N-1 position to see whether the increase in in vivo potency caused by the presence of a fluorine atom at the C-8 position in the N-1 alkyl series can also be applied to the N-1 aryl analogues.

In this paper, we report the regiospecific synthesis and antibacterial activity of 1-aryl-6,8-difluoro-7-(substituted amino)-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (**8**). 3-Amino-1-pyrrolidinyl, 1-piperazinyl, and 4-methyl-1-piperazinyl groups are selected to be introduced at C-7 position of **8** in this study on the basis of our experience with the 6-fluoro-1-arylquinolone antibacterial agents.[1]

Chemistry

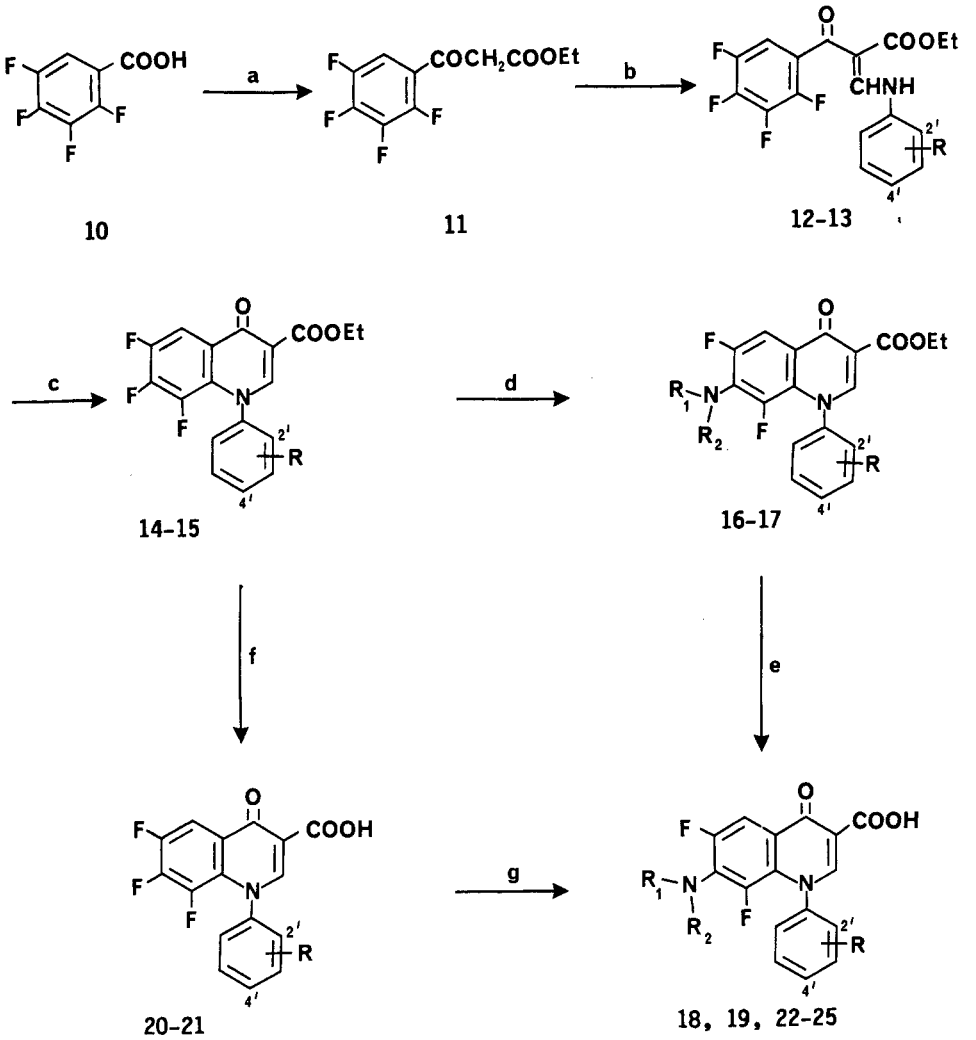
The synthesis of 1-cyclopropyl-6,8-difluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**9**) has recently been reported.[10] It involves the displacement of the 4-fluorine atom of 2,3,4,5-tetrafluoro-1-nitrobenzene by *N*-carbethoxypiperazine to yield the 4-(4-carbethoxypiperazin-1-yl)-2,3,5-trifluoro-1-nitrobenzene. However, this reaction may not be expected to be regiospecific if many different substituted amino groups are required for the introduction at the 4-position. Further, there are potential problems associated with the introduction of substituted anilines to the C-2 position as well as with acid-catalyzed cyclization of the [(*N*,*N*-diarylamino)methylene]malonate. Hence, we investigated other more versatile synthetic routes that can generate many analogues very easily. The 1-aryl-6,8-difluoro-7-(substituted amino)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid derivatives were synthesized as illustrated in Scheme I.

Treatment of 2,3,4,5-tetrafluorobenzoic acid (**10**) with thionyl chloride gave the corresponding acid chloride,

(1) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E.; and Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558.
(2) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
(3) Goueffon, Y.; Montay, G.; Roquet, F.; Pesson, M. *C. R. Seances Acad. Sci.* **1971**, *292*, 37.
(4) Sata, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhaski, S. *Antimicrob. Agents Chemother.* **1982**, *22*, 548.
(5) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.
(6) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H. *J. Med. Chem.* **1984**, *27*, 292.
(7) Fujita, T. In *Drug Design: Fact or Fantasy*; Jolles, G., Woolridge, K. R. H., Eds.; Academic: New York, **1984**; p 19.
(8) Mitscher, L. A.; Sharma, P. N.; Chu, D. T. W.; Shen, L.; Pernet, A. *J. Med. Chem.* **1986**, *29*, 2044.
(9) Kirai, K.; Hosaka, M.; Oomori, Y.; Murayama, S.; Ito, A.; Takagi, K.; Irikura, T.; Mitsuhashi, S. 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct 24–26, 1983, Las Vegas, NV, Abstr. 658.
(10) Ishizaki, T.; Suzue, S.; Irikura, T. *Nippon Kagaku Kaishi* **1985**, 2054.

0022-2623/87/1830-0504$01.50/0

Scheme I

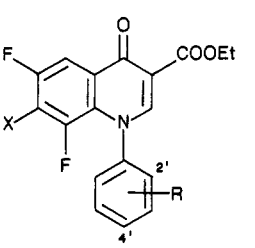


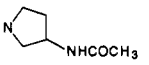
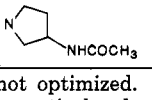
[a] (1) $SOCl_2$. (2) $CH_2(COOEt)/n$-BuLi. (3) H^+. [b] (1) CH-$(OEt)_3/Ac_2O$. (2) $NH_2C_6H_4R$. [c] NaH/THF. [d] HNR_1R_2/pyridine. [e] HCl. [f] CF_3COOH/HCl or NaOH. [g] HNR_1R_2/pyridine.

which, without purification, was reacted with dilithio dianion of monoethyl malonate[11] to give the (2,3,4,5-tetrafluorobenzoyl)acetate **11** (which existed in both keto and enol forms). Treatment of this ester with triethyl orthoformate in acetic anhydride gave the one-carbon homologue enol ether intermediate. This, upon evaporation of the solvent to dryness, was allowed to react with a slight excess of either 4-fluoroaniline or 2,4-difluoroaniline in methylene chloride at room temperature to give the ethyl 3-anilino-2-(2,3,4,5-tetrafluorobenzoyl)acrylates **12** (R = 4′-F) and **13** (R = 2′,4′-F_2). Regiospecific cyclization of compounds **12** and **13** with 1 molar equiv of sodium hydride in tetrahydrofuran (THF) yielded ethyl 1,4-dihydro-4-oxoquinoline-3-carboxylates **14** and **15** (Table I). Displacement of the 7-fluorine atom of the carboxylates **14** and **15** with substituted amines in pyridine yielded the 7-substituted amino derivatives **16** and **17** (Table I). Hydrolysis of the ethyl ester of compounds **16** and **17** with hydrochloric acid gave the desired 1-aryl-7-(substituted amino)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acids **18** and **19** (Table II). Alternately, hydrolysis of the esters of compounds **14** and **15** with sodium hydroxide yielded the 1-aryl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acids **20** and **21** (Table II). Displacement of the 7-fluorine atom of compounds **20** and **21** with an appropriate amine yielded the desired 7-(substituted amino)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acids **22–25** (Table II).

(11) Wierenga, W.; Skulnick, H. I. *J. Org. Chem.* **1979**, *44*, 310.

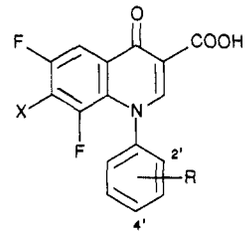
Table I. Ethyl 1-Aryl-6,8-difluoro-7-substituted-1,4-dihydro-4-oxoquinoline-3-carboxylates

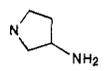
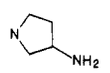
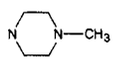
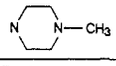


no.	R	X	yield,[a] %	mp, °C	formula[b]
14	4′-F	F	71	190	$C_{18}H_{11}F_4NO_3$
15	2′-F, 4′-F	F	92	178–180	$C_{18}H_{10}F_5NO_3 \cdot {}^1/_5H_2O$
16	4′-F		65	106–109	$C_{24}H_{22}F_3N_3O_4$
17	2′-F, 4′-F		56	114	$C_{24}H_{21}F_4N_3O_4 \cdot {}^1/_3H_2O$[c]

[a] Yields were not optimized. [b] C, H, N, analyses were within ±0.4% of the theoretical values, except otherwise noted. [c] N: calcd, 8.44; found, 7.96.

Table II. 1-Aryl-6,8-difluoro-7-substituted-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids



no.	R	X	yield,[a] %	formula[b,c]
18	4'-F		50	$C_{20}H_{16}F_3N_3O_3 \cdot HCl \cdot 1^1/_2H_2O^d$
19	2'-F, 4'-F		50	$C_{20}H_{15}F_4N_3O_3 \cdot HCl \cdot 1^1/_2H_2O$
20	4'-F	F	50	$C_{16}H_7F_4NO_3 \cdot {}^1/_4H_2O$
21	2'-F, 4'-F	F	72	$C_{16}H_6F_5NO_3 \cdot {}^2/_3H_2O$
22	4'-F		74	$C_{20}H_{16}F_3N_3O_3 \cdot HCl \cdot 2H_2O^e$
23	2'-F, 4'-F		45	$C_{20}H_{15}F_4N_3O_3 \cdot {}^1/_3H_2O$
24	4'-F		69	$C_{21}H_{18}F_3N_3O_3 \cdot H_2O^f$
25	2'-F, 4'-F		12	$C_{21}H_{17}F_4N_3O_3 \cdot {}^2/_3H_2O$

[a,b] See Table I, footnotes *a* and *b*. [c] Melting points of all compounds are >250 °C except **20** (210 °C), **21** (240–243 °C). [d] H: calcd, 4.31; found, 3.80. [e] H: calcd, 4.42; found, 3.69. [f] C: calcd, 57.93; found, 56.66.

Results and Discussion

Table III summarizes the in vitro antibacterial activity of the 1-aryl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acids against five Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* CMX 686B, *Staphylococcus epidermidis* 3519, *Streptococcus faecium* ATCC 8043, and *Streptococcus pyrogenes* 930) and six Gram-negative organisms (*Escherichia coli* Juhl, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* 8045, *Pseudomonas aeruginosa* 5007, *Pseudomonas aeruginosa* K799/WT, and *Acinetobacter* CMX 669). The data for norfloxacin (**2**) and ciprofloxacin (**5**) as well as for **26**, difloxacin (A-56619) (**28**), and A-56620 (**27**) of the 1-aryl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid series[1] are included for comparison.

The effect of mono or difluoro substitutions on the 1-phenyl ring of the 6,8-difluoroquinolones on the in vitro antibacterial potency is shown by comparing the data of compounds **18** vs. **19**, **22** vs. **23**, or **24** vs. **25**. Compounds having substitution with fluorine at the para position or two fluorine atoms at the ortho or para positions generally have similar antibacterial potency. In two out of three cases difluoro substitution on the phenyl group results in a slight increased anti-streptococcal activity.

The structure–activity relationships (SARs) of the C-7 substitution in a series of 6,8-difluoro analogues with the 4'-fluorophenyl group at N-1 (**18**, **22**, and **24**) or a 2',4'-difluorophenyl group at N-1 (**19**, **23**, and **25**) were comparable to those for the corresponding 7-(substituted amino)-6-fluoro-1-aryl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (**1**).[1] With respect to N-1 4'-fluorophenyl or N-1 2',4'-difluorophenyl derivatives, introduction of the C-7 substituent tends to enhance the antibacterial activity. In both series of compounds, the activity against Gram-negative organisms increases in the order 4-methylpiperazinyl ≤ piperazinyl < 3-aminopyrrolidinyl, whereas the Gram-positive activity follows the sequence piperazinyl ≤ 4-methylpiperazinyl < 3-aminopyrrolidinyl. The 6,8-difluoroarylquinolones are generally one tube dilution less active than the corresponding 6-fluoroarylquinolones (**22** vs. **27**, **24** vs. **28**). However, with respect to 3-aminopyrrolidin-1-yl substituent at the 7-position, the 6,8-difluoroarylquinolones are surprisingly much less active than the corresponding 6-fluorarylquinolones (**18** vs. **26**).

Efficacy in systemic infection due to *S. aureus* NCTC 10649, *E. coli* Juhl, and *P. aeruginosa* 5007 in mice of several selected compounds and of ciprofloxacin (**5**), which is one of the leading quinolones under development, is shown in Table IV. The in vivo efficacy on the experimental infection due to *S. aureus* NCTC 10649 of compounds **25** was greater than ciprofloxacin when tested orally. As for compound **22**, it has similar in vivo potency as ciprofloxacin. Compounds **22**–**24** have similar in vivo potency as ciprofloxacin (**5**) on systemic infection caused by *E. coli* Juhl. As for infections caused by *P. aeruginosa*, compounds **22** and **25** were less potent.

A comparison between **22**, a 6,8-difluoroarylquinolone, and its 6-fluoroarylquinolone counterpart **27** in in vivo efficacy is shown in Table V. Even though compounds **22** has higher MIC values than **27** against *E. coli* Juhl and *P. aeruginosa* 5007, its in vivo potency is about the same as **27** upon oral administration of the drugs. Compound **22** is found to be equally potent as **27** in vivo when tested against *S. aureus* subcutaneously, but less potent when tested orally. Hence, these results offer no conclusive evidence that the 6,8-difluoroarylquinolone has better oral absorption than its 6-fluoroarylquinolone counterpart.

Table III. In Vitro Antibacterial Activity of 1-Aryl-6,8-difluoro-7-(substituted amino)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids[a]

	minimum inhibitory concentration (MIC),[b] μg/mL										
no.	S(A)	Sa	Se	Sf	Sp	Ec	Ea	Kp	Pa(5)	Pa(k)	A
2	0.78	0.78	1.56	0.78	3.1	0.1	0.2	0.1	0.39	0.39	6.2
5	0.2	0.39	0.39	0.2	0.39	0.02	0.05	0.02	0.1	0.1	1.56
18	0.2	0.2	0.39	3.1	1.56	0.2	0.2	0.05	3.1	1.56	0.78
19	0.2	0.2	0.39	1.56	0.78	0.1	0.2	0.1	1.56	0.78	0.39
22	0.39	0.39	0.78	6.2	1.56	0.1	0.1	0.05	0.78	0.39	0.39
23	0.39	0.39	0.78	1.56	1.56	0.05	0.2	0.05	0.78	0.78	0.39
24	0.39	0.39	0.78	3.1	3.1	0.39	0.78	0.39	3.1	3.1	0.39
25	0.39	0.39	0.78	6.2	3.1	0.39	0.39	0.2	3.1	1.56	0.39
26	0.05	0.05	0.05	0.78	0.1	0.02	0.05	0.02	0.2	0.2	0.2
27	0.2	0.2	0.39	1.56	0.78	0.05	0.2	0.02	0.39	0.39	0.2
28	0.2	0.2	0.39	1.56	1.56	0.2	0.78	0.1	0.78	1.56	0.78

[a] Structures are shown in Table IV. [b] The MICs were determined by the twofold agar dilution on brain–heart infusion agar. Organisms selected for inclusion in the table as follows: Sa(A), *Staphylococcus aureus* ATCC 6538P; Sa *Staphylococcus aureus* CMX 686B; Se, *Staphylococcus epidermidis* 3519; Sf, *Streptococcus faecium* ATCC 8043; Sp, *Streptococcus pyogenes* 930; Ec, *Escherichia coli* Juhl; Ea, *Enterobacter aerogenes* ATCC 13048; Kp, *Klebsiella pneumoniae* 8045; Pa(5), *Pseudomonas aeruginosa* 5007; Pa(k) *Pseudomonas aeruginosa* K799/WT; A, *Acinetobacter sp.* CMX669.

Table IV. Mouse Protection Test of Selected 6,8-Difluoroarylquinolones in Comparison with Ciprofloxacin (5)

test organism	no.	ED_{50}[a] (95% confidence limits), mg/kg sc	po
S. aureus NCTC 10649	5	1.6 (1.0–2.5)	15.5 (9.9–24.1)
(100 × LD_{50})	22	1.6 (1.0–2.5)	12.5 (7.9–19.7)
	25	3.7 (2.6–5.4)	6.1 (3.7–10.2)
E. coli Juhl (100 × LD_{50})	5	0.2 (0.1–0.2)	1.9 (1.2–3.0)
	22	1.0 (0.6–1.5)	3.2 (2.0–5.0)
	23	0.3 (0.2–0.5)	1.8 (1.2–2.9)
	24	1.3 (1.0–1.8)	2.4 (1.5–3.9)
	25	2.5 (1.6–3.9)	3.9 (2.2–7.0)
P. aeruginosa 5007	5	2.1 (1.1–3.8)	13.3 (6.8–26.2)
(100 × LD_{50})	22	6.3 (4.9–8.1)	25.0 (15.8–19.6)
	25	10.3 (6.6–16.2)	27.9 (17.7–44.1)

[a] See Experimental Section.

This does not support the general observation in the N-1 alkyl quinolones that substitution of a fluorine atom at the C-8 position increases in vivo efficacy.

As a result of this study, it has been shown that 1-aryl-6,8-difluoro-7-(substituted amino)-1,4-dihydro-4-oxoquinoline-3-carboxylic acids are potent antibacterial agents. A few of them possess in vivo potency comparable to that of 6-fluoroarylquinolones and ciprofloxacin. They were synthesized by an efficient and short synthetic route via an intramolecular nucleophilic displacement cyclization reaction.

Experimental Section

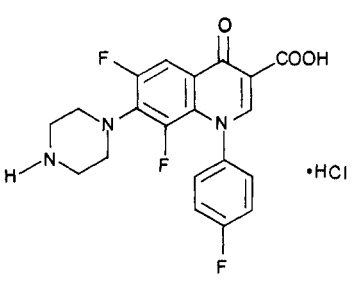
Melting points were taken in a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analyses (unless otherwise specified) were within ±0.4% of the theoretical values. Microanalyses were performed by the Abbott analytical department. The NMR spectra were obtained on a Varian T-60 and HA-100 spectrometers using tetramethylsilane as an internal standard. The NMR peaks were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were recorded on a Kratos MS-50 mass spectrometer. The IR spectra were recorded on a Perkin-Elmer Model 710 A infrared spectrometer. The IR, NMR, and MS data of all compounds were consistent with the assigned structures.

Ethyl (2,3,4,5-Tetrafluorobenzoyl)acetate (11). One drop of dimethylformamide (DMF) was added to a solution of 2,3,4,5-tetrafluorobenzoic acid (1.94 g, 10 mmol) in thionyl chloride (20 mL). After the mixture was heated at 80 °C for 4 h, the solvent was removed by evaporation under reduced pressure, yielding a yellowish mobile oil, 2,3,4,5-tetrafluorobenzoyl chloride. Monoethyl malonate (2.64 g, 20 mmol) and 3 mg of biquinoline were dissolved in 25 mL of dry tetrahydrofuran (THF), and the solution was cooled to −30 °C. A solution of 2.2 M *n*-butyllithium in hexane was added until a pink color remained at −5 °C (16.2 mL). The suspension was then cooled to −50 °C. The acid chloride obtained as described above, dissolved in 20 mL of THF, was added to the suspension dropwise. After 0.5 h, the dry ice bath was removed and the reaction mixture was allowed to warm up to room temperature. The reaction mixture was acidified with 40 mL of 1 N hydrochloric acid and was extracted with ether. The ether fraction was washed with saturated aqueous sodium bicarbonate solution and then water. The ether solution was dried and evaporated to dryness, yielding a yellowish oil. This was purified by use of Kugelrohr distillation to give 2.36 g (89%) of **11**, mp 41–44 °C. NMR ($CDCl_3$): δ (two sets of signals), 1.27 (3 H, t, J = 7.5 Hz, ethyl CH_3), 1.37 (3 H, t, J = 7.5 Hz, ethyl CH_3), 3.82 (1 H, d, J = 13 Hz, CH_2), 4.07 (1 H, d, J = 13 Hz, CH_2), 4.23 (2 H, q, J = 7.5 Hz, ethyl CH_2), 4.27 (2 H, q, J = 7.5 Hz, ethyl CH_2), 5.84 (1 H, s, vinyl H), 7.55 (two sets 1 H, m, aromatic H), 12.57 (1 H, s, enol OH). Anal. ($C_{11}H_8F_4O_3$) C, H.

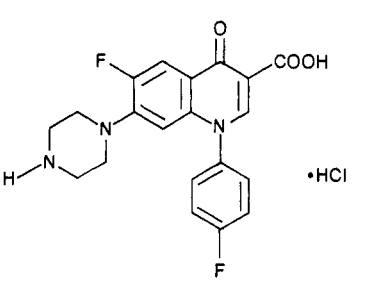
Ethyl 3-(4-Fluoroanilino)-2-(2,3,4,5-tetrafluorobenzoyl)-acrylate (12). A solution of ethyl (2,3,4,5-tetrafluorobenzoyl)-acetate (**11**) (2.36 g, 8.9 mmol) in triethyl orthoformate (2.16 mL, 13 mmol) and acetic anhydride (3.78 mL, 40 mmol) was heated at 130 °C for 1 h with the removal of ethyl acetate formed during the reaction. The solution was evaporated under reduced pressure to a mobile oil, which was then dissolved in methylene chloride (50 mL). 4-Fluoroaniline (1.23 mL, 13 mmol) was added to the solution. After 1 h, the solution was evaporated to dryness and the product was purified through silica gel column, yielding 2.85 g (87%) of **12**, mp 91–94 °C. NMR ($CDCl_3$): δ (two sets of signals), 1.02 (3 H, t, J = 7 Hz, ethyl CH_3), 1.15 (3 H, t, J = 7 Hz, ethyl CH_3), 4.10 (2 H, q, J = 7 Hz, ethyl CH_2), 4.15 (2 H, q, J = 7 Hz, ethyl CH_2), 7.05 (1 H, m, aromatic H), 7.15 (4 H, q, J = 10 Hz, aromatic H), 7.25 (4 H, q, J = 10 Hz, aromatic H), 8.73 (1 H, d, J = 13 Hz, vinyl H), 12.60 (1 H, d, J = 13 Hz, NH). Anal. ($C_{18}H_{12}F_5NO_3$) C, H, N.

Ethyl 3-(2,4-Difluoroanilino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (13). By use of the same procedure described in the preparation of compound **12**, replacing 4-fluoroaniline with 2,4-difluoroaniline, compound **13** was prepared in 73.5% yield, mp 103–105 °C. NMR ($CDCl_3$): δ (two sets of signals), 1.12 (3 H, t, J = 6.5 Hz, ethyl CH_3), 1.15 (3 H, t, J = 6.5 Hz, ethyl CH_3), 4.13 (2 H, q, J = 6.5 Hz, ethyl CH_2), 4.15 (2 H, q, J = 6.5 Hz, ethyl CH_2), 7.00 (2 H, m, aromatic H), 7.15 (1 H, m, aromatic H), 7.40 (1 H, m, aromatic H), 8.50 (1 H, d, J = 13 Hz, vinyl H), 8.62 (1 H, d, J = 13 Hz, vinyl H), 12.50 (1 H, d, J = 13 Hz, NH), 12.75 (1 H, d, J = 13 Hz, NH). Anal. ($C_{18}H_{11}F_6NO_3$) C, H, N.

Table V. Comparison between 6,8-Difluoroarylquinolone and 6-Fluoroarylquinolone on in Vivo Efficacy



22



27

test organism (dose)[a]	no.	MIC, μg/mL	ED_{50}[a] (95% confidence limits), mg/kg sc	po
S. aureus NCTC 10649 (100 × LD_{50})	22		1.6 (1.0–2.5)	12.5 (7.9–19.7)
	27	0.12	1.6 (1.0–2.5)	6.5 (3.8–11.2)
E. coli Juhl (100 × LD_{50})	22	0.1	1.0 (0.6–1.5)	3.2 (2.0–5.0)
	27	0.05	0.6 (0.3–1.3)	4.3 (2.8–6.5)
P. aeruginosa 5007 (100 × LD_{50})	22	0.78	6.3 (4.9–8.1)	25.0 (15.8–39.6)
	27	0.39	1.6 (1.0–2.5)	21.4 (13.4–34.4)

[a] See Experimental Section.

Ethyl 1-(4-Fluorophenyl)-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (14). A 60% sodium hydride-in-oil suspension (14.8 mg, 3.7 mmol) was slowly added to a cold solution of compound **12** (1.18 g, 3.1 mmol) in THF (30 mL). The mixture was heated at 50 °C for 4 h under nitrogen atmosphere and was cooled. Acetic acid (0.5 mL) was added and the mixture was evaporated under reduced pressure to a small volume (2 mL). Water (100 mL) was added and the precipitate was filtered, washed with water, and dried, yielding 800 mg (71%) of **14**, mp 190 °C. NMR ($CDCl_3$): δ 1.38 (3 H, t, J = 6 Hz, ethyl CH_3), 4.38 (2 H, q, J = 6 Hz, ethyl CH_2), 7.25 (2 H, m, aromatic H), 7.40 (2 H, m, aromatic H), 8.17 (1 H, m, C_5-H), 8.35 (1 H, s, C_2-H). Anal. ($C_{18}H_{11}F_4NO_3$) C, H, N.

Ethyl 1-(2,4-Difluorophenyl)-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (15). The previous reaction was repeated, replacing **12** with **13**, to give compound **15** in 92% yield, mp 178–180 °C. NMR ($CDCl_3$): δ 1.40 (3 H, t, J = 7.5 Hz, ethyl CH_3), 4.38 (2 H, q, J = 7.5 Hz, ethyl CH_2), 7.07 (2 H, m, aromatic), 7.53 (1 H, m, aromatic), 8.15 (1 H, m, C_5-H), 8.30 (1 H, s, C_2-H). Anal. ($C_{18}H_{10}F_5NO_3 \cdot {}^1/_5H_2O$) C, H, N.

Ethyl 1-(4-Fluorophenyl)-6,8-difluoro-7-(3-acetamidopyrrolidin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (16). A mixture of **14** (0.5 g, 1.3 mmol) and 3-acetamidopyrrolidine (0.33 g, 2.6 mmol) in pyridine (15 mL) was heated at 40 °C for 6 h. The mixture was cooled and the solvent was condensed to 1 mL by evaporation under reduced pressure. Water (100 mL) was added and the precipitate was filtered and dried. The residue was purified through silica gel column using 5% methanol in methylene chloride as eluent, yielding 400 mg (65%) of **16**, mp 106–109 °C. NMR (Me_2SO-d_6): δ 1.23 (3 H, t, J = 6 Hz, ethyl CH_3), 1.75 (2 H, m, CH_2), 1.78 (3 H, s, $COCH_3$), 2.00 (1 H, m, NCH), 3.25 (1 H, m, NCH_2), 3.57 (1 H, m, NCH_2), 3.65 (2 H, m, NCH_2), 4.17 (2 H, q, J = 6 Hz, ethyl CH_2), 7.40 (2 H, m, aromatic H), 7.65 (1 H, m, C_5-H), 7.75 (2 H, m, aromatic H), 8.07 (1 H, d, J = 4 Hz, NH), 8.17 (1 H, s, C_2-H). Anal. ($C_{24}H_{22}F_3N_3O_4$) C, H, N.

Ethyl 1-(2,4-Difluorophenyl)-6,8-difluoro-7-(3-acetamidopyrrolidin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (17). By use of the same procedure described in the preparation of compound **16**, replacing **14** with **15**, compound **17** was prepared in 55.9% yield, mp 114 °C. NMR (Me_2SO-d_6): δ 1.25 (3 H, t, J = 6 Hz, ethyl CH_3), 1.72 (2 H, m, CH_2), 1.77 (3 H, s, $COCH_3$), 1.97 (1 H, m, NCH), 3.27 (2 H, m, NCH_2), 3.67 (2 H, m, NCH_2), 4.20 (2 H, q, J = 6 Hz, ethyl CH_2), 7.32 (1 H, m, aromatic H), 7.60 (1 H, m, aromatic H), 7.65 (1 H, m, aromatic H), 7.92 (1 H, m, aromatic H), 8.08 (1 H, d, J = 4 Hz, NH), 8.28 (1 H, s, C_2-H). Anal. ($C_{24}H_{21}F_4N_3O_4 \cdot {}^1/_3H_2O$) C, H; N: calcd, 8.44; found, 7.96.

1-(4-Fluorophenyl)-6,8-difluoro-7-(3-aminopyrrolidin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid Hydrochloride (18). A suspension of **16** (0.4 g, 0.85 mmol) in 25 mL of 6 N hydrochloric acid was heated under nitrogen at 110 °C for 6 h. The solvent was removed and the residue was stirred in ethanol at refluxing temperature for 10 min and cooled and filtered, yielding 186 mg (50%) of **18**, mp >250 °C. NMR (Me_2SO-d_6): δ 1.75 (1 H, m, CH_2), 1.97 (1 H, m, CH_2), 2.15 (1 H, m, NCH), 3.55 (2 H, m, NCH_2), 3.80 (1 H, m, NCH_2), 7.45 (2 H, m, aromatic H), 7.78 (2 H, m, aromatic H), 7.82 (1 H, dd, J = 6 Hz, C_5-H), 8.24 (3 H, br s, NH_3^+), 8.44 (1 H, s, C_2-H). Anal. ($C_{20}H_{16}F_3N_3O_3 \cdot HCl \cdot 1^1/_2H_2O$) C, N; H: calcd, 4.31; found, 3.80.

1-(2,4-Difluorophenyl)-6,8-difluoro-7-(3-aminopyrrolidin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid Hydrochloride (19). Hydrolysis of **17** by a similar procedure to the preparation of **18** yielded **19** in 50.4% yield, mp >250 °C. NMR (Me_2SO-d_6): δ 1.97 (2 H, m, CH_2), 2.95 (1 H, m, NCH), 3.60 (2 H, m, NCH_2), 3.80 (2 H, m, NCH_2), 7.35 (1 H, m, aromatic H), 7.65 (1 H, m, aromatic H), 7.87 (1 H, d, J = 8 Hz, C_5-H), 7.90 (1 H, m, aromatic H), 8.65 (1 H, s, C_2-H). Anal. ($C_{20}H_{15}F_4N_3O_3 \cdot HCl \cdot 1^1/_2H_2O$) C, H, N.

1-(4-Fluorophenyl)-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (20). Sodium hydroxide (1 N, 2 mL) was added to a solution of **14** (730 mg, 2 mmol) in THF (10 mL). After the mixture was heated at 70 °C for 3 h, the solvent was distilled off. The residue was dissolved in water (20 mL) and washed with methylene chloride (50 mL). The aqueous phase was acidified with 1 N hydrochloric acid to pH 2. The solid was filtered and washed with water and dried, yielding 340 mg (50%) of **20**, mp 210 °C. NMR ($CDCl_3$): δ 7.27 (2 H, m, aromatic H), 7.44 (2 H, m, aromatic H), 8.22 (1 H, m, C_5-H), 8.67 (1 H, s, C_2-H). Anal. ($C_{16}H_7F_4NO_3 \cdot {}^1/_4H_2O$) C, H, N.

1-(2,4-Difluorophenyl)-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (21). Compound **21** was prepared from **15** by using the same experimental procedure as the preparation of **20**, mp 240–243 °C; 72% yield. NMR ($CDCl_3$): δ 7.12 (2 H, m, aromatic H), 7.52 (1 H, m, aromatic H), 8.22 (1 H, m, aromatic H), 8.62 (1 H, s, C_2-H). Anal. ($C_{16}H_6F_5NO_3 \cdot {}^2/_3H_2O$) C, H, N.

1-(4-Fluorophenyl)-6,8-difluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (22). Piperazine (215 mg, 2.5 mmol) was added to a solution of **20** (337 mg, 1 mmol) in 10 mL of pyridine at 45 °C and the mixture was heated for 5 h. The solvent was removed and the residue was digested in hot ethanol (10 mL) for 5 min. It was cooled, filtered, washed with ethanol (2 × 5 mL) and cold water, and dried, yielding **22**, 299 mg (74%). A portion of it was converted to the hydrochloride salt by dissolving it in hot 1 N hydrochloric acid solution and removing the solvent by distillation under reduced pressure. NMR (Me_2SO-d_6): δ 3.13 (2 H, m, NCH_2), 3.35 (4 H, m, $N(CH_2)_2$), 3.40 (2 H, m, NCH_2), 7.50 (2 H, m, aromatic H), 7.77 (2 H, m, aromatic H), 7.97 (1 H, d, J = 10 Hz, C_5-H), 8.52 (1 H, s, C_2-H), 9.45 (1 H, s, COOH). Anal. ($C_{20}H_{16}F_3N_3O_3 \cdot HCl \cdot 2H_2O$) C, N; H: calcd, 4.42; found, 3.69.

Similarly, by use of an appropriate amine and quinoline-3-carboxylic acid, the following compounds were prepared.

1-(2,4-Difluorophenyl)-6,8-difluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (23): mp >250 °C. NMR (CF_3COOH): δ 3.67 (4 H, m, $N(CH_2)_2$), 3.90 (4 H, m, $N(CH_2)_2$), 7.25 (2 H, m, aromatic H), 7.75 (1 H, m, aromatic H), 8.33 (1 H, d, J = 10 Hz, C_5-H), 9.98 (1 H, s, C_2-H). Anal. ($C_{20}H_{15}F_4N_3O_3 \cdot {}^1/_3H_2O$) C, H, N.

1-(4-Fluorophenyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (24): mp >250 °C. NMR (Me_2SO-d_6): δ 3.14 (3 H, s, CH_3), 3.35 (2 H, m, NCH_2), 3.80 (2 H, m, NCH_2), 3.85 (4 H, m, $N(CH_2)_2$), 7.40 (2 H, m, aromatic H), 7.60 (2 H, m, aromatic H), 8.30 (1 H, d, J = 10 Hz, C_5-H), 9.17 (1 H, s, C_2-H). Anal. ($C_{21}H_{18}F_3N_3O_3 \cdot H_2O$) H, N; C: calcd, 57.93; found, 56.66.

1-(2,4-Difluorophenyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (25): mp >250 °C. NMR (Me_2SO-d_6): δ 2.30 (3 H, s, NCH_3), 2.55 (4 H, m, $N(CH_2)_2$), 3.35 (4 H, m, $N(CH_2)_2$), 7.14 (2 H, m, aromatic H), 7.54 (1 H, m, aromatic H), 7.92 (1 H, m, C_5-H), 8.54 (1 H, s, C_2-H). Anal. ($C_{21}H_{17}F_4N_3O_3 \cdot {}^2/_3H_2O$) C, H, N.

In Vitro Antibacterial Activity. The in vitro antibacterial activity of the test compound was determined in a side-by-side comparison with ciprofloxacin (**5**) by conventional agar dilution procedures. The organisms were grown overnight in brain–heart infusion (BHI) both (Difco 0037-01-6) at 36 °C. Twofold dilutions of the stock solution (2000 μg/mL) of the test compound were made in BHI agar to obtain a test concentration ranging from 200 to 0.005 μg/mL. The plate was inoculated with approximately 10^4 organisms. It was then incubated at 36 °C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compound that yielded no visible growth on the plate.

In Vivo Antibacterial Activity. The in vivo antibacterial activity of the test compounds was determined in CF-1 female mice weighing approximately 20 g. Aqueous solutions of the test compounds were made by dissolving the hydrochloride salt in distilled water or by dissolving the compound in dilute sodium hydroxide and diluting it with distilled water to the desired volume. The median lethal dose of the test organism was determined as follows.

After an 18-h incubation, the cultures of the test organism in BHI broth were serially diluted with 10-fold dilutions in 5% (w/v) hog gastric mucin. One-half milliliter cultures, dilution from 10^{-1} to 10^{-8}, were injected intraperitoneally into mice. The LD_{50} for the test organism was calculated from the cumulative mortalities on the sixth day by using the Reed and Muench procedure.[12]

(12) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493.

The 18-h culture of the above was diluted in 5% (w/v) hog gastric mucin to obtain 100 times the LD_{50} and 0.5 mL was injected intraperitoneally into mice. The mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided equally to be administered at 1 and 5 h after infection. A group of 10 animals each for at least three dose levels were thus treated and the deaths were recorded daily for six days. Ten mice were left untreated as infection control. Fifty percent effective dose values (ED_{50}) were calculated from the cumulative mortalities on the sixth day after infection by using the trimmed version of the Logit method.[13]

Acknowledgment. We thank the staff of the microbiological team for biological testings and the staff of Analytical Department for spectral measurement and elemental analyses.

Registry No. **2**, 70458-96-7; **5**, 85721-33-1; **11**, 94695-50-8; **11** (enol ether), 105859-06-1; **12**, 105859-07-2; **13**, 105859-08-3; **14**, 105859-09-4; **15**, 105859-10-7; **16**, 105859-11-8; **17**, 105859-12-9; **18**, 105859-13-0; **18** (free base), 105859-19-6; **19**, 105859-14-1; **19** (free base), 105859-20-9; **20**, 103994-87-2; **21**, 105859-15-2; **22**, 105859-16-3; **22** (free base), 103995-05-7; **23**, 105859-17-4; **24**, 103995-06-8; **25**, 105859-18-5; **26**, 98106-49-1; **27**, 98105-99-8; **28**, 98106-17-3; 3-acetamidopyrrolidine, 79286-74-1; 2,4-difluoroaniline, 367-25-9; triethyl orthoformate, 122-51-0; 4-fluoroaniline, 371-40-4; monoethyl malonate, 1071-46-1; 2,3,4,5-tetrafluorobenzoyl chloride, 94695-48-4; 2,3,4,5-tetrafluorobenzoic acid, 1201-31-6; piperazine, 110-85-0; 4-methylpiperazine, 109-01-3.

(13) Hamilton, M. A.; Russo, R. C.; Thurston, R. V. *Environ. Sci. Technol.* **1977**, *11*, 714.

Synthesis, Biological Evaluation, and Quantitative Structure–Activity Relationship Analysis of 2-Hydroxy-1*H*-isoindolediones as New Cytostatic Agents[1]

Carcy L. Chan, Eric J. Lien,* and Zoltan A. Tokes[†]

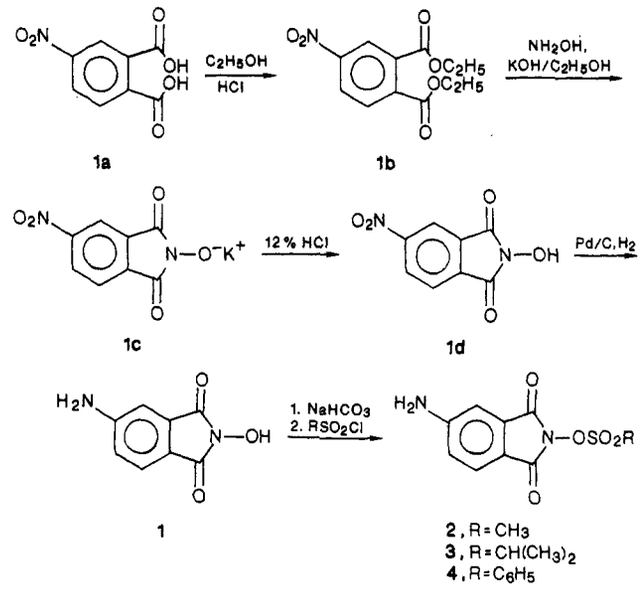
School of Pharmacy and School of Medicine and Department of Biochemistry, Comprehensive Cancer Center, University of Southern California, Los Angeles, California 90033. *Received March 10, 1986*

A series of 16 derivatives of 2-hydroxy-1*H*-isoindole-1,3-diones was designed and synthesized as potential antitumor agents. The cytostatic activity against L1210 cell growth of these compounds was studied, and their IC_{50} values were found to be in the range of 10^{-4} to 10^{-8} M. Quantitative structure–activity relationship analysis of these compounds showed that the inhibitory effect was well correlated with the electronic and the lipophilic parameters. Derivatives having a substituent with strongly electron-donating properties at the 6-position showed enhanced inhibitory activity while compounds having an electron-withdrawing group at the same position showed lower activity.

The necessary functional group in hydroxyurea for the inhibition of ribonucleotide reductase (RR) is known to be the =CNHOH.[2] RR is an important enzyme in DNA synthesis. This enzyme not only catalyzes one of the rate-determining steps in DNA synthesis but its activity is also positively correlated with the proliferation of cells.[3] This correlation is found to be particularly high in fast-growing or malignant cells.[4,5] The activities of three other major enzymes, which are also involved in DNA synthesis, namely, thymidylate synthetase, thymidine kinase, and DNA polymerase, were not increased to such an extent as that of RR.[6,7] Therefore selective inhibition of RR has been used as part of the overall strategy in the design of chemotherapeutic agents. Of all the major known RR inhibitors, only hydroxyurea is currently available for clinical use. Some RR inhibitors, for example, guanazoles and thiosemicarbazones, showed significant in vitro inhibitory activity against cell growth. However, their in vivo toxicities have prevented them from being used in clinical applications.[8,9]

The use of hydroxyurea is limited by its short half-life, which is due to its small molecular size and extremely polar nature. Frequent dosing regimen is required in order to circumvent the inherent problem of hydroxyurea. In the design of new potentially active compounds, the hydrophilic character of the hydroxyurea molecule should be modified such that the optimum balance of lipophilicity and hydrophilicity is achieved while the functional group, =CNHOH is maintained. On the basis of these criteria, a series of 2-hydroxy-1*H*-isoindole-1,3-diones were designed and synthesized. These molecules possess a variety of alkane- and arenesulfonate groups that serve as protective groups for the NOH moiety. The protective group is

Scheme I



[†] Department of Biochemistry.

(1) Portions of this work were presented at APhA Acad. Pharm. Sci. Med. Chem. and Pharmacognosy Section, 132nd APhA Annual Meeting, Feb. 1985, San Antonio, TX.
(2) Hill, R. B.; Gordon, J. A. *Exp. Mol. Pathol.* **1968**, *9*, 71.
(3) Weber, G. *N. Engl. J. Med.* **1977**, *296*, 486.
(4) Takeda, E.; Weber, G. *Life Sci.* **1981**, *28*, 1007.
(5) Witt, L.; Yap, T.; Blakely, R. L. *Adv. Enzyme. Regul.* **1979**, *17*, 157.

0022-2623/87/1830-0509$01.50/0