Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 9, pp. 1021-1026, 1995
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0960-894X/95 $9.50+0.00

0960-894X(95)00160-3

3-BENZYL-QUINOLONES: NOVEL, POTENT INHIBITORS OF MAMMALIAN TOPOISOMERASE II.[1]

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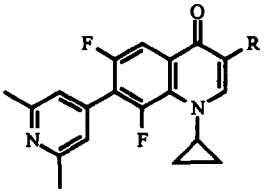
Abstract. Replacement of the 3-carboxy group of quinolone topoisomerase II inhibitors by hydroxy substituted benzyl groups resulted in potent topoisomerase II inhibitors. The 2,6-dihydroxybenzyl analog, Win 64593, had a topo II EC_{50} of 96 nM and had potent in vitro cytotoxicity as well as murine antitumor activity.

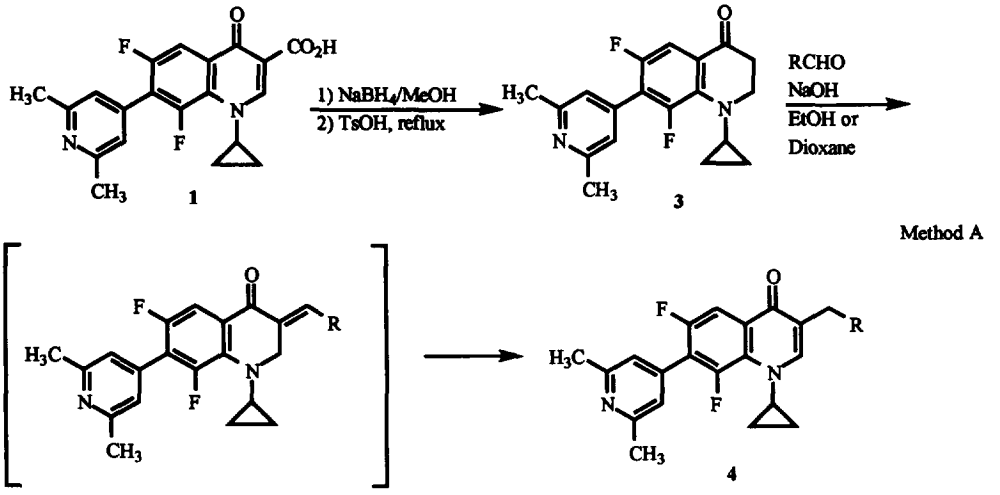
Introduction. We recently described the mammalian topoisomerase (topo) II inhibitory activity of quinolone-carboxylic acid **1** and related analogs.[3] One of the significant aspects of that research was that the descarboxy analog **2** retained significant topo II inhibitory activity. During continued study of analogs of **2** which lacked the carboxy group we have now uncovered a series of hydroxylated benzyl analogs which are very potent topo II inhibitors.



1 R = CO_2H
2 R = H

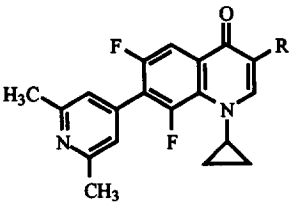
Chemistry. The target quinolone derivatives were prepared using the sequence described in Scheme 1. Reduction of **1** using $NaBH_4$ followed by acid-catalyzed decarboxylation of the ß-keto-acid provided dihydro analog **3**.[4] Aldol condensation of **3** with aldehydes provided exocyclic enones which rearranged to give the endocyclic enones **4** (Method A). The other methods referred to in Table 1 are as follows: B) Hydroxy analogs were prepared by HBr or BBr_3 catalyzed demethylation of the corresponding methyl ethers; C) The phenyl analog was prepared by Stille-type Pd^0 mediated coupling of the bromo analog[5] with PhSn(n-Bu)$_3$; D) The phenethyl analog was prepared via a four-step sequence involving formylation of **3** with ethyl formate, followed by oxidation to the unsaturated aldehyde with MnO_2, Wittig reaction with benzylidene triphenylphosphorane, and hydrogenation over Pd/C; E) Acetamide **18** was heated in the presence of 1 N HCl; F) Curtius rearrangement of acid **26** by treating it with $(PhO)_2PON_3$/t-BuOH followed by hydrolysis of the resulting urethane by heating with 6 N HCl; G) Acetylation of amine **27** with acetic anhydride/pyridine.

Scheme 1



Biological testing.[6] Topo II inhibition (Table 1)- Promotion by test agent of covalent complex formation between [^{32}P]-end-labeled pBR322 DNA and extensively purified HeLa cell topo II was determined by the SDS/K$^+$ precipitation method.[3] The EC_{50} value represents the concentration of test compound at which the amount of DNA precipitated is equivalent to 50% of the maximum precipitated by the reference topo II inhibitor mAMSA. *In vitro* cytotoxicity (Table 1) was measured by quantifying clonogenic survival in soft agar following a 1 hour transient exposure of P388 mouse leukemia cells to drug. The IC_{50} is the concentration of drug which reduced clonogenic survival by 50%. *In vivo* antitumor activity versus Panc 03 (Table 2) was measured at Wayne State University in mice implanted bilaterally s.c. with 30-60 mg of tumor fragments of murine pancreatic adenocarcinoma (Panc 03).[7] Chemotherapy was administered i.v. or s.c. at 3 dose levels starting 3 days after tumor implantation. Treatment was continued daily until lethality or > 10% body weight loss occurred at the top dose. Antitumor activity is reported for the maximum tolerated dosage level (MTD). Antitumor activity was measured as tumor growth inhibition (T/C), where T is the tumor burden in the treatment group and C is the tumor burden in the control group. A T/C value of < 42% is considered significant antitumor activity by the National Cancer Institute (NCI). A T/C < 10% is considered highly active.

Table 1. Method of synthesis and physical, topo II inhibitory, and cytotoxicity properties of quinolones.



Cmpd	R	Methd of Synth	% Yld	mp °C	Formula[a]	Topo II Inh. EC_{50}-uM	in vitro cytox IC_{50}-uM
5	C_6H_5	C	57	226-228	$C_{25}H_{20}F_2N_2O$	>120	-
6	$CH_2C_6H_5$	A	45	190-192	$C_{26}H_{22}F_2N_2O$	3.9	7.3
7	$CH_2CH_2C_6H_5$	D	70	140-141	$C_{27}H_{24}F_2N_2O$	>120	-
8	CH_2-C_6H_{11}	A	30	175-176	$C_{26}H_{28}F_2N_2O$	>230	-
9	CH_2-1-naphthyl	A	35	95-99	$C_{30}H_{24}F_2N_2O \cdot 1/2\ H_2O$	>210	-
10	CH_2-4-pyridyl	A	31	155-157	$C_{25}H_{21}F_2N_3O$	9.1	-
11	CH_2-2-pyridyl	A	30	190-192	$C_{25}H_{21}F_2N_3O \cdot 1/4\ H_2O$	12	-
12	CH_2-2-pyrrolyl	A	46	229 (d)	$C_{24}H_{21}F_2N_3O$	5.1	0.93
13	CH_2-2-imidazolyl	A	40	220 (d)	$C_{23}H_{20}F_2N_4O$	1.1	1.1
14	CH_2-4-imidazolyl	A	20	208-211	$C_{23}H_{20}F_2N_4O$	1.6	0.39
15	CH_2-4-ClC_6H_4	A	49	203-204	$C_{26}H_{21}ClF_2N_2O$	7.7	20
16	CH_2-4-$OCH_3C_6H_4$	A	52	167-168	$C_{27}H_{24}F_2N_2O_2$	14[b]	21
17	CH_2-4-OHC_6H_4	B	76	258-259	$C_{26}H_{22}F_2N_2O_2 \cdot 1/2\ H_2O$	1.2	1.5
18	CH_2-4-$NHCOCH_3C_6H_4$	A	48	265-268.5	$C_{33}H_{27}F_3N_2O_2$	>100	-
19	CH_2-4-$N(CH_3)_2C_6H_4$	A	44	200-202	$C_{28}H_{27}F_2N_3O \cdot 1/4\ H_2O$	>210	-
20	CH_2-4-$NH_2C_6H_4$	E	79	216.5-219.5	$C_{26}H_{23}F_2N_3O$	7.2	-
21	CH_2-3-$OCH_3C_6H_4$	A	45	147-148	$C_{27}H_{24}F_2N_2O_2$	14	20
22	CH_2-3-OHC_6H_4	B	73	144-145	$C_{26}H_{22}F_2N_2O_2 \cdot 1/2\ H_2O$	1.1	2.2
23	CH_2-2-$OCH_3C_6H_4$	A	55	149-150	$C_{27}H_{24}F_2N_2O_2 \cdot 1/2\ H_2O$	14	37
24	CH_2-2-OHC_6H_4	B	66	141-142	$C_{26}H_{22}F_2N_2O_2 \cdot 1/2\ H_2O$	0.33	0.67
25	CH_2-2-$CH_2OHC_6H_4$	A[c]	20	185-187	$C_{27}H_{24}F_2N_2O_2$	6.8	-

26	CH_2-2-$CO_2HC_6H_4$	A	47	>300	$C_{27}H_{22}F_2N_2O_3 \cdot$ 1/4 H_2O	200	-
27	CH_2-2-$NH_2C_6H_4$	F	26	225-227	$C_{26}H_{23}F_2N_3O$	2.1	2.1
28	CH_2-2-$NHCOCH_3C_6H_4$	G	94	222-224	$C_{28}H_{25}F_2N_3O_2$	5.1	-
29	CH_2-2-$NHCOCF_3C_6H_4$	A	70	246.5-247.5	$C_{28}H_{22}F_5N_3O_2$	4.7	-
30	CH_2-2-$NHSO_2CH_3C_6H_4$	A	6	217-218.5	$C_{27}H_{26}F_2N_3O_3S$[d]	3.3	
31	CH_2-2,3-$(OH)_2C_6H_3$	B	66	242-244	$C_{26}H_{22}F_2N_2O_3 \cdot$ 1/4 H_2O	0.36	0.88
32	CH_2-2,4-$(OH)_2C_6H_3$	B	87	276-278	$C_{26}H_{22}F_2N_2O_3 \cdot$ 1/2 H_2O	0.16	0.21
33	CH_2-2,5-$(OH)_2C_6H_3$	B	72	256-258	$C_{26}H_{22}F_2N_2O_3 \cdot$ 3/2 H_2O	0.16	0.36
34	CH_2-2,6-$(OH)_2C_6H_3$	B	52	308-310	$C_{26}H_{22}F_2N_2O_3 \cdot$ 1/4 H_2O	0.096	0.25
35	CH_2-2,4,6-$(OH)_3C_6H_2$	B	90	245-247	$C_{26}H_{23}F_2N_2O_4$[d]	0.098	0.47
1	CO_2H					7.6	29
2	H					17	15
mAMSA						0.72	0.15
VP16						0.81	0.30

[a]Except where noted, C, H, and N analyses were within 0.4% of theoretical values. Spectral data were consistent with structures. [b]Extrapolated value, bell-shaped dose-response curve. [c]Product that resulted from aldol condensation with *o*-phthalaldehyde, apparently via a Cannizarro-type reduction. [d]Structure verified by HRMS, not analysis.

Results and Discussion. A key finding in this work was that the introduction of a benzyl group at the 3-position of **2** gave enhanced topo II inhibitory activity. Indeed, compound **6** was at least as potent as the parent quinolone-carboxylic acid **1**. Topo II inhibitory activity was very sensitive to the distance of the phenyl ring from the quinolone nucleus. Both the lower and higher homologues of **6** were inactive. Also replacement of the benzyl substituent by a cyclohexylmethyl substituent (**8**) destroyed activity. The 1-naphthyl analog **9** was similarly inactive. Heteroaromatic replacements for the phenyl ring were well-tolerated. Pyridyl analogs **10** and **11** were slightly less potent that **6**, pyrrole analog **12** had comparable potency, and imidazoles **13** and **14** were more potent. Regarding substitution of the phenyl ring of **6**, a significant improvement in potency was only obtained by introduction of hydroxy substituents. The *m*-OH (**22**) and *p*-OH (**17**) analogs were approximately 4-fold more potent than **6**, and the *o*-OH analog (**24**) about 12-fold more potent. The various analogs which we prepared to mimic or improve the hydrogen bonding features of the hydroxy compound were no more potent than the unsubstituted analog **6**. Introduction of a second hydroxy group onto analog **24** at the 3-position on the phenyl ring gave no improvement, but hydroxy-substitution at the 4- or 5-position gave a two-fold improvement. Introduction of a second hydroxy at the 6-position gave (**34**), topo II EC_{50} = 96 nM, the most

potent topo II inhibitor in this series and the most potent quinolone topo II inhibitor described to date.[8] Introduction of a third hydroxy group (**35**) gave no further improvement in potency.

The reason for the increased potency of the *o*-hydroxy analogs is by no means clear. It is possible that the hydroxy group is involved in intramolecular hydrogen bonding with the carbonyl as has been suggested for **1**.[3] However in this instance an 8-membered ring would be required. It is also possible that specific new hydrogen bonds are generated between the inhibitor and the enzyme-DNA complex. We speculate that a new aromatic interaction and a specific hydroxy interaction with the enzyme complex provide the high potency observed.

The in vitro cytotoxicity in this series roughly paralleled the topo II inhibitory activity, but the range of activities was narrower. Although the most potent topo II inhibitor (**34**) was also among the most cytotoxic compounds, it was not significantly more cytotoxic than some less potent topo II inhibitors (**14**, mAMSA, VP16).

Activity of several analogs was also measured in a murine antitumor model (Table 2).[9] The most potent topo II inhibitor (**34**) stood out as having excellent activity in this model, with a T/C of 0%. Moderate antitumor activity was observed in other antitumor models (data not shown).

Table 2. *In vivo* murine antitumor activity of selected quinolones vs. Panc 03.[a]

Compound	Dose Schedule	% T/C[b]	MTD[c] (mg/kg)
6	sc, qd 3-12	>100	3749[d]
13	iv, qd 3, bid 4-9; sc bid 10,11	21	88
24	iv, qd 3-11	5	225[d]
32	iv, qd 3, 4, 7, 10	73	20
34	iv, qd 4-11	0	12
1	sc, qd 3-12	31	600
2	sc, qd 3-9	>100	2410[d]
mAMSA	iv, qd 4-9	0	48
VP-16	iv, qd 4, 6, 8, 10, 12, 14, 16	3	96

[a]Conducted at Wayne State University using BDF_1 male mice (5 per treatment group). [b]% T/C is calculated by dividing the tumor burden in the treatment group (T) at the MTD by the tumor burden in the control group (C) and multiplying by 100%. [c]Maximum non-lethal total dose. [d]MTD not reached; highest total dose tested.

Conclusions. We have found that replacing the carboxy group of quinolone carboxylic acid **1** by a 2,6-dihydroxybenzyl group improves topo II potency approximately 80-fold with a corresponding increase in cytotoxicity *in vitro*. This activity also was manifested *in vivo* as antitumor activity in mice. This series of compounds represents a new direction in the SAR of quinolone topoisomerase inhibitors. It is worthy of note that compound **34** was also found to have modest activity against bacterial DNA gyrase (EC_{50} = 0.14 uM, ciprofloxacin = 0.030 uM), and might also represent a new direction to take for quinolone antibiotics.

Acknowledgement. The synthetic contributions of Garry Pilling, Chet Opalka, Mike Reuman, and Bob Perni are gratefully acknowledged as are the contributions of Chiab Panchapor, Susan Pugh, Lisa Polin, Kathryn White, Juiwanna Knight, Lisa Demchik, Julie Jones, and Lynne Jones to generating *in vivo* tumor biology data at Wayne State University (Support by CA 46560).

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4 Kondo, H.; Sakamoyo, F.; Kawakami, K.; Tsukamoto, G. *J. Med. Chem.* **1988,** *31,* 221.
5. The bromo analog was prepared via bromination of **2** using bromine in acetic acid.
6. The biological testing methods have been disclosed previously; Wentland, M. P.; Aldous, S. C.; Gruett, M. D.; Perni, R. B.; Powles, R. G.; Danz, D. W.; Klingbeil, K. K.; Peverly, A. D.; Robinson, R. G.; Corbett, T. H.; Rake, J. B.; Coughlin, S. A. *Bioorg. Med. Chem. Lett.* **1995,** *5,* 405.
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8. The experimental procedure for the synthesis of (**34**) is as follows: To a solution of ketone **3** (400 mg, 1.2 mmol) in 5 ml EtOH and 5 ml 10% NaOH was added 2,6-dimethoxybenzaldehyde (398 mg, 2.9 mmol). The resulting orange suspension was stirred at room temp for 45 min. Five drops of 35% NaOH was added and the mixture warmed to 60 ^{0}C for 2 h to effect conversion to the endocyclic enone. After cooling, the reaction mixture was quenched with sat NH_4Cl and extracted 3x with EtOAc. The EtOAc phase was dried over $MgSO_4$ and concd to an off-white solid which was recrystallized from EtOAc to provide **4** (R = 2,6-dimethoxyphenyl), (416 mg, 73%); mp 191-192 ^{0}C. To a solution of **4** (299 mg, 0.63 mmol) in 15 ml CH_2Cl_2 at 0 ^{0}C was added BBr_3 (1.8 ml, 19 mmol) over 45 min. The resulting suspension was gradually allowed to warm to room temp over 2 h, concd in vacuo and then cooled to 0 ^{0}C. Two ml of MeOH were added and the reaction mixture heated at 100 ^{0}C for 3 h to hydrolyze the borate complex. After cooling the pH was adjusted to 5 using 35% NaOH, and then to 7-8 using sat $NaHCO_3$. The mixture was then filtered and the filtrate extracted 3x with 10% BuOH/$CHCl_3$. The organic phase was combined with the precipitate collected above and the solution dried over $MgSO_4$, and concd to a yellow solid which was recrystallized from $CHCl_3$/hexane to provide **34** (147 mg, 52%); mp 308-310 ^{0}C.
9. Animal use was approved by the Wayne State University IACUC.

(*Received in USA* 6 *February* 1995; *accepted* 30 *March* 1995)