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THE ANTITUMOR ACTIVITY OF NOVEL PYRAZOLOQUINOLINE DERIVATIVES.[1]

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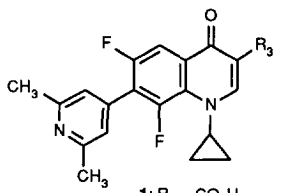
Abstract. Mammalian topoisomerase II inhibition activity has been identified in a series of novel pyrazoloquinoline derivatives; potency for two analogues containing cyclohexyl groups at the 2-position was comparable to the reference agents, mAMSA and VP-16. In several instances, topo II inhibition translated to a high level of *in vitro* cytotoxicity and murine antitumor activity.

Introduction. We recently described the mammalian topoisomerase II inhibitory properties of quinolone derivative **1** and related compounds.[2] The observation that similar potency was seen for **1** and the corresponding decarboxylated derivative **2** prompted us to further modify the carboxylate group of **1** as probes to establish a relationship between structure, topo II inhibitory potency and cytotoxicity. We focussed our efforts on preparing derivatives having a pyrazolo-ring fusion at the 3- and 4-positions of the quinoline ring where the carbonyl group is rigidified in what we had shown was the active conformation.[2] We further modified the 2-position of this new ring system with groups having varied physicochemical properties. We now wish to report our results for this series of pyrazolo-fused quinolones that exhibit sustained or up to 15-fold increased topo II inhibition potency relative to **1**.



1; $R_3 = CO_2H$
2; $R_3 = H$

Chemistry. The target 5-cyclopropyl-6,8-difluoro-7-(2,6-dimethyl-4-pyridinyl)-5*H*-pyrazolo[4,3-c]quinolin-3(2*H*)-ones were prepared in straightforward fashion using the methodology shown in Scheme I. The known quinolone ester **3**[3] was converted to the more reactive thione derivative **4**[4] which was treated with the appropriate hydrazine to give the targets **5-7**, **9-19**, **22**, and **23**. These hydrazines were either commercially available (used to make **5-7**, **9**, **13**, and **14**), known (**10-12**,[5] **16**[6], and **17**[7]) or made using a modification (Pt/H_2 instead of diborane) of a known procedure[8] (**15**, **18/19**, and **22/23**[9]). Compound **8** was made by hydrolyzing **7** in HOAc/12N HCl. Compounds **20** and **21** were made by hydrolysis (2N HCl) of the product from **4** and 4-acetamidocyclohexylhydrazine followed by separation using silica gel chromatography.

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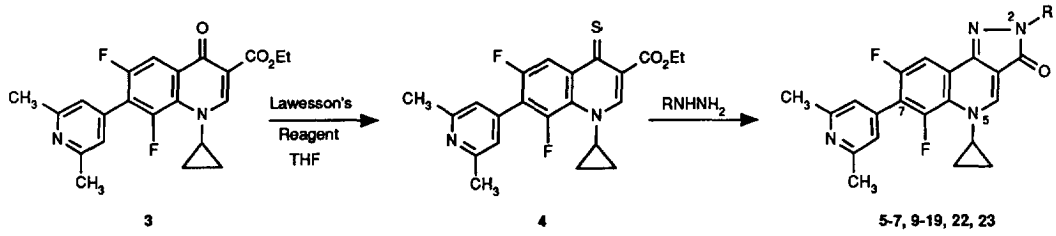
Scheme I



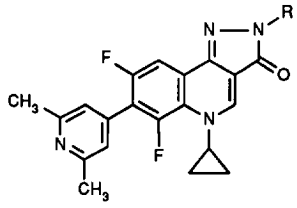
3 4 5-7, 9-19, 22, 23

Results and Conclusions. Biological data and a brief description of the assays used to evaluate the target compounds are found in Tables 1 and 2. Topoisomerase II Inhibition - Good potency was observed for all target compounds which in many instances was greater than the lead compound **1**. The two most potent analogues, **14** and **20**, contain a cyclohexyl group at the 2-position and had comparable (within 2-fold) potency to the two reference compounds, mAMSA and VP-16. Topo II inhibition potency did not correlate with several physicochemical properties of the 2-substituent. For example, compounds with relatively small R groups (e.g., H or CH_3) had roughly the same potency as those with much larger groups (e.g., C_6H_5 or 4-acetamidocyclohexyl). Hydrophilic R groups (e.g., CH_2CH_2OH or $CH_2CO_2^-$ [assuming extensive ionization at pH 7.4]), in general, contributed to activity to the same extent as hydrophobic groups (e.g., CH_3 or C_6H_5). Also, little difference in potency was seen in compounds having inductively electron donating R groups, (e.g., CH_3) and electron withdrawing groups (e.g., CH_2CO_2Et). In several instances potency was increased by the introduction of a cyclohexyl group at the 2-position. Within this subseries **14-23**, however, little correlation between structure (e.g., substitution, stereochemistry, or heteroatom replacement of the 4'-carbon of the cyclohexyl ring) and potency was observed.

In Vitro Cytotoxicity versus P388 - With the exception of **8**, all new compounds showed activity in this assay; potency correlated reasonably well with topo II inhibition potency (EC_{50}/IC_{50} ratios in the range of 1-5) except for analogues with R groups containing basic tertiary amines ($EC_{50}/IC_{50} \geq 10$). This latter observation may be a consequence of enhanced cell permeability due to high aqueous solubility in the ionized form and favorable partitioning of the drug with the lipid components of the cell membrane in the unionized form.

DNA Binding via Intercalation - The only compounds to demonstrate binding to DNA at relevant concentrations were **5**, **10** and **12**. The latter two analogues were quite potent in this assay, 10-fold more so than mAMSA, and have 2-substituents that are known to enhance the DNA binding properties of polyheterocyclic compounds via DNA groove stabilization.[14] As observed with other DNA interactive topo II inhibitors, **10** and **12** exhibited a bell-shaped dose response curve in the topo II assay.[15]

Table 1. Physical, topo II inhibitory, cytotoxicity, and DNA binding properties of pyrazoloquinolines.



cmpd	R	mp °C	formula[d]	topo II inh.[a] EC_{50}-μM	*in vitro* cytox[b] IC_{50}-μM	inter-calation[c] EC_{50}-μM
5	H	>310	$C_{20}H_{16}F_2N_4O$	11	7.9	27
6	CH_3	242-243	$C_{21}H_{18}F_2N_4O \cdot H_2O$	5.9	3.1	>60
7	CH_2CO_2Et	204-206	$C_{24}H_{22}F_2N_4O_3$	6.1	5.1	>60
8	CH_2CO_2H	200 (d)	$C_{22}H_{18}F_2N_4O_3 \cdot HCl$	10	>200	>60
9	CH_2CH_2OH	274-276	$C_{22}H_{20}F_2N_4O_2$	6.4	2.0	>60
10	$(CH_2)_2N(CH_3)_2$	224-226	$C_{24}H_{25}F_2N_5O \cdot C_4H_4O_4$[e]	2.6[f]	0.26	0.93
11	$(CH_2)_3N(CH_3)_2$	169-171	$C_{25}H_{27}F_2N_5O$	1.7	0.16	>50
12	$(CH_2)_2NH(CH_2)_2OH$	158 (d)	$C_{24}H_{25}F_2N_5O_2 \cdot CH_3SO_3H \cdot H_2O$	3.3[f]	1.3	1.2
13	C_6H_5	210 (d)	$C_{26}H_{20}F_2N_4O \cdot 0.25H_2O$	2.7	1.8	>60
14	c-C_6H_{11}	259-261	$C_{26}H_{26}F_2N_4O$	0.90	0.68	>50
15	$CH(CH_2CH_2)_2CHCH_3$ (*cis/trans*)[g]	200-204	$C_{27}H_{28}F_2N_4O$	3.1	1.4	>50
16	$CH(CH_2CH_2)_2O$	282-284	$C_{25}H_{24}F_2N_4O_2$	1.7	0.29	>50
17	$CH(CH_2CH_2)_2NCH_3$	218 (d)	$C_{26}H_{27}F_2N_5O \cdot 0.5H_2O$	3.2	0.094	>50
18	$CH(CH_2CH_2)_2CHNHCOCH_3$ (*cis*)	282 (d)	$C_{28}H_{29}F_2N_5O_2 \cdot 2H_2O$	7.4	1.3	>52
19	$CH(CH_2CH_2)_2CHNHCOCH_3$ (*trans*)	245 (d)	$C_{28}H_{29}F_2N_5O_2 \cdot 2H_2O$	9.8	1.2	>51
20	$CH(CH_2CH_2)_2CHNH_2$ (*cis*)	260-263	$C_{26}H_{27}F_2N_5O$	0.50	0.44	>50
21	$CH(CH_2CH_2)_2CHNH_2$ (*trans*)	225-228	$C_{26}H_{27}F_2N_5O$	4.2	1.5	>50
22	$CH(CH_2CH_2)_2CHN(CH_3)_2$ (*cis*)	137-140	$C_{28}H_{31}F_2N_5O \cdot CH_3SO_3H \cdot 1.5H_2O$	1.7	0.067	>50
23	$CH(CH_2CH_2)_2CHN(CH_3)_2$ (*trans*)	156-159	$C_{28}H_{31}F_2N_5O \cdot CH_3SO_3H \cdot 2H_2O$	4.4	0.26	>50
1				7.6	29	>54
2				17	15	>55
mAMSA				0.72	0.15	11
VP-16				0.81	0.30	>70

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[a]Topoisomerase II Inhibition - 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.[2] EC_{50} values were calculated to be the concentration of test compound at which the amount of DNA precipitated was equivalent to 50% of the maximum precipitated by mAMSA in a concomitant control experiment. [b]*In Vitro* Cytotoxicity 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} value is the concentration of drug which reduced clonogenic survival by 50%. [c]DNA Intercalation -A known ethidium bromide displacement assay was used to determine intercalation potency.[10] The EC_{50} value is the concentration of test agent that causes a 50% reduction in the fluorescence of the calf thymus DNA/ethidium bromide complex. [d]Proton NMR, IR, and MS were consistent with the assigned structures of all new compounds. C, H, and N elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of the theoretical values. [e](*E*)-Butendiote salt. [f]Bell-shaped dose response curve was noted when determining the EC_{50}. [g]Ca. 1:1 mixture.

In Vivo Antitumor Activity versus Panc 03 - In general, analogues with IC_{50} values below 1 μM in the P388 cytotoxicity assay were evaluated for murine solid tumor activity (Table 2). Significant activity (%T/C < 42), in some cases comparable to the reference agents, was seen in a number of analogues. The most efficacious analogues were the 2-dimethylaminoethyl derivative **10** and the pair of isomeric 2 (4-dimethylaminocyclohexyl) derivatives **22** and **23**. Efficacy in this model did not correlate with *in vitro* P388 cytotoxicity or topo II inhibition potency; it should be noted, however, that nearly all compounds in the series with R groups containing basic amines showed significant activity (T/C <42%) suggesting enhanced pharmacodynamic properties.

Table 2. *In vivo* murine antitumor activity vs. Panc 03 for pyrazoloquinolines.[a]

cmpd	% T/C[b]	MTD[c]
5	>100	2500[d]
10	8	34
11	35	64
12	42	1410
13	>100	635[d]
14	18	140
17	24	128
20	21	110
21	42	550
22	5	63
23	2	390
1	31	600[d]
2	>100	1950[e]
mAMSA	0	48
VP-16	3	96

[a]Evaluations of *in vivo* antitumor activity were conducted at Wayne State University in mice implanted bilaterally s.c. with 30-60 mg tumor fragments of murine pancreatic adenocarcinoma (Panc 03). Chemotherapy was administered intravenously at the maximum tolerated dose starting 3 days after tumor implantation. The methods of tumor implantation, end point determination and quantification of tumor cell kill have previously been described.[11-13] Animal use was approved by the Wayne State University IACUC. [b]T/C value = tumor growth inhibition,where T is the median tumor burden in the treatment group X 100 at evaluation and C is the median tumor burden in the control group at evaluation. A T/C value <42% is considered significant antitumor activity. [c]MTD = maximum tolerated total dose administered intravenously in mg/kg. [d]Drug was administered via the subcutaneous route. [e]Highest dose tested via subcutaneous administration - MTD was not reached.

In summary, we have demonstrated that a significant enhancement in topo II inhibition resulted from modification of the lead compound **1** by incorporating the 3-carboxylate and 4-keto functions into a pyrazole ring. Little correlation between structure (i.e., variation of the 2-position) and topo II inhibition potency was observed. In several instances, the observed topo II inhibition translated to a high level of *in vitro* cytotoxicity and murine antitumor activity. Following generation and analysis of additional data, a full account of this work will appear.

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4. Compound **4** was made as follows: Lawesson's reagent (25.2 g, 0.06 mol) was added to a solution of **3** (24.1 g, 0.06 mol) in 720 mL THF and the mixture heated at reflux for 21 h. The solvent was removed in vacuo and the residue purified by column chromatography (silica gel - 97:3/$CHCl_3$:2-propylamine) to give 26.1 g of product. Recrystallization from ethyl acetate gave ethyl 1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-thioxo-3-quinolinecarboxylate (**4**, 21.0 g, 84%); mp 194-195°C. ^{1}H NMR ($CDCl_3$) δ 8.67 (dd, *J*=1.8, 8.8 Hz, 1H), 8.12 (s, 1H), 7.08 (s, 2H), 4.40 (q, *J*=7.2 Hz, 2H), 3.98-3.85 (m, 1H), 2.62 (s, 6H), 1.40 (t, *J*=7.2 Hz, 3H), 1.25-1.05 (m, 4H). Anal. ($C_{22}H_{20}F_2N_2O_2S$) C,H,N.
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9. Compounds **22** and **23** were prepared as follows: A mixture of **4** (0.70 g, 1.69 mmol), 4-dimethylamino-cyclohexylhydrazine (0.83 g, 3.23 mmol) and pyridine (5 mL) was heated to 100°C for 3 h. The reaction was concentrated and the residual oil (0.81 g) purified by MPLC (silical get 98:1:1/$CHCl_3$:MeOH:2-propylamine) to yield the neutral forms of **22** (0.14 g, 17%) and **23** (0.25 g, 30%). Each were converted to their respective methanesulfonic acid salts **22** and **23** by treatment with methanesulfonic acid in EtOH. Compound **22** (2-[*cis*-4-(dimethylamino)cyclohexyl)-5-cyclopropyl-6,8-difluoro-7-(2,6-dimethyl-4-pyridin-yl)-5*H*-pyrazolo[4,3-c]quinolin-3(2*H*)-one; mp 137-140°C; ^{1}H NMR ($CDCl_3$) δ 10.98 (bs, 1H), 8.42 (s, 1H), 7.84 (dd, *J*=1.9, 8.8 Hz, 1H), 7.09 (s, 2H), 4.85-4.74 (m, 1H), 4.10-3.90 (m, 1H), 3.38-3.14 (m, 1H), 2.90 (s, 6H), 2.83 (s, 3H), 2.64 (s, 6H), 2.54-1.80 (m, 8H), 1.39-1.13 (m, 4H). Anal.($C_{28}H_{31}F_2N_5O{\cdot}CH_3SO_3H{\cdot}1.5H_2O$) C,H,N. Compound **23** (*trans* isomer); mp 156-159°C; ^{1}H NMR ($CDCl_3$) δ 11.18 (bs, 1H), 8.40 (s, 1H), 7.87 (dd, *J*=1.8, 8.9 Hz, 1H), 7.10 (s, 2H), 4.57-4.29 (m, J_{aa}=7.8 Hz, 1H), 4.14-3.90 (m, 1H), 3.41-3.18 (m, 1H), 2.86 (s, 6H), 2.84 (s, 3H), 2.65 (s, 6H), 2.45-1.55 (m, 8H), 1.36-1.03 (m, 4H). Anal. ($C_{28}H_{31}F_2N_5O{\cdot}CH_3SO_3H{\cdot}2H_2O$) C,H,N. Assignment of *cis* and *trans* stereochemistry to **22** and **23**, respectively, was based on the chemical shift and/or coupling constant of the cyclohexyl methine H on the carbon attached to pyrazole ring. Assuming the bulky $N(CH_3)_2$ group predominantly occupies an equatorial position, then the methine H of **22** is assigned an equatorial position (*cis*-cyclohexane) based on a downfield chemical shift (4.78 ppm) relative to the higher field chemical shift (4.38 ppm) of the axial methine of the *trans* isomer **23**. Additionally, the coupling constant ($J_{a,a}$=7.8 Hz) of the methine of **23** is consistent with that known for axial cyclohexyl methine couplings (see: Lambert, J.B.; Shurvell, H.F.; Verbit, L.; Cooks, R.G.; Stout, G.H. *Organic Structural Analysis*; Macmillan Publishing Co., Inc.:New York, 1976; p. 39).

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