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RELATIONSHIP OF STRUCTURE OF BRIDGED (2,6-DIMETHYL-4-PYRIDINYL)-QUINOLONES TO MAMMALIAN TOPOISOMERASE II INHIBITION.[1]

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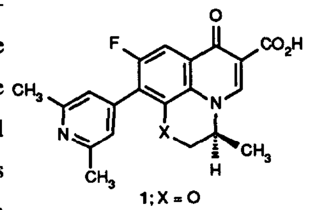
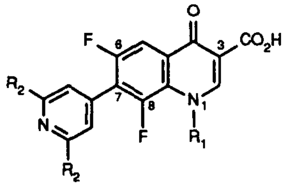
Abstract: Several enantiomerically pure (2,6-dimethyl-4-pyridinyl)quinolones, previously shown to be potent inhibitors of bacterial DNA gyrase, exhibit topoisomerase II inhibitory activity. Among these and other analogues, topoisomerase II inhibitory potency was found to be a sensitive function of the size and substitution of the bridge spanning the 1- and 8-positions of the quinoline ring. The 6-fluoro group was required for activity.

The biochemical target of the quinolone class of anti-infective agents is bacterial DNA gyrase, a type II topoisomerase that is unique to procaryotes.[3] With the exception of some recently reported derivatives,[4-12] quinolones are not generally interactive with mammalian topoisomerase II (topo II) which explains, in part, the low toxicity generally observed in mammals.

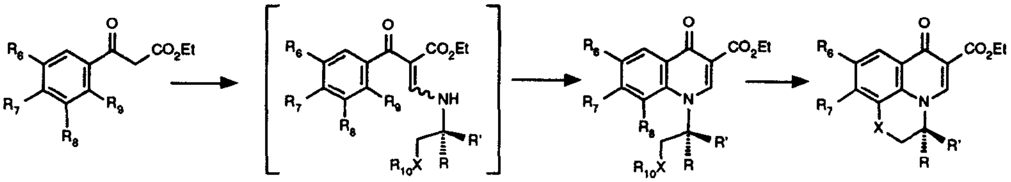
Compounds **1** and **2** are two enantiomerically pure (2,6-dimethyl-4-pyridinyl)quinolones that were recently shown to have outstanding gyrase inhibitory and antibacterial activity, especially against Gram-positive organisms; the latter trait is a consequence of the 2,6-dimethyl-4-pyridinyl (DMP) group.[13,14] These compounds, however, could not be developed as human therapeutics due to an unacceptable in vitro genetic toxicology profile which included potent clastogenic and mutagenic activity in mammalian cells. This profile was consistent with the agents being interactive with topo II; upon subsequent testing, they were found to have modest topo II inhibition potency. Other pyridinylated quinolones reported to have topo II inhibition activity are CP-67,015 (**3**)[4] and the 1-cyclopropyl derivative **4**.[7]


1; X = O
2; X = S

The objective of this study was to determine if topo II inhibitory potency was related to 1) the chirality, size and substitution of the bridge spanning the 1- and 8-positions of the quinoline ring, 2) the degree of fluorine substitution, and 3) the nature of the 7-substituent. As shown in Schemes 1 and 2, novel compounds were prepared from **5**,[15] **7**, or **11**[16] using standard methodology including the palladium-catalyzed Stille coupling of pyridinylstannes with aryl bromides[14,15] and the cycloaracylation procedure.[17] Compound **7** was prepared in straightforward fashion from 2,3,4-trichlorobenzoic acid.[18]


3; $R_1 = CH_2CH_3$, $R_2 = H$
4; $R_1 = \underline{c}\text{-}C_3H_5$, $R_2 = CH_3$

Scheme 1



8 9 10

5; $R_6 = R_8 = R_9 = F$, $R_7 = Br$

a

6; $R_6 = R_8 = R_9 = F$, $R_7 = DMP$

7; $R_6 = H$, $R_7 = R_8 = R_9 = Cl$

for 12· 2 $\xrightarrow{b}$ 12

for 13. 5 $\xrightarrow{c-e}$ 9 ($R_6 = R_8 = F$, $R_7 = Br$, $R_{10}X = HO$, R = H, R' = CH_3) $\xrightarrow{f-i}$ 10 ($R_6 = F$, $R_7 = DMP$, X = NH, R = H, R' = CH_3) $\xrightarrow{j}$ 13

for 15: 5 $\xrightarrow{c,k,l}$ 10 ($R_6 = F$, $R_7 = Br$, X = -OCH_2-, R = H, R' = CH_3) $\xrightarrow{m, j}$ 15

for 16: 5 $\xrightarrow{c,n,l}$ 10 ($R_6 = F$, $R_7 = Br$, X = O, R = CH_3, R' = H) $\xrightarrow{m, j}$ 16

for 17: 5 $\xrightarrow{c,n,e}$ 9 ($R_6 = R_8 = F$, $R_7 = Br$, $R_{10}X = HO$, R = CH_3, R' = H) $\xrightarrow{o-r}$ 10 ($R_6 = F$, $R_7 = DMP$, X = S, R = CH_3, R' = H) $\xrightarrow{j}$ 17

for 18· 6 $\xrightarrow{c,s,t}$ 10 ($R_6 = F$, $R_7 = DMP$, X = O, R = R' = CH_3) $\xrightarrow{u}$ 18

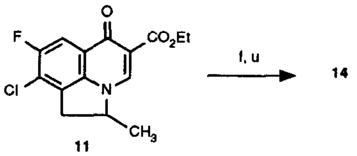
for 19· 5 $\xrightarrow{c,v,l}$ 10 ($R_6 = F$, $R_7 = Br$, X = O, R = R' = H) $\xrightarrow{m, j}$ 19

for 20: 6 $\xrightarrow{c,w,t}$ 10 ($R_6 = F$, $R_7 = DMP$, X = O, R,R' = -CH_2CH_2-) $\xrightarrow{u}$ 20

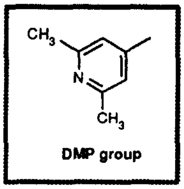
for 23 7 $\xrightarrow{c,d,l}$ 10 ($R_6 = H$, $R_7 = Cl$, X = O, R = H, R' = CH_3) $\xrightarrow{m, j}$ 23

(a) (DMP)Sn(nBu)$_3$, $PdCl_2(PPh_3)_2$, dioxane, (b) oxone®, $EtCO_2H$, H_2O, (c) $(MeO)_2CHNMe_2$, THF, (d) (S)-$HOCH_2CH(Me)NH_2$, (e) $LiCO_3$, DMF; (f) (DMP)$SnMe_3$, $PdCl_2(PPh_3)_2$, EtOH; (g) $MeSO_2Cl$, Et_3N, CH_2Cl_2, (h) NaN_3, DMF, (i) H_2, Pd, DMF; (j) 1N HCl; (k) (S)-$HOCH_2CH_2CH(Me)NH_2$; (l) K_2CO_3, DMF; (m) (DMP)Sn(nBu)$_3$, $PdCl_2(PPh_3)_2$, EtOH; (n) (R)-$HOCH_2CH(Me)NH_2$, (o) (DMP)Sn(nBu)$_3$, $PdCl_2(PPh_3)_2$, DMF; (p) $MeSO_2Cl$, $iPrNEt_2$, CH_2Cl_2, (q) MeCOSK, DMF; (r) EtONa, EtOH; (s) $HOCH_2C(Me)_2NH_2$, (t) NaH, THF, (u) K_2CO_3, EtOH, H_2O, (v) $HO(CH_2)_2NH_2$, (w) $HOCH_2C(-CH_2CH_2-)NH_2\,CF_3CO_2H$ (ref. 19), Et_3N, EtOH

Scheme 2

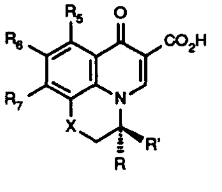


11



DMP group

Table 1. Topoisomerase II inhibitory properties of quinolones.



cmpd	X	R	R'	R_5	R_6	R_7	formula[a]	mp,°C	topo II inh.[b] EC$_{50}$ - μM
1[c,d]	O	H	CH_3	H	F	DMP			31
2[c,e]	S	H	CH_3	H	F	DMP			9.4
12[f]	S→O	H	CH_3	H	F	DMP	$C_{20}H_{17}FN_2O_4S$	294-295	62
13	NH	H	CH_3	H	F	DMP	$C_{20}H_{18}FN_3O_3$	>370 (dec)	730
14	-(*R,S*)	H	CH_3	H	F	DMP	$C_{19}H_{17}FN_2O_3$	344-347 (dec)	>570
15	$-OCH_2-$	H	CH_3	H	F	DMP	$C_{21}H_{19}FN_2O_4$	285-287	150
16[g]	O	CH_3	H	H	F	DMP	$C_{20}H_{17}FN_2O_4$	308-312	>540
17[h]	S	CH_3	H	H	F	DMP	$C_{20}H_{17}FN_2O_3S$	>300	>260
18	O	CH_3	CH_3	H	F	DMP	$C_{21}H_{19}FN_2O_4 \cdot 0.25H_2O$	>300	510
19	O	H	H	H	F	DMP	$C_{19}H_{15}FN_2O_4 \cdot HCl \cdot 1.5H_2O$	>410	>560
20	O	$-CH_2CH_2-$		H	F	DMP	$C_{21}H_{17}FN_2O_4$	299-301	>130
21[c]	O	H	CH_2CH_3	H	F	DMP			420
22[c]	O	H	CH_3	F	F	DMP			50
23	O	H	CH_3	H	H	DMP	$C_{20}H_{18}N_2O_4$	262-264	>290
24[i]	O	H	CH_3	H	F	4-pyridinyl			57
25[j]	O	H	CH_3	H	F	6-isoquinolinyl			58
26[k]	O(*R,S*)	H	CH_3	H	F	4-CH_3-1-piperazinyl			>280
VP-16									0.81

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[a] Proton NMR, IR, and mass spectra were consistent with the assigned structures of all new compounds. Carbon, hydrogen, and nitrogen elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of the theoretical values. [b] Topo II inhibition was measured by an SDS-K^+ precipitation method (see ref. 20) which quantified the amount of highly purified HeLa cell topo II covalently complexed with linearized 3'-[^{32}P]-end labeled pBR322 DNA. The EC$_{50}$ of a test compound was defined to be the concentration with activity equal to 50% of the activity observed with the nearly saturating dose of the reference agent m-AMSA (EC$_{50}$=0.72 μM). [c] see ref. 14. [d] $[\alpha]_D^{25}$= -39.8° ($CHCl_3$). [e] $[\alpha]_D^{25}$= +51.8° ($CHCl_3$). [f] Proton NMR indicated this to be one diastereomer; the stereochemistry was not defined. [g] $[\alpha]_D^{25}$= +40.7° ($CHCl_3$). [h] $[\alpha]_D^{25}$= -48.1° ($CHCl_3$). [i] CP-92,121; see ref. 21. [j] CP-100,964; see ref. 21. [k] Ofloxacin; see ref. 10.

The structures and topo II inhibitory activity of the target compounds are shown in Table 1. The data for the well-known topo II inhibitor and clinically useful antitumor agent VP-16 is included for comparison. A cleavage assay using enzyme isolated from HeLa cells was utilized to assess topo II inhibition. The EC_{50} value represents the concentration of drug that achieves 50% of the maximal effect of m-AMSA; the variance of this assay was such that potency was judged to be similar if the EC_{50} values of test compounds were within 2-fold of each other.

The first six entries in Table 1 summarize the effect on topo II activity upon varying X. The EC_{50} value for the lead compound **1** was 31 µM; this potency level was 38-fold less than that observed for VP-16. Compared to the **1**, the sulfur derivative **2** was 3-fold more potent while sulfoxide **12** was 2-fold less potent. The nitrogen derivative **13** had little or no activity. The racemic ring-contracted analogue **14** was inactive and the ring expanded compound **15** was 5-fold less potent than **1**.

The enantiomers **16** and **17** of **1** and **2**, respectively, were devoid of activity. Using the oxygen case as an example, the large difference in antipodal potency could be a consequence of 1) the (*S*)-methyl group of **1** being an absolute requirement for activity and/or 2) the (*R*)-methyl of **16** being highly detrimental to activity. Two additional derivatives, **18** and **19**, were made and evaluated to help understand these observations. Since both the dimethyl analogue **18** and the desmethyl derivative **19** had little or no activity, it is likely that both rationalizations 1) and 2) are correct. This conclusion, however, does not take into account any potential conformational differences between the four analogues. These results contrast with a study in which the desmethyl analogue of ofloxacin (**26**) had considerably more topo II inhibitory potency than **26** or either of its enantiomers.[10] A related compound, spirocyclopropyl derivative **20**, was inactive; this result is not surprising based on the lack of activity of **18**, although the 1-cyclopropyl group has been shown to impart excellent topo II inhibitory properties to quinolones.[5,7] Replacement of the (*S*)-methyl of **1** with ethyl (example **21**) resulted in substantially diminished potency.

Two analogues of **1** with differing fluorine substitution were evaluated. Relative to **1**, comparable potency was observed when an additional F was attached to the 5-position (**22**) while the des-fluoro derivative **23** was devoid of activity. The influence of fluorine substitution at the 8-position in bicyclic quinolones on topo II activity was recently reported; the 8-F group was found to enhance activity relative to the 8-H derivative.[6]

Comparison of **1** to three known 7-position variants revealed that modification of the DMP group resulted in similar potency when both methyl groups were replaced by hydrogen (example **24**)[21] or when the DMP group was replaced by 6-iosquinolinyl (example **25**)[21]. The 4-methyl-1-piperazinyl group is one of the prototypic 7-substituents of quinolone DNA gyrase inhibitors and when appended to the basic tricyclic racemic framework of **1** gives the commercial antibacterial agent ofloxacin (**26**). Ofloxacin, previously reported to have weak topo II activity,[10] had no activity in our assay at 280 µM, the highest concentration tested.

Within this series, the degree of correlation between gyrase and topo II inhibitory potency varied. For example, compounds **13**, **14**,[22] **15**, and **26** had substantial activity versus gyrase[13] but had little or no topo II activity. Compounds **1**, **2**, **22**,[22] and **24**[22] had substantial activity in both assays although greater potency versus gyrase was seen. Of those compounds tested in both assays, there were none that displayed significant topo II activity but were devoid of or weakly active versus gyrase.

In conclusion, we have found that topo II inhibitory activity within a small series of bridged (2,6-dimethyl-4-pyridinyl)quinolones was a sensitive function of the size and substitution of the bridge spanning the 1- and 8-positions; very high eudismic ratios were observed for analogues **1** and **2**. The 6-fluoro group was required for activity and a pyridinyl group at the 7-position on the quinoline ring was much more beneficial than a methylpiperazinyl group. The sulfur analogue **2** was the most potent in the series, 12-fold less so than VP-16. The modest enzyme inhibitory activity of **2** translated to moderate antitumor activity versus P388.[23] In a clonogenic (one hour exposure) in vitro cytotoxicity assay, the IC_{50} value for **2** was 23 µM (VP-16; IC_{50} = 0.30 µM). In vivo antitumor activity [%ILS (increased life span) = 90 @ 1014 mg/kg - 0/6 long term cures] was observed for **2** in a murine leukemia model in which P388 was implanted ip and drug was administered ip (VP-16; %ILS = 220 @ 180 mg/kg - 5/6 long term cures). The antitumor properties of **2** were judged to be a consequence of promotion of intracellular topo II-DNA covalent complexes based on alkaline elution experiments.[23] Protein-associated DNA strand breaks were seen from exposure of P388 cells to **2** but not to the enantiomer **17** which is devoid of both topo II and cytotoxicity activity.

While this study did not identify any candidate for advancement as a potential antitumor agent, the insights regarding the effect of structure on topo II inhibitory activity were used to design more potent analogues; these results will be the topic of future communications.

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