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POTENT MAMMALIAN TOPOISOMERASE II INHIBITORS: 1-CYCLOPROPYL-6,8-DIFLUORO-1,4-DIHYDRO-7-(2,6-DIMETHYL-4-PYRIDINYL)-4-SUBSTITUTED-QUINOLINES.[1]

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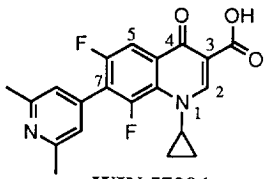
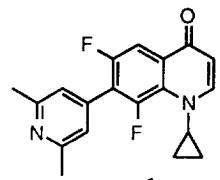
Abstract: A series of novel 4-substituted-1,4-dihydro-quinolines **3** were prepared and found to exhibit moderate to excellent mammalian topo II inhibitory activity. Among the compounds prepared, in general, the nitrogen analogues are the most active compounds and the sulfur analogue is the least active one.

Introduction: The mammalian topoisomerase II enzyme catalyzes the double-strand breakage of DNA to allow the second strand passage and thereby control the topology and conformation of DNA.[2] There are many topoisomerase II inhibitors that demonstrate useful antitumor activity (e.g., m-AMSA, VP-16 and VM-26),[3] and it has been suggested that enhanced topo-II-mediated DNA cleavage is an important mechanism for these antitumor agents.[4] While quinolone-based inhibitors of bacterial topo-II (DNA gyrase) have long been used successfully as antibacterial agents,[5] recent studies have identified some quinolones which also inhibit mammalian topoisomerase II and thus may have potential as antitumor agents.[6]



WIN 57294



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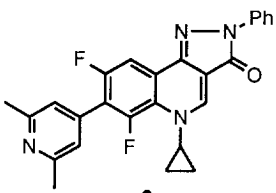

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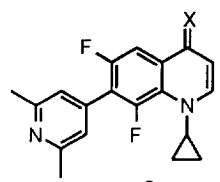

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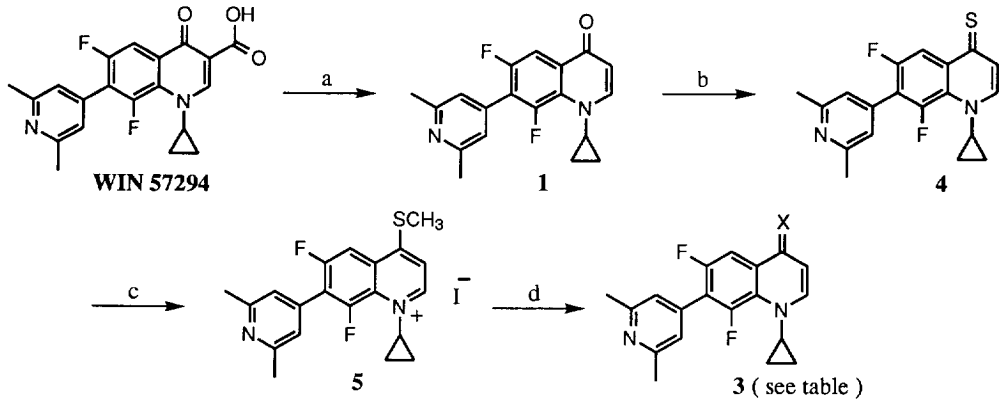
WIN 57294,[7] a potent inhibitor of DNA gyrase, was both clastogenic and mutagenic which precluded its development as a human anti-infective agent. This compound was subsequently found to possess moderate topo II inhibitory activity (EC_{50} = 7.6 μM). Structure activity relationship studies of WIN 57294 resulted in the discovery that the 3-CO_2H group was not a requisite for topo II potency (**1**, EC_{50} = 17 μM).[8] In a recent report, a conformationally rigid quinolone derivative **2** displayed better topo II potency (EC_{50} = 2.7 μM).[9] We thus anticipated that the investigation at the 4-position of the 3-H quinoline, should provide a series of novel potent topo II inhibitors (**3**, Table).

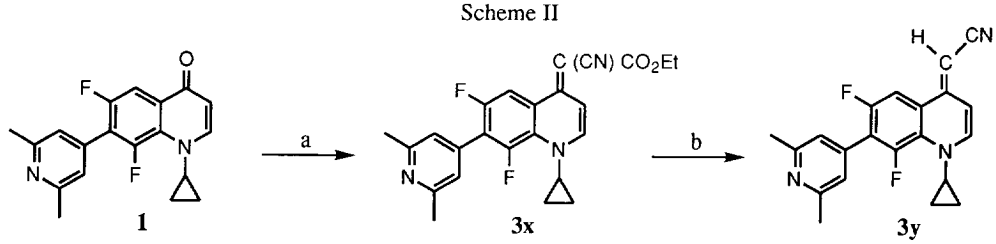
Chemistry: A general route to the 4-substituted-1,4-dihydro-quinolines **3** is outlined in Scheme I. The known WIN 57294[7] was decarboxylated by refluxing in quinoline in the presence of copper to give **1**.[8] Thionation of **1** with Lawesson's reagent afforded the orange crystalline quinolinethione **4.** Alkylation of **4** with iodomethane gave the 4-methylthio-quinolinium iodide salt **5**. Reactions of **5** with either hydrazines ($R^1R^2NNH_2$) or amines (R^3NH_2) afforded the corresponding hydrazones (**3a-3g, 3j**), imines (**3k-3p**), oximes (**3r-3s**) and thioximes (**3t-3u**). The hydrazines and the amines are either commercially available or prepared by known procedures.[10] Acetylation of **3a** with acetic anhydride or phthalic anhydride gave **3h** and **3i** respectively. The synthesis of **3x** is shown in Scheme II. Thermal reaction of **1** with malonitrile or ethyl cyanoacetate in the presence of acetic anhydride afforded the corresponding **3w** and **3x.** Treatment of **3x** with 5% KOH in refluxing dioxane followed by acid workup gave **3y.** Similarly, reaction of **1** with tosyl isocyanate in refluxing toluene afford **3v**, and treatment of **3v** with warm 48% HBr gave **3q**.

Scheme I



Reagents: (a) Cu, Quinoline, 230°C, 2h (92 %); (b) Lawesson's reagent, Toluene, 100°C, 5h (82 %); (c) CH_3I, THF, 20°C, 6h (84 %); (d) i. $R^1R^2NNH_2$ or R^3NH_2, EtOH, 50°C, 18h; ii. $NaHCO_3$

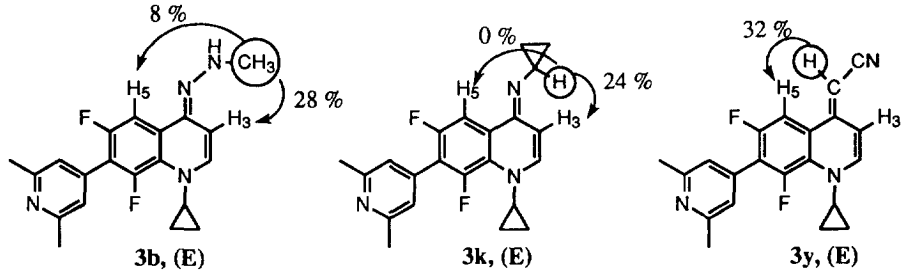
Scheme II



Reagents: (a) $NCCH_2CO_2Et$, $(CH_3CO)_2O$, 110°C, 9h (79 %); (b) 5 % KOH, Dioxane, 105°C, 2h (87%)

1D NOE experiments were done on representative molecules **3b**, **3k** and **3y**, and the structures were confirmed as the E isomers for all three molecules (Scheme III).

Scheme III



1 D NOE Experiments

Results and Discussion: The structures and topo II inhibitory activity of the 4-substituted-quinolines are shown in Table. The data of the reference agents m-AMSA and VP-16 (topo II interactive antitumor agents) are included for comparison. Activity was determined in a cell-free assay of DNA cleavage mediated by purified HeLa cell topo II. The assay measures the amount of topo II covalently linked to pBR322 end-labeled DNA using the SDS-potassium precipitation method of Trask.[11] The EC_{50} value represents the concentration of drug that achieves 50% of the maximal dose-saturating effect of the reference topo II inhibitor, m-AMSA.[12,13]

The lead compound in this series **1**, had an EC_{50} value of 17 μM; this potency was 21-fold less than that of VP-16. The sulfur analogue quinolinethione **4** was poorly active.

The primary hydrazone **3a** shown to have significant topo II inhibitory activity which was 10-fold more potent than **1**. The secondary hydrazone **3b**, tertiary hydrazone **3c** and hydroxyethyl hydrazone **3d** all had similar potencies to **3a.** Within this alkyl-substituted hydrazone subseries, topo II potency is not sensitive to the hydrophobicity of X. The most potent analogue **3e**, the 2-pyridinyl-substituted hydrazone compound, had a topo II potency (0.98 μM) nearly equivalent to VP-16 (0.81 μM). When a bulkier quinoline ring and acridine ring were introduced into **3e** to give **3f** and **3g**, respectively, potency decreased for both **3f** and **3g**. Within this aromatic-substituted hydrazone subset of analogues, activity appears to be a sensitive function of the heterocyclic ring.[14] In the carbonylated hydrazone series, decreasing the electron density of the nitrogen atom (**3h** and **3i**) resulted in 11-fold decreased in potency as compared to **3a**. A less electron-withdrawing carbonylated hydrazone **3j** was predicted to be more potent than **3h** and **3i**. Indeed, **3j** was only slightly less potent than **3a**.

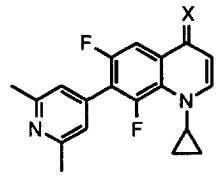
Compared to hydrazones **3a-3d**, comparable potency were observed for aliphatic-substituted imines (**3k** and **3m**), and aromatic-substituted imines (**3n-3p**). The unsubstituted imine **3q** was less potent.

Oximes (**3r** and **3s**) and thioximes (**3t** and **3u**) displayed similar potency as the hydrazones (**3a-3d**). Potency seems not related to the hydrophobicity of X in the oxime series. The reduced potency of **3v** relative to that of **3t** and **3u** may be related to the electron-withdrawing effect of the sulfonyl group in **3v**. The electronic effect observed here is consistent to the previous observation in the subseries **3h-3j**.

Electron-withdrawing substituted carbon can be a bioisostere of nitrogen.[15] Indeed, the nitrile or carboxylic ester substituted carbon analogues (**3w-3y**) were only 2-3 fold less potent than **3a.**

In Vitro Cytotoxicity and In Vivo Antitumor Activity: Cytotoxicity was determined by quantitating P388 murine leukemia cells on a Coultier Counter following a 48 hour exposure of cells to test compounds. P388

Table: 4-Substituted-Quinolines



3

Compound	X	mp°C	Topo II inhibition EC_{50} (μM)
1	O		17
4	S	240-243	76[a]
3a	NNH_2	> 238 (dec)[c]	1.7
3b	$NNHCH_3$	171-174	1.9
3c	$NN(CH_3)_2$	173-175	2.3
3d	$NNH(CH_2)_2OH$	> 154 (dec)	3.2
3e	NNH-2-pyridinyl	130-133	0.98
3f	NNH-3-quinolinyl	234-236	92
3g	NNH-9-acridinyl	> 275 (dec)	21
3h	$NNHCOCH_3$	265-268	20
3i	NNPhth	244-246	16
3j	$NNHCONH_2$	190-192	3.1
3k	N-c-C_3H_5	> 230 (dec)	2.4
3l	$N(CH_2)_2N(CH_3)_2$	> 155 (dec)	14
3m	$N(CH_2)_3N(CH_3)_2$	72-74	5.5
3n	N-2-pyridinyl	189-190	3.6
3o	N-2-OH-Ph	151-155	1.6
3p	N-4-NH_2-Ph	106-110	7.6
3q	NH	> 270 (dec)	12
3r	NOH	> 240 (dec)	1.4
3s	$NOCH_3$	197-199	2.6[b]
3t	NS-4-Cl-Ph	222-224	9.4
3u	NS-2-pyridinyl	234-236	3.8
3v	NSO_2-4-Me-Ph	204-205	45[b]
3w	$C(CN)_2$	263-265	6.3[b]
3x	$C(CN)CO_2Et$	235-238	4.8[b]
3y	CHCN	164-165	4.0
VP-16			0.81
m-AMSA			0.72

[a] Extrapolated value - 50 % inhibition was not observed at the highest concentration of compound tested.

[b] Bell - shaped dose response curve. [c] Hydrochloride salt

cells were maintained in log phase throughout the duration of cytotoxicity assays. The IC_{50} is defined as that concentration of drug which reduced the population of viable cells to 50% that of an untreated control. The in vitro cytotoxicity of the 4-substituted quinolines described in this paper in most instances did not correlate well with the topo II potency in comparison to VP-16. For example, in P388 cytotoxicity assay, the IC_{50} value for selected analogues **1, 3a, 3c** and **3e** were 9.7 μM, 3.0 μM, 0.27 μM and 1.0 μM (VP-16, 0.12 μM).

Compound **3a, 3c** and **3e** were evaluated for in vivo antitumor activity in mice implanted subcutaneously with murine pancreatic ductal adenocarcinoma No. 03 (Panc 03).[16] The quantitative end point used to assess antitumor activity was percent Tumor Growth Inhibition (% T/C). % T/C = T/C x 100 where T and C are median tumor weights of the treatment and control groups, respectively. Modest in vivo antitumor activity was observed for **3e** (% T/C = 37 at 2345 mg/kg administered iv, sc). For comparison, VP-16 was much more potent (% T/C = 3 at 96 mg/kg administered iv).[17]

Conclusions: A novel series of 4-substituted-1,4-dihydro-quinolines was prepared and found to exhibit moderate to excellent mammalian topo II potency showing that there is considerable latitude for substitution of the carbonyl of the quinolones. The most potent analogue **3e,** had a topo II potency nearly equivalent to VP-16, a clinically useful topo II interactive antitumor agent. However, the in vivo antitumor activity of **3e** was significantly less compared to VP-16.

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