Topoisomerase I-Mediated Antiproliferative Activity of Enantiomerically Pure Fluorinated Homocamptothecins

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Homocamptothecin (hCPT) is an E-ring modified camptothecin (CPT) analogue bearing a methylene spacer between the alcohol and carboxyl functions of the CPT lactone. Combining pronounced inhibitory activity of topoisomerase I (Topo I) with enhanced plasma stablility, hCPT constitutes an attractive template for the elaboration of new anticancer agents. Fluorinated hCPT analogues, prepared in enantiomerically pure form, were assayed by their stimulation of Topo I-mediated DNA cleavage. Translation into cytotoxicity against tumor cells was evaluated on HT29 human colon adenocarcinoma and on the multidrug resistant lung and bladder tumor cell lines, A549 and T24r. Good correlation is observed between the ability of the drugs to stimulate Topo I-mediated DNA cleavage and the respective 50% inhibitory concentrations (IC_{50} values) of the HT29, A549, and T24r cell growth. Fluorine substitution in the A-ring of hCPT was found to have a pronounced influence on biological activity, providing several compounds which are up to 100-fold more potent than CPT in terms of IC_{50}. Among these, 10,11-difluoro-hCPT has been selected for further development.

Camptothecin derivatives (CPTs) constitute a promising class of anticancer agents, exerting potent cytotoxicity by an original mode of action which converts the ubiquitous nuclear enzyme topoisomerase I (Topo I) into a cellular poison via the stabilization of Topo I−DNA covalent complexes.[1] Based on the CPT template, the current generation of compounds suffers, however, from the intrinsic instability of the highly electrophilic α-hydroxylactone which, under physiological conditions, undergoes rapid hydrolysis to an equilibrium mixture in which the biologically inactive carboxylate form predominates.[2]

We have previously reported the insertion of a methylene spacer between the alcohol and carboxyl functions of CPT to give hCPT.[3] This change to the CPT lactone uniquely conserves Topo I-mediated activity[4] and was found to promote additional DNA breaks which are not seen with CPT.[5] Another advantage comes from the diminished electrophilicity of the lactone, providing hCPT with a slow hydrolysis instead of the fast interconversion observed with CPT. Furthermore, even under mildly acidic conditions, this hydrolysis is *irreversible*, thus avoiding the possibility of haemorrhagic cystitis, a known limiting toxicity of some CPT analogues. Subsequent synthesis and screening of racemic analogues showed fluorinated hCPTs to be worthy of further investigation.[6] Fluorine substitution of the CPT skeleton, although reported in several instances,[7−11] has not been previously underlined as a key feature for activity enhancement. The changes in sequence-specificity of the drug-induced DNA cleavage by Topo I imply somewhat different interactions within the cleavable complexes[5] and, hence, that the SAR of CPTs may not be totally applicable to hCPTs. This paper describes our approach to select an optimal fluorine substitution pattern.

The biologically active enantiomers of CPT and hCPT were presumed to have identical spatial arrangement for their ethyl and hydroxyl groups, based on their similarity of their stereospecific Topo I inhibition and positive rotation of polarized sodium D emission. The biologically active counterpart of (*S*)-CPT was therefore (*R*)-hCPT, since homologation of the lactone changes the substituent priority ruled by chemical nomenclature. Our previously established synthetic strategy was amenable to the preparation of enantiopure compounds via a classical resolution. As shown in Scheme 1, racemic *β*-hydroxyacid **2** was resolved with quinidine to give the desired (*R*)-form in an enantiomeric excess (ee) of 70%, and a structural analysis by X-ray diffraction of the crystalline quinidium salt confirmed the absolute configuration assignment. Removal of the benzyl protecting group, lactonization, and demethylation gave pyridone **7**, whose enantiomeric purity was brought above 99% ee by recrystallization from acetone/water. The preparation was completed by coupling **7** with a fluorinated quinoline, and subsequent formation of the C-ring by intramolecular Heck reaction.[12] The required quinolines were obtained from the corresponding fluoroanilines by the Meth−Cohn method,[6] except for hCPT **8a** which was prepared from the corresponding fluoroanthranilic acid.

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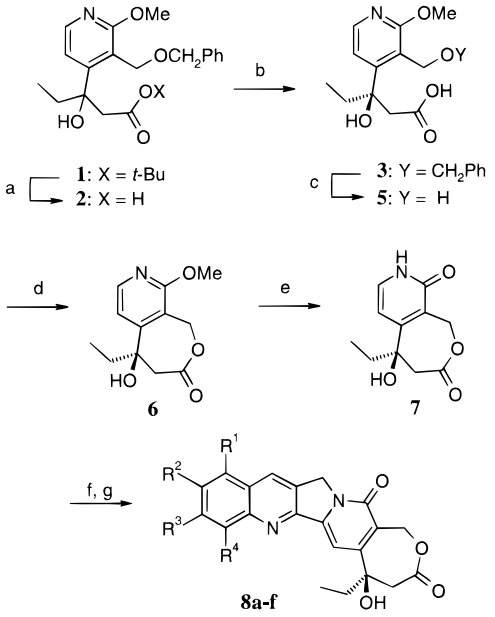
Scheme 1[a]

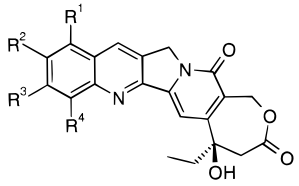


[a] Reagents and conditions: (a) TFA, rt, 90%; (b) quinidine, *i*-PrOH, 40%; (c) Pd/C $HCOONH_4$, MeOH, rt, 88%; (d) DCC, THF, 50 °C; (e) TMSCl, NaI, MeCN, reflux, 34% from **5**, then recrystallization from *i*-PrOH, 71%; (f) fluorinated hydroxymethylquinoline, DEAD, PPh_3, DMF, rt, 40−50%; (g) $Pd(OAc)_2$, PPh_3, KOAc, TBAB, MeCN, reflux, 40−65%.

The activity of these fluorinated hCPTs was assayed with human Topo I on supercoiled plasmid pKMp27, and the products were analyzed on an agarose gel containing ethidium bromide.[4] In the presence of the drugs, the nicked DNA fraction was significantly increased in a dose-dependent manner, reflecting the stabilization of Topo I−DNA cleavable complexes. Quantification of this effect in the presence of 0.1 μM of test compound is reported in Table 1. Stimulation of DNA cleavage is more efficient with **8c**, fluorinated at R^3, than with compounds **8a** and **8b**, with a fluorine at R^1 or R^2, respectively. At 0.1 μM, the stimulation of DNA cleavage by **8d** is about 3 times lower than with **8c**, R^4 being the least favorable position for fluorine substitution. The DNA cleavage values obtained for unsubtituted hCPT are comparable with that of **8a** or **8b**, while difluorinated compounds **8e** and **8f** are, like **8c**, highly efficient in stimulating DNA cleavage.

Translation of the Topo I inhibitory activity into antitumor cytotoxicity was determined by antiproliferative assays on three human cancer cell lines. HT29 colon adenocarcinoma and A549 non-small cell lung cancer were obtained from the American Type Culture Collection, while T24r resulted from prolonged exposure of T24 bladder carcinoma to a cocktail of three anticancer drugs comprising vinorelbine, an investigational alkylating agent PE1001, and doxorubicin.[13] A549 and T24r were selected for their multidrug resistance characteristics, established by flow cytometry and RT-PCR to be due to the overexpression of MRP for A549,[14] and Pg-P for T24r.[15] Cultured cells were incubated for 72 h with the drugs, and the viable cells were quantified by WST-1 colorimetric assay.[4] The 50% inhibitory concentrations (IC_{50}s) are presented in Table 1. The antiproliferative activities of the compounds were found to be in agreement with their stimulation of DNA cleavage

Table 1. Biological Data for Fluorinated hCPTs



test compd	substituents R1	R2	R3	R4	Topo I clvd DNA[a]	cytotoxicity against tumor cells (IC_{50}, nM)[b] HT29[c]	A549[c]	T24r[c]
8a	F				18	20	17	1.6
8b		F			17	27	22	4.5
8c			F		29	11	2.9	0.17
8d				F	7.3	190	130	22
8e	F		F		30	10	1.9	0.32
8f		F	F		32	8.4	3.4	0.40
hCPT					21	30	26	3.3
CPT					8.2	80	67	88

[a] Stimulation of pKMp27 DNA cleavage by human Topo I in the presence of 0.1 μM test compound, calculated with the formula $(N-N_0)/N_0$ where N and N_0 correspond to the fraction of nicked DNA in the drug-treated and the drug-free runs, separated by electrophoresis on an ethidium bromide containing agarose gel.[5] Data were compiled from quantitative analysis of three gels and must be considered as a set of averaged values. [b] Mean values of 50% inhibitory concentration determined by WST-1 (soluble tetrazolium) assay in triplicate.[4] [c] HT29 and A549 cell lines were obtained from the ATCC (Rockville, MD), and the T24r cell line[13] was a gift from Dr. Robert Kiss, Université Libre de Bruxelles, Belgium.

(Figure 1). Graph A illustrates the trend of the IC_{50} values on HT29, in decreasing order when going from **8d** to **8f** and matching the increasing capacity of the compounds to stimulate DNA cleavage. Cell lines which overexpress P-gp have been previously shown to have a certain degree of cross-resistance to hydroxylated CPT analogues such as topotecan and SN-38.[6] The remarkable levels of activity of compounds **8a**−**f** on the A549 and T24r cell lines demonstrate, on the contrary, that the multidrug resistant phenotypes of these cells do not recognize fluorinated hCPTs. Here again, a good correlation is observed between the relative DNA cleavage activities of compounds **8a**−**f** and their IC_{50} values on A549 and T24r (Graphs B and C), indicating that fluorine substitution does not introduce any bias with respect to multidrug resistance.

In summary, fluorine substitution in the A-ring of hCPT is found to have pronounced influence on biological activity, and, depending on the substitution pattern, cytotoxic activity relative to CPT may be increased considerably on multidrug resistant tumor cell lines. The good correlation that exists between the stimulation of DNA cleavage and cytotoxicity is a rather critical aspect of structure−activity studies of CPT derivatives[16,17] which has not always been verified,[18] due to physicochemical differences which alter cellular penetration and intracellular concentration of the drugs.[19] That such a good match is found for fluorinated hCPTs confirms Topo I poisoning as their principal mechanism of action, and may be due to the small elemental radius of fluorine and the narrow range of lipophilicities involved. Within the series of compounds presented here, hCPTs **8c**, **8e**, and **8f**, all bearing a fluorine atoms at R^3, constitute a group of highly active Topo I poisons, which are also more resistant to hydrolysis because of

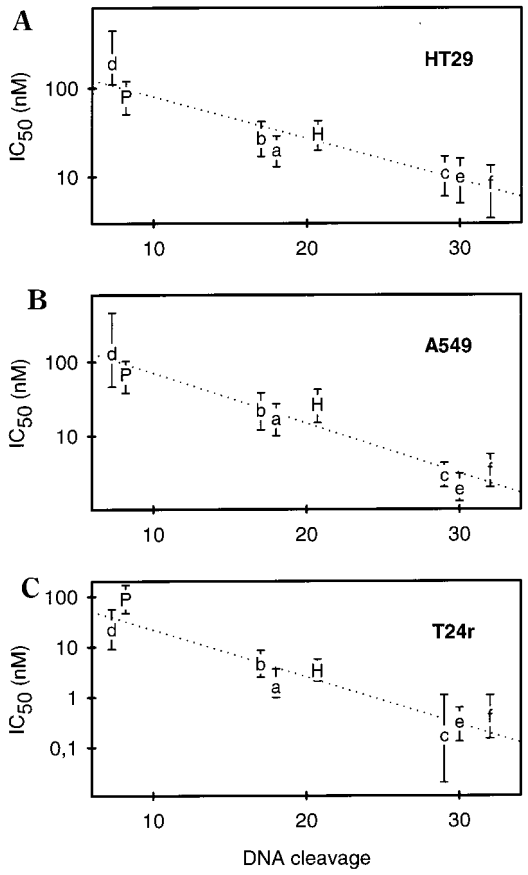


Figure 1. Correlations, by least-squares linear regression, between the ability for the drugs to stimulate Topo I-mediated DNA cleavage at 0.1 μM and their 50% inhibitory concentrations logarithm values on HT29, A549, and T24r ($r^2 = 0.90$, 0.93, and 0.89, respectively). The error bars correspond to the confidence intervals of the IC_{50} determinations. The symbols used are: **P** for CPT, **H** for hCPT, and **a** to **f** for compounds **8a**−**f**.

their modified lactone. After 2 h of incubation in human plasma at 37 °C, more than 50% of difluorinated hCPT **8f** remained in the biologically active lactone form, whereas CPT decayed exponentially to reach an equilibrium of less than 10% lactone within 30 min. Further work has shown **8f**, also known as BN 80915, to be orally active at very low doses in a variety of human tumor xenografts.[20]

Experimental Section

Resolution of 3-(3-Benzyloxymethyl-2-methoxy-4-pyridyl)-3-hydroxy-pentanoic Acid (2). Racemic *tert*-butyl ester **1**[6] (40 g, 100 mmol) was treated with trifluoroacetic acid (150 mL) at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure, and the residue was taken up in dichloromethane (200 mL) and extracted with saturated sodium bicarbonate (2 × 100 mL). The combined aqueous extracts were washed with dichloromethane (100 mL) and acidified to pH 1 with 6 N HCl. Extraction with dichloromethane (2 × 200 mL), drying of the combined extracts over $MgSO_4$, and concentration under reduced pressure gave racemic acid **2** which was engaged in the subsequent resolution without further purification. A mixture of acid **2** (31.1 g, 90 mmol) and quinidine (29.2 g, 90 mmol) was stirred in isopropyl alcohol (60 mL) at 50 °C. The clear solution obtained was allowed to cool to room temperature and then stored at 4 °C until crystal seeds appeared (16 h). Crystallization was then completed with stirring at room temperature for 2 h. The crystals, collected by filtration, were further purified by crystallization from isopropyl alcohol to give the diastereomerically pure quinidine salt of acid **4**, suitable for X-ray diffraction to determine its absolute configuration: mp >250 °C; $[\alpha]^{D}_{20} = +168$ (*c* 0.16, MeOH); IR (KBr) 837, 1067, 1230, 1241, 1391, 1508, 1552, 1591, 1619 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.66 (t, 3H), 1.23 (m, 1H), 1.58 (m, 2H), 1.81 (m, 2H), 1.85 (m, 1H), 2.05 (t, 1H), 2.35 (q, 1H), 2.54 (d, 1H), 2.77 (m, 1H), 2.82 (d, 1H), 2.95 (m, 2H), 3.35 (m, 4H), 3.80 (s, 3H), 3.91 (s, 3H), 4.53 (s, 2H), 4.75 (d, 1H), 5.13 (t, 2H), 5.62 (s, 1H), 6.04 (m, 2H), 7.05 (d, 1H), 7.35 (m, 7H), 7.54 (d, 1H), 7.94 (d, 1H), 7.98 (d, 1H), 8.70 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 8.35, 20.88, 21.76, 24.81, 27.51, 34.70, 38.41, 46.18, 47.93, 48.69, 53.58, 55.73, 59.84, 63.36, 68.97, 72.21, 75.86, 102.17, 115.45, 116.25, 117.62, 119.04, 121.38, 126.52, 127.44, 127.71, 128.24, 131.36, 138.78, 139.96, 143.96, 145.28, 147.61, 157.28, 157.30, 163.55, 174.51. Anal. ($C_{20}H_{24}N_2O_2 \cdot C_{19}H_{23}NO_5 \cdot H_2O$) C, H, N. The filtrate was evaporated under reduced pressure, and the residue, taken up in dichloromethane (270 mL), was washed with 1 N HCl (270 mL), dried, and concentrated to give the desired enantiomerically enriched acid **3** (13.5 g, 70% ee, (5μ-Chiral-AGP 4 × 100 mm, 1.2 mL/min isopropyl alcohol/water/10 mM (pH 6.5) phosphate buffer 30/920/50, UV 280 nm)) as an oil: IR (neat) 699, 737, 825, 1064, 1453, 1556, 1594, 1712, 1952 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.67 (t, 3H), 1.88 (m, 2H), 2.76 (d, 1H), 2.98 (d, 1H), 3.85 (s, 3H), 4.53 (s, 2H), 4.76 (s, 2H), 5.75 (s, 1H), 7.04 (d, 1H), 7.31 (m, 5H), 8.03 (d, 1H), 12 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 8.27, 34.66, 46.09, 53.79, 63.31, 72.22, 76.11, 116.44, 117.49, 127.63, 127.86, 128.40, 138.77, 145.58, 156.66, 163.58, 172.79. A crystal of the quinidine salt of **4** was analyzed on a CAD4 (Enraf-Nonius, Bohemia, NY) spectrometer using Cu Kα emission, and the structure was solved and refined with the SHELXS86 and SHELXL93 (Sheldrick, G.M., University of Gottingen, Germany) least-squares algorithms. The resulting three-dimensional structure revealed compound **4** to be a 3*S*-3-hydroxy-pentanoic acid and, by deduction, compound **3** to be a 3*R*-3-hydroxy-pentanoic acid.

(5*R*)-5-Ethyl-1,4,5,8-tetrahydro-5-hydroxy-oxepino[3,4-*c*]pyridine-3,9-dione (7). To a solution of acid **3** (13.5 g, 39 mmol) in methanol (90 mL) was added 10% palladium on charcoal (50% wet, 28 g), and the resulting suspension was degassed and treated under nitrogen with a solution of ammonium formate (11.5 g, 183 mmol) in methanol (135 mL). The reaction mixture was stirred at room temperature for 30 min, then heated to 40 °C for 30 min, and allowed to cool to room temperature. The supported catalyst was removed by filtration, and the filtrate was evaporated under reduced pressure. Removal of residual traces of methanol was achieved by the addition of toluene (40 mL) and evaporation under reduced pressure. The resulting oil was dissolved in dry THF (50 mL) and treated with dicyclohexylcarbodiimide (7.2 g, 34.5 mmol) in THF (20 mL), and the solution was heated at 50 °C for 1 h before cooling to room temperature. The urea byproduct was removed by filtration, and the filtrate was concentrated under reduced pressure. The oily residue was taken up in dry acetonitrile (46 mL), treated with sodium iodide (6 g, 40.5 mmol) and trimethylsilyl chloride (5.2 mL, 40.5 mmol), and stirred for 5 h at room temperature. The reaction mixture was then diluted with acetonitrile (28 mL) and water (5.6 mL) to give a precipitate which was collected by filtration and taken up in water (10 mL). The resulting suspension was neutralized to pH 7.5 with ammonia, and the precipitate was collected by filtration and dried under reduced pressure to give the desired product (4.2 g, 34% yield, 77% ee (Chirosebond Cl-5μ 4.6 × 250mm, 1 mL/min dichloromethane/isopropyl alcohol 88/12, UV 300 nm)) as an off-white solid. Recrystallization from acetone (40 mL) and water (150 mL) provided enantiomerically pure pyridone **7** (3g, 99.7% ee) as white crystals: mp >250 °C; $[\alpha]^{D}_{20} = +125$ (*c* 0.154, DMSO); IR (KBr) 812, 1041, 1181, 1284, 1530, 1563, 1626, 1746 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.77 (t, 3H), 1.66 (m, 2H), 3.12 (dd, 2H), 5.24 (dd, 2H), 5.65 (br, 1H), 6.30 (d, 1H), 7.29 (d, 1H), 11.59 (br, 1H); ^{13}C NMR

(DMSO-d_6) δ 8.23, 35.89, 42.44, 61.15, 72.84, 105.02, 122.73, 133.86, 155.78, 161.31, 172.04. Anal. ($C_{11}H_{13}NO_4$) C, H, N.

Fluorinated hCPTs. The following compounds were prepared by coupling under Mitsunobu conditions of **7** and the appropriate fluorinated hydroxymethylquinoline, followed by an intramolecular Heck reaction, according to the general method previously reported for racemic compounds.[6] It was checked by HPLC on chiral stationary phase that the tertiary alcohol did not epimerize.

(5*R*)-5-Ethyl-11-fluoro-5-hydroxy-4,5,13,15-tetrahydro-1*H*,3*H*- oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (8a): mp >250 °C; IR (KBr) 815, 1205, 1612, 1629, 1660, 1734 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.86 (q, 2H), 3.07 (d, 1H), 3.46 (d, 1H), 5.28 (s, 2H), 5.40 (d, 1H), 5.53 (d, 1H), 6.03 (s, 1H), 7.43 (s, 1H), 7.55 (t, 1H), 7.85 (q, 1H), 8.01 (d, 1H), 8.82 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.35, 36.40, 42.49, 50.86, 61.37, 73.25, 100.13, 111.50 (d), 118.30 (d), 123.12, 124.80 (d), 125.41 (d), 130.28 (d), 130.50, 144.19, 148.79, 153.89, 155.83, 157.55 (d, *J* 253 Hz), 159.15, 171.93. Anal. ($C_{21}H_{17}FN_2O_4\cdot0.25H_2O$) C, H, N.

(5*R*)-5-Ethyl-10-fluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (8b): mp > 250 °C; IR (KBr) 828, 1201, 1508, 1585, 1651, 1754 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3H), 1.83 (q, 2H), 3.05 (d, 1H), 3.47 (d, 1H), 5.26 (s, 2H), 5.39 (d, 1H), 5.52 (d, 1H), 6.05 (s, 1H), 7.38 (s, 1H), 7.78 (m, 1H), 7.96 (d, 1H), 8.21 (m, 1H), 8.65 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.47, 36.50, 42.53, 50.70, 61.44, 73.35, 99.70, 111.90 (d), 120.72 (d), 122.77, 129.00 (d), 130.95, 131.24 (d), 131.96 (d), 144.49, 145.28, 152.61 (d), 155.92, 159.23, 160.46 (d, *J* 247 Hz), 172.11. Anal. ($C_{21}H_{17}FN_2O_4\cdot0.5H_2O$) C, H, N.

(5*R*)-5-Ethyl-9-fluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′ :6,7]indolizino[1,2-*b*]quinoline-3,15-dione (8c): mp > 250 °C; IR (KBr) 837, 1226, 1505, 1587, 1653 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3H), 1.84 (q, 2H), 3.04 (d, 1H), 3.47 (d, 1H), 5.24 (s, 2H), 5.39 (d, 1H), 5.52 (d, 1H), 6.06 (s, 1H), 7.39 (s, 1H), 7.65 (t, 1H), 7.89 (d, 1H), 8.23 (dd, 1H), 8.72 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.35, 36.41, 42.51, 50.56, 61.39, 73.24, 99.97, 112.47 (d), 118.03 (d), 122.91, 125.35, 129.62 (d), 131.29 (d), 131.94, 144.33, 148.99 (d), 153.78, 155.83, 159.16, 162.93 (d, *J* 249 Hz), 171.95. Anal. ($C_{21}H_{17}FN_2O_4$) C, H, N.

(5*R*)-5-Ethyl-8-fluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (8d): mp > 250 °C; IR (KBr) 763, 1211, 1508, 1613, 1659, 1731 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H), 1.87 (q, 2H), 3.08 (d, 1H), 3.47 (d, 1H), 5.29 (s, 2H), 5.40 (d, 1H), 5.53 (d, 1H), 6.06 (s, 1H), 7.44 (s, 1H), 7.69 (m, 2H), 7.96 (m, 1H), 8.75 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.34, 36.37, 42.44, 50.62, 61.38, 73.29, 100.03, 114.52 (d), 123.00, 124.61 (d), 127.72 (d), 129.70 (d), 131.16, 131.70, 138.06 (d), 144.27, 153.08, 155.93, 157.45 (d, *J* 255 Hz), 159.15, 171.95. Anal. ($C_{21}H_{17}FN_2O_4\cdot0.25H_2O$) C, H, N.

(5*R*)-5-Ethyl-9,11-difluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (8e): mp 227 °C (dec); IR (KBr) 854, 1245, 1505, 1638, 1748 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.85 (q, 2H), 3.07 (d, 1H), 3.46 (d, 1H), 5.26 (s, 2H), 5.40 (d, 1H), 5.52 (d, 1H), 6.03 (s, 1H), 7.42 (s, 1H), 7.73 (t, 1H), 7.81 (d, 1H) 8.82 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.32, 36.07, 42.49, 50.79, 61.34, 73.21, 100.43, 103.33 (dd), 109.29 (dd), 115.81 (d), 123.38, 125.28 (d), 130.19, 143.87, 148.79 (dd), 155.03, 155.80, 158.23 (dd, *J* 255, 15 Hz), 159.09, 162.20 (dd, *J* 248, 14 Hz), 171.90. Anal. ($C_{21}H_{16}F_2N_2O_4\cdot0.25H_2O$) C, H, N.

(5*R*)-5-Ethyl-9,10-difluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (8f): mp > 250 °C; IR (KBr) 871, 1261, 1512, 1579, 1654, 1746 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.85 (m, 2H), 3.08 (d, 1H), 3.44 (d, 1H), 5.26 (s, 2H), 5.39 (d, 1H), 5.52 (d, 1H), 5.99 (s, 1H), 7.38 (s, 1H), 8.14 (dd, 1H), 8.23 (dd, 1H), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.23, 36.33, 42.50, 50.54, 61.33, 73.14, 99.80, 114.32 (d), 115.30 (d), 122.87, 125.38 (d), 130.42, 131.32, 144.09, 145.24 (d), 149.58 (dd, *J* 250, 16 Hz), 151.81 (dd, 253, *J* 16 Hz), 153.32, 155.77, 159.05, 171.80. Anal. ($C_{21}H_{16}F_2N_2O_4\cdot0.75H_2O$) C, H, N.

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