ORGANIC LETTERS

2000
Vol. 2, No. 1
33−36

Outer-Ring Stereochemical Modulation of Cytotoxicity in Cephalostatins[1]

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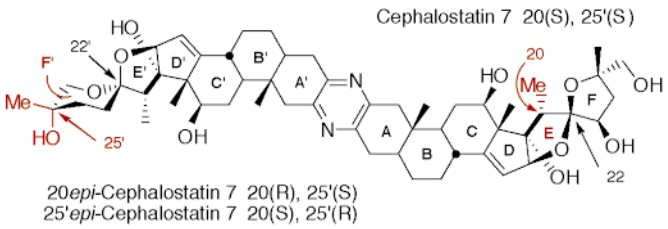
Received October 16, 1999

ABSTRACT



20- and 25′-epimers of cephalostatin 7, prepared by directed unsymmetrical pyrazine synthesis, address outer-ring topographical and stability questions and intimate an oxacarbenium ion rationale for the role in bioactivity of the spiroketal (E/F, E′/F′) rings of this class of antitumor agents.

The cephalostatins[1,2] and ritterazines[3] comprise a family of 45 structurally unprecedented marine products with extreme cytotoxicity against human tumors. Cephalostatin 1 (**1**) and ritterazine B display ∼1 nM GI_{50}s in two-day tests in the NCI 60-line screen and 10^{-14} M GI_{50}s in six-day tests in the Purdue 6-line minipanel.[4] We recently reported total syntheses of **1**[5a] via directed unsymmetrical pyrazine formation[5b] and cephalostatin 7 (**2**) in biomimetic fashion.[5c] Nearly 50 analogues have been disclosed along with partial SAR rationales.[3−6,7] The mechanism of action of the bissteroidal pyrazines, apparently shared by monosteroidal glycosides such as OSW-1,[8] remains unknown. Beside a steroidal platform, critical features implicated in the pharmacophore include a set of covalently linked polar and nonpolar domains and the spiroketals (or C22 equivalent).[2−8]

Criteria for the latter seem unclear beyond their evident necessity for high activity. Pending identification of a mechanism or binding site, empirical studies on the type, substituent effects, and reactivity of steroidal spiroketals may facilitate emergence of an intelligible SAR pattern. A role as a network of hydrogen bond donors/acceptors has been envisioned.[9] E-Ring oxacarbenium ions (E-ox.) have been proposed as active intermediates relating the OSW and cephalostatin classes,[10] and F-ring ions (F-ox.) merit consideration.[7] We therefore sought to illuminate relevant parameters for the outer rings of cephalostatin cytotoxins.

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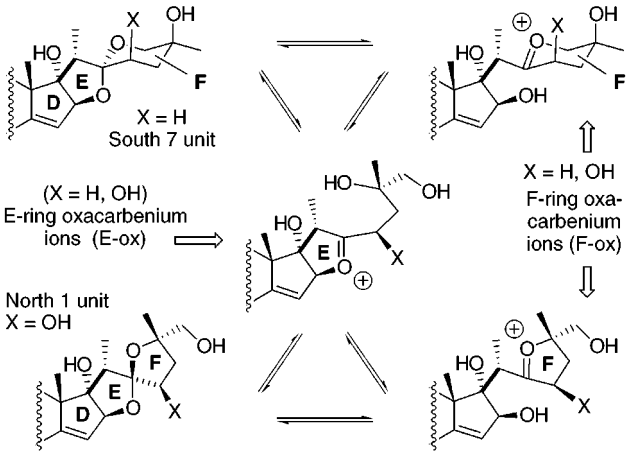
10.1021/ol991153y CCC: $19.00
Published on Web 12/15/1999

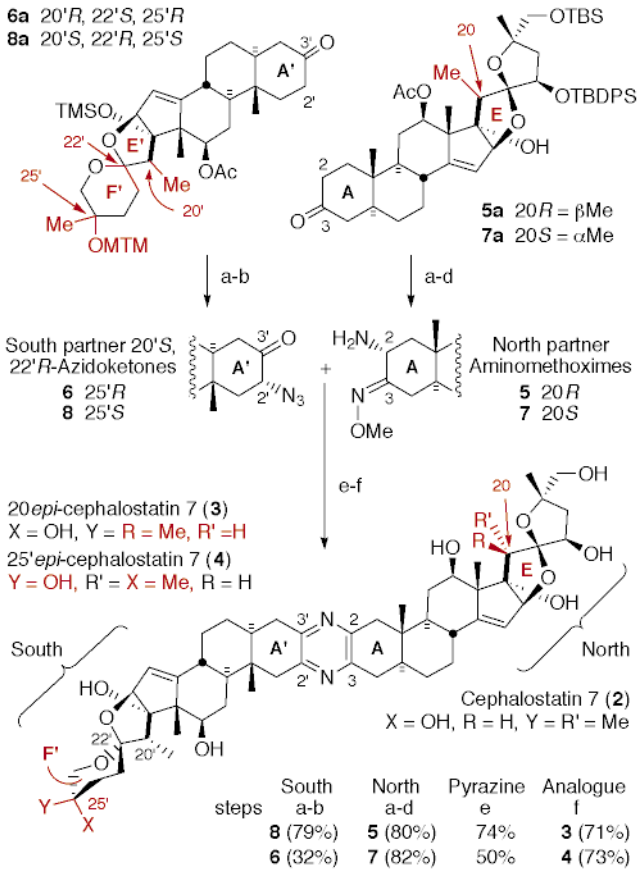


Limited information regarding stereochemical effects on activity appear among C22 epimeric ritterazines.[3,4] To examine the impact of E- and F-ring stereochemistry on activity, we designed analogues **3** and **4** with modified units 20-*epi*-North 1 (**5**) and 25′-*epi*-South 7 (**6**), derived from isomers **5a** and **6a** obtained during syntheses of the North 1 (**7**)[11a] and South 7 (**8**)[11b] units, respectively.

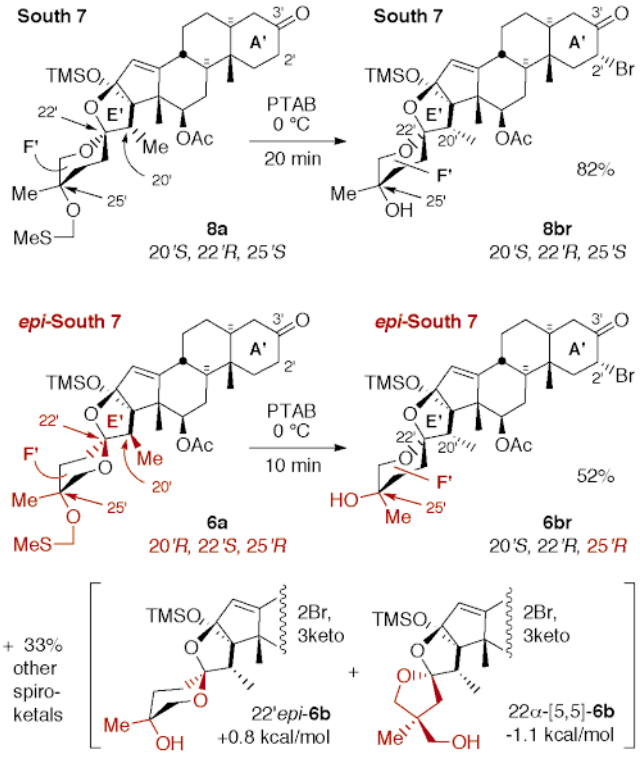
Coupling partners **5**−**8** (Scheme 1) were approached from the 3-ketosteroids in the usual manner.[5,11] Bromination of **5a**−**8a** and azide substitution gave 2α-azido-3-ketones (**6** and **8** were utilized as such). Subsequent methoxime formation and azide reduction afforded epimeric 2α-amino-3-methoximes **5** and **7**. Analogues 20-*epi*-cephalostatin 7 (**3**) and 25′-*epi*-cephalostatin 7 (**4**) ascended from these partners via our protocol for unsymmetrical pyrazine synthesis (catalytic Sn^{2+}, benzene, 80 °C) followed by routine deprotection.

The 20-*epi*-North 1 series **5a-5** proved as stable to all manipulations as North 1 **7a-7**, but the 25′-*epi*-South 7 series **6a-6** betrayed a promiscuous bent. Bromination of **8a** effected concomitant 25′-OMTM deprotection to give **8br** with complete spiroketal fidelity, and azide substitution proceeded in near quantitative yield.[11b] By contrast, wanton **6a** surrendered to C20′/22′ isomerization and bred further 5,6- and 5,5-spiroketal progeny, with several likely candidates lying close in energy to **6br**. Similar material losses accompanied the azidation and coupling steps.

Scheme 1. Synthesis of Analogues **3** and **4**



(a) PTAB; (b) TMGA; (c) MeONH₃Cl; (d) PPh₃/H₂O; (e) cat. Bu₂SnCl₂, 4Å sieves, PVP; (f) (i) TBAF, (ii) K₂CO₃.



Analogues **3** and **4** were comparatively evaluated in the National Cancer Institute's 60-line panel of human tumors alongside parent **2** and benchmark **1**. Cytotoxicity data for **2**−**4** is summarized in Tables 1 and 2.[12] Surprisingly, inversion at either C20 (as in the North 1 unit E-ring of **3**) or at C25′ (as in the South 7 unit F′-ring **4**) similarly diminished the activity. In addition to a 5−100-fold increase

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(12) (a) Boyd, M. R. In *Anticancer Drug Development Guide*; Teicher, B., Ed.; Humana Press: Totowas, NJ, 1997; p 23. (b) GI_{50}s were normalized to those of the benchmark cephalostatin 1 (**1**, average 1.1 nM).

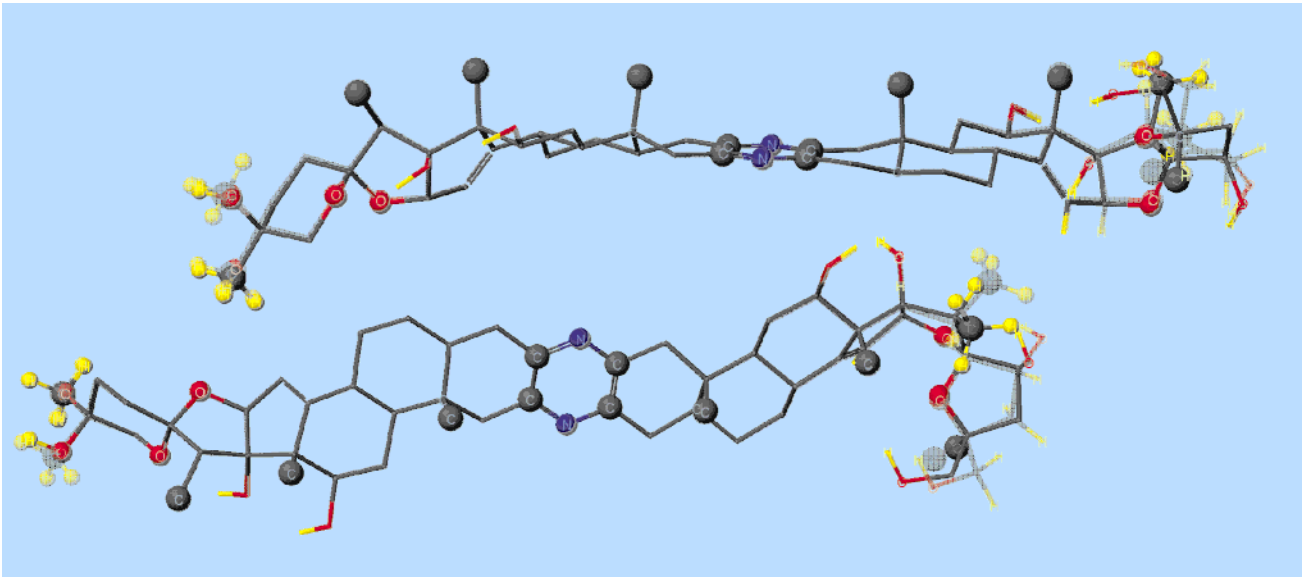


Figure 1. Overlays of **4** and **3** with **2**. Top: edge view of the pyrazine core and F-rings; **4** in bold, **3/2** in shadow. Bottom: canted top view of the pyrazine core providing a view of the F′-rings as chairs, an edge view of the D/E bicyclic moiety, and bottom view of the F-rings; **3** in bold, **2/4** in shadow.

in many GI_{50}s relative to **2**, the number and kinds of tumor lines affected by **3** and **4** was considerably reduced, and in strikingly similar fashion. The possibility that mere coincidence explained such consequences in rather different variants of **2** seemed remote.

Polarity matching or functionality alteration rationales do not apply here, while topographical changes in **3** and **4** relative to **2** seem unlikely to bear responsibility for their similar loss of activity. No conformational difference between **2** and **4** (essentially superimposable) was evident in molecular models[13] (Figure 1). In **3**, minor changes arose in the E/F-ring conformations and disposition of the F-ring substituents relative to **2** and **4** to accommodate an outward (not really *endo*, which the 13Me prevents) orientation of the 20βMe. Little added hindrance to the F-ring oxygen and greater access to 17OH was evident in **3**, but the opposite obtained in **4**. A simple explanation based on hydrogen bonding therefore also appears untenable.

Table 1. Cytotoxicity (GI_{50} NM)[12] of **2**−**4** vs Representative Human Tumors (NCI-60) and Activity Factor[a]

	2	**3** (20-*epi*-**2**)	**4** (25′-*epi*-**2**)
leukemia: MOLT-4	16.5	195	177
lung: HOP-92	56.1	204	234
colon: HCT-116	24.5	427	380
CNS: SF-295	10.7	251	93
breast: MCF-7	4.8	112	98
skin: SK-MEL-2	120	>1000	>1000
ovary: IGROV1	468	>1000	>1000
renal: 786-0	7.9	186	120
prostate: PC-3	18.6	229	339
overall	49 (±15)	>420[b]	>400[b]
a.f. (activity factor)[a]	0.97	0.80	0.78

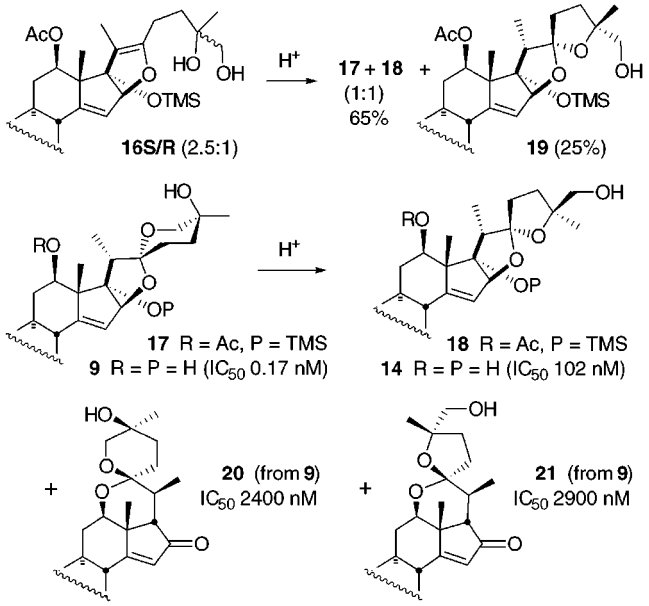
[a] Fraction of cell lines indicating a measurable $GI_{50} \leq 10^{-6}$ M. [b] Lower limit only; insufficient lines affected for a true average.

One might assume that hampered protonation of the F′-(F)-ring oxygens in **4**(**3**) relative to **2** would retard formation of an active E′(E)-ox., accounting for the loss of activity. However, stereoelectronics suggest that the axial lone pair of O26′ (F′-ring oxygen) in South 7 units, the one "more hindered" in **4**, should be kinetically less basic than either the equatorial or O16′ (E′-ring) lone pairs, as it is aligned for participation into the O16′−C22′ antibonding orbital.[14] Moreover, the 25′-*epi*-South 7 series **6a-6** proved far more labile than the already acid-sensitive South 7[5a,11b] series **8a-8**. We note that 25(S) precursor **16S** cyclized to 5,6- and 5,5-spiroketals **17** and **18** (1:1), while **16R** gave only the 5,5-spiroketal **19**, in accord with mechanics[13] relative energies (Scheme 2).[11b] Facile diversion of **6a-6** during bromination (0 °C, HBr coproduct) to spiroketals analogous to **19**, with further losses during azidation (25 °C, weak acid, polar solvent) and coupling (Sn^{2+}, molecular sieves, higher temperature), are telling. A kinetic protonation argument thus seems inadequate.

The sapogenin 5,6-spiroketal is destabilized by 20βMe, 22βO, or 25βMe arrangements, which usually facilitate ring opening reactions and equilibration to a more stable form.[15] Related 20βMe and 22βO consequences apply to 5,5-systems.[5a] Calculations predict similar destabilization of highly oxygenated cephalostatin subunit spiroketals, with experimental verification available in most cases.[3,5,11,16]

We have noted that the most active compounds feature at least one spiroketal in a less stable form.[5a] Members with a higher energy spiroketal form often display cytotoxicities superior to otherwise identical or related members with lower energy variants. For instance, ritterazine B **9** (22β) is calculated (mechanics)[13] to lie 2 kcal/mol above less active

Scheme 2. Some E-ox. and F-ox. Mediated Spiroketal Chemistry



ritterazine F **10** (22α). The modified units in (similarly) *less* active **3** and **4** are calculated to lie (dissimilarly) 2.7 and 1.3 kcal/mol, respectively, *above* those of the "parent" units of **2**, dispelling this simple SAR notion.

Access to both kinds of oxacarbenium ions in South 7 type units has been indicated experimentally. Facile isomerizations of **17** to **18**[11b] and of natural products ritterazine B **9** to ritterazine C **14**[17] likely proceed via E′-ring oxacarbenium ions. Interestingly, **14** was much less potent against P388 than **9** although they could ultimately afford the same E-ox. Head-to-head testing of ritterazines in the NCI panel was performed[12] as for **2**−**4** and confirms a corresponding large drop in potency against human cancers for "analogue" **14** vs "parent" **9** (Figure 2, Table 2). The intermediacy of an F′-ox. or corresponding protonated 22′-ketone is consistent with production of the even less cytotoxic translocated spiroketals **20** and **21** upon longer exposure of **9** to the influence of acid in polar solvent.[3]

If spiroketal-furnished oxonium or oxacarbenium ions contribute to the activity of these antitumor agents, we speculated that some indication thereof might appear in their energies. Since the relative stabilities of neutral compounds which could eventually achieve the same ion failed to correlate with bioactivity, semiempirical calculations were carried out on the charged species. Initial results suggest that equipotent **3** and **4** suffer equally diminished E-ox. access ($\Delta\Delta H_f$ +3 kcal/mol) compared to that of **2**. This relationship, if general, should prove valuable for interpreting the SAR of this class and help guide further work aimed at elucidating the mechanism of action and/or binding site.

We conclude that epimeric cephalostatins **3** and **4** provide useful new SAR clues further implicating a role for the spiroketals. These and a number of other bissteroidal pyrazines isomerized at outer-ring postions display intriguing relative cytotoxicities not amenable to explanation by any simple topography, hydrogen bonding, protonation, or spiroketal stability arguments. A rationale based on the currently remaining viable postulate, namely modulated access to oxacarbenium ions, is under active exploration to determine its generality and potential utility.

Table 2. Cytotoxicities (nM) of **2**−**15** vs P388 (Mouse Leukemia, ED_{50})[1,3] or Human (NCI-60, GI_{50}, and Activity Factor)[12] Tumors

	2	**9**	**10**	**11**	**12**	**13**	**14**	**15**
P388	$<10^{-4}$	0.17	0.81	0.81			102	
NCI60	49	3.2			$>46^b$	$>150^b$	$>116^b$	12
a.f.[a]	0.97	0.98			0.90	0.88	0.77	0.98

[a,b] Notes to activity as in Table 1.

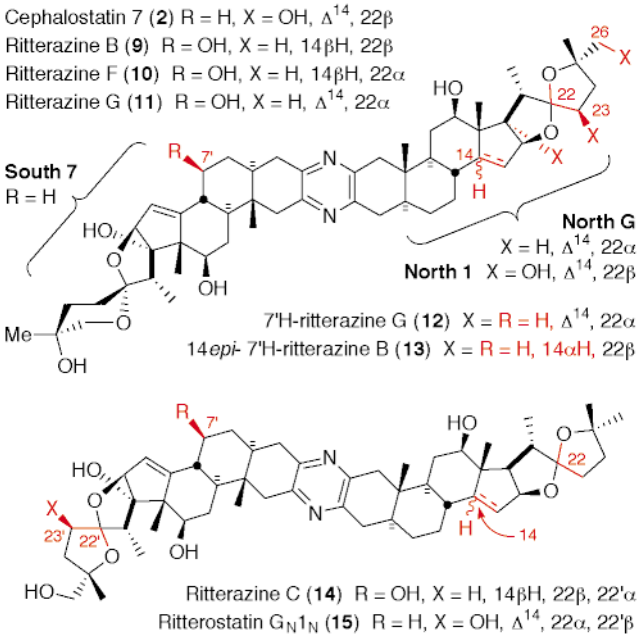


Figure 2. Other related epimeric or isomeric cytotoxins.

Acknowledgment. We warmly thank Professors Fusetani and Matsunaga for samples of ritterazines B and C for comparative testing and Proctor & Gamble (T.L. fellowship) and the NIH (CA 60548) for funding.

Supporting Information Available: Experimental and spectral details for **3**−**8**, NMR comparisons, and bioactivity tables for **1**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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