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AN EXCEPTIONALLY POTENT ANALOG OF SANDRAMYCIN

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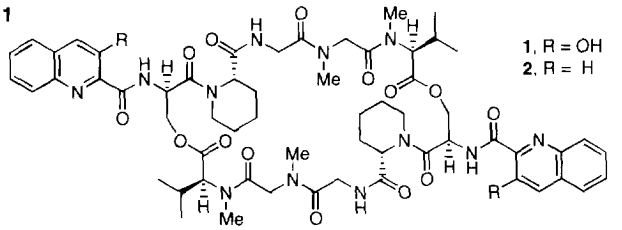
Abstract. The preparation and preliminary evaluation of **2**, an analog of sandramycin, are detailed. Although **2** was typically 4–10× less potent than sandramycin against leukemia cell lines, it was found to be 1–10,000× more potent against melanomas, carcinomas, and adenocarcinomas exhibiting typical IC_{50} values of 200–1 pM and placing it among the most potent agents identified to date. © 1997 Elsevier Science Ltd.

Sandramycin (**1**), a potent antitumor antibiotic[1] structurally characterized through spectroscopic and chemical degradation studies,[2] constitutes one of the newest members of a growing class of cyclic decadepsipeptides including luzopeptins A-C and E_2,[3] quinaldopeptin[4] and quinoxapeptins A and B[5] which possess potent antitumor, antiviral, and antimicrobial activity (Figure 1).[3-5] Characteristic of this class of agents, sandramycin possesses a two fold axis of symmetry and two heteroaromatic chromophores that results in sequence-selective DNA bis-intercalation spanning two base-pairs preferentially at 5′-AT sites.[6-9] In this respect, the agents are functionally related to the quinoxaline antitumor antibiotics,[10] including echinomycin and triostin A which also bind to DNA by bis-intercalation but with a substantially different sequence selectivity (5′-CG versus 5′-AT).[11,12]

Figure 1

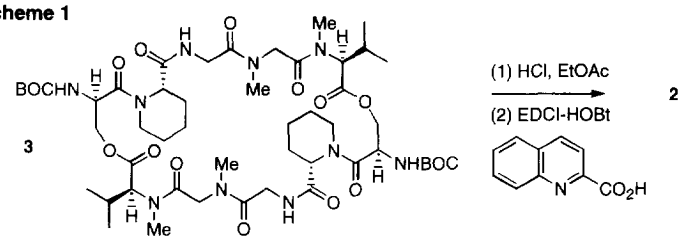


We recently reported a convergent total synthesis of sandramycin in which the heteroaromatic chromophores were introduced in the final stages.[6] This not only provided sufficient quantities of the natural product to define its DNA binding properties, but also provided the key partial structures lacking one or both of the pendant chromophores. This strategy has now been extended to the preparation of a series of analogs[13] differing only in the structure of the pendant chromophore and, as described herein, the identification of **2** as an exceptionally potent cytotoxic agent (Figure 1).

Preparation of 2. The synthesis of **2**[14] required *N*-BOC deprotection of **3**[6] ($[\alpha]_D^{23}$ –53 (*c* 1.5, $CHCl_3$), 3 M HCl–EtOAc, 25 °C, 30 min) and coupling of the resulting bis amine with quinoline-2-carboxylic acid (4 equiv of EDCI, 6.0 equiv of HOBt, 10 equiv of $NaHCO_3$, DMF, 25 °C, 48–72 h, 76–94%), Scheme 1.

In Vitro Cytotoxic Activity. In preceding studies, sandramycin (**1**) and luzopeptin A were established to exhibit comparable cytotoxic potencies, luzopeptin A was determined to be 100–300× more potent than

Scheme 1



echinomycin, and the potency of the luzopeptins smoothly declined in the series A > B > C.[6] Removal of one chromophore from sandramycin reduced the cytotoxic potency 500–1000× and **3** in which both chromophores of sandramycin were removed was inactive.[6] These latter studies established the essential role of the chromophores for observation of the potent cytotoxic activity. The examination of **2** (Table 1) further established this important and remarkable role. Simply removing the chromophore acidic phenol from sandramycin provided an exceptionally potent cytotoxic agent. In the leukemia cell lines, it was found that sandramycin was typically 4–10× more potent than **2** with the notable exception of HL-60 where **2** was unusually potent. In the remaining cell lines, **2** was found to be equipotent or more potent than sandramycin (1–10,000×). Thus, the removal of the acidic phenol from the chromophore of sandramycin provided an agent that is substantially more potent than the natural product in a range of tumor cell lines. This cytotoxic potency is exceptional and typically **2** exhibits IC_{50} values in the 200–1 pM range placing it among the most potent agents defined to date.

Table 1. In vitro cytotoxic activity, IC_{50} (nM)

cell line	sandramycin	**2**
L1210 (mouse leukemia)	0.02	0.2
Molt-4 (human T-cell leukemia)	0.7	3
HL-60 (human promyelomic leukemia)	80	0.001
B16 (mouse melanoma)	0.4	0.06
SK-MEL28 (human melanoma)	1	0.001
M24-MET (human metastatic melanoma)	0.4	0.04
BT-549 (human breast carcinoma)	1	0.01
MCF-7 (human breast carcinoma)	300	0.04
OVCAR-3 (human ovarian carcinoma)	4	1
PC-3 (human prostate carcinoma)	0.2	0.001
SIHA (human cervix carcinoma)	0.3	0.001
786-O (human perirenal carcinoma)	8	0.01
H322 (human lung adenocarcinoma)	20	10
UCLA-P3 (human lung adenocarcinoma)	10	0.002
HT-29 (human colon adenocarcinoma)	1	0.01

Although many explanations may account for such observations, it is not due to discernible differences in the relative DNA binding affinity or selectivity of **2** and sandramycin.[13] Rather, it may likely be due simply to removal of the acidic phenol providing better target delivery without adversely affecting the DNA binding properties of the agent. These and related issues are under investigation and will be reported in due course.

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14. (*N*-(Quinolinyl-2-carbonyl)-D-Ser-Pip-Gly-Sar-NMe-Val)$_2$ (Serine Hydroxyl) Dilactone (**2**): white powder: R_f = 0.38 (10% CH_3CN–EtOAc); $[\alpha]_D^{23}$ −139 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.42 (d, 2H, *J* = 6.3 Hz), 8.53 (d, 2H, *J* = 4.6 Hz), 8.29 (s, 4H), 7.97 (d, 2H, *J* = 8.3 Hz), 7.87 (dd, 2H, *J* = 1.0, 8.3 Hz), 7.72 (ddd, *J* = 1.4, 7.1, 8.3 Hz), 7.60 (ddd, 2H, *J* = 1.0, 7.1, 8.3 Hz), 5.56 (d, 2H, *J* = 7.1 Hz), 5.55 (d, 2H, *J* = 16.3 Hz), 5.31 (d, 2H, *J* = 6.3 Hz), 4.97 (dd, 2H, *J* = 1.5, 12.0 Hz), 4.86 (d, 2H, *J* = 11.0 Hz), 4.46 (dd, 2H, *J* = 2.9, 12.0 Hz), 4.43 (dd, 2H, *J* = 4.5, 12.8 Hz), 4.08 (m, 2H), 4.04 (d, 2H, *J* = 16.8 Hz), 3.76 (d, 2H, *J* = 13.3 Hz), 3.55 (d, 2H, *J* = 16.3 Hz), 3.14 (s, 6H), 2.93 (s, 6H), 2.03 (d split septet, 2H, *J* = 6.6, 11.0 Hz), 1.82–1.52 (m, 12H), 0.92 (d, 6H, *J* = 6.6 Hz), 0.78 (d, 6H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7, 169.3, 169.1, 167.7, 166.9, 163.8, 149.4, 146.6, 137.3, 129.9, 129.7, 129.4, 128.7, 127.8, 118.8, 62.7, 62.0, 52.4, 50.8, 49.3, 43.8, 41.9, 34.9, 30.4, 28.8, 26.3, 24.9, 20.2, 19.4, 18.8; IR (KBr) ν_{max} 3328, 2939, 1743, 1669, 1636, 1497, 1425, 1286, 1136, 1015, 847, 777 cm^{-1}; FABHRMS (NBA) *m/z* 1189.5685 ($M + H^+$, $C_{60}H_{76}N_{12}O_{14}$ requires 1189.5682).

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