Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1019–1020

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A New Class of Highly Cytotoxic Diketopiperazines

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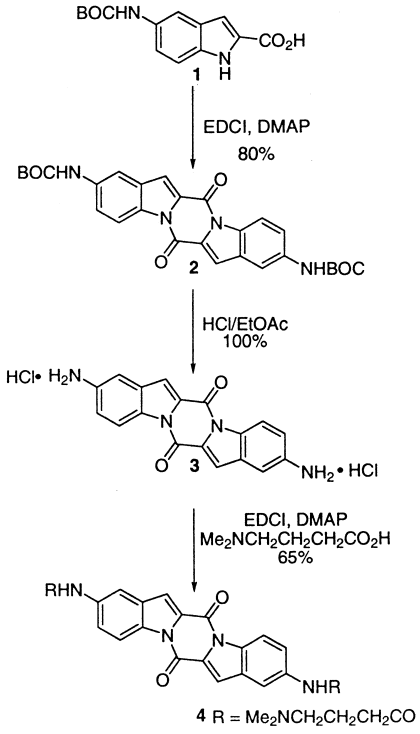
Received 1 February 2000; accepted 25 February 2000

Abstract—The discovery of a novel class of diketopiperazines possessing potent cytotoxic activity is described.

DNA binding/alkylating agents that exhibit selective cytotoxic activity are commonly used as therapeutics for the treatment of cancer. During the course of preparing a combinatorial library of DNA minor groove binding agents, a novel class of diketopiperazines possessing potent cytotoxic activity and DNA binding affinity was discovered.[1] Herein is reported the discovery, synthesis and biological properties of these agents.

Synthesis

During the course of preparing a library of heterocyclic dipeptides by solution phase parallel synthesis,[1] the indole carboxylic acid **1**[1] was found to provide the diketopiperazine **2**, rather than the expected dipeptide (Scheme 1). Further elaboration of this core unit through BOC deprotection (4.0 M HCl/EtOAc, 25 °C, 3 h) afforded the free diamine **3**, which was converted to the more water soluble bis(dimethylamino)diketopiperazine **4** (EDCI/DMAP, 25 °C, 24 h). Along with the core diketopiperazine, two extended agents were prepared in order to probe the possibility of developing more extended agents with increased biological activity and/or better DNA binding properties. Because of the low solubility of the diketopiperazine core itself and to facilitate the characterization of intermediates, an alternative route was employed for these agents that constructed the core diketopiperazine at the final step allowing the isolation of more soluble intermediates at each step (Scheme 2). The aminoindole **5**[1] was coupled to two different aromatic amino acids used extensively in the preparation of previously reported DNA binding agents to afford **6** and **7**. BOC deprotection (4.0 M HCl/EtOAc, 25 °C, 2 h) and coupling to 4-(dimethylamino)butyric acid



Scheme 1.

afforded the two amines **8** and **9**. Hydrolysis of the esters (LiOH, 1:1 MeOH/H_2O, 60 °C, 4 h) and treatment of the crude acids with EDCI/DMAP afforded the two extended diketopiperazines **10** and **11**.

Biological Activity

The diketopiperazines were assayed for cytotoxic activity using a standard L1210-cell based cytotoxicity assay

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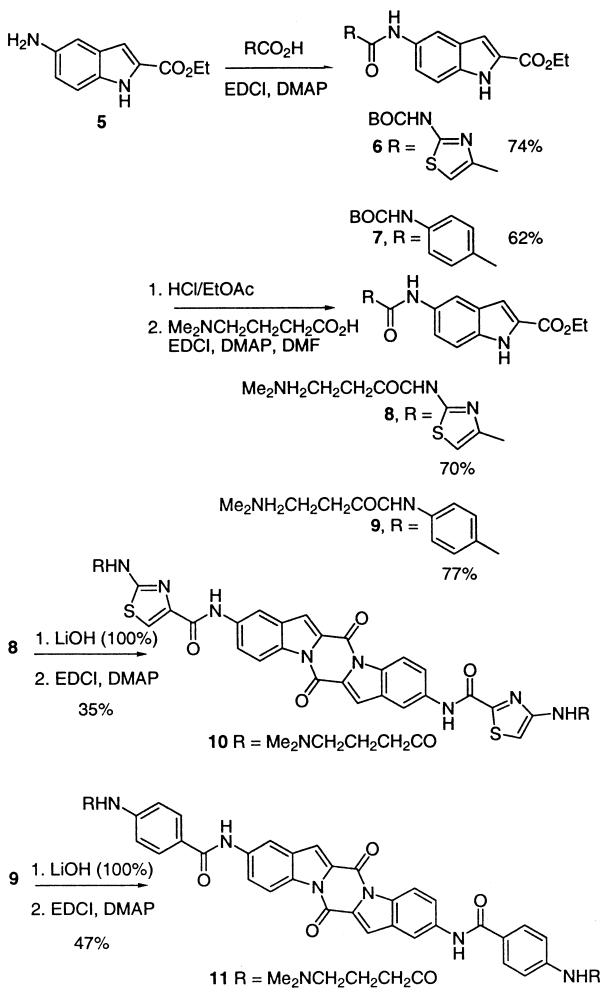

Scheme 2.

(Table 1). While both the BOC-protected and free amino derivatives **2** and **3** as well as the two extended agents **10** and **11** showed low activity, $IC_{50}=29$, 30, 4, and 7 μM, respectively, the dimethylamino compound **4** showed remarkably potent activity, $IC_{50}=34$ nM.

Table 1. Cytotoxic activity and DNA binding affinity

	IC_{50} (nM) (L1210)	K ($\times 10^5$)[a] poly[dA]-poly[dT]
2	29,000	ND[b]
3	30,000	ND
4	34	2.03
10	4000	NA[c]
11	7000	NA

[a]Binding constants calculated from $K=K$etbr[EtBr]/[Agent].
[b]Not determined due to poor solubility of agent.
[c]Demonstrated no decrease in fluorescence up to 10 mM.

While the striking difference in cytotoxic activity may be in part due to the low solubility of **2** and **3** compared to **4**, it may also arise from the increased affinity of **4** for the polyanionic backbone of DNA. Analysis of the DNA binding properties of **4**, **10** and **11** using quantitative titration with displacement of prebound ethidium bromide showed that only **4** bound to DNA to an appreciable extent (Table 1).[2,3]

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA78045), Novartis, and the award of a NIH postdoctoral fellowship (BEF, F32 CA76718, 1998–2000)

References and Notes

1. Boger, D. L.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* submitted for publication.
2. Boger, D. L.; Invergo, B. J.; Coleman, R. S.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. *Chem–Biol. Interact.* **1990**, *73*, 29. Boger, D. L.; Sakya, S. M. *J. Org. Chem.* **1992**, *57*, 1277.
3. Spectroscopic data. **2**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.62 (s, 1H), 8.30 (d, 1H, $J=8.9$ Hz), 8.06 (s, 1H), 7.76 (s, 1H), 7.56 (d, 1H, $J=8.9$ Hz), 1.50 (s, 9H). **4**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.23 (s, 1H), 8.41 (d, 1H, $J=9.2$ Hz), 8.34 (d, 1H, $J=1.9$ Hz), 7.87 (s, 1H), 7.70 (dd, 1H, $J=8.8$, 1.9 Hz), 2.45 (t, 4H, $J=7.4$ Hz), 2.30 (s, 6H), 1.84 (pent, 2H, $J=7.2$ Hz). FABHRMS (DHB) m/z 543.2716 ($M+H^+$, $C_{30}H_{34}N_6O_4$ requires 543.2720. **6**: ^{1}H NMR (acetone-d_6, 500 MHz) δ 9.39 (s, 1H), 8.24 (s, 1H), 7.86 (s, 1H), 7.58 (dd, 1H, $J=8.8$, 1.8 Hz), 7.50 (d, 1H, $J=8.8$ Hz), 4.34 (q, 2H, $J=7.4$ Hz), 1.52 (s, 9H), 1.35 (t, 3H, $J=7.0$ Hz). **7**: ^{1}H NMR (DMF-d_7, 500 MHz) δ 10.30 (s, 1H), 9.48 (s, 1H), 8.33 (s, 1H), 7.80 (d, 2H, $J=11.1$ Hz), 7.65 (s, 2H), 7.47 (d, 2H, $J=11.0$ Hz), 3.78 (s, 3H), 1.23 (s, 9H); FABHRMS (NBA) m/z 412.1519 ($M+H^+$, $C_{21}H_{21}N_3O_6$ requires 412.1519). **8**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.92 (s , 1H), 7.74 (s , 1H), 7.35 (m , 2H), 7.06 (s, 1H), 4.28 (d, 2H, $J=7.0$ Hz), 2.42 (m, 4H), 2.25 (s , 6H), 1.83 (pent, 2H, $J=7.4$ Hz), 1.30 (t, 3H, $J=7.0$ Hz). **9**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.97 (δ, 1H, $J=1.8$ Hz), 7.93 (d, 2H, $J=12.4$ Hz), 7.73 (d, 2H, $J=11.0$ Hz), 7.50 (dd, 1H, $J=2.5$, 11.4 Hz), 7.48 (d, 1H, $J=11.0$ Hz), 7.15 (s, 1H), 4.38 (q, 2H, $J=7.4$ Hz), 2.52 (t, 2H, $J=7.6$ Hz), 2.48 (t, 2H, $J=7.4$ Hz), 2.36 (s, 6H), 1.92 (pent, 2H, $J=7.6$ Hz), 1.41 (t, 3H, $J=7.0$ Hz). **10**: ^{1}H NMR (CF_3CO_2D, 500 MHz) δ 8.78 (br s, 2H), 8.43 (br s, 2H), 8.15 (br s, 2H), 7.80 (br s, 4H), 3.68 (br s, 4H), 3.35 (s, 16H), 2.62 (br s, 4H); FABHRMS (DHB) m/z 795.2495 ($M+H^+$, $C_{38}H_{38}N_{10}O_6S_2$ requires 795.2495). **11**: ^{1}H NMR (DMF-d_7, 500 MHz) δ 8.60 (m, 12H), 8.02 (s, 2H), 7.46 (s, 2H), 2.87 (t, 4H, $J=7.6$ Hz), 2.78 (s, 12H), 2.68 (t, 4H, $J=7.1$ Hz), 2.32 (pent, 4H, $J=7.0$ Hz); FABHRMS (DHB) m/z 781.3455 ($M+H^+$, $C_{44}H_{44}N_8O_6$ requires 781.3462).