Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1850–1854
Bioorganic & Medicinal Chemistry Letters

Darpones and water-soluble aminobutoxylated darpone derivatives are distinguished by matrix COMPARE analysis

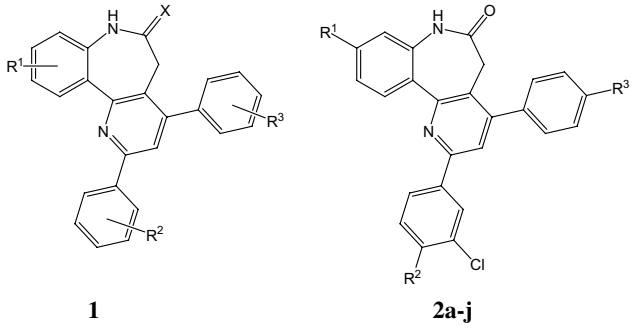
Christian Prühs and Conrad Kunick*

Technische Universität Braunschweig, Institut für Pharmazeutische Chemie, Beethovenstraße 55, D-38106 Braunschweig, Germany

Received 1 December 2006; revised 12 January 2007; accepted 13 January 2007
Available online 24 January 2007

Abstract—Darpones are a class of compounds with antiproliferative activity for cancer cells in vitro and in mouse models. In order to improve the solubility of the compounds, darpones with aminobutoxy side chains were synthesized. The new derivatives showed retained antiproliferative activity for cultured cancer cell lines. However, a change of the selectivity pattern in the in vitro cell line screening project of the American National Cancer Institute indicates that the solubilized derivatives might act through a different biological mechanism. A matrix COMPARE analysis of the cancer cell line screening data clearly distinguished darpones with and without solubilizing aminobutoxy side chains.

The darpones (general formula 1, Fig. 1) constitute a class of compounds[1,2] which have demonstrated antiproliferative activity both in the in vitro cell line screening project (IVCLSP)[3–5] of the National Cancer Institute (NCI) and in vivo in the murine hollow fiber tumor model.[6] Structure–activity relationship (SAR) studies have shown that for the antiproliferative activity the secondary lactam structure and both aryl substituents in 2- and 4-position on the basic heterocyclic ring system are required.[7] The molecular mechanism of the antiproliferative activity of the title compounds is still unknown. An isolated remark in the literature reported the observation that the darpone 682765 (general formula **1**, R^1, R^3 = H, R^2 = 4′-Cl, X = 0) showed a correlation with the expression of the EGFR ligand TGF-α in the cancer cell line panel of the IVCLSP.[8]



1 **2a-j**

Figure 1. General formula **1** of darpones (X = O, S) and modified darpones **2a**–**j**. For definition of R^1–R^3, see Table 1.

Up to date, a straightforward development of the darpone class is not only hampered by the lack of knowledge regarding the biological targets but also by their low water solubility. We therefore designed and synthesized water-soluble darpone derivatives by attaching aminobutoxy side chains to the darpone basic structure **2a**.

The parent template **2a** for structure modification was chosen to meet three requirements: simple synthesis, several options for structure modifications, and a maximum 'darpone-like' behavior in the IVCLSP. In the IVCLSP a test compound is characterized by three parameters for approximately 60 human cancer cell lines: (1) the GI_{50} value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), (2) the TGI value (TGI, molar concentration of the compound leading to total inhibition of net cell growth), and (3) the LC_{50} value (LC_{50} = molar concentration of the compound leading to 50% net cell death). Based on these data mean values are calculated indicating the growth inhibitory activity averaged over all cell lines. The mean-graph midpoint $\log_{10}GI_{50}$ (MG-MID $\log_{10}GI_{50}$) is the mean of all 60 $\log_{10}GI_{50}$ values for the tumor cell lines

Keywords: Antiproliferative activity; Darpones; Compare analysis; Solubility.
* Corresponding author. Tel.: +49 531 391 2754; fax: +49 531 391 2799; e-mail: c.kunick@tu-braunschweig.de

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.01.043

from the panel. The inhibition profile ('fingerprint') resulting from the selectivity of a compound over the 60 cell lines is typical for the molecular mechanisms of the growth inhibition. The analysis of such a pattern is an established method to identify mechanisms or molecular targets underlying growth inhibitory activity[9–11] and might be used to discover prototypes of novel anticancer drugs.[12] The characteristic pattern generated by darpones is clearly different from the patterns of established antitumor drugs.[2] A frequently used tool for the comparison of inhibition profiles in the IVCLSP is COMPARE, a program which generates pairwise correlation coefficients (PCC) between compounds.[5,13] In the 'matrix COMPARE' mode the tool is suitable to detect relationships between a multitude of antiproliferative compounds and to group compounds with similar biological mechanisms. Employing 'matrix COMPARE' for the 44 darpone entities included in the NCI database of compounds tested in the IVCLSP revealed many high pairwise intercorrelations within the compound family (Fig. 2). Successive deletion of darpones with comparatively low correlations from the matrix ended with a collection of 15 darpones showing exclusively pairwise correlations with PCCs between 0.4 and 0.9. Of these, compound **2a** (R^1, R^2, R^3 = H) was selected as parent structure for aminoalkyl-substituted derivatives because of the additional two criteria given above. Since the molecular darpone targets are unknown and side chains at the wrong position were expected to prevent binding between the darpone derivative and its target, the solubilizing aminoalkoxy side chains were attached to three different positions around the darpone molecule (represented by R^1, R^2, R^3 in formula **2**, Fig. 1).

The synthesis of the new darpone derivatives is exemplified in Scheme 1 for the preparation of **2g**, **2j**, and **2m**. In the first step, the cyclic ketone **3** is reacted with an appropriately substituted chalcone **4** in the presence of lithium hydroxide in THF yielding the Michael adduct **5**. The latter is cyclized under oxidative conditions by heating with ammonium ferric sulfate and ammonium acetate in glacial acetic acid to furnish the darpone **2d**.[1] After BBr_3-induced ether cleavage[14] the resulting phenol **6** is treated with an excess of 1,4-dibromobutane. The so obtained bromo derivative **7** is reacted with morpholine or *N*-methylpiperazine, respectively, to give tertiary amines which are converted to the hydrochlorides **2j** and **2m** by treatment with hydrochloric acid. The primary amine **2g** is obtained by a classical Gabriel synthesis procedure in which the bromo derivative **7** is reacted with the potassium salt of phthalimide in DMSO and subsequent cleavage of the resulting phthalimide derivative by hydrazinolysis. The other methoxy- and aminobutoxy-substituted darpones of Table 1 were prepared following similar procedures starting with educts bearing the methoxy group at appropriate positions.

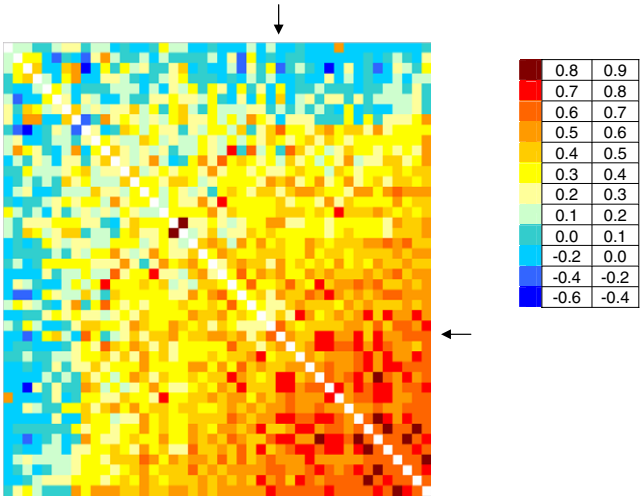
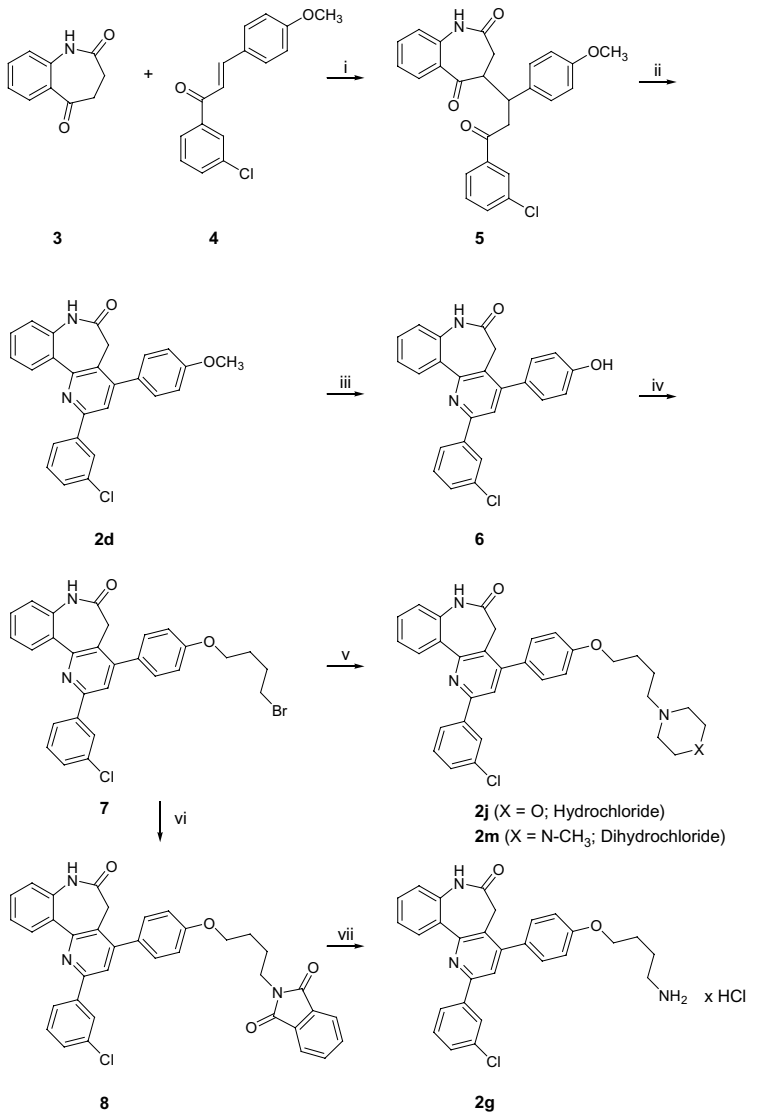


Figure 2. Matrix COMPARE analysis of the 44 darpones in the NCI database of compounds tested in the IVCLSP. Darpones are sorted from left to right and from top to bottom by increasing 'darpone-likeliness' (the cumulative pairwise correlation coefficient (PCC) with all other darpones). Individual PCCs are color coded (For PCC limits refer to color code legend). The arrows indicate the data lines of compound **2a**.

The determination of solubility in phosphate buffer (pH 4.5) revealed poor solubility (<0.005 gL^{-1}) of the primary amine hydrochlorides **2e**–**2g**. In contrast, the hydrochlorides of the morpholino derivative **2j** and the dihydrochlorides of the *N*-methylpiperazino compounds **2k** and **2m** showed good solubility (>0.5 gL^{-1}). Obviously, not only the attachment position of the solubilizing side chains but also the structure of the amino moiety has a high impact on solubility. For instance, while the compound **2g** with an aminobutoxy side chain R^3 shows low solubility, the corresponding morpholinobutoxy analog **2j** is much better soluble. The cancer cell line screening in the IVCLSP revealed that all compounds with aminobutoxy side chains retained antiproliferative activity (Table 1). With the exception of compound **2f** these derivatives exhibited an averaged growth inhibition by single-digit micromolar concentrations. This bioactivity is comparable to that of the parent compound **2a**.

Of note, the aminobutoxy side chain at R^1 enhances the potency of the compounds. This becomes obvious by the comparison of the methoxy compound **2b** and its aminobutoxy analogs **2e**, **2h**, and **2k**. The latter are one order of magnitude more potent as antiproliferative agents. In contrast, no such enhancement is found for the two other sets of compounds which bear the solubilizing side chains at R^2 or R^3, respectively. Another interesting feature of the test results is that the aminobutoxy-substituted derivatives seem to show a changed selectivity pattern in the IVCLSP. This shift is not obvious on first sight, but is disclosed by a matrix COMPARE analysis performed with the compounds **2b**–**2m** of Table 1 and the 15 most 'darpone-like' darpones from the NCI database (Fig. 3). The matrix COMPARE analysis assorted the darpones with and without basic side chains into two groups which are represented by the orange/yellow squares in the upper left and the lower right corner of the matrix. While the three chloro-methoxy-substituted darpones **2b**–**2d** (section B) still showed significant profile correlations with the most characteristic 15 known darpones (section A), no such correlation is found between darpones with (section C) and without



Scheme 1. Synthesis of darpones with aminoalkyl side chains. Reagents and conditions: (i) LiOH, THF, 25 °C, 2 h (65%); (ii) NH_4OAc, $NH_4Fe(SO_4)_2$, AcOH, Δ, 2 h (73%); (iii) (1) BBr_3, CH_2Cl_2, 25 °C, 2 h; (2) H_2O (61%); (iv) 1,4-dibromobutane, K_2CO_3, acetone, Δ, 2 h (58%); (v) (1) morpholine or *N*-methylpiperazine, DMF, 80 °C; (2) HCl, EtOH (35% and 28%, respectively); (vi) potassium phthalimide, DMSO, 90 °C, 30 min (59%); (vii) (1) H_2NNH_2, EtOH, Δ, 1 h; (2) NaOH, 1 h; (3) HCl (23%).

(sections A and B) aminobutoxy side chains (blue areas).Within the group of aminobutoxy-substituted derivatives clear correlations are found, regardless of the attachment position for the side chain. Two hypotheses can be deduced from these results: First, the introduction of basic side chains apparently modifies the mode of antiproliferative activity in the darpone compound class. Second, the attachment point of the basic side chain is not as relevant as initially considered. For instance, the *N*-methylpiperazinobutoxy derivatives **2k**, **2l**, and **2m** all exhibit averaged growth inhibition in the single-digit micromolar concentration range with selectivity for distinct cell lines, for example, the colon carcinoma cell line HCT-15. Consequently, it is questionable whether the antiproliferative activity of the darpones is resulting from a specific interaction with a particular target, because in this case it is likely that at least one subgroup of modified derivatives would have exhibited diminished growth inhibition. Another explanation for the changed selectivity pattern of the modified darpones is the possibility that the amino functions influence cellular uptake or cellular efflux transport mechanisms.

These results also have consequences for the determination of molecular drug targets by affinity approaches. Biologically active drugs decorated by linker chains with terminal amino groups (e.g., **2e**–**2g)** can easily be bound to solid matrices. These immobilized molecules then can be employed for the isolation of proteins that are the potential molecular targets of the basic drug molecule. In a number of cases, targets of antiproliferative and other drugs have been successfully identified by this methodology.[15,16] The findings with the darpones show that care has to be exercised when interpreting the results of affinity experiments, since the introduction of linker chains might substantially change the molecular target repertory.

Table 1. Antiproliferative activity of the darpones **2**

Entry[a] (NSC-number)	R^1	R^2	R^3	Solubility[b]	GI_{50} HCT-15[c] (μM)	GI_{50} MG-MID[d] (μM)
2a (684480)	H	H	H	n.t.	2.7/4.3	2.2/4.4
2b (737718)	Methoxy	H	H	n.t.	>100	22.39
2c (731691)	H	Methoxy	H	n.t.	3.0	3.3
2d (731692)	H	H	Methoxy	n.t.	1.9	2.4
2e (737724)	Aminobutoxy × HCl	H	H	(−)	1.8	2.2
2f (737723)	H	Aminobutoxy × HCl	H	(−)	13.8	13.8
2g (737722)	H	H	Aminobutoxy × HCl	(−)	6.6	6.3
2h (740585)	Morpholinobutoxy × HCl	H	H	(++)	1.6	5.1
2i (740583)	H	Morpholinobutoxy × HCl	H	(+)	2.3	7.1
2j (740581)	H	H	Morpholinobutoxy × HCl	(++)	1.2	5.1
2k (740586)	*N*-Methylpiperazinobutoxy × 2HCl	H	H	(++)	0.81	1.7
2l (740584)	H	*N*-Methylpiperazinobutoxy × 2HCl	H	(−)	0.87	4.0
2m (740582)	H	H	*N*-Methylpiperazinobutoxy × 2HCl	(++)	0.91	1.3

[a] NSC-number (NCI Identification number) in brackets. Two values indicate repeated testing.
[b] Solubility in phosphate buffer (pH 4.5) determined by HPLC; (−) indicates solubility <0.005 gL^{-1}; (+) indicates solubility between 0.005 gL^{-1} and 0.5 gL^{-1}; (++) indicates solubility >0.5 gL^{-1}; n.t., not tested, minimal solubility in aqueous systems.
[c] Colon carcinoma cell line.
[d] MG-MID, meangraph midpoint: averaged value of the concentrations needed for 50% growth inhibition over all cell lines in the IVCLSP.

Figure 3. Matrix COMPARE analysis of the 15 'most characteristic' darpone derivatives (A); the chloro-methoxy-substituted darpone derivatives **2b**, **2c**, **2d** (B), and the novel darpones with amine side chains **2e**–**2m** (C). Individual PCCs are color coded (for PCC limits refer to color code legend).

Acknowledgments

Funding of this project by the Deutsche Forschungsgemeinschaft (to C.P. and C.K., Grant Nos. KU1371/1-1 and KU1371/1-2) is gratefully acknowledged. We are grateful to the National Cancer Institute for testing the compounds in the IVCLSP and especially to Mark Kunkel and Dan Zaharevitz for introducing us into the Matrix COMPARE methodology.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.043.

References and notes

1. Link, A.; Kunick, C. *J. Med. Chem.* **1998**, *41*, 1299.
2. Link, A.; Zaharevitz, D. W.; Kunick, C. *Pharmazie* **1999**, *54*, 163.
3. Monks, A.; Scudiero, D. A.; Johnson, G. S.; Paull, K. D.; Sausville, E. A. *Anti-Cancer Drug Des.* **1997**, *12*, 533.
4. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
5. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
6. Hollingshead, M.; Alley, M.; Camalier, R.; Abbott, B.; Mayo, J.; Malspeis, L.; Grever, M. *Life Sci.* **1995**, *57*, 131.
7. Schultz, C.; Link, A.; Kunick, C. *Arch. Pharm. Pharm. Med. Chem.* **2001**, *334*, 163.
8. Wosikowski, K.; Schuurhuis, D.; Johnson, K.; Paull, K. D.; Myers, T. G.; Weinstein, J. N.; Bates, S. E. *J. Natl. Cancer Inst.* **1997**, *89*, 1505.

9. Rabow, A.; Shoemaker, R.; Sausville, E.; Covell, D. *J. Med. Chem.* **2002**, *45*, 818.
10. Keskin, O.; Bahar, I.; Jernigan, R.; Beutler, J.; Shoemaker, R.; Sausville, E.; Covell, D. *Anti-cancer Drug Des.* **2000**, *15*, 79.
11. Weinstein, J. N.; Myers, T. G.; O'Connor, P. M.; Friend, S. H.; Fornace, A. J., Jr.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sausville, E. A.; Zaharevitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. *Science* **1997**, *275*, 343.
12. Kunick, C. *Curr. Med. Chem. Anti-Cancer Agents* **2004**, *4*, 421.
13. Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
14. McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron* **1968**, *24*, 2289.
15. Bach, S.; Knockaert, M.; Reinhardt, J.; Lozach, O.; Schmitt, S.; Baratte, B.; Koken, M.; Coburn, S. P.; Tang, L.; Jiang, T.; Liang, D.-C.; Galons, H.; Dierick, J.-F.; Pinna, L. A.; Meggio, F.; Totzke, F.; Schaechtele, C.; Lerman, A. S.; Carnero, A.; Wan, Y.; Gray, N.; Meijer, L. *J. Biol. Chem.* **2005**, *280*, 31208.
16. Knockaert, M.; Wieking, K.; Schmitt, S.; Leost, M.; Grant, K. M.; Mottram, J. C.; Kunick, C.; Meijer, L. *J. Biol. Chem.* **2002**, *277*, 25493.