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Bioorganic & Medicinal Chemistry Letters 17 (2007) 5063–5067
Bioorganic & Medicinal Chemistry Letters

Cytotoxic small molecule dimers and their inhibitory activity against human breast cancer cells

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Received 18 May 2007; revised 3 July 2007; accepted 6 July 2007
Available online 13 July 2007

Abstract—Small molecules based upon natural product dimers that exhibit cytotoxic activity were synthesized and evaluated for their anti-proliferative activity in human breast cancer cell lines. A central isophthalic core structure linking aromatic amines containing 3,5-disubstitutions produced the most active compounds. This series of compounds was found to be more active against the estrogen receptor positive cell line MCF-7 than the estrogen receptor negative cell line, SKBr3.

Numerous proteins responsible for cell proliferation and differentiation exist either as hetero- or homodimers or become activated through dimerization as the initial step in their respective signaling cascade. For example, the receptor tyrosine kinases VEGF and PDGF are important cellular growth factors activated as homodimers by ligand binding.[1,2] Certain cytokines, including human growth hormone and erythropoietin, have been shown to bind simultaneously to two receptors and create a receptor–ligand–receptor complex.[3,4] The estrogen receptor isoforms ERα and ERβ form homo- or heterodimers upon ligand binding.[5–7] Hsp90, a molecular chaperone responsible for the maturation of numerous signaling proteins, exists predominantly as a cytosolic homodimer.[8] The existence of such dimeric proteins as therapeutic targets suggests that dimeric small molecules can be appropriate building blocks for disruption of these and related protein complexes.

Many natural product dimers and their analogues have been studied for cytotoxic activity (Fig. 1). Torreyanic acid, a quinone dimer, demonstrated cytotoxic activity in numerous human cancer cell lines and was determined to exhibit selective toxicity against cell lines that demonstrate susceptibility to agonists of protein kinase C.[9] The dicerandrols were isolated and characterized as 2,2′-dimeric tetrahydroxanthones that display potent cytotoxic activity against lung and colon cancer cell lines.[10] Coumermycin A1, a dimeric member of the coumarin family of antibiotics exhibits potent anti-proliferative activity in human breast cancer cell lines and structural analogues exhibit low micromolar anti-proliferative activity.[11,12] In addition to these representative examples of naturally occurring dimers, numerous groups have synthesized dimeric structures of natural products in an effort to simultaneously inhibit both monomeric units of a dimeric protein. Several series of artemisinin-derived trioxane dimers have yielded derivatives that demonstrate submicromolar anti-proliferative activity.[13–16] Geldanamycin dimers,[17] estrogen dimers,[18] and nonsteroidal bifunctional dimers[19] that mimic tamoxifen have also been prepared and retain potent anti-proliferative activity against various breast cancer cell lines. Based upon the dimeric nature of these compounds we sought to identify and optimize a simple scaffold that exhibits anti-proliferative activity against human breast cancer cells. In particular, we desired a small molecule dimer structurally related to the coumermycin A1 aglycon.

Coumermycin A1 (Fig. 1) is a natural product dimer that contains the general structure of novobiocin linked at the 2- and 4-positions of the central 3-methyl pyrrole bisester. We chose to prepare dimeric compounds that linked simplified amines mimicking the coumarin ring

Keywords: Dimers; Cytotoxic; Modulators; Inhibitors.
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doi:10.1016/j.bmcl.2007.07.014

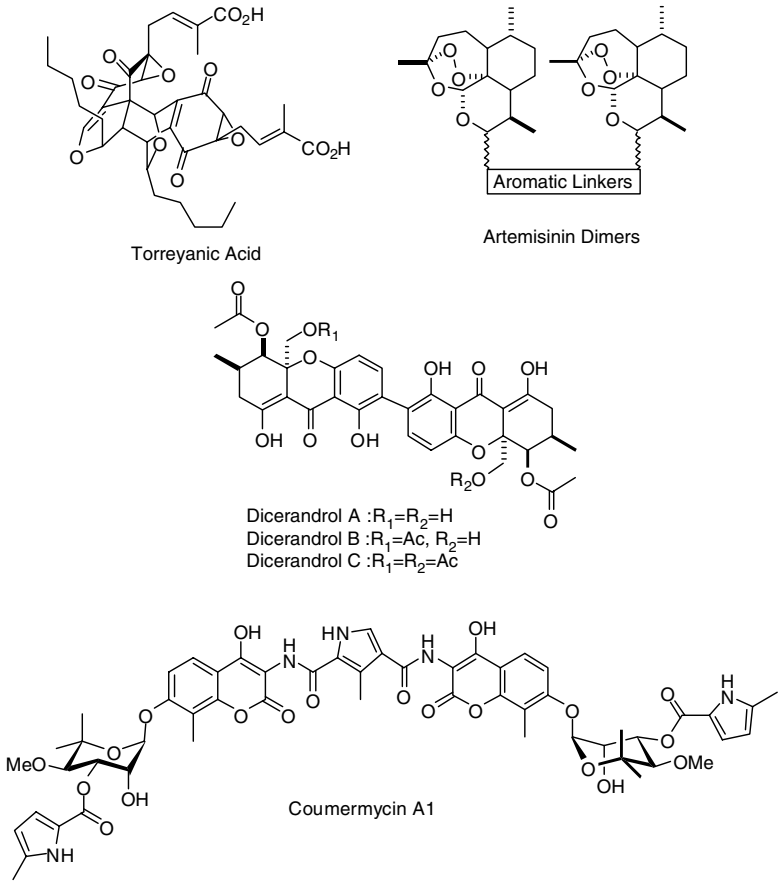


Figure 1. Structures of various natural product and synthetically prepared cytotoxic dimers.

of coumermycin A1 through 5- or 6-membered aryl rings that were closely related to that of the central pyrrole. The diacids and amines used in the preparation of these analogues were chosen to investigate the role of hydrogen bonding, lipophilicity, and steric features important for manifesting anti-proliferative activity.

The first series of dimeric analogues was synthesized in an effort to optimize the aromatic appendages connected to the central diacid. This series contained 2,5-pyridine dicarboxylic acid (**A**, Fig. 2) as the central unit and was coupled with a variety of amines that contained hydrophobic, hydrophilic, and hydrogen-bonding substituents (**DM1**–**DM17**). A second series contained 3,4-ethylenedioxyaniline (**1**, Fig. 3) and was coupled to various diacids that represented analogues of the core acid (**DM18**–**DM27**). These dimers were prepared using the protocol shown in Scheme 1.[20]

The analogues prepared via this protocol (**DM1**–**DM27**, Table 1) were tested for their anti-proliferative activity against two distinct human breast cancer cell lines, an estrogen receptor positive cell line, MCF-7, and an estrogen receptor negative, Her2 over-expressing cell line, SKBr3. The MTS cell proliferation kit (Promega) was utilized to determine cell viability following a 72-h incubation of the compound in each cell line as described previously.[21] In this assay, a novel tetrazolium dye (MTS) is bioreduced by dehydrogenase enzymes in viable cells into a soluble formazan product, the absorbance of which is directly proportional to the number of living cells present. Coumermycin A1 was used as a positive control in these assays and the IC_{50} values obtained were comparable to those previously reported.[12] Each dimer was incubated with the dye at the highest

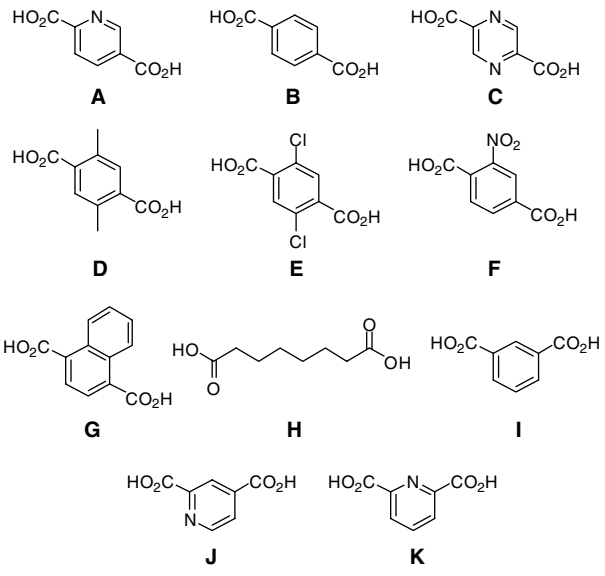


Figure 2. Diacids used for dimer synthesis.

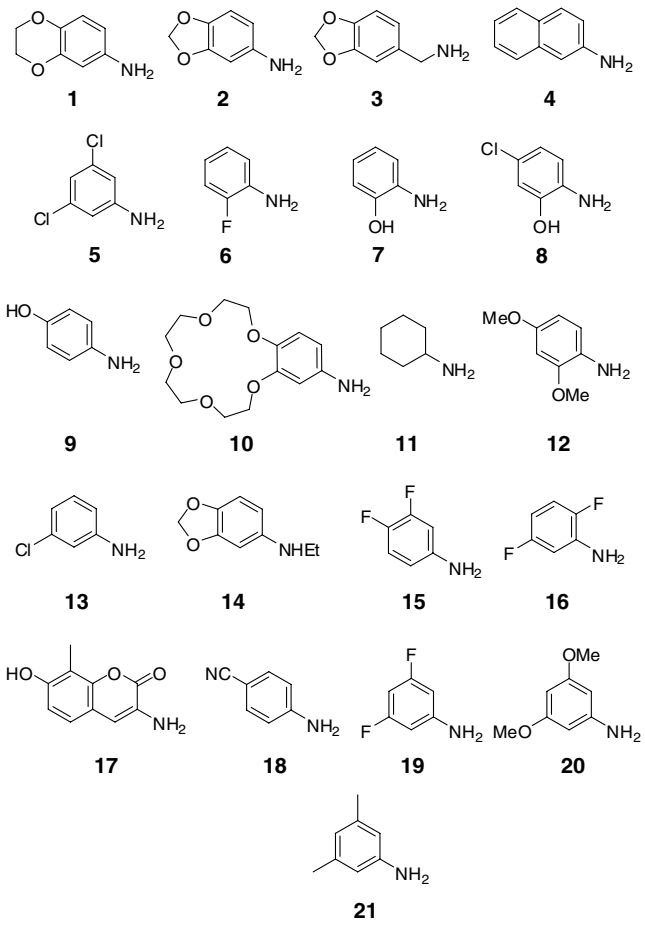
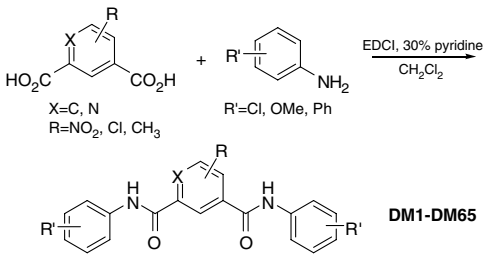


Figure 3. Amines used for dimer synthesis.

concentration tested in the anti-proliferative assays as a control for potential dimer-induced reduction.

Of this initial series that contained the 2,5-pyridine dicarboxylic acid as the central structure, only **DM5**, **DM10**, and **DM13** exhibited anti-proliferative activity in both cell lines (Table 1). The most active compound, **DM5** ($IC_{50} = 4.1$ and 2.3 μM in MCF-7 and SKBr3 cells, respectively), incorporated the 3,5-dichloroaniline side chains. **DM13**, which contained a single chlorine in the 3-position on the aniline, exhibited an ~7-fold reduction in anti-proliferative activity, suggesting a chlorine at each position is important for optimal activity.

Only one compound in the second series utilizing 3,4-ethylenedioxyaniline as the amine, **DM27**, exhibited



Scheme 1. General scheme for the synthesis of **DM1**–**DM65**.

Table 1. Anti-proliferative activity (μM) for the first two series of dimeric compounds

Compound	Diacid[a]	Amine[b]	MCF-7	SKBr3
Coumermycin A1	—	—	6.7 ± 0.2[c]	3.2 ± 1.5
DM1	A	1	>100	>100
DM2	A	2	>100	>100
DM3	A	3	>100	>100
DM4	A	4	>100	>100
DM5	A	5	4.1 ± 0.8	2.3 ± 0.1
DM6	A	6	>100	>100
DM7	A	7	>100	>100
DM8	A	8	>100	>100
DM9	A	9	>100	>100
DM10	A	10	7.2 ± 1.8	4.5 ± 1.6
DM11	A	11	>100	>100
DM12	A	12	>100	>100
DM13	A	13	27.3 ± 8.0	28.3 ± 1.1
DM14	A	14	>100	>100
DM15	A	15	>100	>100
DM16	A	16	>100	>100
DM17	A	17	>100	>100
DM18	B	1	>100	>100
DM19	C	1	>100	>100
DM20	D	1	>100	>100
DM21	E	1	>100	>100
DM22	F	1	>100	>100
DM23	G	1	>100	>100
DM24	H	1	>100	>100
DM25	I	1	>100	>100
DM26	J	1	>100	>100
DM27	K	1	27.5 ± 8.5	>100

[a] Diacid from Figure 2.
[b] Amines from Figure 3.
[c] Values are means ± standard error of two separate experiments performed in triplicate.

anti-proliferative activity in this assay. Interestingly, this compound was only active in MCF-7 cells, suggesting the potential for future development of this scaffold as a cell-type specific inhibitor.

Based on the anti-proliferative activity of **DM5**, we synthesized a third series of dimers that incorporated the most active amine (**5**) with varying diacids (**DM28**–**DM35**, Table 2) in an attempt to further optimize the central moiety. Interestingly, the active compounds observed in this series contained variations of isophthalic acid (**DM28**, **DM29**, and **DM35**), suggesting that

Table 2. Anti-proliferative activity (μM) for the third series of dimeric compounds

Compounds	Diacid[a]	Amine[b]	MCF-7	SKBr3
DM28	J	5	4.2 ± 1.3[c]	10.5 ± 2.3
DM29	K	5	10.2 ± 3.5	16.3 ± 6.5
DM30	E	5	>100	>100
DM31	D	5	>100	>100
DM32	B	5	>100	>100
DM33	H	5	>100	>100
DM34	G	5	>100	>100
DM35	I	5	2.7 ± 0.2	4.5 ± 0.2

[a] Diacid from Figure 2.
[b] Amines from Figure 3.
[c] Values are means ± standard error of two separate experiments performed in triplicate.

orientation of the anilines is important for the inhibition of cell growth. While the selectivity of **DM27** for MCF-7 cells was lost when the 3,4-ethylenedioxyaniline was replaced with aniline **5** (**DM29**), the overall anti-proliferative activity was increased ~3-fold. In addition, the three active dimers were 2- to 5-fold more active against the MCF-7 cell line.

Based upon the increased anti-proliferative activity associated with the central isophthalic core structure, we expanded the amines coupled to these diacids to give rise to a fourth series of dimers (**DM36**–**DM53**). As expected, this series contained numerous dimers with anti-proliferative activity (Table 3). Two anilines (**4** and **6**) that were inactive in the original series of dimers demonstrated anti-proliferative activity when coupled with isophthalic acid derivatives. In addition, the dimer containing 3,5-dichloroaniline coupled to isophthalic acid (**DM35**) was 25-fold more active than the corresponding dimer containing a single chlorine atom (**DM41**), further supporting the importance of substituents in both positions for optimal activity. All dimers in this series were more active against MCF-7 than SKBr3 cells, consistent with the trend observed with the previous generations of compounds.

A final generation of dimers that incorporated anilines with varying substituents at the 3- and 5-positions (**19**-**21**) coupled with the four active diacids (**A**, **I**–**K**) was synthesized and evaluated (Table 4). These anilines were chosen based upon the size, hydrogen bonding, and electronic capabilities of the moieties at these two positions. This series produced no discernible structure-activity relationships for the 3,5-disubstituted anilines. Only **DM65**, containing 3,5-dimethylaniline linked through isophthalic acid exhibited anti-proliferative activity (IC_{50} = 2.1 and 12.2 μM for MCF-7 and SKBr3, respectively) comparable to the most active compounds from the previous series. Because chlorine and methyl groups are generally considered isosteric,[22] these data suggest that the size of the substituents at these two positions is a determining factor for optimal activity.

Table 3. Anti-proliferative activity (μM) for the fourth series of dimeric compounds

Compounds	Diacid[a]	Amine[b]	MCF-7	SKBr3
DM36	J	4	81.8 ± 6.7[c]	>100
DM37	J	13	2.4 ± 0.4	9.0 ± 5.3
DM38	J	6	40.4 ± 3.2	87.7 ± 10.6
DM39	J	18	>100	>100
DM40	I	4	4.6 ± 2.4	15.2 ± 1.3
DM41	I	13	56.5 ± 0.2	>100
DM42	I	6	>100	>100
DM43	I	18	>100	>100
DM44	I	10	>100	>100
DM45	K	17	>100	>100
DM46	I	17	>100	>100
DM47	J	17	>100	>100
DM48	K	15	>100	>100
DM49	J	15	>100	>100
DM50	I	15	12.5 ± 1.6	>100
DM51	K	16	>100	>100
DM52	I	16	>100	>100
DM53	J	16	>100	>100

[a] Diacid from Figure 2.
[b] Amines from Figure 3.
[c] Values are means ± standard error of two separate experiments performed in triplicate.

Table 4. Anti-proliferative activity (μM) for the final series of dimeric compounds

Compounds	Diacid[a]	Amine[b]	MCF-7	SKBr3
DM54	K	19	>100[c]	>100
DM55	K	20	52.1 ± 3.5	>100
DM56	K	21	>100	>100
DM57	A	19	>100	>100
DM58	A	20	22.8 ± 5.0	34.9 ± 1.9
DM59	A	21	16.3 ± 1.5	104 ± 14
DM60	J	19	14.7 ± 0.6	>100
DM61	J	20	>100	>100
DM62	J	21	>100	>100
DM63	I	19	>100	>100
DM64	I	20	>100	>100
DM65	I	21	2.1 ± 0.8	12.2 ± 0.7

[a] Diacid from Figure 2.
[b] Amines from Figure 3.
[c] Values are means ± standard error of two separate experiments performed in triplicate.

In conclusion, a dimeric small molecule scaffold that displayed anti-proliferative activity was explored for the purpose of generating more efficacious compounds. The synthesis and evaluation of several generations of dimeric compounds provided structure–activity relationships for this series, indicating a central isophthalic core linking aromatic amines containing 3,5-disubstitutions is important for anti-tumor activity. Several compounds exhibited low micromolar activity and these dimers were generally more effective against MCF-7 cells, compared to SKBr3 cells.

Acknowledgment

The authors gratefully acknowledge support of this project by the NIH (R01 CA114393 and P50 GM069663).

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