Bioorganic & Medicinal Chemistry Letters 20 (2010) 6620–6623

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Tailored α-methylene-γ-butyrolactones and their effects on growth suppression in pancreatic carcinoma cells

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ARTICLE INFO

Article history:
Received 13 May 2010
Revised 3 September 2010
Accepted 7 September 2010
Available online 21 September 2010

Keywords:
Pancreatic cancer
Anti-proliferation
Alpha-methylene-gamma-butyrolactones
Allylboration
Parthenolide

ABSTRACT

A selected series of racemic α-methylene-γ-butyrolactones (AMGBL) were synthesized via allylboration and screened against three human pancreatic cancer cell lines (Panc-1, MIA PaCa-2, and BxPC-3). This systematic study established a discernible relationship between the substitution pattern of AMGBL and their anti-proliferative activity. β,γ-diaryl-AMGBLs, particularly those with a *trans*-relationship exhibited higher potency than parthenolide and LC-1 against all three cell lines.

Pancreatic adenocarcinoma is the fourth leading cause of cancer-related deaths in the United States.[1] An effective treatment for this cancer, with mortality nearly equal to incidence, remains a challenge. Medicinal chemists have profiled naturally occurring α-methylene-γ-butyrolactones (AMGBL),[2] such as sesquiterpene lactones, for their anti-cancer properties for several decades.[3] Among them, parthenolide, the active ingredient of feverfew (*Chrysanthemum parthenium*) (PT, Fig. 1), is currently actively pursued for the potential treatment of a variety of cancers.[4] It is believed that the Michael acceptor PT influences the intracellular redox pathways by interacting with exofacial thiols.[2,5] A recent study found that parthenolide does not have a global effect on all free exofacial thiols, but rather a specific effect on 12 kDa and 22 kDa proteins.[6] A novel combination therapy with arsenic trioxide and PT against pancreatic cancer cells has also been reported.[7] In addition, it has been shown that PT and the non-steroidal anti-inflammatory drug sulindac (Merck: Clinoril) cooperate to mediate growth suppression and inhibit the transcription factor nuclear factor-kappa B (NF-κB) pathway in pancreatic carcinoma cells.[8] The bio-available amino-derivative of PT, dimethylaminoparthenolide (LC-1),[9] is believed to undergo deamination via metabolic

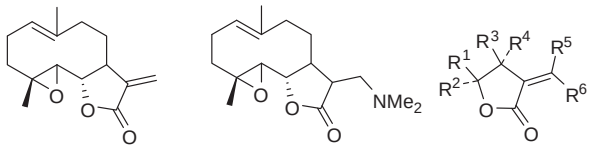

Figure 1.

N-oxidation, followed by Cope-elimination[10] to the bio-active PT. Moreover, PT as well as LC-1 have been shown to leave normal hematopoietic stem and progenitor cells apparently unharmed.[11] All of these have led to a renewed interest in synthetic AMGBLs[12,13] to understand their mode of action and possibly identify potent small molecules with anti-proliferative effects.

Our recently designed allylboration–cyclization protocol[14] to tailor a diverse set of regio- and stereospecific alkyl- and aryl-substituted AMGBLs provided a unique opportunity to evaluate them against carcinoma cells. The synthesis and structure–activity relationship (SAR) of a novel series of AMGBLs as growth inhibitors of three human pancreatic cancer cell lines (BxPC-3, Panc-1, and MIA PaCa-2) in comparison to PT and LC-1 are described herein. The data provide pointers on substitution patterns of AMGBL for cytotoxic activity.

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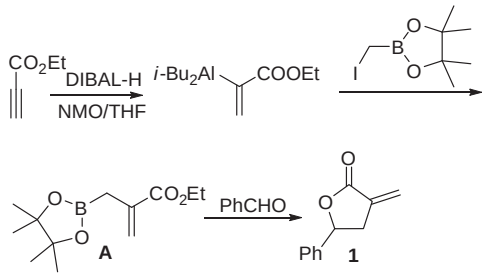
doi:10.1016/j.bmcl.2010.09.022

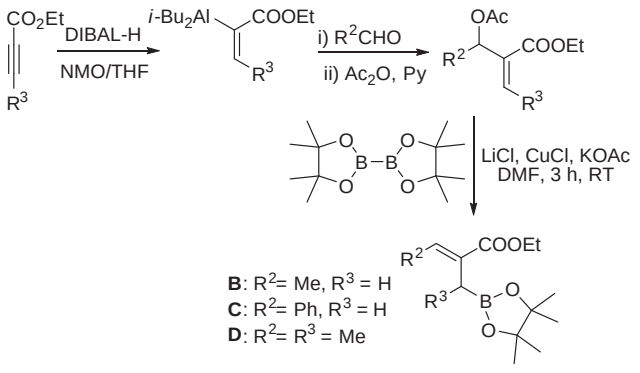


Scheme 1. Preparation of allylborating agent **A** and γ-phenyl-AMGBL **1**.

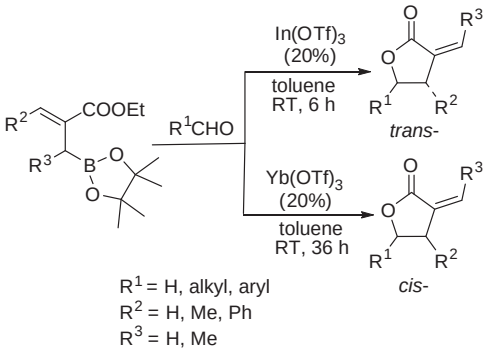
The parent allylborating agent **A** was prepared from (3-ethoxy-3-oxoprop-1-en-2-yl)diisobutylaluminum and iodomethylboronate as described by Villieras (Scheme 1).[15] The required vinylaluminum reagent was prepared from ethyl propiolate and diisobutylaluminum hydride according to our published protocol.[16] Reagents **B** and **C** were prepared as reported earlier[14] via the nucelophilic addition–elimination of borylcopper to acetates derived from alkenylalumination of appropriate aldehydes (R^2CHO) (Scheme 2). Reagent **D** bearing an α-methyl group (R^3 = Me) was prepared using a similar protocol starting with ethyl but-2-ynoate, followed by the treatment of the alkenylaluminum reagent with acetaldehyde ($R^2 = CH_3$), acetylation, and borylation.

The design of the AMGBLs with differing substitutions was on the basis of allylboration of appropriate aldehydes (R^1CHO) using reagents **A**–**D**. The required *cis* or *trans*-stereochemistry was achieved by the choice of either $Yb(OTf)_3$ or $In(OTf)_3$, respectively, to catalyze the allylborations (Scheme 3).[17] Pancreatic cancer cell lines, Panc-1, MIA PaCa-2, and BxPC-3 cells were plated in triplicate in 96-well plates. Twenty-four hours later, the cells were treated with 10 μM concentrations of lactones. After 72 h of treatment, cell growth was determined by the addition of the CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) reagent (Promega Inc., Madison, WI). Percent cell growth was determined relative to control treated cells (100%). If significant anti-proliferative activity was observed, the experiments were repeated with decreasing concentrations (1.0, 0.1, and 0.01 μM) of the lactones.

To elucidate the determinants of potency, initially, we investigated the importance of substitution at the β- and γ-positions of the AMGBL structure. While the γ-phenyl lactone **1**, prepared from reagent **A** and benzaldehyde, showed no cytotoxicity at 10 μM concentrations, the β-phenyl lactone **2**, prepared from **C** and formaldehyde, possessed activity at that concentration in all three cell lines, *albeit* less than parthenolide. This may be expected due to the increased Michael acceptor property attributed to the phenyl group β- to the methylene moiety in **2**.



Scheme 2. Preparation of allylborating agents **B**–**D** via alkenylalumination.



Scheme 3. Syntheses of *cis*- and *trans*-AMGBL via allylboration.

To ascertain the influence of the Michael addition, substitutions were introduced at both β- and γ-positions. Thus, β-phenyl-γ-cyclohexyllactone **3**, prepared from cyclohexanecarboxaldehyde and **C** was examined, which revealed that the substitution at the γ-position with an alkyl group had deleterious effect on the cytotoxic activity. The aryl and alkyl groups were swapped in **4**, prepared from benzaldehyde and **B**, and upon assay revealed increased activity at 10 μM concentration, for all the cell lines. Lactone **5**, bearing two aliphatic groups, prepared from isobutyraldehyde and **B**, showed no toxicity at 10 μM concentration, suggesting that an aryl group at the γ-position is important for activity, provided the β-position is also substituted. The cytotoxicity effect of AMGBL bearing an electron-donating (OMe, **6**) and electron-withdrawing (F_5, **7**) groups compared to the parent lactone **4** revealed higher potency for the lactone bearing the electron-donating group.

To further verify the effect of the Michael acceptor on the potency, the methylene group was replaced by an ethylidene group in **8** and **9**, which were prepared from 4-methoxy- and 4-nitrobenzaldehyde, respectively, using **D**. Unlike the methylene lactones, the alkylidene lactones failed to arrest cell growth at 10 μM concentration, probably a direct result of decreased Michael addition. The results of cell proliferation assay are summarized in Table 1. As can be seen from the table, altering the sterics and electronics at the γ-position by substituting phenyl with a 2-naphthyl group (**10**) provided an AMGBL with increased potency, similar to PT against all the three cell lines.

Following the trend, the substituent effects were further probed with AMGBLs bearing aryl substituents at both β-, and γ-positions. To this end, **11** was prepared from benzaldehyde using **C**. The assay revealed that the potency is enhanced by the presence of aromatic groups on the adjacent positions. Examination of the substituent's stereochemical effect with **12**, prepared with **C** and benzaldehyde under the influence of a strong Lewis acid,[17] showed that the *trans*-lactone is more potent than the *cis*-isomer, particularly against Panc-1 cell line. Again the electronic influence was examined with **13**, **14**, and **15**, which confirmed that the phenyl group bearing the electron-donating methoxy or methyl substituent provides a modest contribution to potency when compared to the electron-withdrawing trifluoromethyl group in **15**. Lactones **12**, **13**, and **14** showed increased anti-proliferative activity than parthenolide for all three cell lines even at 1 μM concentration.

Having identified that two aromatic groups at the β- and γ-positions are essential for increased potency, and coupling the results achieved with **10**, the effect of a 2-naphthyl group at γ-position was again examined by preparing **16** and **17** from 2-naphthaldehyde and **C**. Analysis showed that the *trans*-lactone **17** has superior activity compared to the *cis*-isomer **16** with IC_{50} ~1 μM concentration against all three cell lines.

Table 1
Inhibition of pancreatic cell lines by AMGBL[a]

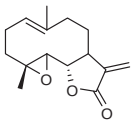
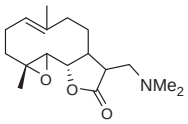
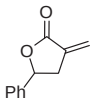
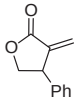
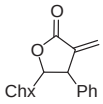
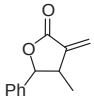
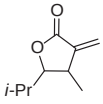
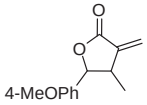
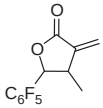
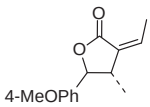
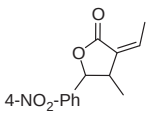
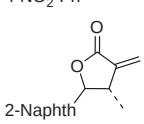
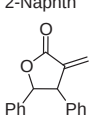
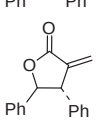
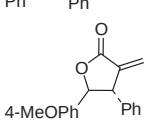
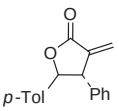
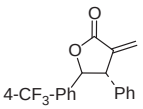
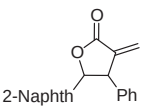
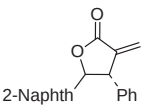
Lactone		% Cell growth			
#	Structure	Concn (μM)	Panc-1	MIA PaCa-2	BxPC-3
PT		10	12	3	6
		1	132	78	92
LC		10	17	2	7
		1	117	73	92
1		10	135	106	136
2		10	31	14	40
3		10	104	81	95
4		10	62	14	41
		1	143	114	98
5		10	119	143	153
6		10	32	9	13
		1	130	109	128
7		10	70	53	89
		1	86	120	98
8		10	139	124	179
9		10	106	110	117
10		10	22	4	1
		1	139	74	104
11		10	38	3	8
		1	108	32	51
12		10	1	1	1
		1	116	30	86
13		10	0	0	0
		1	80	54	73

Table 1 (*continued*)

Lactone		% Cell growth			
#	Structure	Concn (μM)	Panc-1	MIA PaCa-2	BxPC-3
14		10	0	2	0
		1	105	26	67
15		10	33	5	13
		1	152	142	138
16		10	33	6	27
		1	98	125	113
17		10	13	3	1
		1	53	29	60

[a] All cells were treated with the indicated lactones for 72 h and cell growth determined by a colorimetric proliferation assay. Percent cell growth is expressed relative to control cells (0), set equal to 100%. Mean was determined from at least two independent experiments performed in triplicate.

The concentration-dependency of the growth suppression for the three cell lines with the more potent lactones (**12**, **13**, **14**, and **17**) was compared with that of parthenolide and LC-1. As can be seen from Charts 1–3,[18] **12**, **14**, and **17** reveal greater antiproliferative activity relative to parthenolide, especially at the higher concentrations tested. Of the compounds tested, **12** appears to be the most effective in all three lines. In addition, although the compounds dose-dependently inhibit growth of all three pancreatic cancer cell lines, PaCa-2 cells are the most responsive to treatment.

The effect of the selected potent lactones on normal hematopoietic cells isolated from human cord blood was also examined.[11] Treatment with 1 μM of LC-1, **12**, **14** or **17** for 18 h did not significantly affect the total number of erythroid and myeloid colonies formed compared to DMSO (vehicle)-treated controls.

In conclusion, we have synthesized a series of AMGBLs with varying substitutions at the β- and γ-positions and examined them for growth suppression activity against three human pancreatic carcinoma cell lines, Panc-1, MIA PaCa-2, and BxPC-3. The potency depends on the substitution pattern of the AMGBL and the follow-

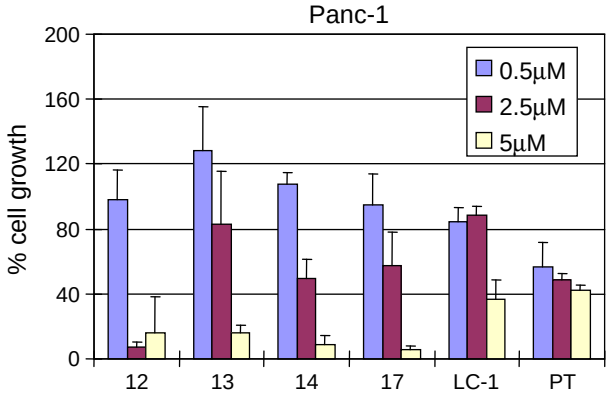


Chart 1. Comparison of proliferation assay for Panc-1 cells with selected potent AMGBLs.

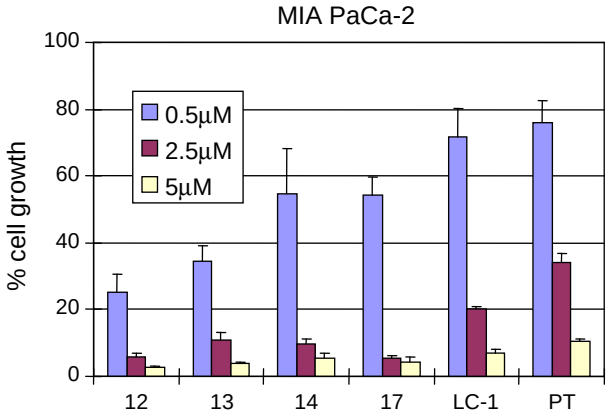


Chart 2. Comparison of proliferation assay for Mia PaCa-2 cells with selected potent AMGBLs.

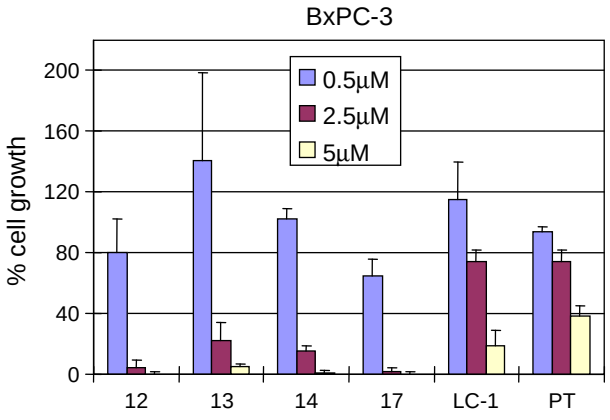


Chart 3. Comparison of proliferation assay for BxPc-3 cells with selected potent AMGBLs.

ing observations were made from the data obtained: (i) substitutions at β, and γ-positions of AMGBL are necessary; lack of substitution at either position results in reduced activity, (ii) the α-methylene group is critical for anti-proliferative activity; an α-alkylidene group is not as effective, (iii) aromatic groups at both β- and γ-positions reveal increased potency, (iv) the cytotoxicity increased further with the bulkier aromatic group (naphthyl vs phenyl) at the γ-position, (v) electron-donating groups on the aromatic moiety at the γ-position increases the potency, and (vi) the stereochemistry of the substitutions also affect the cytotoxicity; β,γ-*trans*-disubstituted α-methylene-γ-butyrolactones are more potent than the corresponding *cis*-isomers.

Our analysis has identified **12**, **13**, **14**, and **17** to be as potent or slightly superior inhibitors than parthenolide for all three human pancreatic cancer cell lines examined. Among the lactones examined, *trans*-β,γ-diphenyl- and *trans*-γ-2-naphthyl-β-phenyl-α-methylene-γ-butyrolactones, **12** and **17**, respectively, showed excellent inhibitory activity for all three cell lines.

The possibility of large-scale synthesis of these lactones with readily available materials makes this study attractive. Further work to introduce heteroaryl groups as well as chirality in the lactones is in progress. We are also examining the ability of the β,γ-diaryl lactones to inhibit NF-κB-DNA binding.[19] Other assays to determine whether the lactones induce apoptosis or affect other growth/survival signaling pathways are also under way.

Acknowledgments

Financial support from the Purdue University Herbert C. Brown Center for Borane Research (P.V.R.), American Cancer Society grant # RSG-06-267-01-CCE (C.M.S. and M.Y-S.) and The Indiana Genomics Initiative (INGEN) of Indiana University, supported in part by Lilly Endowment Inc. are gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.022.

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