Bioorganic & Medicinal Chemistry Letters 18 (2008) 5171–5173

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

γ-Lactones α,β- and β,γ-fused to carbocycles as novel antiproliferative drugs

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ARTICLE INFO

Article history:
Received 23 July 2008
Revised 25 August 2008
Accepted 26 August 2008
Available online 29 August 2008

Keywords:
γ-Lactones
Anticancer drugs
Drug discovery
Privileged structures
Ring closing metathesis

ABSTRACT

A set of γ-lactones α,β-fused and β,γ-fused to carbocycles have been synthesized and evaluated for their in vitro antiproliferative activities using the human cancer cell lines SW1573 (lung), T-47D (breast) and WiDr (colon). The compounds are obtained by intramolecular ring closing metathesis of the corresponding dienes. Active compounds exhibited GI_{50} values in the range 8–18 μM. A structure–activity relationship is also discussed.

γ-Lactones are widely distributed in nature as secondary metabolites displaying a broad biological profile including antibiotic, antihelmintic, antifungal, antitumor, antiviral, anti-inflammatory, and cytostatic properties.[1] Therefore, γ-lactones represent remarkable lead structures and have attracted our interest for the development of new drugs.[2] The γ-lactone unit is generally present in medium size rings.

Of particular interest to us are the naturally occurring α,β- and β,γ-fused γ-lactones acetoxycrenulide (**1**),[3] ambrosin (**2**),[4] vernolepin (**3**),[5] 10-epi-8-deoxycumambrin B (**4**),[6] dehydroleucodin (**5**),[7] and ludartin (**6**)[8] (Fig. 1). The development of new methodologies for their preparation plays a major role in the synthesis of these biologically active products.

Herein, we report on the antiproliferative activity of a series of bicyclic γ-lactones α,β- or β,γ-fused to carbocycles. The synthetic monocyclic precursors were also evaluated for their biological activity in order to obtain structure–activity relationships. As a model for the anticancer activity we used the representative panel of human solid tumor cell lines SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon).

In our group, we have developed a method based on the cyclization of enantiomerically enriched α-[(phenylthio)acyl]-β,γ-unsaturated esters **7a–c** to obtain γ-lactones **8a–c** with a high degree of substitution and a total stereochemical control.[9] As shown in Scheme 1, compounds **8a–c** were hydrolyzed under basic conditions to give carboxylic acids **9a–c**, which were reduced with borane to the corresponding primary alcohol **10a–c**. General precursor **10a–c** were easily prepared using such strategy and served as the common intermediates for the synthesis of both γ-lactones α,β- and β,γ-fused to carbocycles.

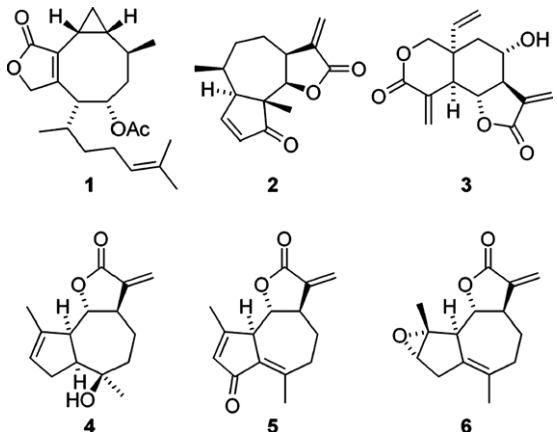

Figure 1. Structure of representative naturally occurring γ-lactones α,β- and β,γ-fused to carbocycles.

The general procedures to obtain γ-lactones α,β-fused[10] and β,γ-fused[11] to carbocycles is shown in Scheme 2. Alcohols **10a–b** were transformed into alkenes **11a–b** (80% average yield) by the

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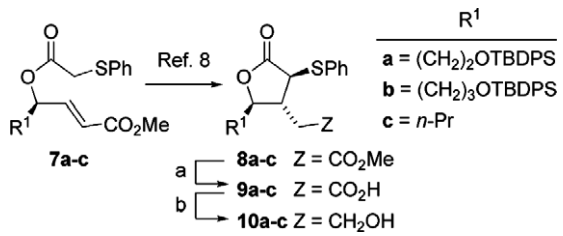
doi:10.1016/j.bmcl.2008.08.098



Scheme 1. Reagents and conditions: (a) NaOH, THF/H_2O (4:1), 92%; (b) $BH_3{\cdot}SMe_2$, THF, 0 °C, 88%.

sequence oxidation-Wittig homologation. Deprotection of the silyl protecting group led to the primary alcohols **12a**–**b** (90% yield of average yield). Similarly, the oxidation-Wittig strategy was used to transform alcohols **12a**–**b** into alkenes **13a**–**b**. Finally, ring closing metathesis using second generation Grubbs' catalyst afforded in high yields the corresponding γ-lactones β,γ-fused to carbocycles **14a**–**b**.

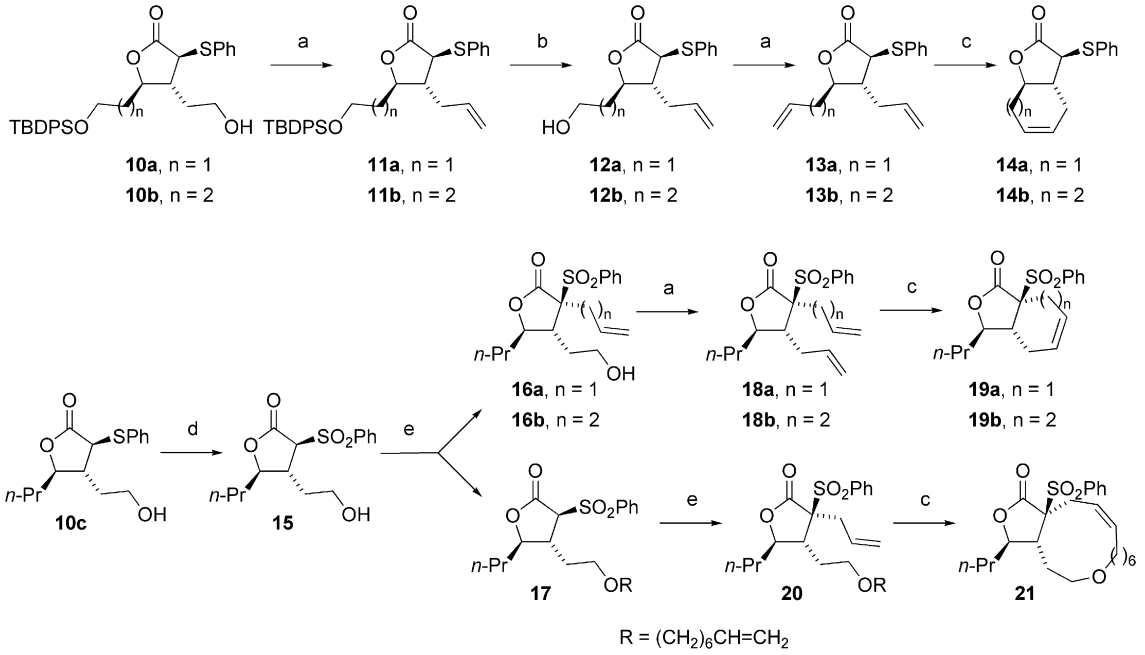
For the synthesis of γ-lactones α,β-fused to carbocycles the common precursor **10c** needs to be transformed into the corresponding sulfone **15**. The oxidation is mediated by Oxone® and proceeds in almost quantitative yields. Sulfone **15** was alkylated in α-position with stereochemistry retention using short chain alkyl bromides to yield compounds **16a**–**b** (70%). Once again, the oxidation-Wittig strategy was applied to obtain compounds **17a**–**b**. Ring closing metathesis under the aforementioned conditions led the corresponding γ-lactones α,β-fused to carbocycles **18a**–**b**. However, for long carbon chain alkyl bromides the alkylation of sulfone **15** produced exclusively the O-alkylated derivative. Thus, compound **19** was obtained in 55% yield. Alkylation of **19** with allyl bromide gave intermediate **20**, which after ring closing metathesis afforded the bicyclic compound **21**.[12]

The in vitro antiproliferative activity of the isolated intermediates was evaluated using the National Cancer Institute (NCI) protocol[13] after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[14] In addition to the antitumor activity, the lipophilicity (Clog*P*) of the compounds was evaluated by in silico calculation based on their chemical structure.[15] Clog*P* values were calculated to correlate lipophilicity with antitumor activity. The results are shown in Table 1. The Clog*P* values for the compounds reported in this study are within a wide range 0.5–8.3 (Table 1). Taken as

Table 1
Lipophilicity and in vitro antiproliferative activity against a representative panel of human solid tumor cell lines[a]

Compound	Clog*P*[b]	Cell line		
		SW1573 (NSCLC)	T-47D (breast)	WiDr (colon)
8a	6.6	>100	>100	>100
8b	6.7	>100	>100	>100
8c	3.0	>100	>100	>100
9a	6.8	16 (±1.4)	14 (±1.6)	16 (±1.4)
9b	6.8	18 (±1.0)	15 (±1.4)	17 (±1.9)
9c	2.5	>100	>100	>100
10a	6.2	7.8 (±1.2)	8.0 (±4.4)	13 (±3.4)
10b	6.3	13 (±2.4)	7.4 (±1.6)	13 (±1.8)
10c	2.0	>100	>100	>100
11a	8.3	>100	>100	>100
11b	8.3	>100	>100	>100
12a	2.1	>100	>100	>100
12b	2.0	>100	>100	>100
13a	3.5	49 (±11)	34 (±14)	33 (±8.3)
13b	4.0	57 (±6.2)	35 (±12)	40 (±6.3)
14a	2.3	30 (±8.4)	18 (±6.3)	21 (±3.7)
14b	2.8	36 (±2.9)	17 (±3.1)	21 (±3.2)
15	0.5	>100	>100	>100
16a	1.6	>100	>100	>100
16b	2.1	>100	>100	>100
17a	3.6	34 (±15)	23 (±8.8)	27 (±8.7)
17b	4.1	21 (±11)	16 (±3.8)	25 (±7.8)
18a	2.9	28 (±21)	26 (±13)	19 (±3.5)
18b	2.9	16 (±3.6)	17 (±6.9)	15 (±5.6)
19	3.2	7.5 (±0.5)	22 (±9.6)	28 (±5.6)
20	4.3	>100	5.7 (±1.5)	24 (±13)
21	4.4	79 (±33)	76 (±23)	>100

[a] Values expressed as GI_{50} (50% growth inhibition) are given in μM and are means of two to four experiments, standard deviation is given in parentheses.
[b] Ref. 15.



Scheme 2. Reagents and conditions: (a) i—$SO_3{\cdot}$py, DMSO, CH_2Cl_2, rt; ii—$Ph_3P^+CH_3Br^-$, KHMDS, THF, 80%; (b) *n*-$Bu_4N^+F^-$, THF, rt, 90%; (c) 2nd generation Grubbs' catalyst, CH_2Cl_2, 40°C, 85% yield for **14a**, **18a**–**b**, 89% yield for **14b**, 15% yield for **21**; (d) Oxone®, MeOH/H_2O (1:2), rt, 93%; (e) i—NaH, DMF; ii—RX, 70% yield for **16a**–**b**, 55% yield for **19**, 75% yield for **20**.

a whole, lipophilicity is not sufficient to explain the observed differences in growth inhibition.

The data on antiproliferative activity shown in Table 1 allow to classify the compounds in two groups. A first group is comprised of the inactive compounds, that is, those derivative with GI_{50} values >100 μM against all cell lines. The second group includes the remaining derivatives, which show GI_{50} values in the range 5.7–79 μM. With the exception of compounds **20** and **21**, all active products induced growth inhibition in the three cell lines. Overall, the active compounds did not show a clear trend in terms of specificity for a particular cell line. However, compound **20** was inactive against the lung cancer cells SW1573 whilst showed good activity against the T-47D breast (5.7 μM) and WiDr colon (24 μM) cell lines. On the contrary, bicyclic derivative **21** was inactive against WiDr cells and showed a weak activity against the other cell lines (76–79 μM).

The most active compounds of the set are **10a**–**b** with GI_{50} values in the range 7.8–13 μM. However, the non-silyl-protected equivalent **10c** is inactive. This result is also observed for active compounds **9a**–**b** and inactive **9c**. The difference may be explained by the increased lipophilicity of the silyl-protected derivatives when compared to the compound**s** lacking silyl groups, thus facilitating drug diffusion through the cell membrane. In our group, this strategy has been applied successfully to substituted tetrahydropyrans[16] and naturally occurring catalpol.[17] The presence of a silyl group is not enough premises to induce growth inhibition. That is the case for compounds **8a**–**c** and **11a**–**b**, which are all inactive. A direct comparison between compounds **8**–**11** indicates that the carboxylic acid group in **9a**–**b** and the hydroxyl group in **10a**–**b** play a role in the biological effect. Furthermore, the deprotection of the silyl group in **11a**–**b** leads to inactive derivatives **12a**–**b**. However, the dialkene analogs **13a**–**b** showed modest GI_{50} values in the range 33–57 μM. This antiproliferative effect was slightly improved in the corresponding β,γ-fused γ-lactones **14a**–**b**, which showed GI_{50} values in the range 17–36 μM.

Oxidation of sulfide **10c** to give sulfone **15** did not induce changes in activity, both compounds being inactive. The α-alkylated derivatives **16a**–**b** are also inactive. However, dialkenes **17a**–**b** and bicycles **18a**–**b** showed GI_{50} values in the range 16–34 μM and 15–28 μM, respectively. When comparing the subset of compounds **16**–**18** with that of analogs **12**–**14**, a parallelism in activity is observed. Thus, hydroxy-alkenes (**12** and **16**) are inactive; dialkenes (**13** and **17**) induce cell growth inhibition; and bicycles (**14** and **18**) show a subtle enhancement in activity. The data seem to indicate that a sulfone group induces more activity than a sulfide group.

While the alkylation of **15** in α position leads to inactive compound **16**, the O-alkylation produces active analog **19**. Thus, the O-alkylated derivative **19** shows GI_{50} values in the range 7.5–28 μM, with selectivity against the lung cancer cells SW1573. Further alkylation in α-position of **19** leads to **20**, which losses activity toward the lung cancer cells. However, it shows an enhancement of the effect on T-47D cells. The cyclization of derivative **20** produces a substantial loss in activity.

Despite the large antitumor activity reported for naturally occurring lactones,[1] we show that our set of γ-lactones exhibit a moderate activity. We speculate that the absence of a α,β-unsaturated group may explain this effect. Work is currently conducted to determine the exact role in activity of the sulfide and the sulfone group. Furthermore, we are using in-house methodology for the transformation of the sulfide and sulfone groups into exo- or endocyclic double bonds. These findings will be reported elsewhere.

In summary, we have synthesized a set of γ-lactones α,β-fused and β,γ-fused to carbocycles, using ring closing metathesis as the cycling strategy. The analysis of the antiproliferative data gives a structure–activity relationship with the substituents on the γ-lactone modulating the biological activity. Some derivatives showed promising activity against the lung, breast, and colon cancer cell lines. Privileged structures such as γ-lactones, with their inherent drug-likeness, represent an ideal source of core synthons and capping fragments for the design and synthesis of drugs targeted at various receptors. The general methodology reported herein allows the quick production of a variety of γ-lactones that are useful for the discovery of novel drug leads.

Acknowledgments

This research was supported by the Spanish MEC co-financed by the European Regional Development Fund (CTQ2005-09074-C02-01/BQU), and the Canary Islands Government, and the Fundación Canaria de Investigación y Salud (PI 1/06 and PI 35/06). L.G.L. thanks the Spanish MSC-FIS for a postdoctoral contract. J.M.P. thanks the Spanish MEC-FSE for a Ramón y Cajal contract.

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