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Synthesis and antiproliferative activity of (2*R*,3*R*)-disubstituted tetrahydropyrans

Romen Carrillo,[a] Leticia G. León,[a,b] Tomás Martín,[a,c,*] Víctor S. Martín[a] and José M. Padrón[a,b,*]

[a]*Instituto Universitario de Bio-Orgánica 'Antonio González' (IUBO-AG), Universidad de La Laguna, C/Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain*
[b]*BioLab, Instituto Canario de Investigación del Cáncer (ICIC), C/Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain*
[c]*Instituto de Productos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas (CSIC), C/Astrofísico Francisco Sánchez 3, 38206 La Laguna, Spain*

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Abstract—In this study, we synthesized a series of enantiomerically pure (2*R*,3*R*)-disubstituted tetrahydropyrans with diverse functional groups. The in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780 (ovarian cancer), SW1573 (non-small cell lung cancer), and WiDr (colon cancer). Overall, the results show the relevance for antiproliferative activity of the α,β-unsaturated ester side chain at position 2 of the THP ring.

The marine environment is an endless source of new molecular scaffolds that form the basis for the development of new anticancer drugs.[1] Within our program directed at the development of novel antitumor compounds based on heterocyclic scaffolds (Fig. 1), marine drugs containing cyclic ethers have attracted considerably our interest.[2]

Recently, we have reported on the antiproliferative activity of a series of enantiomerically pure (2*R*,3*S*)-disubstituted tetrahydropyrans (*trans*-THPs).[3] In this study we explore the biological activity of a series of (2*R*,3*R*)-disubstituted tetrahydropyrans (*cis*-THPs). The growth inhibition was tested against a panel of three representative human solid tumor cells: A2780 (ovarian cancer), SW1573 (non-small cell lung cancer, NSCLC), and WiDr (colon cancer). The results are compared with those obtained for the corresponding *trans*-THPs. A structure–activity relationship is also discussed.

In order to accomplish the synthesis of the *cis*-THP derivatives (Scheme 1), we used as starting material the diol **1**,[4] which can be obtained from the commercially available tri-*O*-acetyl-D-galactal.[5] Silyl protection of **1** affords **2**, which after selective cleavage of the exocyclic silyl ether leads to the key intermediate *cis*-alcohol **3**.[6] Two-carbon homologation of the *cis*-alcohol **3** to the benzyl (*E*)-α,β-unsaturated ester **4** is achieved using a Swern oxidation and subsequent Horner–Emmons reaction. Further removal of the *tert*-butyl dimethyl silyl (TBS) group yields the secondary alcohol **5**. A similar two-step strategy to obtain compound **4** is used to prepare the corresponding benzyl (*Z*)-α,β-unsaturated ester **6**. This time the Horner–Emmons reaction is run under Ando conditions.[7] In addition to the benzyl esters, the methyl esters can be prepared by the choice of the appropriate phosphonoacetate in the Horner–Emmons step. Thus, methyl (*Z*)- and (*E*)-α,β-unsaturated esters

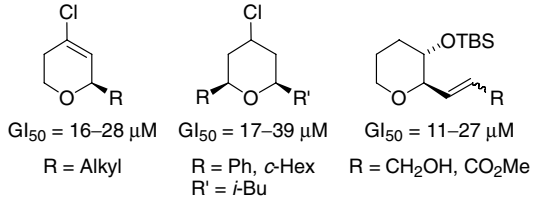

Figure 1. Heterocyclic scaffolds with antiproliferative activity.

Keywords: Marine products; Anticancer drugs; Cyclic ethers; Solid tumors; Structure–activity relationship.
* Corresponding authors. Tel.: +34 922 318 580; fax: +34 922 318 571; e-mail addresses: tmartin@ipna.csic.es; jmpadron@ull.es

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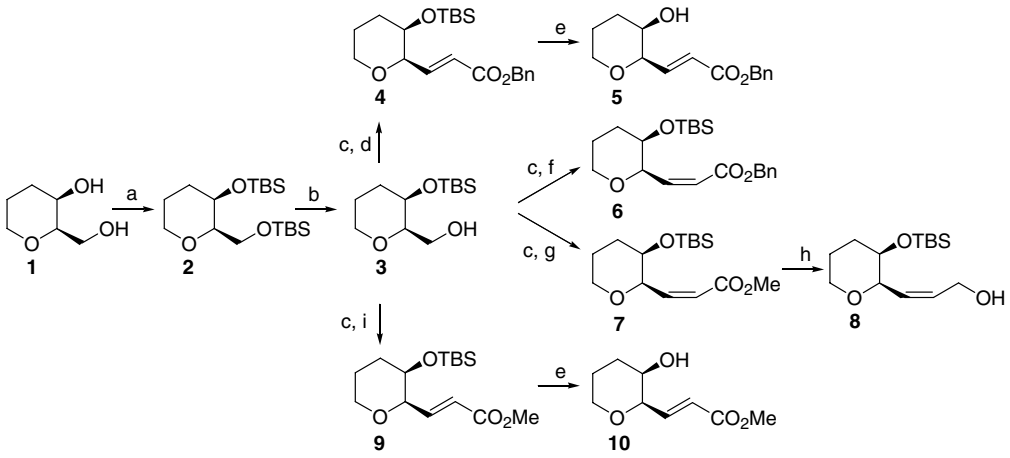
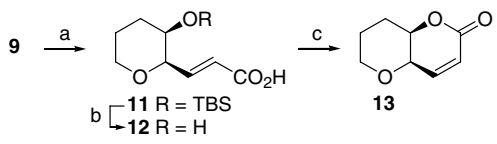


Scheme 1. Reagents and conditions: (a) TBSCl, imidazole, CH_2Cl_2; (b) TFA/THF/H_2O (1:1:1), 76% yield from **1**; (c) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N; (d) $(MeO)_2POCH_2CO_2Bn$, C_6H_6, NaH, rt, 85% yield from **3**; (e) HF, CH_3CN, for **5** 96%, for **10** 95%; (f) $(PhO)_2POCH_2CO_2Bn$, THF, NaH, −78 °C, 78% yield from **3**; (g) $(PhO)_2POCH_2CO_2Me$, THF, NaH, −78 °C, 80% yield from **3**; (h) DIBAL-H, THF, 0 °C, 90%; (i) $(MeO)_2POCH_2CO_2Me$, C_6H_6, NaH, rt, 92% yield from **3**.

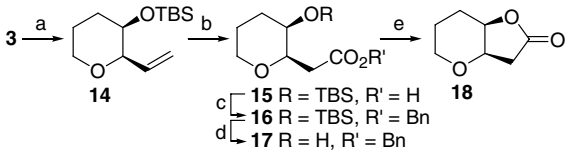
7 and **9** are synthesized, respectively. The reduction of compound **7** with DIBAL-H in THF at 0 °C provides the (*Z*) allylic alcohol **8**. Removal of the TBS group from methyl (*E*)-α,β-unsaturated ester **9** yields the secondary alcohol **10**.

Further derivatization of compound **9** is shown in Scheme 2. The methyl (*E*)-α,β-unsaturated ester **9** is transformed into the (*E*)-α,β-unsaturated acid **11** by saponification of the methyl ester. Subsequent deprotection of the TBS group affords the (*E*)-α,β-unsaturated acid **12**. When compound **12** is treated under modified Yamaguchi conditions,[8] the (*Z*)-α,β-unsaturated δ-lactone **13** is the only obtained product as the result of a concomitant isomerization of the double bond followed by lactonization.[6b]

A final set of derivatives is prepared from *cis*-alcohol **3** after a one-carbon homologation performed by a simple four-step sequence. Thus, oxidation to aldehyde by Swern reaction, Wittig methylenation ($Ph_3P{=}CH_2$) to afford alkene **14**, hydroboration with 9-BBN in THF, and oxidative cleavage of the alkyl borane, yielding the primary alcohol which is oxidized to the corresponding carboxylic acid **15** (Scheme 3). Esterification of the carboxylic acid **15** with benzyl alcohol provides the benzyl ester **16**. Further removal of the TBS group affords the hydroxy ester **17**. Finally, catalytic hydrogenation followed by treatment with 2-chloro-1,3-dimethylimidazolinium chloride, NaH, and DMAP[9] yields the γ-lactone **18**.[6b]



Scheme 2. Reagents and conditions: (a) LiOH, THF, H_2O, Δ, 94%; (b) HF, CH_3CN; (c) 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, CH_2Cl_2 (0.1 M), 42% yield from **11**.



Scheme 3. Reagents and conditions: (a) i—$(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N; ii—$Ph_3P{=}CH_2$, THF, 88%; (b) i—9-BBN, THF; ii—H_2O_2, NaOH; iii—$NaIO_4$, $RuCl_3$ (cat.), $CH_3CN/CCl_4/H_2O$ (2:2:3), 88%; (c) BnOH, DMAP, CSA, DCC, CH_2Cl_2; (d) HF, CH_3CN, 55%; (e) i—H_2, $Pd(OH)_2$, AcOEt; ii—2-chloro-1,3-dimethylimidazolinium chloride, NaH, DMAP, CH_2Cl_2, 0 °C to rt, 85% yield from **17**.

The in vitro antiproliferative activity was evaluated using the National Cancer Institute (NCI) protocol.[10] We screened growth inhibition and cytotoxicity against the panel of human solid tumor cell lines A2780, SW1573, and WiDr after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[11] In addition to the biological activity, for each compound the lipophilicity expressed as Clog *P*[12] was calculated to correlate lipophilicity with antitumor activity.[13] The Clog*P* values together with the growth inhibition data are listed in Table 1. Overall, lipophilicity is not sufficient to explain the observed differences in growth inhibition.

The growth inhibition results allow us to classify the compounds according to their activity profile. A first group of active compounds comprises five *cis*-THPs: **4**, **6**, **7**, **13**, and **16**. The remaining products are considered inactive ($GI_{50} > 80$ μM). Derivatives **7** and **13** are the most antiproliferative compounds of the series with GI_{50} values in the range 5.7–8.3 μM against A2780 and SW1573 cells. However, the activity decreases in WiDr cells (38–39 μM). Interestingly, compounds **6** and **16** show selectivity for the highly refractory cancer cell line WiDr with GI_{50} values in the range 19–20 μM. The ovarian cancer cells A2780 and the NSCLC cells SW1573 are the most sensitive to the drugs. On the contrary, the colon cancer cell line WiDr is the least sensitive to the drugs. This observation is consistent with a

Table 1. Lipophilicity and in vitro antiproliferative activity of (2*R*,3*R*)-disubstituted tetrahydropyrans against human solid tumor cells[a]

Compound	Clog *P*[b]	A2780			SW1573			WiDr		
		GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
2	5.72	>100			>100			>100		
3	2.34	>100			>100			>100		
4	5.26	32 (±2.7)			37 (±5.7)			90 (±14)		
5	1.87	>100			>100			>100		
6	5.26	29 (±8.7)	77 (±19)		26 (±6.2)	92 (±16)		20 (±2.9)	64 (±31)	
7	3.52	7.3 (±1.7)	39 (±20)	86 (±20)	6.3 (±2.9)	68 (±45)		38 (±10)		
8	2.54	>100			>100			>100		
9	3.52	98 (±2.6)			>100			>100		
10	0.14	>100			>100			>100		
11	3.03	>100			>100			>100		
13	−0.55	8.3 (±1.0)	35 (±1.1)		5.7 (±0.4)			39 (±7.6)		
14	3.79	86 (±13)			>100			>100		
16	4.97	23 (±4.5)			30 (±2.9)			19 (±1.6)		
17	2.17	>100			>100			>100		
18	−0.53	>100			>100			>100		

[a] Values representing GI_{50} are given in μM and are means of two to four experiments, standard deviation is given in parentheses. TGI and LC_{50} values are given only if they are less than 100 μM, which is the maximum concentration test.
[b] Ref. 13.

previous study in which colon cancer cells showed more drug resistance than ovarian cancer cells.[14] When considering total growth inhibition (TGI) values, compounds **6**, **7**, and **13** appear as the most potent products of the series. However, cytotoxicity (expressed as LC_{50}) is only observed for compound **7** against the most sensitive cell line A2780. Therefore, these *cis*-THPs may be considered as cytostatic drugs. Cytostatic drugs play an important role in antiangiogenic therapy because they do not kill cells in the same way as cytotoxic drugs do. In addition, they could be used in cancer therapy in combination with cytotoxic drugs or radiotherapy.

A direct comparison of the growth inhibition parameters allows identifying the following structure–activity relationship. The role of the TBS group in the enhancement of tumor cell growth inhibition was established in a series of *trans*-THP derivatives.[3] In this new series of *cis*-THPs we find similar results, being the compounds bearing TBS ethers more active than the corresponding free hydroxyl derivatives (**4** vs **5**, and **16** vs **17**). That condition is not sufficient for activity as shown with the inactive compounds **9** (OTBS) and **10** (OH). The substituent at position 2 of the tetrahydropyran ring is responsible for the activity exerted by the compounds. In general, α,β-unsaturated esters together with saturated benzyl ester **16** give the best results. Other functional groups such as silyl ether (**2**), primary hydroxyl (**3**), (*Z*)-allylic alcohol (**8**), and α,β-unsaturated acid (**11**) proved inactive. In this particular context, vinyl derivative **14** has a low activity against A2780 ovarian cells. A similar 2-vinyl-6-alkyl-THP showed GI_{50} values of 18 and 24 μM against A2780 and SW1573 cells, respectively.[15] We speculate that the steric hindrance of the TBS group in compound **14** may induce a negative effect on the activity of the vinyl group.

The stereochemistry of the double bond seems to play an important role in activity. Thus, (*Z*)-α,β-unsaturated esters are more active than the corresponding (*E*) products (**6** vs **4**, and **7** vs **9**). This result is the opposite of that found in *trans*-THPs, where the (*E*)-α,β-unsaturated esters showed to be more active.[3] In addition, the (*Z*)-α,β-unsaturated methyl ester **7** is more potent than the corresponding benzyl ester **6**, whilst the (*E*)-α,β-unsaturated benzyl ester **4** is more active than the corresponding methyl ester **9**. It is noteworthy that the α,β-unsaturated-δ-lactone **13**, which lacks the TBS group and has a very low Clog *P* value, exhibits similar biological activity as derivative **7**. On the contrary, the γ-lactone **18** is inactive signifying the role of the unsaturation in the biological activity.

Table 2 shows the antiproliferative activities of some *cis*-THPs compared to those of their corresponding *trans*-THPs reported earlier. The results do not point out a clear correlation between biological activity and the stereochemistry of the substituent at position 3 of the THP ring. A common feature to both series is that the most active *cis*- and *trans*-THPs bear an unsaturated side chain at position 2 of the THP ring.

Table 2. Comparison of in vitro antiproliferative activity between (2*R*,3*R*)- and (2*R*,3*S*)-disubstituted THPs[a]

Compound[b]	Cell line		
	A2780	SW1573	WiDr
7	7.3	6.3	38
trans-**7**	54[c]	94[c]	96[c]
8	>100	>100	>100
trans-**8**	11[c]	20[c]	17[c]
9	98	>100	>100
trans-**9**	28	47[c]	84[c]

[a] Values representing GI_{50} are given in μM.
[b] The nomenclature *trans* refers to the (2*R*,3*S*)-disubstituted THP isomers.
[c] Ref. 3a.

In summary, we have synthesized a series of (2*R*,3*R*)-disubstituted THPs in a simple and direct way from a common precursor. On the basis of growth inhibition parameters, a structure–activity relationship was obtained. Although the results are preliminary, we found that these synthetic derivatives considerably induced growth inhibition in a panel of three human solid tumor cell lines of diverse origin. Overall, the results show the relevance of the α,β-unsaturated ester at position 2 of the THP ring.

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References and notes

1. Mayer, A. M. S.; Gustafson, K. R. *Eur. J. Cancer* **2004**, *40*, 2676, and references therein.
2. (a) Miranda, P. O.; Padrón, J. M.; Padrón, J. I.; Villar, J.; Martín, V. S. *Chem. Med. Chem.* **2006**, *1*, 323; (b) Miranda, P. O.; León, L. G.; Padrón, J. I.; Martín, V. S.; Padrón, J. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3135; (c) Crisóstomo, F. R. P.; Padrón, J. M.; Martín, T.; Villar, J.; Martín, V. S. *Eur. J. Org. Chem.* **2006**, 1910.
3. (a) Padrón, J. M.; Donadel, O. J.; León, L. G.; Martín, T.; Martín, V. S. *Lett. Drug Des. Discov.* **2006**, *3*, 29; (b) Donadel, O. J.; Martín, T.; Martín, V. S.; Villar, J.; Padrón, J. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3536.
4. Snatzke, G.; Raza, Z.; Habus, I.; Sunjic, V. *Carbohydr. Res.* **1988**, *182*, 179.
5. Tri-*O*-acetyl-D-galactal was purchased from Aldrich Chemical Company (Milwaukee, WI).
6. (a) Carrillo, R.; Martín, V. S.; Martín, T. *Tetrahedron Lett.* **2004**, *45*, 5215; (b) Carrillo, R.; Martín, V. S.; López, M.; Martín, T. *Tetrahedron* **2005**, *61*, 8177.
7. Ando, K. *Tetrahedron Lett.* **1995**, *36*, 4105.
8. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
9. Garcia, D. M.; Yamada, H.; Hatakeyama, S.; Nishizawa, M. *Tetrahedron Lett.* **1994**, *35*, 3325.
10. In this method, for each drug a dose–response curve is generated and three levels of effect can be calculated, when possible. The effect is defined as percentage of growth (PG), where 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% cell killing (LC_{50}) represent the drug concentration at which PG is +50, 0, and −50, respectively Skehan, P.; Storeng, P.; Scudeiro, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
11. Cells were inoculated at densities of 7000 (A2780), 6000 (SW1573), and 12,000 (WiDr) cells per well, based on their doubling times. Pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration, that is 100 μM. Control cells were exposed to an equivalent concentration of DMSO. Each agent was tested in duplicate at five different tenfold dilutions. Drug incubation times were 48 h, after which cells were precipitated with 25 μL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 492 nm using Bio-Tek's PowerWave XS 96-well plate reader. The percentage growth was calculated at each of the drug concentration levels based on the difference in OD at the start and end of drug exposure. Values were corrected for background OD from wells only containing medium.
12. In a recent comparative study, the computer program ClogP® appeared the most accurate predictor of Clog*P* values Machatha, S. G.; Yalkowsky, S. H. *Int. J. Pharm.* **2005**, *294*, 185.
13. Software-predicted lipophilicity of the compounds was calculated with the program ClogP® accessible via Internet (www.daylight.com/daycgi/clogp) working with the Hansch–Leo's 'fragment constant' method.
14. Pizao, P. E.; Peters, G. J.; van Ark-Otte, J.; Smets, L. A.; Smitskamp-Wilms, E.; Winograd, B.; Pinedo, H. M.; Giaccone, G. *Eur. J. Cancer* **1993**, *A*, 1566.
15. Crisóstomo, F. R. P.; Carrillo, R.; León, L. G.; Martín, T.; Padrón, J. M.; Martín, V. S. *J. Org. Chem.* **2006**, *71*, 2339.