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One-pot synthesis and SAR study of *cis*-2,6-dialkyl-4-chloro-tetrahydropyrans

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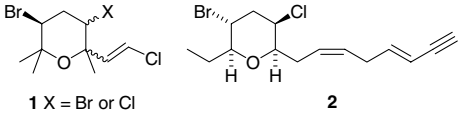
Abstract—A series of *cis*-2,6-dialkyl-4-chloro-tetrahydropyrans were prepared by means of an iron(III)-catalyzed process. The in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780, SW1573, and WiDr. The results show that the presence of bulky substituents favors the Prins cyclization leading to new products with better activity profile against all cell lines tested.

The marine environment is a source of natural products that have demonstrated significant and extremely potent biological activities including: cytotoxic, antibiotic, anti-inflammatory and antispasmodic, antiviral, cardiotonic and cardiovascular, antioxidant, and enzyme inhibition.[1] The major drawbacks of marine compounds are the very low amounts in which they are present in their natural sources and the difficulty to collect the organisms that originate them. This makes impractical the direct use of the marine reservoir to isolate products to be used in therapy. Thus, the synthetic chemistry is required to develop high yield synthetic methods, which are able to produce the drugs in large quantities preserving the natural sources.

Marine organisms have proven to be an endless source of novel organohalogen secondary metabolites.[2] Of particular interest to us are halogen-containing cyclic ethers. In our group we have developed new methodologies to accomplish their total synthesis.[3] More recently, we have started a program directed at the development of novel antitumor compounds based on these scaffolds.[4]

Aplysiapyranoids A–D **1** and srilankenyne **2** are representative examples of halogenated marine compounds with a tetrahydropyran (THP) scaffold (Fig. 1). Aplysiapyranoids A–D **1**, which were isolated from the sea hare *Aplysia kurodai*,[5] have shown modest in vitro cytotoxicity in the range 60–300 μM. Aplysiapyranoid D was the most active compound of the series against Moser cells (human colon cancer) with an IC_{50} of 46 μM. Srilankenyne **2** was isolated from the sea hare *Aplysia oculifera*[6] but no data have been reported on its biological activity.

Herein we report on the one-pot synthesis of marine product analogs containing the common structural scaffold of 4-chlorotetrahydropyran. In addition, the biological activity was tested against a panel of three



1 X = Br or Cl **2**

Figure 1. Structures of natural halogen-containing tetrahydropyrans.

Keywords: Marine drugs; Halogenated tetrahydropyrans; Solid tumors; Drug design; Structure–activity relationship.
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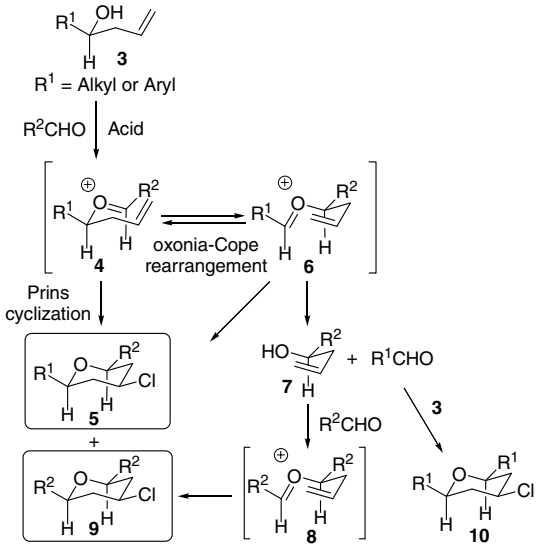
representative human solid tumor cells: A2780 (ovarian cancer), SW1573 (non-small cell lung cancer, NSCLC), and WiDr (colon cancer). A structure–activity relationship is also discussed.

The Prins cyclization is a powerful tool to obtain substituted THPs.[7,8] When secondary homoallylic alcohols or α-acetoxyethers are used to generate the oxocarbenium ion intermediate, an oxonia-Cope rearrangement can take place as competitive process with the Prins cyclization. The two competitive processes could yield a mixture of products (Scheme 1). For instance, this process could generate four different products (**5**, **7**, **9**, and **10**), if we assume in the newly generated 2,6-dialkyl-4-halotetrahydropyran all substituents allocated equatorial.[7b,9] Otherwise, the mixture will be even more complex.

Direct evidence for the oxonia-Cope rearrangement was given by Speckamp–Hiemstra.[10] An important consequence derived from the rearrangement is the partial or total racemization of the final products when enantiomeric chiral starting materials are being reacted.[7d,f,11] Several authors, studying different types of substrates, have evaluated the influence of this rearrangement on the regio- and stereochemical outcome of the cyclization.[7d,f,12] A full study including factors to modulate these competitive processes has been recently reported by Rychnovsky et al.[8a]

We have recently described the reaction between but-3-en-1-ol (**3**, R^1 = H) and several aldehydes using $FeCl_3$ as promoter.[13] The reaction proceeded satisfactorily, affording the corresponding *cis*-4-halo-2-alkyl THP (**5**, R^1 = H) in high yields (Table 1, entries 1–3). In the present work, we studied the influence of diverse alkyl groups (R^1) on the course of the reaction between secondary homoallylic alcohols **3** and 3-methylbutanal (R^2 = *i*-Bu). Table 1 summarizes the results obtained in this study.



Scheme 1. Plausible mixture originated by competition of Prins cyclization and oxonia-Cope rearrangement in the coupling of homoallylic alcohols and aldehydes.

Table 1. Cyclization of homoallylic alcohols and aldehydes promoted by iron(III) chloride

Entry	R^1	R^2	Ratio **5**:**9**	Yield (%)
1	H	*i*-Bu	100:0	93
2	H	Ph	100:0	97
3	H	*p*-NO_2Ph	100:0	83
4	Me	*i*-Bu	50:50	80
5	Et	*i*-Bu	60:40	75
6	*i*-Bu	*i*-Bu	—[a]	76
7	*c*-Hex	*i*-Bu	100:0[b]	70
8	Ph	*i*-Bu	50:50	70
9	*p*-NO_2Ph	*i*-Bu	85:15	57
10	*p*-OCH_3Ph	*i*-Bu	75:25	55

[a] Compounds **5** and **9** are identical.
[b] Within the NMR detection limit, neither **7** nor **10** was observed.

For secondary homoallylic alcohols **3**, the increment in bulkiness of the R^1 group (methyl to cyclohexyl) is concomitant with an increase in the formation of **5** and a subsequent decrease of **9** (entries 4–7). This steric factor favors the Prins cyclization to **5** minimizing the 2-oxonia-Cope rearrangement. In the case of benzylic homoallylic alcohols, the reaction is dependent upon the substituents on the aromatic ring (entries 8–10). The presence of electron-deficient aromatic rings favors the Prins cyclization yielding a non-symmetrical 2,4,6-trisubstituted THP **5** as the major product (entries 9 and 10). In all the cases, the stereochemical configuration of the THPs was all *cis*.[14] No traces of the symmetrical THP **10** were observed in any case.

To prove the formation of intermediate **7**, we reacted pent-4-en-2-ol (**3**, R^1 = Me) and 3-methylbutanal with a substoichiometric amount of $FeCl_3$ (0.1 equiv). The homoallylic alcohol **7** (R^2 = *i*-Bu) was obtained as a single product (100% yield, 50% conversion). This compound was isolated and fully characterized. When treated with more $FeCl_3$ and 3-methylbutanal, THP **9** (R^2 = *i*-Bu) was yielded as the only product. This result is consistent with the experimental data (entry 4), where a mixture of THPs **5** and **9** was obtained.

Table 2 shows the lipophilicity, expressed as C log *P*,[15,16] of a series of 2-alkyl and 2,6-dialkyl 4-chloro-tetrahydropyrans. C log *P* values were computed to correlate lipophilicity with antitumor activity. In addition, the in vitro anticancer activity was evaluated using the National Cancer Institute (NCI) protocol.[17] 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.[18] The resulting biological activities for each compound are reported in Table 2.

From the results of lipophilicity we observe that those compounds with C log *P* values lower than 4 were inactive. On the contrary, all active products show C log *P* values in the range 4.37–5.61. These results are consistent with prior studies on the cytotoxicity of structurally related substituted THPs.[4b,c]

Table 2. Lipophilicity and in vitro antiproliferative activity against human solid tumor cells[a]

Compound	C log P[b]	Substituent		A2780			SW1573			WiDr		
		R^1	R^2	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
5a	3.06	H	*i*-Bu	>100			>100			>100		
5b	2.65	H	Ph	>100			>100			>100		
5c	2.39	H	*p*-NO_2Ph	>100			>100			>100		
5d	3.58	Me	*i*-Bu	>100			>100			>100		
5e	5.04	*i*-Bu	*i*-Bu	63 (±41)	90 (±24)		67 (±38)	89 (±23)		>100		
5f	5.61	*c*-Hex	*i*-Bu	20 (±4.1)	38 (±5.7)	73 (±10)	22 (±1.2)	43 (±3.2)	82 (±12)	26 (±2.3)	63 (±21)	
5g	4.62	Ph	*i*-Bu	17 (±1.4)	35 (±1.3)	72 (±5.9)	24 (±2.2)	48 (±8.7)	90 (±18)	39 (±19)	88 (±20)	
5h	4.37	*p*-NO_2Ph	*i*-Bu	21 (±9.4)	64 (±19)		30 (±8.0)			38 (±13)		
5i	4.54	*p*-MeOPh	*i*-Bu	25 (±6.3)	57 (±19)	95 (±7.7)	30 (±11)	80 (±24)		36 (±19)	73 (±32)	95 (±11)

[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 tested.
[b] Ref. 15.

The growth inhibition results allow us to classify the compounds in two groups according to their activity profile. A first group is comprised of the inactive derivatives **5a**–**d**. The remaining derivatives constitute the group of active products. From this set, compound **5e** is the least active and exhibits significantly decreased activity as shown by GI_{50} values over 50 μM. The remaining active products (**5f**–**i**) show GI_{50} values in the range 17–25, 22–30, and 26–39 μM against A2780, SW1573, and WiDr cells, respectively. Overall, these synthetic derivatives seem to show better activity profiles than the natural products aplysiapyranoids **1**.[19]

When considering TGI and LC_{50} values (Table 2), compounds **5f** and **5g** appear as the most active products of the series. The presence of either an electron-withdrawing group (**5h**) or an electron-donating group (**5i**) at *p*-position of the phenyl ring produces a decrease in activity when compared to the parent compound **5g**. Similarly to conventional and investigational anticancer drugs,[20] the ovarian cancer cell line is more sensitive to these THP derivatives than NSCLC and colon cancer cells.

From the obtained dose–response parameters the following structure–activity relationship is obtained. In general, those compounds bearing two alkyl groups at position 2 and 6 of the THP ring showed significant cytotoxicity in all cell lines, whereas the monoalkylated compounds were found inactive. The exception is given by compound **5d**, which is dialkylated but is inactive. Another conclusion is drawn from these results. Compounds with branched (R^2 = *t*-Bu **5e**) or cyclic (R^2 = *c*-Hex **5f**, Ph **5g**, *p*-NO_2Ph **5h**, and *p*-MeOPh **5i**) substituents on the THP ring were more active than the corresponding linear derivatives (R^2 = Me **5d**). Thus, the antiproliferative activities were in the order *c*-Hex ≈ Ph > *p*-MeOPh > *p*-NO_2Ph > *i*-Bu > Me. In view of these results, it appears that the biological activity of the 2,6-dialkyl THPs does not correlate with the calculated C log *P* values. However, the substitution pattern and steric hindrance of the alkyl side chains are important.

In summary, we have prepared a series of *cis*-2,6-dialkyl-4-chloro-tetrahydropyrans in a simple and direct way. The key step is a regioselective iron(III) chloride-catalyzed Prins-type cyclization. This general methodology allows the quick and large production of a variety of synthons that are valuable for the syntheses of novel bioactive compounds. We can access non-symmetrical 2,4,6-substituted THPs like **5** in one step using the suitable secondary homoallylic alcohol and $FeCl_3$ as an inexpensive, environmentally friendly, and stable Lewis acid. The bulkiness of the alkyl group, the presence of electron-deficient aromatic rings, and the nature of the Lewis acid are some of the factors implicated in the control of 2-oxonia-Cope rearrangement versus Prins cyclization. On the basis of growth inhibition parameters, a structure–activity relationship was obtained. Although preliminary, the results show that modifications on the THP ring by the addition of diverse substituents might lead to new products with better activity profiles.

Acknowledgments

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