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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3087–3090
Bioorganic & Medicinal Chemistry Letters

# Antiproliferative activity of 4-chloro-5,6-dihydro-2*H*-pyrans. Part 2: Enhancement of drug cytotoxicity☆

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Received 30 January 2007; revised 12 March 2007; accepted 14 March 2007
Available online 19 March 2007

**Abstract**—The Prins reaction was the basis to synthesize functionalized alkyl chlorodihydropyran derivatives. The inexpensive, stable, and environmentally friendly $FeCl_3$ promotes the cyclization. The method represents an efficient and regioselective manner to obtain in a single step chlorovinyl–TMS oxacycles. The in vitro antiproliferative activities of the compounds 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 an enhancement in the cytotoxicity exhibited by the new analogs when compared to their parental compounds.


The Prins reaction allows the synthesis of functionalized dihydro- and tetrahydropyran derivatives in a single step by the coupling between unsaturated alcohols and aldehydes catalyzed by Lewis acid.[1] These oxacyclic structures are common scaffolds present in diverse halogen-containing marine products with antitumor activity such as aplysiapyranoids, A–D.[2] We have recently reported on the synthesis of the novel marine product analogs 2-alkyl-4-chloro-5,6-dihydro-2*H*-pyrans by an iron(III) catalyzed Prins-type cyclization.[3] These halogen-containing oxacycles (Fig. 1) showed larger in vitro antitumor activity than the natural aplysiapyranoids. The chlorovinyl group was established as the pharmacophore, which is the moiety responsible for the activity exerted by these compounds.[4]

Herein, we describe further modifications on the heterocyclic ring by the inclusion of diverse substituents that lead to new products with enhanced activity profile. We based the strategy to introduce new functional or alkyl groups in the chlorodihydropyran scaffold by means of our iron(III) catalyzed silyl alkyne-Prins cyclization.[5]

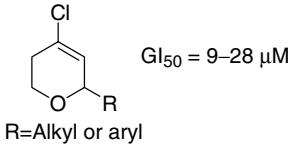


**Figure 1.** General structure of cytotoxic 2-alkyl-4-chloro-5,6-dihydro-2*H*-pyrans.

The biological activity of the compounds prepared was studied against the representative human solid tumor cells A2780 (ovarian cancer), SW1573 (non-small cell lung cancer, NSCLC), and WiDr (colon cancer). The results are compared to the previously reported antitumor activities.

In a previous study, we discovered that the silyl alkyne-Prins reaction of trimethylsilyl homopropargyllic alcohol (**1a**) with 3-methylbutanal (**2**) led to the chlorodihydropyran derivative **3a** (Scheme 1).[5] However, this product was obtained in a 1:1 mixture with the corresponding chlorodihydropyran **3b**, which lacks the TMS group. This side product was prepared in a straightforward manner by the reaction of unprotected homopropargyllic alcohol (**1b**). A totally different result occurred when reacting secondary homopropargyllic alcohols with aldehydes under the aforementioned iron(III) catalysis. As reported earlier, the reaction of

*Keywords*: Antitumor agents; Iron(III) chloride; Organohalogen drugs; Prins reaction; Structure–activity relationships.
☆ Part 1, see Ref. 4.
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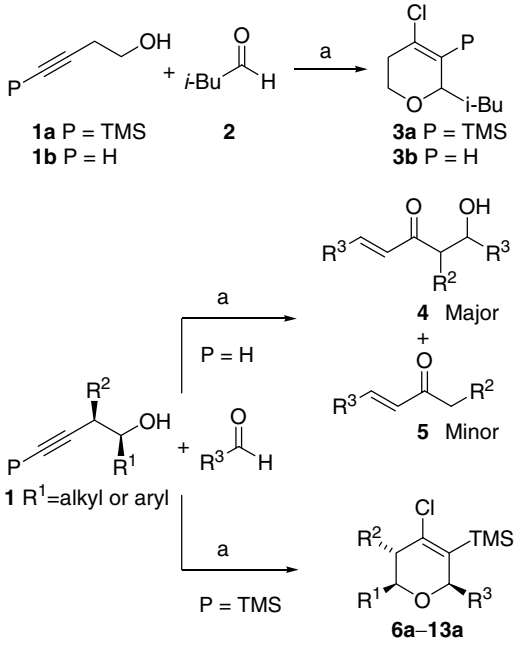
doi:10.1016/j.bmcl.2007.03.045



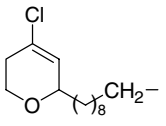

**Scheme 1.** Reagents and condition: (a) $FeCl_3$, $CH_2Cl_2$, rt, 1 min, 88% for **3a**–**3b**, 40–70% for **4**–**5**, 55–82% for **6a**–**13a**.

secondary homopropargyllic alcohols (**1**, $R^1$ = alkyl or aryl) gave the cytotoxic ketones **4** and **5**.[6] Interestingly, trimethylsilyl protected secondary homopropargyllic alcohols submitted to the described conditions gave the corresponding chlorodihydropyran derivative as sole product (Scheme 1).

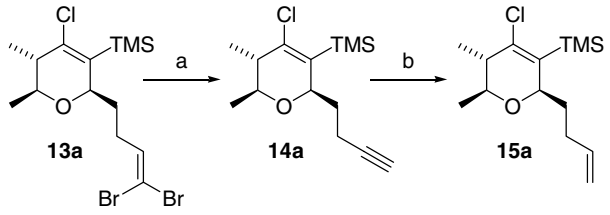
Therefore, we applied this alkyne silyl-Prins cyclization methodology to prepare the set of tetra- and pentasubstituted dihydropyrans **6a**–**13a** shown in Table 1.

In addition, we prepared two unprecedented derivatives by the transformation of compound **13a**. Thus, the reaction of derivative **13a** with *n*-butyl lithium led to the desired alkyne **14a** in 86% yield (Scheme 2). The subsequent hydrogenation of **14a** using Lindlar's catalyst gave the corresponding alkene **15a** in almost quantitative yield.

**Table 1.** Structure of tetra- and pentasubstituted dihydropyrans

| Compound | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **6a** | Me | H | *s*Bu |
| **7a** | Me | H | *c*Hex |
| **8a** | Et | H | *c*Hex |
| **9a** | *c*Hex | H | *c*Hex |
| **10a** | Bn | H | *c*Hex |
| **11a** | Bn | H |  |
| **12a** | Me | Me[a] | *c*Hex |
| **13a** | Me | Me[a] | $Br_2C{=}CH(CH_2)_2$– |
| **14a** | Me | Me[a] | but-3-ynyl |
| **15a** | Me | Me[a] | but-3-enyl |

[a] (±)-Erythro **1** gave dihydropyrans with a *trans*-relative configuration of $R^2$ group with respect to $R^1$ and $R^3$.



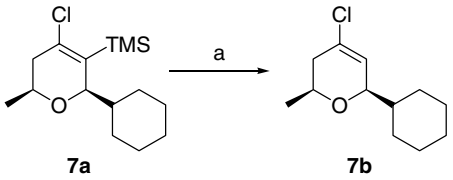

**Scheme 2.** Reagents and condition: (a) *n*-BuLi, $Et_2O$, −78 °C, 86%; (b) $H_2$, Pd/C Lindlar, EtOAc, 98%.

The cleavage of the TMS group offers an alternative to the production of derivatives with alkyl groups at positions 2 and 6 of the ring, such as compound **7b** (Scheme 3). As shown earlier, these products cannot be obtained by the direct reaction between aldehydes and unprotected secondary homopropargyllic alcohols (**1**, P = H, Scheme 1).[7]

The antitumor activity of compounds **3a**–**b**, **6a**–**15a**, and **7b** was evaluated against the three human solid tumor cell lines using the National Cancer Institute (NCI) protocol.[8] The growth inhibition parameters $GI_{50}$, TGI, and $LC_{50}$ together with the calculated lipophilicity values given as *C*log *P*[9] are given in Table 2. Overall, the results showed that the majority of the compounds are able to induce not only growth inhibition but also net cell kill, even in the resistant cancer cell line WiDr. The ovarian cancer cells were the most sensitive to the drugs. This observation is consistent with a previous study in which colon cancer cells showed more drug resistance than ovarian cancer cells.[10]

The lipophilicity values are not sufficient to explain the observed activity profiles. The *C*log *P* values for all the compounds are within a large range, 3.3–10.5 (Table 2). We found earlier that 2-alkyl-4-chloro-5,6-dihydro-2*H*-dihydropyran derivatives with *C*log *P* values larger than 4 were the most active products.[4] In the present study, this behavior was also observed.

When considering the growth inhibition parameters we found that compounds **3b** and **6a** are the only inactive products against all cell lines. The direct comparison of activity profiles between compounds **3a** and **3b** indicates that the introduction of the TMS group at position 3 of the oxacyclic ring enhances the activity against all cell lines. The effect is observed for the pair of compounds **7a** and **7b** against the colon cancer cells (WiDr). No improvement in activity was observed for 1,1-dibromovinyl derivative **13a** when compared to the analog **16** (Fig. 2). Perhaps in these compounds the effect of the




**Scheme 3.** Reagents and condition: (a) $HI/H_2O$ (1:1), $CH_2Cl_2$, reflux, 86%.

**Table 2.** Lipophilicity and growth inhibition parameters for the in vitro screening of dihydropyrans against human solid tumor cells[a]

| Compound | *C*log *P*[b] | A2780 (ovarian cancer) | | | SW1573 (non-small cell lung cancer) | | | WiDr (colon cancer) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $GI_{50}$ | TGI[c] | $LC_{50}$[c] | $GI_{50}$ | TGI[c] | $LC_{50}$[c] | $GI_{50}$ | TGI[c] | $LC_{50}$[c] |
| **3a** | 4.96 | 27 ± 5.1 | 66 ± 32 | 92 ± 11 | 10 ± 7.4 | 28 ± 10 | 65 ± 18 | 20 ± 13 | 39 ± 17 | 79 ± 15 |
| **3b** | 3.32 | >100 | | | >100 | | | >100 | | |
| **6a** | 5.48 | >100 | | | >100 | | | >100 | | |
| **7a** | 6.05 | 17 ± 5.9 | 32 ± 7.5 | 61 ± 6.9 | 16 ± 5.2 | 31 ± 7.8 | 62 ± 11 | 15 ± 8.8 | 33 ± 16 | 74 ± 24 |
| **7b** | 4.41 | 19 ± 9.2 | 38 ± 14 | 79 ± 18 | 12 ± 2.6 | 43 ± 13 | | 65 ± 29 | | |
| **8a** | 6.58 | 16 ± 2.3 | 35 ± 2.4 | 75 ± 4.0 | 22 ± 4.9 | 49 ± 13 | 91 ± 10 | 29 ± 6.5 | 73 ± 38 | 95 ± 7.7 |
| **9a** | 8.09 | 22 ± 3.7 | 46 ± 2.2 | 90 ± 8.5 | 23 ± 6.3 | 48 ± 15 | 88 ± 11 | 30 ± 9.6 | 81 ± 33 | 97 ± 4.6 |
| **10a** | 7.62 | 34 ± 14 | 78 ± 35 | | 30 ± 5.8 | | | 62 ± 36 | 88 ± 20 | |
| **11a** | 10.46 | 23 ± 0.6 | 47 ± 0.2 | 96 ± 1.6 | 30 ± 6.9 | 68 ± 9.7 | | 67 ± 4.8 | | |
| **12a** | 6.57 | 19 ± 3.9 | 38 ± 6.1 | 77 ± 11 | 20 ± 4.9 | 41 ± 10 | 76 ± 19 | 42 ± 22 | 84 ± 28 | |
| **13a** | 5.65 | 16 ± 1.1 | 33 ± 1.2 | 70 ± 1.7 | 22 ± 3.6 | 43 ± 5.8 | 87 ± 11 | 25 ± 14 | 90 ± 18 | |
| **14a** | 4.92 | 21 ± 2.3 | 39 ± 4.5 | 75 ± 13 | 19 ± 2.4 | 37 ± 6.1 | 75 ± 19 | 24 ± 7.9 | 68 ± 38 | 85 ± 19 |
| **15a** | 6.93 | 17 ± 1.7 | 36 ± 4.1 | 19 ± 9.8 | 23 ± 6.1 | 55 ± 22 | 92 ± 11 | 37 ± 2.3 | | |
| **16** | 4.25 | 16 ± 5.5 | 53 ± 41 | 76 ± 23 | 30 ± 7.0 | 92 ± 16 | | 23 ± 3.5 | 57 ± 21 | 96 ± 5.9 |
| **17** | 2.24 | 37 ± 7.2 | | | 41 ± 6.4 | | | >100 | | |
| **18** | 3.89 | 20 ± 2.6 | | | 26 ± 3.6 | | | >100 | | |
| **19** | 7.26 | 8.6 ± 6.7 | 25 ± 6.1 | 65 ± 3.3 | 14 ± 6.2 | 36 ± 10 | 85 ± 17 | 20 ± 2.3 | 61 ± 5.6 | 89 ± 15 |

[a] Values are given in μM and are means of two to four experiments, standard deviation is given in parentheses.
[b] Ref. 9.
[c] TGI and $LC_{50}$ values are given only if they are less than 100 μM, which is the maximum test concentration.

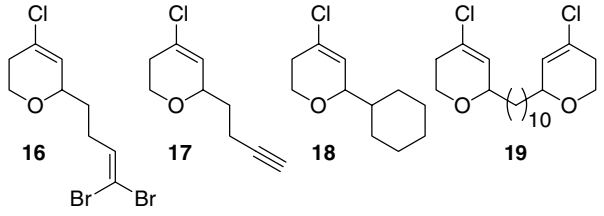



**Figure 2.** Structures of previously reported cytotoxic alkyl chloro dihydropyrans.

1,1-dibromovinyl group is prevailing. On the contrary, compound **14a** was to a large extent more potent against all cell lines than its analog **17** ($GI_{50}$ = 37, 41, and >100 μM against A2780, SW1573 and WiDr cells, respectively).[4] This outcome is also consistent with our previous studies where we described that the TBS (tert-butyldimethylsilyl) group enhanced the cytotoxic activity of 2,3-disubstituted-tetrahydropyrans in leukemia and solid tumor cells.[11] By means of the regioselective addition of TBS and TBDPS (*tert*-butyldiphenylsilyl) groups, inactive naturally occurring catalpol was transformed into antiproliferative analogs.[12] Furthermore, cisplatin[13] and camptothecin[14] analogs containing a silyl group have been reported to give a better activity profile than their respective parental anticancer drugs.

Compounds **7a** and **7b** were more active than **18** (analog of **7b** lacking the methyl group at $R^1$), which showed a modest activity against the ovarian and the lung cancer cells, and was inactive against WiDr cells.[4] Thus, in addition to the TMS group we found that the introduction of alkyl chains at position 6 of the ring ($R^1$) seems to favor the cytotoxic effect. Alkyl groups that produce similar biological results are Me (**7a**), Et (**8a**), and cHex (**9a**), whilst Bn (**10a**) gave less potent derivatives.

The results also indicate that the nature of the alkyl side chain at position 2 of the ring ($R^3$) may be relevant for the modulation of the biological activity. For instance, a cHex group at position 2 of the oxacyclic ring (**7a**–**10a**) leads to more active compounds when compared to other groups such as *s*Bu (**6a**) or linear alkyl substituents (**11a** and **13a**–**15a**). In this context, we found that the heterodimer **11a** showed reduced activity when compared to a previously reported homodimer **19** bearing two 4-chloro-5,6-dihydro-2*H*-pyran fragments.[4] We cannot discard that other factors than described are involved in this unexpected result.

In conclusion, we have shown that the addition of TMS and alkyl groups to the chlorodihydropyran scaffold leads to derivatives with enhanced cytotoxic profile when compared to 2-alkyl-4-chloro-5,6-dihydro-2*H*-pyrans. Based on these results, it is anticipated that these chlorovinyl–TMS oxacyclic compounds will be active against both sensitive and resistant solid tumors. All the cyclic products can be prepared in a single step from linear precursors by means of a silyl alkyne-Prins cyclization reaction. This general methodology will allow the quick production of oxacyclic synthons for the discovery of novel bioactive compounds.

## Acknowledgments

This research was supported by the EU INTERREG IIIB-MAC initiative (05/MAC/2.3/C14 BIOPOLIS), the Ministerio de Educación y Ciencia of Spain (MEC), co-financed by the European Regional Development Fund (CTQ2005-09074-C02-01/BQU) and the Canary Islands Government. P.O.M. thanks the Spanish MEC for a FPI fellowship. J.I.P. and J.M.P. thank the Spanish MEC for a Ramón y Cajal contract.

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7. All intermediates and final products gave satisfactory analytical and spectroscopic data in full accord with their assigned structures. Compound **14a**: $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ = 4.27 (m, 1H), 3.13 (m, 1H), 2.24 (m, 2H), 2.09 (m, 1H), 1.91–1.84 (m, 2H), 1.52 (m 1H), 1.16 (d, $J$ = 6.1 Hz, 3H), 1.02 (d, $J$ = 7.0 Hz, 3H), 0.19 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ = 145.1 (C), 135.4 (C), 84.0 (C), 76.0 (CH), 75.2 (CH), 68.1 (CH), 44.2 (CH), 34.4 ($CH_2$), 19.6 ($CH_3$), 15.3 ($CH_3$), 14.0 ($CH_2$), 0.1 ($3CH_3$); FTIR ($CHCl_3$): 2931.5, 1667.5, 1595.6, 1250.8, 840.0 cm$^{-1}$; elemental analysis calcd (%) for $C_{14}H_{23}ClOSi$ (270.87): C 62.08H 8.56; found: C 62.09 H 8.40. Compound **15a**: $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ = 5.78 (m, 1H), 4.96 (dd, $J_1$ = 17.1, $J_2$ = 1.7 Hz, 1H), 4.96 (dd, $J_1$ = 17.1, $J_2$ = 1.7 Hz, 1H), 4.89 (d, $J$ = 10.1 Hz, 1H), 4. 20 (m, 1H), 3.14 (m, 1H), 2.13 (m, 3H), 1.74 (m, 1H), 1.47 (m, 1H), 1.19 (d, $J$ = 6.1 Hz, 3H), 1.03 (d, $J$ = 7.0 Hz, 3H), 0.18 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ = 144.7 (C), 138.3 (CH), 135.9 (C), 114.3 ($CH_2$), 76.8 (CH), 75.3 (CH), 44.2 (CH), 34.8 ($CH_2$), 29.0 ($CH_2$), 19.7 ($CH_3$), 15.4 ($CH_3$), 0.1 ($3CH_3$); FTIR ($CHCl_3$): 2976.5, 1671.5, 1595.6, 1250.9, 840.2 cm$^{-1}$; elemental analysis calcd (%) for $C_{14}H_{25}ClOSi$ (272.89): C 61.62 H 9.23; found: C 61.79 H 9.30.
8. 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.
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