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Antiproliferative activity of 2-alkyl-4-halopiperidines and 2-alkyl-4-halo-1,2,5,6-tetrahydropyridines in solid tumor cell lines

Leticia G. León,[a,b] Rubén M. Carballo,[a] María C. Vega-Hernández,[a,b] Víctor S. Martín,[a] Juan I. Padrón[a] and José M. Padrón[a,b,*]

[a]*Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG), Universidad de La Laguna, Cl Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain*
[b]*BioLab, Instituto Canario de Investigación del Cáncer (ICIC), Cl Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain*

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Abstract—A series of *trans*-2-alkyl-4-halopiperidines and 2-alkyl-4-halo-1,2,5,6-tetrahydropyridines 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 (ovarian cancer), SW1573 (non-small cell lung cancer), and WiDr (colon cancer). The results on the biological activity revealed that, in general, the 2-alkyl-4-halo-1,2,5,6-tetrahydropyridine analogs are more potent than the *trans*-2-alkyl-4-halopiperidine derivatives. A remarkable selectivity of the aza compound **5f** for the resistant cell line WiDr was observed. Cell cycle studies revealed a G_2/M phase arrest for **5f**.

Marine organisms have proven to be an endless source of novel biologically active natural products.[1] In particular, marine secondary metabolites appear as promising molecules for the development of anticancer drugs.[2] Within our program directed at the development of novel antitumor drugs based on heterocyclic scaffolds from marine origin (Fig. 1), this type of compounds has attracted our interest.[3]

A major drawback of marine compounds is the very low amounts in which they are present in their natural sources. This fact forbids the direct use of the marine reservoir to isolate drugs to be used in chemotherapy. Therefore, chemists are encouraged to develop high yield synthetic methods to produce these compounds in a limited number of chemical steps and in large quantities. In our group, we have developed diverse methodologies for the synthesis of cyclic ethers.[4] Of particular interest is our approach to the synthesis of oxacycles based on a one-pot Prins-type cyclization, which is promoted by the inexpensive and environmentally friendly iron(III) chloride.[5]

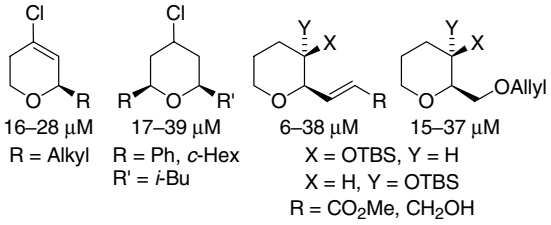

Figure 1. Heterocyclic marine scaffolds and antiproliferative activity against the human solid tumor cell lines A2780 (ovarian), SW1573 (non-small cell lung), and WiDr (colon).

In an effort to find more active analogs, we considered azacyclic bioisosteres of the previously reported oxacyclic derivatives. Herein we report on the antiproliferative activity of a set of 12 *trans*-2-alkyl-4-halopiperidines **3** and twelve 2-alkyl-4-halo-1,2,5,6-tetrahydropyridines **5** against a panel of three representative human solid tumor cells: A2780 (ovarian cancer), SW1573 (non-small cell lung cancer, NSCLC), and WiDr (colon cancer). We performed cell cycle studies to assess the effect of the most potent compound **5f** in SW1573 and WiDr cells.

Keywords: Marine drugs; Anticancer drugs; Halogenated piperidines; Halogenated tetrahydropyridines; Solid tumors; Structure–activity relationship.
* Corresponding author. Tel.: +34 922 318 580; fax: +34 922 318 571; e-mail: jmpadron@ull.es

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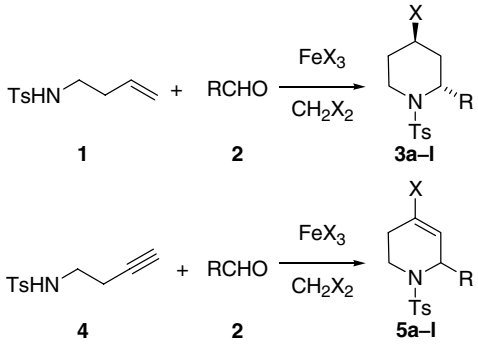
The compounds were prepared by means of the so-called aza-Prins-type cyclization.[6] This fast, simple, and versatile method is based on the consecutive generation of a γ-unsaturated-iminium ion and further nucleophilic attack by the unsaturated carbon–carbon bond. In this process, homoallyl tosylamine (**1**) leads to the corresponding *trans*-2-alkyl-4-halo-1-tosylpiperidine **3** as the major isomer, while homopropargyl tosylamine (**4**) gives 2-alkyl-4-halo-1-tosyl-1,2,5,6-tetrahydropyridine **5** as only product (Scheme 1). The choice of the iron(III) salt-solvent system $FeCl_3/CH_2Cl_2$ or $FeBr_3/CH_2Br_2$ leads to the 4-chloro or 4-bromo derivatives, respectively.[7] Thus, we have prepared the set of *trans*-2-alkyl-4-halopiperidines **3a–l** (Table 1) and their corresponding analogs 2-alkyl-4-halo-1,2,5,6-tetrahydropyridines **5a–l** (Table 2).



Scheme 1. Synthesis of substituted 4-halopiperidines and 4-halo-1,2,5,6-tetrahydropyridines.

The in vitro antiproliferative activity was evaluated using the National Cancer Institute (NCI) protocol[8] after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[3b] For *trans*-2-alkyl-4-halopiperidines **3a–l**, the results showed that the majority of the compounds were able to induce growth inhibition in the resistant cancer cell line WiDr (Table 1). With the exception of compound **3f** (R = Bn), A2780 and SW1573 cells were less sensitive to the derivatives than WiDr cells. This is a remarkable effect, since the general observation for conventional antitumor drugs is that WiDr colon cancer cells are more drug resistant than A2780 ovarian

Table 1. Lipophilicity and in vitro antiproliferative activity of *trans*-2-alkyl-4-halopiperidines against human solid tumor cells[a]

Compound	Clog P[b]	Substituent		A2780			SW1573			WiDr		
		X	R	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
3a	3.16	Cl	H	>100			>100			>100		
3b	5.14	Cl	*i*-Bu	36 (±6.7)			19 (±6.9)			22 (±7.8)		
3c	6.86	Cl	*n*-Hep	>100			>100			54 (±28)		
3d	5.71	Cl	*c*-Hex	72 (±16)			53 (±25)			15 (±6.8)		
3e	4.92	Cl	Ph	>100			>100			37 (±10)		
3f	5.25	Cl	Bn	19 (±1.7)	50 (±1.0)		12 (±0.3)	66 (±47)		20 (±1.6)		
3g	3.30	Br	H	>100			>100			>100		
3h	5.28	Br	*i*-Bu	39 (±11)			22 (±0.8)			14 (±1.3)		
3i	7.00	Br	*n*-Hep	>100			>100			>100		
3j	5.85	Br	*c*-Hex	>100			82 (±20)			14 (±5.0)		
3k	5.06	Br	Ph	>100			>100			36 (±2.4)		
3l	5.39	Br	Bn	63 (±17)			73 (±2.4)			37 (±15)		

[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. 10.

Table 2. Lipophilicity and in vitro antiproliferative activity of 2-alkyl-4-halo-1,2,5,6-tetrahydropyridines against human solid tumor cells[a]

Compound	Clog P[b]	Substituent		A2780			SW1573			WiDr		
		X	R	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
5a	3.42	Cl	H	>100			>100			>100		
5b	5.39	Cl	*i*-Bu	35 (±8.3)			31 (±8.5)			21 (±6.9)		
5c	7.11	Cl	*n*-Hep	92 (±11)			49 (±12)			50 (±9.7)		
5d	5.97	Cl	*c*-Hex	39 (±17)			62 (±0.6)			24 (±7.1)		
5e	5.18	Cl	Ph	17 (±3.8)	42 (±14)	86 (±25)	16 (±5.4)	37 (±8.1)	87 (±8.3)	18 (±5.6)	77 (±41)	91 (±16)
5f	5.51	Cl	Bn	4.8 (±1.1)			6.6 (±4.5)			3.3 (±0.4)	14 (±0.3)	79 (±30)
5g	3.51	Br	H	>100			>100			>100		
5h	5.48	Br	*i*-Bu	36 (±14)			26 (±7.5)			16 (±2.5)		
5i	7.20	Br	*n*-Hep	>100			49 (±30)			40 (±26)		
5j	6.06	Br	*c*-Hex	44 (±10)			65 (±3.9)			18 (±3.4)		
5k	5.27	Br	Ph	49 (±8.3)			54 (±9.4)			29 (±13)		
5l	5.60	Br	Bn	27 (±8.2)	77 (±31)		21 (±9.5)	78 (±38)		24 (±5.7)	71 (±25)	

[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. 10.

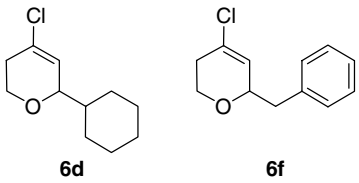


Figure 2. Structures of antiproliferative six-membered oxacycles.

Table 3. Comparison of in vitro antiproliferative activity between oxa- and azacycles against human solid tumor cells[a]

Compound	Substituent	Cell line		
	R	A2780	SW1573	WiDr
5d	*c*-Hex	39	62	24
6d	*c*-Hex	20[b]	26[b]	>100[b]
5f	Bn	4.8	6.6	3.3
6f	Bn	36[b]	58[b]	>100[b]

[a] Values representing GI_{50} are given in μM.
[b] Ref. 3.

cancer cells.[9] Analog **3f** was the most potent compound of the series with GI_{50} values in the range 12–20 μM against the three cell lines. This compound was the only piperidine analog able to induce total growth inhibition (TGI) in A2780 and SW1573 cells. However, cytotoxicity (expressed as LC_{50}) was not observed for any of the *trans*-2-alkyl-4-halopiperidine derivatives.

The results on the biological activity of the 2-alkyl-4-halo-1,2,5,6-tetrahydropyridine analogs are shown in Table 2. Majority of the compounds induced antiproliferative effect on the three cell lines tested. Similarly to the *trans*-2-alkyl-4-halopiperidine derivatives, WiDr was the most sensitive cell line. The most potent 2-alkyl-4-halo-1,2,5,6-tetrahydropyridine derivative was compound **5f** (R = Bn), which produced GI_{50} values against the three cell lines in the range 3.3–6.6 μM. When considering TGI and LC_{50} values (Table 2) compounds **5e**, **5f**, and **5l** appeared as the most antiproliferative products of the series.

Lipophilicity is a major determinant of pharmacokinetic and pharmacodynamic properties of drug molecules. The general trend of lipophilicity expressed as C log *P* values was found to be C log *P* < 4 for inactive derivatives and 4.92 < C log *P* < 7.20 for active compounds.[10] In view of these results, it appears that the biological activity does not correlate with the calculated C log *P* values. Differences in activity between chlorinated and brominated derivatives did not follow a clear trend. Finally, the endocyclic insaturation at C_3 appears as responsible for the larger activity of 2-alkyl-4-halo-1,2,5,6-tetrahydropyridine analogs. This result is consistent with our previous findings in 2-alkyl-4-chloro-5,6-dihydro-2*H*-pyrans (Fig. 2), in which the chlorovinyl group was established as the pharmacophore. The new azacycles only showed a significant selectivity for the resistant colon cancer cell line when compared to the corresponding oxacyclic derivatives (Table 3).

Cell cycle control is the major regulatory mechanism of cell growth. Many cytotoxic agents and/or DNA damaging agents arrest the cell cycle at the G_0/G_1, S, or G_2/M phase and then induce apoptotic cell death. We examined cell cycle phase distribution by flow cytometry to determine if cell growth inhibition involved cell cycle

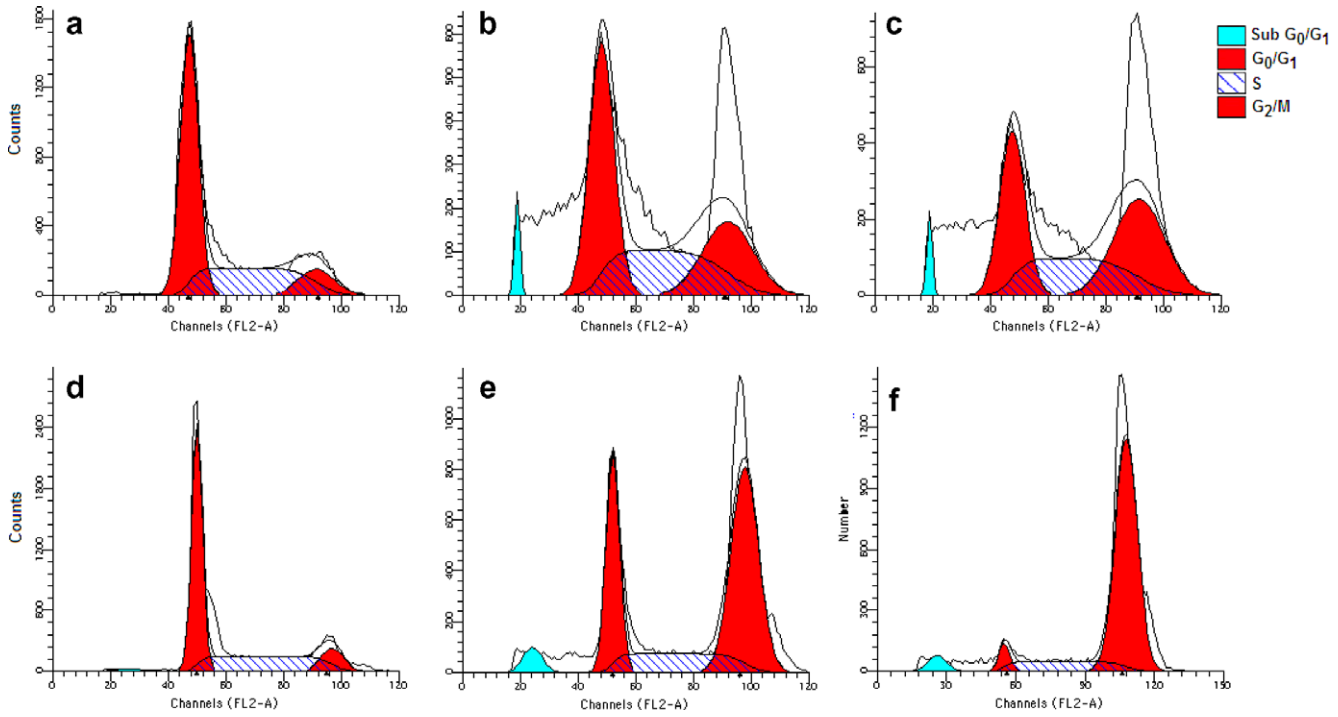


Figure 3. Effects of compound **5f** on cell cycle distribution of SW1573 NSCLC cells (a–c) and WiDr colon cancer cells (d–f). Cells were cultured for 24 h in the absence (control, a and d) or presence of **5f** at 10 μM (b and e) and 20 μM (c and f).

changes. Therefore, SW1573 and WiDr cells were exposed for 24 h to the most active derivative **5f** at drug doses of 10 and 20 μM.[11] The results are shown in Figure 3. For SW1573 cells exposed to 10 μM of derivative **5f**, it was possible to observe a slight increase in the percentage of cells in the G_2/M phase (25%) with respect to control cells (11%). At the higher dose, the amount of NSCLC cells in G_2/M phase augmented to 38%. The rise was concomitant with a decrease in the G_0/G_1 phase compartment. Interestingly, the cell cycle arrest was more evident for WiDr cells exposed to compound **5f**. Thus, the percentage of cells at G_2/M was 53% and 81% after exposure to 10 and 20 μM, respectively. These values are much larger than that of non-treated WiDr cells, which was 11%. The results are in agreement with those obtained in the antiproliferative study, showing a remarkable selectivity of the new compound for the more resistant colon cancer cell line WiDr.

In summary, we have determined the antiproliferative activity of a series of *trans*-2-alkyl-4-halopiperidines and 2-alkyl-4-halo-1,2,5,6-tetrahydropyridines. Active compounds showed a significant selectivity for the more resistant cancer cell line WiDr. The halovinyl group seems responsible for the enhanced activity of the pyridine analogs when compared to piperidine derivatives. A clear structure–activity relationship for chlorinated and brominated derivatives was not observed. Cell cycle arrest at G_2/M was confirmed for the most active derivative. Ongoing studies on the mechanism of action will be reported elsewhere.

Acknowledgments

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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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10. Software-predicted lipophilicity of the compounds was calculated with the program New C log *P* accessible via internet (http://intro.bio.umb.edu/111-112/OLLM/111F98/newclogp.html).
11. Cells were seeded in six-well plates at a density of $0.7–1 \times 10^6$ cells/well. After 24 h, the products were added to the respective well and incubated for an additional period of 24 h. Cells were trypsinized, harvested, transferred to test tubes (12.75 mm), and centrifuged at 1500 rpm for 10 min. The supernatant was discarded and the cell pellets were resuspended in 200 μl of cold PBS and fixed by the addition of 3 mL ice-cold 70% ethanol. Fixed cells were incubated overnight at −20 °C after which time were centrifuged at 1500 rpm for 10 min. The cell pellets were resuspended in 500 μl PBS. Then, 20 μl of DNAse-free RNAse (200 μl/ml) and 10 μl of propidium iodide (40 μl/ml) were sequentially added. The mixture was incubated in the dark at 37 °C for 30 min. Flow cytometric determination of DNA content (25,000 cells/sample) was analyzed by FACSCalibur Flow Cytometer (Becton Dickinson, San José, CA, USA). The fractions of the cells in G_0/G_1, S, and G_2/M phase in addition to apoptosis were analyzed using cell cycle analysis software, ModFit LT 3.0 (Verity Software House, Topsham, ME, USA).