Synthesis of Furonaphth[1,3]oxazine and Furo[1,3]oxazinoquinoline Derivatives as Precursors for an o-Quinonemethide Structure and Potential Antitumor Agents

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The synthesis of dihydro furonaphth[1,3]oxazine derivatives 3 was performed through a Mannich-type condensation between 2-cyano-5-hydroxy-3-methylphenoth[1,2-b]furan 2a, 1.5 eq of a primary amine and 3 eq of formaldehyde. Similarly, 2-cyano-5-hydroxy-3-methylfuro[2,3-f]quinoline 2b gave the dihydro furo[1,3]oxazinoquinoline compounds 4. Heating a mixture of the naphthofuran 2a, tert-butylamine and formaldehyde at toluene reflux led to the furonaphthoxazine 3e, which decomposes to afford an o-quinonemethide intermediate 5. The latter was trapped with 1-morpholinopropane to give a dihydro furonaphthopyran derivative 6. All compounds 2, 3, 4 and 6 were assayed for in vitro cytotoxic activity toward L 1210, MDA-MB 231 and PC3 tumor cells. Among them, furonaphth[1,3]oxazines 3b, 3c, and furo[1,3]oxazinoquinolines 4c, 4d showed significant activity against L 1210 cells, while furoquinoline 2b was the most cytotoxic compound towards all three cell lines.

Key words dihydro-1,3-oxazine; furonaphth[1,3]oxazine; furo[1,3]oxazinoquinoline; o-quinonemethide; cytotoxicity

As part of our investigations on the biological properties of fused 3-methylfurans derivatives, we developed an efficient synthesis of 3-methyl-5-hydroxynaphthofurans and 3-methyl-5-hydroxyfurquinolines through an addition-cyclization process of 2-ethoxybut-2-enal N,N-dimethylhydrazone with naphthoquinones or quinolinones.1 Evaluation of the in vitro cytotoxic activity of these compounds was performed on murine lymphocytic leukemia cells (L 1210), human mammary adenocarcinoma cells (MDA-MB 231) and human prostate cells (PC3).2 Several naphthofurans and furquinolines showed significant IC50 values towards L 1210 cells while cytotoxicity against MDA-MB 231 and PC3 tumor cells was only retained in the furquinoline series. On the other hand, some N-substituted dihydro[1,3]oxazines condensed with aromatic rings were reported to possess cytotoxic or antifungal activities.3-6 In order to check whether aminomethylation of the 5-hydroxynaphthofuran and 5-hydroxyfurquinoline skeletons would influence the inhibition of tumor cell proliferation, we planned to synthesize and test a series of furonaphth[1,3]oxazine and furo[1,3]oxazinoquinoline derivatives.

Synthesis

The hydrazone function of 1a or 1b was converted to a cyano group by the use of magnesium monoperoxyphthalate hexahydrate (MMPP) according to a known procedure.7 Thus, compounds 2a and 2b9b) were obtained in excellent yields (94 and 90% respectively). Then, the dihydro[1,3]oxazine derivatives 3 and 4 were prepared through a Mannich-type condensation by heating an ethanol solution of the corresponding phenol 2 with a primary amine (1.5 eq) and three equivalents of a 37% aqueous solution of formaldehyde (Chart 1 and Table 1).

Mannich bases of phenols and dihydro[1,3]oxazines are known to be thermally unstable and to decompose to o-quinonemethide and the respective amine or imine.9-11 Heating to reflux a tolucne solution of the naphthofuran 2a and a mixture of tert-butylamine (1 eq) and formaldehyde (2 eq) in the same solvent gave the furonaphthoxazine 3e, which decomposed quickly to an o-quinonemethide 5 through a retro Diels–Alder reaction. Due to its instability, 5 was not isolated, but was trapped with trans-1-morpholinopropane. Thus, the furonaphthopyran 6 was obtained as a single stereoisomer in 71% yield through a one-pot procedure. Formation of compound 6

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resulted from a regiospecific and stereospecific [4 + 2]
cycloaddition with an inverse electron demand (Chart 2).
Its relative trans configuration was established from its
$^1$H-NMR spectrum at 300 MHz. Indeed, H-2 gave a
doublet at 4.44 ppm with a 9.4 Hz $J$-value which is in
accord with literature data for $J$-trans coupling in
analogous dihydropyran derivatives.$^{12}$

**Pharmacology**

All of compounds 2, 3, 4 and 6 were assayed for in vitro
cytotoxic activity against the three tumor cell lines
mentioned above. The IC$_{50}$ values are reported in Table 2.

The IC$_{50}$ values given in Table 2 show that the
furonaphth[1,3]oxazine derivatives are more cytotoxic
towards L 1210 cells than the starting naphthofuran 2a.
In the furafuro[1,3]oxaxinoquinolines, the best IC$_{50}$ values
against L 1210 cells are observed when R is an allyl (4c)
or a dimethylaminomethyl group (4d). But, these two
compounds remain less active than the parent furaoxazine
2b. Finally, the furanophophorphan 6 is inactive. A
comparison of the IC$_{50}$ values of 2a and 2b indicated
that the furaoxazine structure is essential for significant
cytotoxicity against the three tumor cell lines used.
Moreover, aminomethylation of 2a slightly reduces the
cytotoxicity of compounds 3b and 3c to L 1210 cells,
whereas the cytotoxicity of 4 is weaker than that of 1b.

**Experimental**

Melting points were measured on a Büchi apparatus (capillary tube).

<table>
<thead>
<tr>
<th>3</th>
<th>X</th>
<th>R</th>
<th>Time (h)</th>
<th>Yield (%)</th>
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</thead>
<tbody>
<tr>
<td>1a</td>
<td>CH</td>
<td>CH$_2$CH$_3$</td>
<td>1</td>
<td>80</td>
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<tr>
<td>2b</td>
<td>CH</td>
<td>CH$_2$C$_6$H$_5$</td>
<td>0.5</td>
<td>90</td>
</tr>
<tr>
<td>3c</td>
<td>CH</td>
<td>CH$_2$C$_6$H$_4$OCH$_3$</td>
<td>0.5</td>
<td>80</td>
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<tr>
<td>3d</td>
<td>CH</td>
<td>CH$_2$C$_6$H$_4$CH$_3$</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>4e</td>
<td>CH</td>
<td>CH$_2$C$_6$H$_4$N(CH$_3$)$_2$</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CH</td>
<td>C(CH$_3$)$_3$</td>
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<td>60</td>
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</table>

<table>
<thead>
<tr>
<th>4</th>
<th>X</th>
<th>R</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
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<tr>
<td>2a</td>
<td>CH</td>
<td>CH$_2$CH$_3$</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>2b</td>
<td>CH</td>
<td>CH$_2$C$_6$H$_5$</td>
<td>0.5</td>
<td>90</td>
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<tr>
<td>3c</td>
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<td>3d</td>
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<tr>
<td>4e</td>
<td>CH</td>
<td>CH$_2$C$_6$H$_4$N(CH$_3$)$_2$</td>
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</tr>
<tr>
<td>5</td>
<td>CH</td>
<td>C(CH$_3$)$_3$</td>
<td>0.75</td>
<td>60</td>
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</table>

**Table 1.** Synthesis of Furonaphth[1,3]oxazines 3 and Furafuro[1,3]oxaxinoquinolines 4

<table>
<thead>
<tr>
<th>Compounds</th>
<th>L 1210</th>
<th>MDA-MB-231</th>
<th>PC3</th>
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<tr>
<td>2a</td>
<td>18.071$	imes10^{-6}$</td>
<td>17.009$	imes10^{-6}$</td>
<td>30.941$	imes10^{-6}$</td>
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<tr>
<td>2b</td>
<td>0.459$	imes10^{-6}$</td>
<td>0.950$	imes10^{-6}$</td>
<td>2.007$	imes10^{-6}$</td>
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<tr>
<td>3a</td>
<td>10.002$	imes10^{-6}$</td>
<td>&gt;28$	imes10^{-6}$</td>
<td>&gt;28$	imes10^{-6}$</td>
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<tr>
<td>3b</td>
<td>2.995$	imes10^{-6}$</td>
<td>&gt;26$	imes10^{-6}$</td>
<td>&gt;28$	imes10^{-6}$</td>
</tr>
<tr>
<td>3c</td>
<td>2.256$	imes10^{-6}$</td>
<td>16.612$	imes10^{-6}$</td>
<td>14.127$	imes10^{-6}$</td>
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<tr>
<td>3d</td>
<td>4.958$	imes10^{-6}$</td>
<td>7.512$	imes10^{-6}$</td>
<td>10.481$	imes10^{-6}$</td>
</tr>
<tr>
<td>4a</td>
<td>6.0$	imes10^{-6}$</td>
<td>10.137$	imes10^{-6}$</td>
<td>10.932$	imes10^{-6}$</td>
</tr>
<tr>
<td>4b</td>
<td>3.013$	imes10^{-6}$</td>
<td>12.286$	imes10^{-6}$</td>
<td>10.260$	imes10^{-6}$</td>
</tr>
<tr>
<td>4c</td>
<td>1.499$	imes10^{-6}$</td>
<td>7.073$	imes10^{-6}$</td>
<td>4.596$	imes10^{-6}$</td>
</tr>
<tr>
<td>4d</td>
<td>2.261$	imes10^{-6}$</td>
<td>4.754$	imes10^{-6}$</td>
<td>16.319$	imes10^{-6}$</td>
</tr>
<tr>
<td>6</td>
<td>&gt;28$	imes10^{-6}$</td>
<td>&gt;28$	imes10^{-6}$</td>
<td>&gt;28$	imes10^{-6}$</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.035$	imes10^{-6}$</td>
<td>0.082$	imes10^{-6}$</td>
<td>0.515$	imes10^{-6}$</td>
</tr>
</tbody>
</table>

The infrared (IR) spectra were obtained on a Perkin-Elmer 1310
spectrophotometer. The proton nuclear magnetic resonance spectra were
recorded at 300 MHz on a Bruker AM 300 apparatus. Chemical shifts are
reported in ppm (δ) from tetramethylsilane (TMS) as an internal
reference. Elemental analysis was done at the Centre de Microanalyse
du CNRS at Solaize, France.

All of the amines were freshly distilled before use. Compounds 1a,11b1)
and 2b11) were prepared according to the cited procedures. The
naphthofuran derivative 2a was obtained as reported.8 1-Morpholinopropane
was also prepared as reported.13)

2-Cyano-5-hydroxy-3-methylphthalonaphth[1,2-b]furan (2a) A solution of
the hydrazone 1a (0.223 g, 1 mol) in 3 ml of methanol was added under
stirring to magnesium monoperoxyphthalate hexahydrate (1.224 g,
2.5 mmol) in the same solvent (8 ml) cooled to 0°C. At the end of
the addition (5 min), stirring was continued for 5 min at 0°C. Then,
dichloromethane (25 ml) and water (25 ml) were added. The organic layer
was washed twice with a saturated aqueous solution (25 ml) of sodium
chloride. The solution was dried over magnesium sulfate and evaporated
under vacuum. The pink solid obtained was recrystallized from ethanol
to yield compound 2a (94%), mp 167–170°C. IR (KBr): 3400,
2200 cm$^{-1}$. 1H-NMR (CDCl$_3$): δ: 10.41 (1H, s, OH), 8.25 (1H, dd, $J$=7.7, 1.1 Hz, H-6 or H-9), 8.18 (1H, dd, $J$=7.7, 1.1 Hz, H-6 or H-9),
7.70 (1H, dt, $J$=7, 1.3 Hz, H-7 or H-8), 7.63 (1H, dt, $J$=7, 1.3 Hz, H-7 or H-8), 6.93 (1H, s, H-4), 2.50 (3H, s, CH$_3$). Anal. Caled for C$_{14}$H$_8$N$_2$:O$_2$;
C, 75.82; H, 4.06; N, 6.27. Found: C, 75.02; H, 4.14; N, 6.36.

General Procedure for the Synthesis of 3-Substituted-6-cyano-5-methyl-
3,4-dihydro-2H-furo[3′:2′:3′4′]napthal[2′:1′]1,1,2,3 ]oxazines (3) The
primary amine (1.5 mmol) was added to a 37% aqueous solution
of formaldehyde (3 mmol) in ethanol (15 ml) previously cooled in an
ice bath. The resulting mixture was stirred at 0°C for 30 min. Then,
compound 2a (1 mmol) was added and the reaction mixture was heated
at 50°C for a variable time, the evolution of the reaction being followed
by TLC. Precipitates of the corresponding dihydrofuranoxazines
3 were formed. They were collected and recrystallized from ethanol.

3-Benzyl-6-cyano-5-methyl-3,4-dihydro-2H-furo[3′:2′:3′4′]napthal[2′:1′]:
l[1,1,2,3]oxazine (3a) Compound 3a was obtained as a white solid (80% yield), mp 190°C. IR (KBr): 2220 cm$^{-1}$. 1H-NMR (CDCl$_3$): δ: 8.23 (2H,
m, H-8 and H-11), 7.62 (2H, m, H-9 and H-10), 7.32 (3H, m, H aromat.),
5.07 (2H, s, H-2), 4.35 (2H, s, H-4), 4.0 (2H, s, N·CH$_2$-C$_6$H$_5$), 2.42
(3H, s, CH$_3$). Anal. Caled for C$_{14}$H$_8$N$_2$O$_2$: 0.33 H$_2$O: C, 76.65; H,
5.22; N, 7.77. Found: C, 76.56; H, 5.09; N, 8.01.
6-Cyano-3,5-dimethyl-2-morpholin-3,4-dihydro-2H-furo[3′,2′:3,4]-naphth[2,1-c]oxazin-3(2H)-one (3b).

Synthesis of 6-Cyano-3,5-dimethyl-2-morpholin-3,4-dihydro-2H-furo[3′,2′:3,4]napth[1,2-b]pyran (6a) 

tert-Butylamine (0.08 ml, 0.75 mmol) was added to a cooled, aqueous solution of 37% formaldehyde (0.14 ml, 1.55 mmol) in toluene (5 ml). The mixture was stirred for 30 min. A solution of compound 2a (0.168 g, 0.75 mmol) in 5 ml of toluene was added and the reaction mixture was heated at reflux for 15 min. Then, a solution of 1-morpholinopropene (0.143 g, 1.125 mmol) in toluene (1 ml) was added and heating was continued for 45 min. A white precipitate was formed by cooling. It was recovered and recrystallized from toluene. Yield 71%. mp 270°C (IR (KBr): 2220 cm⁻¹; H-NMR (CDCl₃): δ: 8.21 (2H, d, J = 6.2 Hz, H-8 and H-11), 7.64 (2H, m, H-9 and H-10), 7.33 (2H, d, J = 8.6 Hz, H aromat.), 6.92 (2H, d, J = 8.6 Hz, H aromat.), 5.07 (2H, s, H-2), 4.40 (2H, s, H-4), 3.99 (2H, m, 2H, CH₂, 2H, 2H, OCH₂), 3.34 (8H, s, OCH₂), 2.44 (3H, s, CH₂-5). Anal. Calc. for C_{29}H_{30}N_{12}O_{12}: 74.98; H, 5.24; N, 7.29. Found: C, 75.0; H, 5.2; N, 7.37.


Vitro Cytotoxicity Assays

All compounds were dissolved in DMSO (Certo, final solution concentration 0.2%) and were tested at various concentrations on three tumor cell systems. Assays included solvent and reference controls.

In Vitro Cytotoxic Activity towards L1210 Cells

L1210 murine leukemia cells were cultured in suspension in RPMI 1640 medium (Eurobio) with 10% heat-inactivated fetal calf serum (Boehringer, Mannheim), 2-mercaptoethanol (Sigma, 10 μM), L-glutamine (Boehringer, Mannheim, 2 mM) and 1% of penicillin-streptomycin (Sigma, 25 μg/ml). Cells (10⁶ cells/ml) were cultured in a humidified, 5% CO₂ air atmosphere. For the screening, the cell suspension (cells in an exponential growth phase) was adjusted to 10⁶ viable cells per ml (cell viability was estimated by the Trypan blue exclusion test). Cells were distributed in wells of a microtiter culture plate (Falcon, 225 ml per well) before introducing the test compounds or the solvent (25 μl). Four days later, cells were counted with a Coulter Counter (Coultronics, France). IC₅₀ was defined as the concentration inhibiting by 50% the cell growth compared to the control after 96 h of culture, and was determined from the regression line of percentage cell growth inhibition against the logarithm of the dose.

In Vitro Cytotoxic Activity towards Human Cancer Cell Lines

MDA-MB-231 and PC3 MDA-MB-231 cells were maintained in DMEM (Gibco) supplemented with 10% heat-inactivated fetal calf serum (Eurobio), insulin 1 μU/ml, L-glutamine (2 mM) and antibiotics. PC3 cells were cultured in a solution composed of 65% F12 Nutrient Mixture Ham (Gibco), 25% DMEM and 10% heat-inactivated fetal calf serum, L-glutamine (2 mM) and antibiotics. All the cell suspensions were maintained at 37°C in a humidified, 5% CO₂ air atmosphere. The cytotoxic activity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) colorimetric method based on that of Mosmann [7]. Cells (10⁴ cells/ml) were seeded in 96-well tissue culture plates (153 μl per well). After 24 h of culture, 15 μl of each compound dissolved in 15 μl of medium with DMSO (controls) or 15 μl of medium containing a compound solubilized in this solvent. After 5 days of culture, each well received 15 μl of a sterile solution of MTT in phosphate-buffered saline (PBS) solution at 5 mg/ml and the plate was incubated for 4 h. Then, the culture medium was removed and 100 μl of DMSO was added to each well for quantitation of blue formazan by reading the absorbance at 540 nm on a Tintek Multiskan II Flow (Laboratories, France).

The IC₅₀ values were calculated from the regression lines in plots of the percentage decrease in absorbance versus controls against the logarithm of the concentration.

References and Notes

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