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## Report

# The *in vitro* antitumor assay of 5-(*Z*)-arylidene-4-imidazolidinones in screens of AIDS-related leukemia and lymphomas

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**Thirty-one different 5-(*Z*)-arylidene-4-imidazolidinones were tested on six AIDS-related lymphoma (ARL) tumor cell lines, one leukemia CCRF-CEM cell culture and five different lymphoma cell lines: RL, KD-488, AS283, PA682 and SU-DHL-7. The investigated compounds showed remarkable activity against ARL, compounds 3d and 5c proved to inhibit AS283 and SU-DHL-7 cell lines, respectively, both at a $GI_{50}$ value of 0.03 $\mu$M. The 2-(2-carboxyphenylamino) series proved to be the most active members in this investigation. Compounds 6b and 6d showed $GI_{50}$ (MGMID) values of 6.1 and 8.7 $\mu$M, respectively, against the studied six ARL. [© 2001 Lippincott Williams & Wilkins.]**

*Key words*: Antitumor screening, AIDS-related leukemia and lymphoma, 2-thioxo-4-imidazolidinone, 5(*Z*)-arylidene-4-imidazolidinone.

## Introduction

Retroviruses are viral agents which are natural inducers of leukemias and solid tumors among animals and man. In man, they are implicated as ethiological agents of a specific type of leukemia, adult-T cell leukemia.[1] HIV (AIDS virus), is capable of leading to the formation of several types of opportunistic tumors,[2–7] such as non-Hodgkin lymphomas and Kaposi's sarcoma.[8–13] AIDS-related lymphoma (ARL) is almost exclusively of the non-Hodgkin's lymphoma type.[14] Patients with ARL responded to azidothymidine (AZT) and interferon-$\alpha$; lymphoma cells were believed to undergo apoptosis upon the treatment with those two drugs.[15] Half-mustard-type phenothiazines were synthesized and tested on seven ARL tumor cell lines, they proved to affect the growth and inhibited the growth rate of such tumor cell lines.[16]

In the course of identifying new chemical structures which may serve as leads for designing novel antitumor agents, we were particularly interested in imidazolinones. In this respect, the linking of this heterocycle to hydrophilic and lipophilic moieties such as hydroxymethylpiperidine, morpholine, piperidine and aminobenzoic acid produced a series of active antitumor agents,[17] screened at the National Cancer Institute's (NCI) *in vitro* disease-oriented antitumor screen, which determines a test agent's effect on growth against a panel of human tumor cell lines.[18–20] As a continuation to our previous efforts,[17,21] we would like to report herein the antitumor screening results, obtained from the NCI facility, of 31 different 5-(*Z*)-arylidene-4-imidazolidinones (Figure 1), against six ARL tumor cell lines.

## Materials and methods

### Source of compounds

The 5-(*Z*)-arylidene-4-imidazolidinone derivatives used in the present study were previously synthesized and characterized.[17] Their chemical names are shown in the following list and their chemical structures are presented in Figure 1. 5-(*Z*)-(4-methylbenzylidene)-2-thioxo-4-imidazolidinone (**1a**); 5-(*Z*)-(4-chlorbenzylidene)-2-thioxo-4-imidazolidinone (**1b**); 5-(*Z*)-(2-Thienylidene)-2-thioxo-4-imidazolidinone (**1c**); 5-(*Z*)-(4-methylbenzylidene)-2-methylmercapto-4-imidazolidinone (**2a**); 5-(*Z*)-(4-methoxybenzylidene)-2-methylmer-

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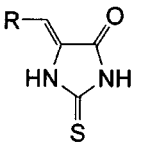
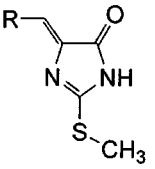
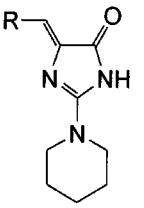


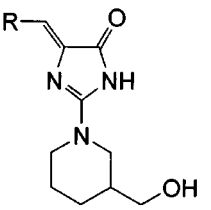
**1a:** R = 4-$CH_3$Ph, **1b:** R = 4-ClPh,
**1c:** R = 2-thienyl.



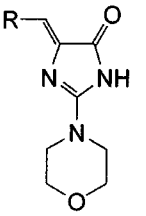
**2a:** R = 4-$CH_3$Ph, **2b:** R = 4-$CH_3$OPh.



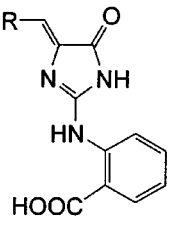
**3a:** R = Ph, **3b:** R = 4-$CH_3$Ph,
**3c:** R = 4-$CH_3$OPh, **3d:** R = 4-ClPh
**3e:** R = 2-thienyl, **3f:** R = 2-furyl.



**4a:** R = Ph, **4b:** R = 4-$CH_3$Ph,
**4c:** R = 4-$CH_3$OPh, **4d:** R = 4-ClPh,
**4e:** R = 4-HOPh, **4f:** R = 2-thienyl.



**5a:** R = Ph, **5b:** R = 4-$CH_3$Ph,
**5c:** R = 4-$CH_3$OPh, **5d:** R = 4-ClPh,
**5e:** R = 4-HOPh, **5f:** R = 2-thienyl,
**5g:** R = 2-furyl.



**6a:** R = Ph, **6b:** R = 4-$CH_3$Ph,
**6c:** R = 4-$CH_3$OPh, **6d:** R = 4-ClPh,
**6e:** R = 4-HOPh, **6f:** R = 2-thienyl,
**6g:** R = 2-furyl.

**Figure 1.** Structures of 5-*Z*-arylidene-4-imidazolidinone analogs.

capto-4-imidazolidinone (**2b**); 5-(*Z*)-(benzylidene)-2-piperidino-4-imidazolidinone (**3a**); 5-(*Z*)-(4-methylbenzylidine)-2-piperidino-4-imidazolidinone (**3b**); 5-(*Z*)-(4-methoxybenzylidine)-2-piperidino-4-imidazolidinone (**3c**); 5-(*Z*)-(4-chlorobenzylidine)-2-piperidino-4-imidazolidinone (**3d**); 5-(*Z*)-(2-thienylidene)-2-piperidino-4-imidazolidinone (**3e**); 5-(*Z*)-(2-furylidene)-2-piperidino-4-imidazolidinone (**3f**); 5(*Z*)-(benzylidene)-2-(3-hydroxymethyl)piperidino-4-imidazolidinone (**4a**); 5-(*Z*)-(4-methylbenzylidine)-2-(3-hydroxymethyl)piperidino-4-imidazolidinone (**4b**); 5-(*Z*)-(4-methoxybenzylidene)-2-(3-hydroxymethyl)piperidino-4-imidazolidinone (**4c**); 5-(*Z*)-(4-chlorobenzylidene)-2-(3-hydroxymethyl)piperidino-4-imidazolidinone (**4d**); 5-(*Z*)-(4-hydroxybenzylidene)-2-(3-hydroxymethyl)piperidino-4-imidazolidinone (**4e**); 5-(*Z*)-(2-thienylidene)-2-(3-hydroxymethyl)piperidino-4-imidazolidinone (**4f**); 5-(*Z*)-(benzylidene)-2-morpholino-4-imidazolidinone (**5a**); 5-(*Z*)-(4-methylbenzylidene)-2-morpholino-4-imidazolidinone (**5b**); 5-(*Z*)-(4-methoxybenzylidene)-2-morpholino-4-imidazolidinone (**5c**); 5-(*Z*)-(4-chlorobenzylidene)-2-morpholino-4-imidazolidinone (**5d**); 5-(*Z*)-(4-hydroxybenzylidene)-2-morpholino-4-imidazolidinone (**5e**); 5-(*Z*)-(2-thienylidene)-2-morpholino-4-imidazolidinone (**5f**); 5-(*Z*)-(2-furylidene)-2-morpholino-4-imidazolidinone (**5g**); 5-(*Z*)-(benzylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6a**); 5-(*Z*)-(4-methylbenylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6b**); 5-(*Z*)-(4-methoxybenylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6c**); 5-(*Z*)-(4-clorobenylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6d**); 5-(*Z*)-(4-hydroxybenylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6e**); 5-(*Z*)-(2-thienylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6f**); 5-(*Z*)-(2-furylidene)-2-(2-carboxyphenylamino)-4-imidazolidinone (**6g**).

### Antitumor testing and data analysis

Compounds **1a–6g** were subjected to the NCI *in vitro* disease-oriented human cells screening panel assay as reported.[18–20] Six ARL tumor cell lines were used, one leukemia CCRF-CEM cell culture and five different lymphoma cell lines: RL, KD-488, AS283, PA682 and SU-DHL-7. Each cell line was incubated with five concentrations (0.01–100 $\mu$M) of each agent and used to create log concentration–percent growth inhibition curves. Three response parameters, i.e. $GI_{50}$ (50% inhibition of growth), TGI (0% growth of tumor growth inhibition) and $LC_{50}$ (concentration for 50% lethality to cells) ($-50$% growth), were calculated for each cell line. The $GI_{50}$ value corresponds to the agent's concentration causing 50% decrease in net cell growth, the TGI value is the agent's concentration causing total growth inhibition and the $LC_{50}$ value is the agent's concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h). Mean-graph midpoint values (MG-MID) for a certain agent are the average of individual real and default $GI_{50}$, TGI or $LC_{50}$ values of all cell lines involved in the screen.[20]

## Results and discussion

### Antitumor screening

The NCI antitumor drug discovery screen has been designed to distinguish between broad spectrum antitumor compounds and tumor-selective agents.[20] In the present study, the 4-imidazolidinone analogs **1a–6g** showed a distinctive potential of selectivity as well as broad spectrum activity. With regard to sensitivity against the activity of ARL cell lines, compound **6b** showed effectiveness toward CCRF-CEM leukemia and KD488 lymphoma cell lines at concentrations of 3.7 and 0.8 $\mu$M, respectively. Compound **3d** proved to be active against AS283 lymphoma cell line at a concentration of 0.03 $\mu$M. SU-DHL-7 lymphoma cell line proved to be sensitive toward compounds **5a**, **5c** and **5g** at concentrations of 0.03, 0.03 and 0.6 $\mu$M, respectively (Table 1).

With regard to broad-spectrum antitumor activity, compounds **1b**, **3d**, **5e** and **6a–e** showed $GI_{50}$ and TGI (MG-MID) $<100$ $\mu$M against the six ARL cell lines used. Compounds **1c**, **2a** and **b**, **3a–c**, **3e**, **4b–f**, **5a–d**, **5f** and **g** and **6f** and **g** showed (MG-MID) values $<100$ $\mu$M at only the $GI_{50}$ level. Compounds **1a**, **3f** and **4a** proved to be inactive, showing $GI_{50}$ (MG-MID) values $>100$ $\mu$M, while compounds **2b**, **4b–c** and **5f** are the least effective agents with $GI_{50}$ (MG-MID) values in the range of 90.2–97.6 $\mu$M (Tables 1 and 2).

## Structure–activity relationship (SAR)

Based on MG-MID values (Tables 1 and 2), the activity of the tested imidazolidinone analogs could be correlated to the structure variations and modifications. A tentative SAR could be deduced as follows:

(1) In the 2-thioxo-4-imidazolidinone series (**1a–c**), 4-substitution of the benzylidene function manipulates the activity. The electron donating $4CH_3$ group gave the inactive compound **1a**. Replacing the $4\text{-}CH_3$ function by a chlorine atom (electron withdrawing) increased the activity by almost 4-fold (**1b**, $GI_{50}$; MG-MID=25.1 $\mu$M), while the replacement of the entire 5-benzylidene moiety by its 5-(2-thienylidene) isostere (**1c**) caused a marginal increase in activity.

(2) In the mercapto-4-imidazolidinone series (**2a** and **b**), replacing the $4\text{-}CH_3$-benzylidene group (**2a**, $GI_{50}$; MG-MID=54.8 $\mu$M) by a $4\text{-}CH_3O$-benzylidene (**2b**, $GI_{50}$; MG-MID=95.2 $\mu$M) decreased the activity by 2-fold.

(3) In the 2-piperidino-4-imidazolidinone series (**3a–f**), the type of substituent at the 5-position also controls the magnitude of activity. An electron-withdrawing group such as a chlorine atom produced an active compound (**3d**, $GI_{50}$; MG-MID=16.1 $\mu$M); removal of the chlorine atom or its replacement with an electron-donating group such as in **3a**, **3b** or **3c**, caused a dramatic decrease in the antitumor potency. The same concept could also be detected in the 2-(3hydroxymethyl)-piperidino-analogs **4a–f**. In the other two series 2-morpholino (**5a–f**) and 2-(2-carboxyphenylamino) (**6a–g**), the type of substituent at position 5 did not really affect the magnitude of activity.

(4) Correlating the antitumor activity to the type of the substituent at position 2 of the imidazolidinone analogs allowed the deduction of the following order of decreasing activity: 2-carboxyphenylamino > piperidino > morpholino > 3-hydroxymethylpiperidino; in other words, **6a–g** > **3a–f** > **5a–g** > **4a–f**.

The findings of the present investigation showed that the most potent members of this series are compounds **6b** (6.1 $\mu$M) and **6d** (8.7 $\mu$M). The broad-spectrum antitumor activity as well as

**Table 1.** $GI_{50}$ concentration ($\mu$M) of *in vitro* ARL cell lines by 5-(*Z*)-arylidene-4-imidazolidinone anaogs **1a**–**6g**[a]

| Agents[b] | CCRF-CEM[c] | RL[d] | KD488[d] | AS283[d] | PA682[d] | SU-DHL-7[d] | MG-MID[e] |
|---|---|---|---|---|---|---|---|
| **1b** | 24.4 | 32.7 | 11.1 | 19.8 | 49.5 | 13.2 | 25.1 |
| **1c** | –[f] | –[f] | 80.9 | –[f] | –[f] | 45.7 | 87.8 |
| **2a** | 68.5 | –[f] | 21.0 | 55.0 | 61.1 | 23.0 | 54.8 |
| **2b** | –[f] | –[f] | 85.1 | –[f] | –[f] | 86.2 | 95.2 |
| **3a** | –[f] | –[f] | 62.4 | 20.0 | –[f] | –[f] | 80.4 |
| **3b** | 61.5 | –[f] | 22.9 | 19.7 | 71.6 | 33.5 | 51.5 |
| **3c** | –[f] | –[f] | 35.8 | –[f] | –[f] | 74.7 | 85.1 |
| **3d** | 10.2 | 44.5 | 4.5 | 0.03 | 23.3 | 14.3 | 16.1 |
| **3e** | 51.6 | 87.2 | 40.0 | 87.6 | 45.2 | 31.0 | 57.1 |
| **4b** | –[f] | –[f] | 70.6 | –[f] | –[f] | 92.3 | 93.8 |
| **4c** | –[f] | –[f] | –[f] | –[f] | –[f] | 41.2 | 90.2 |
| **4d** | 69.6 | –[f] | 16.9 | 23.6 | –[f] | NT[g] | 62.0 |
| **4e** | –[f] | 45.1 | 6.6 | –[f] | –[f] | –[f] | 75.3 |
| **4f** | –[f] | –[f] | 3.1 | –[f] | –[f] | 13.4 | 69.4 |
| **5a** | –[f] | –[f] | 15.5 | 41.7 | –[f] | 0.3 | 59.6 |
| **5b** | –[f] | –[f] | 3.4 | –[f] | –[f] | 17.5 | 70.2 |
| **5c** | –[f] | –[f] | 21.8 | 7.8 | –[f] | 0.03 | 54.9 |
| **5d** | –[f] | –[f] | –[f] | –[f] | –[f] | 31.0 | 88.5 |
| **5e** | –[f] | 13.8 | 26.2 | –[f] | 94.8 | 33.0 | 61.3 |
| **5f** | –[f] | –[f] | 88.1 | –[f] | –[f] | NT[g] | 97.6 |
| **5g** | –[f] | –[f] | 28.6 | –[f] | –[f] | 0.6 | 71.5 |
| **6a** | 5.2 | 54.8 | 4.3 | 4.1 | 18.3 | 1.4 | 14.7 |
| **6b** | 3.7 | 12.5 | 0.8 | 1.3 | 15.8 | 2.2 | 6.1 |
| **6c** | 18.2 | 68.8 | 9.5 | 13.3 | 35.7 | 3.9 | 24.9 |
| **6d** | 7.1 | 17.4 | 6.1 | 3.7 | 15.9 | 5.1 | 8.7 |
| **6e** | 11.0 | 21.3 | 12.3 | 9.1 | 15.8 | NT[g] | 13.9 |
| **6f** | 21.1 | –[f] | 24.9 | 38.9 | –[f] | 12.8 | 49.6 |
| **6g** | 86.4 | –[f] | 5.4 | 27.2 | –[f] | 27.0 | 57.7 |

[a]Data obtained from the NCI *in vitro* AIDS-related lymphoma screen.
[b]Compounds **1a**, **3f** and **4a** showed $GI_{50}$ values $>100$ $\mu$M.
[c]Leukemia cell line.
[d]Lymphoma cell lines.
[e]$GI_{50}$ mean-graph midpoint.
[f]$GI_{50}$ values $>100$ $\mu$M.
[g]NT, not tested.

**Table 2.** TGI concentration ($\mu$M) of *in vitro* cell lines by 5-(*Z*)-arylidene-4-imidazolidinone analogs **1a**–**6g**

| Agents[a] | CCRF-CEM[b] | RL[c] | KD488[c] | AS283[c] | PA682[c] | SU-DHL[c] | MG-MID[d] |
|---|---|---|---|---|---|---|---|
| **1b** | –[e] | –[e] | –[e] | 85.1 | –[e] | 72.8 | 92.9 |
| **3d** | –[e] | –[e] | –[e] | 4.0 | –[e] | 83.2 | 81.2 |
| **5e** | –[e] | 73.5 | –[e] | –[e] | –[e] | –[e] | 95.6 |
| **6a** | 50.7 | 97.6 | 44.4 | 42.0 | 61.3 | 16.1 | 52.0 |
| **6b** | 25.6 | 61.9 | 47.2 | 31.0 | 87.5 | 25.9 | 46.5 |
| **6c** | 71.9 | –[e] | 67.7 | 95.9 | –[e] | 33.4 | 78.2 |
| **6d** | 40.6 | –[e] | 55.3 | 43.1 | 54.8 | 37.2 | 55.2 |
| **6e** | 38.9 | 85.4 | 44.4 | 51.8 | 46.2 | 21.2 | 48.0 |

[a]The rest of the compounds showed a TGI value $>100$ $\mu$M.
[b]Leukemia cell line.
[c]Lymphoma cell lines.
[d]TGI mean-graph midpoint.
[e]TGI values $>100$ $\mu$M.

potential cytotoxic effects of the lead compounds **6b** and **6d** will be of interest for future derivative design in the hope of finding more active compounds.

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