Some Antagonists of Platelet Activating Factor Are Cytotoxic for Human Malignant Cell Lines

Susanne Danhauser-Riedl, Stephan B. Felix, William J. Houlihan, Monica Zafferani, Gabriela Steinhauser, Dorothea Oberberg, Hildegard Kalvelage, Raymonde Busch, Johann Rastetter, and Wolfgang E. Berdel

Department of Medicine I, Division of Hematology and Oncology [S. D.-R., S. B. F., M. Z., G. S., D. O., H. K., J. R., W. E. B.], and Department of Medical Statistics and Epidemiology [R. B.], Technische Universität München, 8000 Munich 80, Federal Republic of Germany, and Sandoz Research Institute, East Hanover, New Jersey 07936 [W. J. H.]

ABSTRACT

Nine new platelet activating factor (PAF) antagonists from 4 different chemical classes (thiopyrimidines: SDZ 59–015; thioimidazolines: SDZ 61–813; imidazoisoquinolines: SDZ 62–434, SDZ 62–759, SDZ 63–135, SDZ 63–596; and imidazopiperidines: SDZ 61–638, SDZ 62–293, SDZ 62–694) have been tested for cytostatic/antiproliferative (tritiated thymidine uptake) and cytotoxic (trypan blue dye exclusion) activity in neoplastic human cell lines of different histology in vitro. The antiproliferative activity of 3 of the 9 PAF antagonists (SDZ 61–638, SDZ 61–813, SDZ 62–694) was not stable after freezing and thawing. SDZ 59–015 showed only minor cytotoxic or antiproliferative effects in a dose range of 2–40 μM after 24, 48, and 72 h of incubation. SDZ 62–434 showed varying activity. There were no significant differences between the activities of the other 3 PAF antagonists from the imidazoisoquinoline class, which showed drug concentrations inhibiting 50% of the activity studied (IC50) and drug concentrations yielding a 50% decrease of trypan blue dye exclusion (LC50) of ≥20 μM at ≥48 h, even in the K-562 cell line, which is known to be rather resistant for a variety of cytotoxic drugs related to PAF. SDZ 62–293 showed the best antineoplastic properties with IC50 and LC50 values ≥10 μM at ≥48 h including K-562, SDZ 62–434, SDZ 62–759, SDZ 63–135, SDZ 63–596, and SDZ 62–293 have been further tested in a human tumor cloning assay in 5 cell lines. Colony formation was reproducibly suppressed to <30% of the controls only by SDZ 63–135 (≤40 μM) and SDZ 62–293 (≤20 μM) during continuous exposure. There was no correlation between the IC50 values for the antiproliferative activity of the test compounds and their IC50 values for PAF-induced human platelet aggregation. Furthermore, the antiproliferative activity of the most active compound, SDZ 62–293, could not be antagonized by preincubation with the specific PAF antagonists WEB 2170 or WEB 2086 or PAF itself in noncytotoxic doses. In addition, since we were not able to find a correlation between the presence of PAF specific binding sites on neoplastic cells and their respective sensitivity for the cytotoxic effects of the PAF antagonists tested, binding to PAF specific binding sites does not seem to be a prerequisite for the cytotoxic/antiproliferative activity of these test compounds. This study shows antineoplastic activity of some PAF antagonists in vitro and we recommend some of these drugs for further investigation as experimental drugs in cancer therapy.

INTRODUCTION

During the last years, there has been increasing interest in certain ether lipids and derivatives as experimental anticancer drugs (1–3). Although it seems to be too early to judge their clinical potential, three of these compounds have recently been tested in clinical trials with some encouraging activity in a variety of tumor types (1–6). In addition, one compound is being tested clinically as a purging agent in autologous bone marrow transplantation because of its selective cytotoxicity against leukemic cells in comparison to normal bone marrow progenitor cells (7, 8).

Ether lipids exert part of their activity via direct cytotoxicity due to accumulation in tumor cells and membrane destruction. However, the mechanisms finally leading to cell death are still unknown and controversial (for discussion, see Refs. 1–3). Structural similarities of some of these lipids with PAF, a biologically active intermediate of lipid metabolism (9), are obvious. In an investigation to test the hypothesis that PAF specific binding sites present on cell membranes are important for the cytotoxicity of PAF related ether lipids, we surprisingly have found cytotoxic activity of some PAF antagonists (10). Thus, we have screened 9 new PAF antagonists from 4 different chemical classes for antineoplastic activity in vitro and in vivo.

Here we report on the in vitro cytotoxicity of these investigational compounds and on some aspects of their mechanism of action.

MATERIALS AND METHODS

Drugs. Nine PAF antagonists from 4 different chemical classes (thio- orimidazolines: SDZ 59–015; thioimidazolines: SDZ 61–813; imidazo isoquinolines: SDZ 62–434, SDZ 62–759, SDZ 63–135, SDZ 63–596; and imidazopiperidines: SDZ 61–638, SDZ 62–293, SDZ 62–694) were supplied by the Sandoz Research Institute (East Hanover, NJ). Their chemical structures are depicted in Fig. 1. The PAF antagonists WEB 2086 (11) and WEB 2170 (12) were kindly provided by Boehringer (Ingelheim, FRG). C6,P-PAF (1-O-hexadecyl-2-acetyl-sn-glycerol-3-phosphocholine) and C12,P-PAF (1-O-octadecyl-2-acetyl-sn-glycerol-3-phosphocholine) were purchased from Betchold (Bern, Switzerland). Tritiated [3H]PAF was purchased from Amersham-Buchler (Braunschweig, FRG). The PAF antagonist CV 3988 (13) was obtained from Takeda Chemicals, Inc. (Osaka, Japan). The compounds were dissolved in RPMI 1640 medium (Gibco, no. 240, Glasgow, Scotland, UK), sterilized as solutions by microspar filtration (0.22 μM, Millex; Millipore, Molsheim, France). Stock solutions (1 mM) were used freshly or after storage at −20°C.


For the HTCA we used the HL-60, K-562, CCL-121, HTB-38, and 85-HG-63 cell lines. The "competition assay" and the studies concerning PAF specific binding sites were done with the HL-60 and HTB-38 cell lines.

Received 7/10/90; accepted 9/21/90.

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1 Recipient of Grant Be 822/2-6 from Deutsche Forschungsgemeinschaft and by Sandoz Research Institute, East Hanover, NJ.

2 Recipient of Grant Be 822/2-6 and a Heisenberg-Scholarship from Deutsche Forschungsgemeinschaft. To whom reprint requests should be addressed, at Department of Hematology and Oncology, Klinikum Steglitz, Freie Universität, Berlin, Hindenburgdamm 30, 1000 Berlin 45, FRG.
The hematological cell lines were supplied by Dr. W. R. Vogler (K-562, HL-60; Emory University, Atlanta, GA) and by Dr. G. Roos (DHL-4, U-698, Li-A; University of Umea, Umea, Sweden). The solid tumor cell lines were purchased from the American Type Culture Collection (CCL-121, CCL 218, HTB-38, HTB-9; Rockville, MD) or supplied by Dr. D. Stavrou (85-HG-59, 85-HG-63; University of Hamburg, Hamburg, FRG). These cells were stored in liquid nitrogen after in vitro passaging procedures have been recently described in this journal (14). A decrease in colony formation within triplicate cultures were <±20%.

IC₅₀ values in the different assays were obtained from the respective dose-response plots. For the trypsin blue dye exclusion which primarily measures single cell viability and not the growth of a cell population we have used the term IC₅₀ instead.

### Competition Assay

The objective of this assay was to block specific PAF binding sites on the cells (9, 16) in order to investigate whether binding of the PAF antagonists to specific PAF binding sites is a prerequisite for their cytotoxic activity. Cells were preincubated for 2 or 24 h at 37°C in 5% CO₂ and high humidity with C₄₅₀PAF, C₄₅₀PAF, or the PAF antagonists WEB 2086 and WEB 2170 in noncytotoxic doses (C₄₅₀PAF: 2, 10, 20, and 50 μM; C₄₅₀PAF: 2, 10, and 20 μM; WEB 2086: 20 and 100 μM; WEB 2170: 2, 20, and 100 μM). After 2 of 24 h (as indicated) the preincubated cells were further coincubated with SDZ 62–293 (0.5, 2, 10, and 20 μM) and [³²P]Thymidine uptake which primarily measures single cell viability and not the growth of a cell population was measured to assay cytostatic/antiproliferative activity of the latter compound (14).

### PAF Binding Studies

The method was modified from procedures published before (16). To obtain information concerning the presence of specific PAF binding sites in the cell lines studied (9, 16), cells were collected in Hanks’ buffered salt solution (Seromed, no. 2023, Berlin, FRG) containing 0.25 g/dl essentially fatty acid free bovine serum albumin (Sigma A-6003, Deisenhofen, FRG). Viability before the binding assays was always >85% as assessed by trypan blue dye exclusion. In a total assay volume of 0.5 ml, 5 x 10⁶ cells were incubated for 1 h at 4°C or at room temperature with 2 nm [³²P]PAF (10,000 cpm) and varying amounts (0.1 nM-10 μM) of unlabeled PAF and PAF antagonists WEB 2086 and CV 3988. Bound [³²P]PAF was separated from unbound [³²P]PAF by centrifuging the assay mixture in a microfuge for 2 min and removing the supernatant. The cell sediment was resuspended in 300 μl water and counted in microvials containing 2 ml scintillation cocktail (Szintigel-Roth, Karlsruhe, FRG). All binding assays were performed in triplicate.

### Platelet Aggregation Inhibition

The method used for the experiments concerning platelet aggregation inhibition was described recently (17). Briefly, human species subjects were kept aspirin free for 1 week and fasted overnight. Platelet rich plasma was prepared from freshly obtained blood anticoagulated with 0.38% sodium citrate. Platelet count contained blood anticoagulated with 0.38% sodium citrate. Platelet count.

Cytotoxicity Studies. The trypsin blue dye exclusion assay and the [³²P]Thymidine uptake assay were performed as reported recently by our group (14). The trypsin blue dye exclusion assay was only used in the hematopoietic cell lines growing as suspension cultures, whereas the [³²P]Thymidine uptake assay was used in all cell lines. Briefly, in both assay systems the cells were coincubated with the test compounds at final concentrations of 2, 10, 20, and 40 μM in 10% fetal calf serum (Gibco, no. 014-06300) containing culture medium (14) for 24, 48, and 72 h. Cytotoxic activity of the compounds on a single cell level was assayed as a decrease in trypsin blue dye exclusion of the cells given as a percentage of the controls. Comparison of simultaneous evaluations by different individuals revealed a reproducibility with deviations of <±10%. Cytostatic/antiproliferative activity of the compounds was measured as an inhibition of [³²P]Thymidine uptake into the cells after freezing and thawing given as a percentage of the controls. Standard deviations within triplicate cultures were <±20%.

HTCA was performed using the capillary tube system first described by Maurer and Ali-Osman (15), with the following modifications. Glass capillaries (1.38 x 126 mm) were sonicated for 30 min in double distilled water, dried, and sterilized at 180°C. The cells were continuously exposed to the compounds at a concentration of 2, 20, or 40 μM during the complete assay period. The capillary incubation mixture consisted of 300 μl cell suspension (5 x 10⁶ cells/ml) in double enriched CMRL-1066 medium (Gibco, no. 041-01535; for ingredients see Ref. 14, with the exception of mercaptoethanol being at a 5 x 10^{-4} M concentration), 705 μl RPMI 1640 medium, 225 μl heat-inactivated horse serum (Gibco, no. 220-06350), and 270 μl 3% agar (Agar-Noble, no. 014-01-01; Difco Laboratories, Detroit, MI) diluted (1:3) in RPMI 1640 medium at 37°C. This capillary incubation mixture (75 μl) was injected into one capillary. The system was evaluated under an inverted microscope before and after an incubation period of 10 days under the conditions recently described (14). A decrease in colony formation under the influence of the test drugs was interpreted as cytotoxicity on the level of the self-generating capacity of the cells. Standard deviations within triplicate cultures were <±20%.

Table 1. Chemical structures of the PAF antagonists tested.

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>I.C.</th>
<th>SDZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazoisoquinolines</td>
<td>CH₃ C₆H₂O</td>
<td>61-813</td>
</tr>
<tr>
<td>Thiopyrimidine</td>
<td>C₈H₁₄O₂</td>
<td>59-015</td>
</tr>
<tr>
<td>Imidazopiperidines</td>
<td>CH₃ C₆H₁₄</td>
<td>61-638</td>
</tr>
<tr>
<td>R</td>
<td>R’</td>
<td>n</td>
</tr>
<tr>
<td>C₃H₇</td>
<td>C₆H₄</td>
<td>2</td>
</tr>
<tr>
<td>C₃H₇</td>
<td>C₆H₄</td>
<td>1</td>
</tr>
<tr>
<td>C₁₀H₂₃</td>
<td>C₆H₁₄</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical structures of the PAF antagonists tested.
Table 1 ([H]Thymidine uptake and trypan blue dye exclusion after an incubation time of 48 h at a concentration of 20 μM of the different PAF antagonists in malignant human cell lines)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>SDZ 62-293</th>
<th>SDZ 63-135</th>
<th>SDZ 63-596</th>
<th>SDZ 62-759</th>
<th>SDZ 62-434</th>
<th>SDZ 59-015</th>
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<tbody>
<tr>
<td></td>
<td>T</td>
<td>H</td>
<td>T</td>
<td>H</td>
<td>T</td>
<td>H</td>
</tr>
<tr>
<td>CEM*</td>
<td>0</td>
<td>0.1</td>
<td>9.0</td>
<td>3.2</td>
<td>42.6</td>
<td>4.1</td>
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<td>HL-60</td>
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<td>0.2</td>
<td>1.6</td>
<td>0.2</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>K-562</td>
<td>2.8</td>
<td>20.0</td>
<td>30.2</td>
<td>55.8</td>
<td>46.3</td>
<td>39.6</td>
</tr>
<tr>
<td>Li-A</td>
<td>0</td>
<td>4.6</td>
<td>7.9</td>
<td>79.8</td>
<td>12.5</td>
<td>33.7</td>
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<tr>
<td>U-698</td>
<td>0</td>
<td>0</td>
<td>4.6</td>
<td>57.7</td>
<td>—</td>
<td>26.2</td>
</tr>
<tr>
<td>DHL-4</td>
<td>4.7</td>
<td>10.8</td>
<td>15.7</td>
<td>29.7</td>
<td>61.0</td>
<td>29.5</td>
</tr>
<tr>
<td>CCL-218</td>
<td>0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>85-HG-59</td>
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<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>18.3</td>
<td>26.7</td>
</tr>
<tr>
<td>85-HG-63</td>
<td>0</td>
<td>0.6</td>
<td>2.5</td>
<td>25.1</td>
<td>18.9</td>
<td>67.4</td>
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<td>HTB-9</td>
<td>0</td>
<td>0.1</td>
<td>1.2</td>
<td>2.7</td>
<td>3.1</td>
<td>103.1</td>
</tr>
<tr>
<td>HTB-38</td>
<td>0</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>18.3</td>
<td>26.7</td>
</tr>
</tbody>
</table>

* T, trypan blue dye exclusion; H, [H]thymidine uptake; for details of the assays and values depicted see "Materials and Methods."

For histology see "Materials and Methods.

Percentage of control.

—, not determined.

The response was quantitated by determining the area under the curve using a plane polarimeter. The percentage of inhibition of the aggregation response was determined by dividing the area under the curve generated in the presence of the test compound by the area under the curve of PAF alone, multiplying by 100, and subtracting from 100.

Statistical Analysis. Data from the trypan blue dye exclusion assay, [H]thymidine uptake assay, and HTCA have been examined in the Wilcoxon matched pairs signed ranks test (two tailed) and in the Friedman two way analysis according to their applicability. P values <0.05 were interpreted as significant, and P values <0.01 were highly significant differences.

RESULTS

In a first set of [H]thymidine uptake studies using the HTB-38 cell line, we looked at whether the 9 different PAF antagonists have freezing stability with regard to their antiproliferative activity after 48 h of incubation. We compared the antiproliferative activity of a compound immediately after preparation of the solution (A, see below), or freezing (Ix) for 24 h (B, see below), or storing at —20°C for at least 4 weeks (C, see below). IC₅₀ values show that 3 of the 9 PAF antagonists are not stable after freezing and storage at —20°C. These are the thioimidazoline SDZ 61–813 (IC₅₀ values in μM for A, 5.3; B, 22.1; C, >40.0) and the imidazopiperidines SDZ 61–638 (IC₅₀ values in μM for A, 6.7; B, 6.0; C, >40.0) and SDZ 62–694 (IC₅₀ values in μM for A, 6.3; B, >40.0; C, 26.8). These compounds were eliminated from further testing.

All tested compounds showed time- and dose-dependent cytotoxic/antiproliferative activity in the tested dose and time range. Table 1 summarizes a comparison of the data concerning trypan blue dye exclusion (T) (hematopoietic cell lines only) and [H]thymidine uptake ([H]) (all cell lines) at a concentration of 20 μM after an incubation time of 48 h. Ranking of the compounds (Friedman analysis, two tailed) showed decreasing cytotoxic and antiproliferative activity from the left side (SDZ 62-293) to the right side (SDZ 59-015) of Table 1 (P = 0.0002).

Within the imidazopiperidines SDZ 62–293 showed strong dose- and time-dependent cytotoxic and antiproliferative activity with IC₅₀ values ≤10 μM at ≤48 h including the K-562 cell line (Fig. 2). Compared on an equimolar basis SDZ 62–293 showed significantly higher cytotoxic activity (trypan blue dye exclusion) in human hematological malignancies on the dose levels of 10, 20, and 40 μM after 48 h of incubation (P < 0.05) than the other 5 tested PAF antagonists. In human solid tumor cell lines SDZ 62–293 showed significantly higher antiproliferative activity ([H]thymidine uptake) on the dose levels of 10, 20, and 40 μM (P < 0.05) in comparison with SDZ 59-015. However, in the solid tumor cell lines, there were no significant differences when this compound was compared with the compounds of the imidazoisoquinoline class.

The imidazoisoquinolines SDZ 63–135, SDZ 63–596, and SDZ 62–759 showed strong time- and dose-dependent cytotoxic and antiproliferative activity in a dose range of 2–40 μM after 24, 48, and 72 h of incubation in cell lines of hematological malignancies and solid tumors of human origin (Table 1). These compounds showed cytotoxic activity even in the K-562 cell line which is known to be rather resistant for a variety of cytotoxic drugs related to PAF (18). Fig. 2 depicts dose- and time-dependent cytotoxic activity of the most active compound of the imidazoisoquinoline class SDZ 63–135 in K-562 cell line as an example. The imidazoisoquinoline SDZ 62–434 showed strong antiproliferative activity (2–40 μM) after 24, 48, and 72 h of incubation in 4 of 5 solid tumor cell lines (except HTB 9) (Table 1). SDZ 62–434, however, showed little cytotoxic effect in the K-562 cell line and no effect in the other 5 tested cell lines of human hematological malignancies (Table 1).
that this compound has a significantly lower cytotoxic activity than the compound on the x axis at the same dose level. P values between 0.05 and 0.10 are given to
on the x axis shows a significantly higher cytotoxic effect than the compound on the y axis at the same dose level. P values <0.05 for compounds on the y axis indicate

in a dose range of 2–40 \( \mu M \) after 24, 48, and 72 h of incubation (Table 1).

With the exception of SDZ 59–015 all compounds (SDZ 62–293, SDZ 63–135, SDZ 63–596, SDZ 62–759, and SDZ 62–434) have been further tested in an HTCA in 5 cell lines. During continuous exposure colony formation was reproducibly suppressed to <30% of the controls only by SDZ 63–135 (≤40 \( \mu M \)) and SDZ 62–293 (≤20 \( \mu M \)) (Fig. 3). There was no significant difference between the cytotoxic activity of the latter two compounds (Table 2), but both were significantly more active than the other 3 PAF antagonists compared on an equimolar dose level (Table 2).

In an attempt to correlate PAF antagonist activity of the 5 compounds to their cytotoxic/antiproliferative effects, the IC\(_{50}\) values for inhibition of PAF-induced human platelet aggregation are compared with the IC\(_{50}\) values for their antiproliferative activity ([\( ^{3} \)H]thymidine uptake) in Table 3. No correlation between both activities could be seen (Table 3).

In the competition assay we tried to antagonize the antiproliferative activity ([\( ^{3} \)H]thymidine uptake) of SDZ 62–293 by preincubation of the HL-60 and HTB-38 cell lines with the specific PAF antagonists WEB 2170 or WEB 2086 or PAF itself in noncytotoxic doses. Examples of these experiments are depicted in Fig. 4. The antiproliferative activity of SDZ 62–293 could not be antagonized by preincubation with one of the PAF antagonists or PAF itself.

Furthermore, direct studies, analyzing specific binding of labeled PAF to binding sites of this molecule possibly present on HL-60 cells in Scatchard plots, were impossible due to the high metabolism of PAF (16), which prevented PAF uptake to measurable levels of saturation (data not shown). However, specific binding in single point situations could be defined as the difference between total binding of labeled PAF (at 2 × 10\(^{-10}\) M assay concentration) and nonspecific binding of labeled PAF at a higher concentration (2 × 10\(^{-4}\) M). Under these conditions usually 25% of total binding was specific (Table 4). Subsequently, we attempted to characterize the binding of PAF through studies using unlabeled PAF and two specific PAF antagonists with different chemical structures (WEB 2086 and CV 3988) as inhibitors of the binding of radioactively labeled PAF. Unlabeled PAF (dose range, 1 × 10\(^{-11}\)–5 × 10\(^{-8}\) M) and PAF antagonists (dose range, 5 × 10\(^{-8}\)–1 × 10\(^{-4}\) M) were added, and IC\(_{50}\) values were estimated from the resulting plots. As shown in Table 4, CV 3988 competes with PAF for binding sites in the HL-60 cell line, whereas WEB 2086 had no effect whatsoever. At higher concentrations (1 × 10\(^{-10}\)–1 × 10\(^{-8}\) and 1 × 10\(^{-8}\)–1 × 10\(^{-4}\) M, respectively), both PAF and the antagonist

The thiopyrimidine SDZ 59–015 showed no or only minor cytotoxic or antiproliferative activity in various cell lines of hematological malignancies and solid tumors of human origin in a dose range of 2–40 \( \mu M \) after 24, 48, and 72 h of incubation (Table 1).

Table 2 Significant P values (Wilcoxon test) for each compound compared with each other at equimolar dose levels in the HTCA

<table>
<thead>
<tr>
<th>Compounds</th>
<th>SDZ 62–293</th>
<th>SDZ 62–135</th>
<th>SDZ 62–759</th>
<th>SDZ 62–596</th>
<th>SDZ 62–434</th>
<th>Dose level of test compounds (( \mu M ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDZ 62–434</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>40</td>
</tr>
<tr>
<td>SDZ 62–596</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>20</td>
</tr>
<tr>
<td>SDZ 62–759</td>
<td>( P = 0.04 )</td>
<td>( P = 0.08 )</td>
<td>( P = 0.08 )</td>
<td>( P = 0.08 )</td>
<td>( P = 0.08 )</td>
<td>20</td>
</tr>
<tr>
<td>SDZ 62–135</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>20</td>
</tr>
<tr>
<td>SDZ 62–293</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>( P = 0.04 )</td>
<td>20</td>
</tr>
</tbody>
</table>

\( ^{a} \) Compounds were ranked according to their antiproliferative activity as compared in the Friedman analysis.

Table 3 Lack of correlation of IC\(_{50}\) values (\( \mu M \)) for inhibition of PAF induced aggregation of human platelets and antiproliferative activity ([\( ^{3} \)H]thymidine uptake) of the different PAF antagonists in the HL-60 cell line after 48 h of incubation

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Antiproliferative activity*</th>
<th>Inhibition of PAF induced human platelet aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDZ 62–293</td>
<td>&lt;2.0</td>
<td>57.0</td>
</tr>
<tr>
<td>SDZ 63–135</td>
<td>6.0</td>
<td>1.3</td>
</tr>
<tr>
<td>SDZ 63–596</td>
<td>6.1</td>
<td>0.25</td>
</tr>
<tr>
<td>SDZ 62–759</td>
<td>7.0</td>
<td>1.6</td>
</tr>
<tr>
<td>CV 3988</td>
<td>36.7</td>
<td>32.0</td>
</tr>
<tr>
<td>CV 3988</td>
<td>&gt;40.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Compounds were ranked according to their antiproliferative activity as compared in the Friedman analysis.

In the competition assay we tried to antagonize the antiproliferative activity ([\( ^{3} \)H]thymidine uptake) of SDZ 62–293 by preincubation of the HL-60 and HTB-38 cell lines with the specific PAF antagonists WEB 2170 or WEB 2086 or PAF itself in noncytotoxic doses. Examples of these experiments are depicted in Fig. 4. The antiproliferative activity of SDZ 62–293 could not be antagonized by preincubation with one of the PAF antagonists or PAF itself.

Furthermore, direct studies, analyzing specific binding of labeled PAF to binding sites of this molecule possibly present on HL-60 cells in Scatchard plots, were impossible due to the high metabolism of PAF (16), which prevented PAF uptake to measurable levels of saturation (data not shown). However, specific binding in single point situations could be defined as the difference between total binding of labeled PAF (at 2 × 10\(^{-10}\) M assay concentration) and nonspecific binding of labeled PAF at a higher concentration (2 × 10\(^{-4}\) M). Under these conditions usually 25% of total binding was specific (Table 4). Subsequently, we attempted to characterize the binding of PAF through studies using unlabeled PAF and two specific PAF antagonists with different chemical structures (WEB 2086 and CV 3988) as inhibitors of the binding of radioactively labeled PAF. Unlabeled PAF (dose range, 1 × 10\(^{-11}\)–5 × 10\(^{-8}\) M) and PAF antagonists (dose range, 5 × 10\(^{-8}\)–1 × 10\(^{-4}\) M) were added, and IC\(_{50}\) values were estimated from the resulting plots. As shown in Table 4, CV 3988 competes with PAF for binding sites in the HL-60 cell line, whereas WEB 2086 had no effect whatsoever. At higher concentrations (1 × 10\(^{-10}\)–1 × 10\(^{-8}\) and 1 × 10\(^{-8}\)–1 × 10\(^{-4}\) M, respectively), both PAF and the antagonist

The thiopyrimidine SDZ 59–015 showed no or only minor cytotoxic or antiproliferative activity in various cell lines of hematological malignancies and solid tumors of human origin in a dose range of 2–40 \( \mu M \) after 24, 48, and 72 h of incubation (Table 1).

With the exception of SDZ 59–015 all compounds (SDZ 62–293, SDZ 63–135, SDZ 63–596, SDZ 63–759, and SDZ 62–434) have been further tested in an HTCA in 5 cell lines. During continuous exposure colony formation was reproducibly suppressed to <30% of the controls only by SDZ 63–135 (≤40 \( \mu M \)) and SDZ 62–293 (≤20 \( \mu M \)) (Fig. 3). There was no significant difference between the cytotoxic activity of the latter two compounds (Table 2), but both were significantly more active than the other 3 PAF antagonists compared on an equimolar dose level (Table 2).

In an attempt to correlate PAF antagonist activity of the 5 compounds to their cytotoxic/antiproliferative effects, the IC\(_{50}\) values for inhibition of PAF-induced human platelet aggregation are compared with the IC\(_{50}\) values for their antiproliferative activity ([\( ^{3} \)H]thymidine uptake) in Table 3. No correlation be-
that binding to specific PAF binding sites on cells or PAF agonistic/antagonistic properties of these structures correlate with their cytotoxic effect (10). The same holds true for the cytotoxic PAF antagonists reported here. There was neither a correlation between their inhibition of PAF induced platelet aggregation and their antiproliferative activity (Table 3), nor could we block the antiproliferative effects by preincubation of the cells with other nontoxic PAF antagonists (Fig. 4). Furthermore, the presence of specific PAF binding sites (receptor structures) (9, 16) was not even a prerequisite for the cytotoxicity of these PAF antagonists (Table 4). Our data on PAF binding sites in the nondifferentiated HL-60 cell line are in accordance with observations reported by Vallari et al. (20).

In conclusion, there is antineoplastic cytotoxicity of some new PAF antagonists from different chemical classes in vitro. These structures have not been studied for anticancer activity before. Comparative testing of these compounds in vitro with bone marrow cells in the setting of bone marrow purging might be of interest. Additionally, there is also some therapeutic in vivo activity, further investigation of these compounds as experimental drugs in cancer therapy is recommended.

**REFERENCES**


Some Antagonists of Platelet Activating Factor Are Cytotoxic for Human Malignant Cell Lines


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