Nonredox 5-Lipoxygenase Inhibitors Require Glutathione Peroxidase for Efficient Inhibition of 5-Lipoxygenase Activity

OLIVER WERZ, DAGMAR SZELLAS, MARGARETE HENSELER, and DIETER STEINHILBER
Institute of Pharmaceutical Chemistry, University of Frankfurt, D-60439 Frankfurt, Germany

ABSTRACT
Nonredox type 5-lipoxygenase (5-LO) inhibitors, such as ZM 230487, its methyl analogue ZD 2138, or the Merck compound L-739,010, suppress cellular leukotriene synthesis of ionophore stimulated granulocytes with IC50 values of about 50 nM. However, in cell homogenates or in preparations of purified enzyme, up to 150-fold higher concentrations are required for similar inhibition of 5-LO activity. This loss of 5-LO inhibition in cell homogenates was reversed by addition of glutathione or dithiothreitol, which increased the inhibitory potency of ZM 230487 or L-739,010 by about 150-fold so that 5-LO inhibition was comparable with that of intact cells. In the presence of thiols, addition of hydroperoxide [13(S)-HpODE], glutathione-peroxidase inhibition by iodacetate or selenium-deficiency lead to impaired 5-LO inhibition by ZM 230487 in cell homogenates. Moreover, addition of glutathione peroxidase was required for efficient inhibition of purified human 5-LO by ZM 230487. The data suggest that low hydroperoxide concentrations are important for efficient 5-LO inhibition by ZM 230487. The kinetic analysis revealed a noncompetitive inhibition of 5-LO by ZM 230487 at low hydroperoxide levels, whereas it acted as a competitive inhibitor with low affinity under nonreducing conditions in granulocyte homogenates. No such redox-dependent effects were observed with the 5-LO inhibitor BWA4C, the 5-LO activating protein-inhibitor MK-886 or the pentacyclic triterpene acetyl-11-keto-β-boswellic acid. These data suggest that physiological conditions associated with oxidative stress and increased peroxide levels lead to impaired efficacy of nonredox type 5-LO inhibitors like ZM 230487 or L-739,010. This could explain the reported lack of activity of this class of 5-LO inhibitors in chronic inflammatory processes.

Leukotrienes are important mediators of inflammatory and allergic reactions which are produced by granulocytes, monocytes/macrophages, and mast cells after stimulation (Samuelsson et al., 1983). The activity of the enzyme that has been purified from various sources depends on Ca2+ and ATP (Jakschik et al., 1980; Rouzer and Samuelsson, 1987). 5-LO catalyzes the two initial steps in leukotriene biosynthesis from arachidonic acid (Samuelsson, 1983). The activity of the enzyme that has been purified from various sources depends on Ca2+ and ATP (Jakschik et al., 1980; Rouzer and Samuelsson, 1987). In intact cells, 5-LO requires the presence of the membrane-bound FLAP for leukotriene synthesis (Miller et al., 1990). It has been shown that the enzyme activity is further regulated by the cellular redox status (Bryant et al., 1982) and that a threshold level of hydroperoxides is required for the activation of the enzyme (Rouzer and Samuelsson, 1986). Recently, it was found that selenium-dependent peroxidases are responsible for suppression of 5-LO activity in B-lymphocytes and immature myeloid cells (Werz and Steinhilber, 1996). In vitro, 5-LO is activated under conditions that promote lipid peroxidation (Riendeau et al., 1989) e.g., by increasing the levels of hydroperoxides via depletion of glutathione (Hatzelmann and Ullrich, 1987; Hatzelmann et al., 1989) or selenium (Weitzel and Wendel, 1993). In vivo, there is an enhanced formation of activated oxygen species at inflammatory sites which, among other signals, are involved in 5-LO activation and subsequent leukotriene synthesis in activated leukocytes.

The biological profile of leukotrienes suggests that inhibitors of the 5-LO pathway may have therapeutic potential in a variety of inflammatory and allergic diseases. 5-LO inhibitors can be classified according to the mechanism of enzyme inhibition. Redox type inhibitors like phenidone or BW-755C reduce the active site iron of the enzyme into the ferrous form (Fe2+) and keep the enzyme in its inactive state. However, redox-active compounds also interact with other biological redox systems, which leads to side-effects like methaemoglobin formation (Lau et al., 1992). Iron ligand inhibitors represent a class of drugs that inhibit leukotriene synthesis by chelating the iron in the catalytic center of 5-LO. Most of the compounds of this class are hydroxamic acid or N-hydroxyurea derivatives, such as the orally active compounds Zileuton...
and BWA4C (Tateson et al., 1988; Falgueyret and Riehenda, 1993). N-hydroxyureas and hydroxamates are weak redox active compounds and it is presumed that the 5-LO inhibitory action of these drugs might be related in part to these properties (Riehenda et al., 1991; Rouzer et al., 1991).

The search for specific and highly potent nonredox 5-LO inhibitors led to the development of the methoxysterathydro-
pyran derivatives, such as ZD 2138, its ethyl analogue ZM 230487, or L-739,010, which inhibit 5-LO activity in ex vivo whole-blood assays with IC_{50} values of 20–50 nM (Crawley et al., 1993).

However, despite its strong potency in several ex vivo and in vitro models, ZD 2138 failed to strongly inhibit leukotriene synthesis at sites of chronic inflammation (Kusner et al., 1993). The extract was diluted with 120 μl of water and 100 μl of the diluted extract were analyzed by HPLC as described (Steinhilber et al., 1989).

When cell homogenates were assayed, the cells were finally resuspended in 1 ml of PBS (with 1 g/liter of glucose, without Ca^{2+} and Mg^{2+}) and cooled on ice. After addition of EDTA (1 mM), sonication (3 × 5 sec), addition of 1 mM ATP and the test compounds (5–10 min at 4°), the samples were precultured for 30 sec at 37° and the incubation was started by the addition of Ca^{2+} and arachidonic acid (2 mM and 40 μM final concentrations, respectively). After 10 min at 37°, the incubation was stopped with 1 ml of methanol and the formed 5-LO products were extracted and analyzed by HPLC as described for the intact cells. 5-LO activity is expressed as nanograms of 5-LO products per 10^6 cells, which includes the all-trans isomers of LTB4, LT4, and 5-hydroxy-6,8,11,14-eicosatetraenoic acid.

Expression of 5-LO in Escherichia coli. Expression of 5-LO was performed in E. coli JM 109 cells, transfected with pT3-5-LO, as described by Zhang et al. (1992, 1993). The cells were harvested by centrifugation at 10,000 × g for 10 min and the pellet was lysed by incubation in 50 ml of 50 mM triethanolamine/HCl, pH 8.0, 5 mM EDTA, soybean trypsin inhibitor (60 μg/ml), 1 mM phenylmethylsulfonyl fluoride and lysozyme (500 μg/ml) for 5 min at room temperature followed by 25 min on ice. Lysed cells were homogenized by sonication (3 × 15 sec) using a Bandelin Sonopuls Disruptor HD 200 at 50% and centrifuged at 19,000 × g for 15 min. The supernatant was collected, whereas the pellet was subjected to a second round of lysis and sonication in 25 ml of buffer as described above. Supernatants were pooled and proteins (including 5-LO) were precipitated with 50% saturated ammonium sulfate during stirring on ice for 40 min. The precipitate was collected by centrifugation at 16,000 × g for 25 min and the pellet was stored at −20° or resuspended in 20 ml of PBS-buffer containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride.

Purification of 5-LO from E. coli and granulocytes. Homog-
enate from granulocytes were prepared as described above and centrifuged for 20 min at 10,000 × g and 4°. The resulting supernatant or the resuspended pellet of the (NH_4)_2SO_4 precipitate of the E. coli proteins were centrifuged at 100,000 × g for 70 min at 4°.

The 100,000 × g supernatant was applied to an ATP-agarose column (Sigma A2767; bed volume, 2 ml) equilibrated with PBS containing 1 mM EDTA (equilibration buffer). The column was washed with equilibration buffer (7 ml), followed by 50 mM phosphate buffer, pH 7.4, 0.5 mM NaCl, 1 mM EDTA (10 ml), 50 mM phosphate buffer, pH 7.4, and 1 mM EDTA (10 ml). 5-LO enzyme was eluted with 50 mM phosphate buffer, pH 7.4, 1 mM EDTA/20 mM ATP (10 ml). The ATP eluate was concentrated by ultrafiltration (YM 10 membrane; Amicon) to 2.5 ml. Then, the buffer was changed to 50 mM phosphate buffer, pH 7.4, 1 mM EDTA by gel filtration on a prepacked column (PD-10; Pharmacia, Freiburg, Germany). The resulting sample was further purified by anion-exchange chromatography using a ResourceQ column (Pharmacia; bed volume, 1 ml) equilibrated in 50 mM Tris buffer, pH 7.9, 1 mM EDTA (ResourceQ-
equilibration buffer). After the elution of unabsorbed material at a flow rate of 1 ml/min, the flow rate was increased to 2.5 ml/min and the column was developed with a linear gradient of 0–0.3 M NaCl in the ResourceQ-equilibration buffer over 10 min. Fractions containing 5-LO were combined and used for the 5-LO assay. Purity of the 5-LO preparations was about 50% as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, respectively. Granulocyte preparations contained a 37-kDa protein, whereas two proteins (25 and 72 kDa) were the major contaminants in the E. coli preparations.

Results

ZM 230487 and L-739,010 are significantly less potent 5-LO inhibitors in broken cell preparations than in intact cells. The efficacy of compounds that represent different types of leukotriene synthesis inhibitors were tested in intact cells and homogenates of human granulocytes or HL 60 cells. Identical arachidonic acid concentrations were used in the intact cell and the homogenate assay. As expected, the FLAP inhibitor MK-886 was only active in intact cells but not in the corresponding cell homogenates (Table 1). BWA4C, a redox active hydroxamic acid derivative, had a 3-fold lower IC_{50} value in intact cells than in broken cell preparations of differentiated HL 60 cells (0.05 and 0.15 μM, respectively). Similar differences were found for AKBA, a novel type 5-LO inhibitor (Safayhi et al., 1992, 1995) (IC_{50} values of 15 and 50 μM, respectively). The nonredox 5-LO inhibitors ZM 230487 and L-739,010 inhibited cellular 5-LO activity in granulocytes at the nanomolar range with IC_{50} values of 50 nM and 20 nM, respectively. Interestingly, 50- to 150-fold higher concentrations of these drugs were required for similar inhibition of 5-LO activity in the corresponding cell homogenates (Table 1).

Thiols are required for the strong 5-LO inhibitory potency of ZM 230487 and L-739,010 in broken cell preparations. The cellular redox status is an important parameter for 5-LO activation in intact cells. Recently, we and others showed that selenium-dependent peroxidases are involved in the regulation of cellular 5-LO activity in a variety of cell types (Bryant et al., 1982; Weitzel and Wendel, 1993; Werz and Steinhiber, 1996). Because peroxidases require millimolar concentrations of thiols as cosubstrates, 1 mM GSH or DTT was added as a cosubstrate to obtain peroxidase activity in the homogenate assays. As shown in Fig. 1, addition of thiols increased the inhibitory potency of ZM 230487 and L-739,010 by about 100-fold.

Thus, in the presence of thiols, 5-LO inhibition in cell homogenates is comparable with the inhibition observed in intact cells (Fig. 1). In contrast to ZM 230487 and L-739,010, similar IC_{50} values were found for AKBA and BWA4C in the absence or presence of thiols (Fig. 2).

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>ZM 230487</th>
<th>L-739,010</th>
<th>BWA4C</th>
<th>AKBA</th>
<th>MK-886</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact cells</td>
<td>0.05</td>
<td>0.02</td>
<td>0.05</td>
<td>15</td>
<td>0.16</td>
</tr>
<tr>
<td>Homogenates</td>
<td>5.5</td>
<td>1</td>
<td>0.15</td>
<td>50</td>
<td>inactive</td>
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* HL-60 cells.

Hydroperoxide addition or inhibition of peroxidase by iodoacetate or selenium deficiency leads to impaired efficacy of ZM 230487. To study the role of glutathione peroxidases, 13-HpODE was added to homogenates to counteract peroxidase-mediated effects. Fig. 3 shows that addition of 3 μM 13(S)-HpODE to DTT-supplemented homogenates resulted in a shift of the IC_{50} value of ZM 230487 from 50 to 450 nM. The corresponding alcohol, 13(S)-HODE, only marginally affected the IC_{50} value (70 nM). Almost identical dose response curves were obtained with 15-hydroperoxy-6,8,11,14-eicosatetraenoic acid and 15-hydroxy-6,8,11,14-eicosatetraenoic acid, respectively. Thus, in the presence of increased peroxide levels, ZM 230487 is less potent in blocking 5-LO activity.

Fig. 1. Influence of thiols on 5-LO inhibition by ZM 230487 (top) and L-739,010 (bottom) in granulocyte homogenates. Granulocytes were isolated as described in Experimental Procedures. Intact cells were preincubated for 5 min with the indicated concentrations of ZM 230487 and the 5-LO reaction was started by addition of 10 μM ionophore and 40 μM arachidonic acid. 5-LO activity of purified 5-LO and cell homogenates was determined after preincubation with the indicated concentrations of inhibitor for 10 min in the absence or presence of the indicated thiols. Results are expressed as mean ± standard error of three independent experiments.

Fig. 2. Effect of 13(S)-HpODE on 5-LO inhibition. Granulocytes were isolated as described in Experimental Procedures. 5-LO activity was determined after preincubation with ZM 230487 (top) and L-739,010 (bottom) in granulocyte homogenates. Granulocytes were isolated as described in Experimental Procedures. Intact cells were preincubated for 5 min with the indicated concentrations of ZM 230487 and the 5-LO reaction was started by addition of 10 μM ionophore and 40 μM arachidonic acid. 5-LO activity of purified 5-LO and cell homogenates was determined after preincubation with the indicated concentrations of inhibitor for 10 min in the absence or presence of the indicated thiols. Results are expressed as mean ± standard error of three independent experiments.

TABLE 1

IC_{50} values of leukotriene inhibitors in intact cells and homogenates of granulocytes.

<table>
<thead>
<tr>
<th>Granulocytes</th>
<th>Inhibitor</th>
<th>ZM 230487</th>
<th>L-739,010</th>
<th>BWA4C</th>
<th>AKBA</th>
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<td>50</td>
<td>inactive</td>
<td></td>
</tr>
</tbody>
</table>

* HL-60 cells.
Next, the effects of glutathione-peroxidase inhibition were investigated. Iodacetate is known to inactivate glutathione peroxidases in the presence of thiols by alkylation of the selenol moiety at the active site of the enzymes (Ursini et al., 1985). As shown in Fig. 4, iodacetate at a concentration of 2 mM strongly reduces 5-LO inhibition by ZM 230487 in DTT-supplemented homogenates and shifts the IC₅₀ value from 0.05 to 3 μM.

To further confirm our hypothesis, that selenium-containing peroxidases are involved, the thiol effects in homogenates of selenium-supplemented BL41-E95-A cells and in cell preparations from selenium-deficient cultures were studied. Identical IC₅₀ values (0.1 μM) for ZM 230487 were observed in the absence of GSH, irrespective of the selenium status and in selenium-deficient cells in the presence of GSH (1 mM). However, the efficacy of ZM 230487 was increased by 6-fold when GSH was added to homogenates derived from selenium-containing cells. Taken together, these data clearly demonstrate that the 5-LO inhibitory activity of ZM 230487 depends on the peroxide level, which is controlled by selenium-dependent peroxidases.

Fig. 2. Dose response curves of the 5-LO inhibitors AKBA (top) and BW4C (bottom). Granulocyte homogenates were preincubated for 10 min with the indicated concentrations of ZM 230487 in the absence and presence of 1 mM DTT. Then, 5-LO activity was determined as described in Experimental Procedures. Values are given as mean ± standard error of three independent experiments.

Fig. 3. Effects of hydroperoxides on 5-LO inhibition by ZM 230487 in granulocyte homogenates. Incubation mixtures consisting of PBS, pH 7.4, 1 mM EDTA, 2 mM CaCl₂, 1 mM ATP, 20 μg/ml phosphatidylincholine, 1 mM DTT, and 40 μM arachidonic acid with or without 3 μM 13(S)-HpODE or 13(S)-HODE were prepared at RT with the indicated concentrations of ZM 230487 (1 μl, final volume). The 5-LO reaction was started by the addition of aliquots (40 μl) of homogenates. After 10 min at 37°, the incubation was stopped with 1 ml of methanol and the formed 5-LO products were extracted and analyzed by HPLC as described. Values are given as mean ± standard error of three independent experiments.

Fig. 4. Effects of iodacetate on the peroxidase-dependent inhibition of 5-LO by ZM 230487 in homogenates of granulocytes. Granulocyte homogenates were preincubated for 10 min with the indicated concentrations of inhibitor and 1 mM DTT in the presence or absence of 2 mM iodacetate. Then, 5-LO activity was determined as described in Experimental Procedures. Values are given as mean ± standard error of three independent experiments.
Glutathione peroxidase strongly enhances the inhibitory potency of ZM 230487 on purified human 5-LO. Human recombinant 5-LO was expressed in E. coli and purified as described in Experimental Procedures. Inhibition of the recombinant enzyme by ZM 230487 was checked in the absence or presence of purified glutathione peroxidase (Fig. 5). In the absence of glutathione peroxidase, the IC$_{50}$ value of ZM 230487 was 0.6 $\mu$m, which is comparable with data reported in the literature (Smith et al., 1995). GSH alone did not significantly affect 5-LO inhibition by ZM 230487. However, in the presence of glutathione peroxidase (20 milliunits/ml), an enhanced sensitivity of 5-LO against ZM 230487 was observed and the resulting IC$_{50}$ value was 30 nM. A similar pattern was found for the purified LO from human granulocytes (data not shown). Thus, it can be concluded that glutathione peroxidase is required for efficient inhibition of purified 5-LO by ZM 230487.

Noncompetitive and competitive inhibition of 5-LO activity by ZM 230487 depends on the 5-LO redox status. Nonredox inhibitors are considered to be competitive, active, site-directed 5-LO inhibitors that bind to an acid-binding pocket distal to the active site of the enzyme. Studies with the isolated 5-LO suggested a competitive inhibition of 5-LO by these compounds. However, stimulation of granulocytes with ionophore (10 $\mu$m) in the presence of arachidonic acid (40 $\mu$m) lead to a strong increase in cellular 5-LO activity compared with ionophore stimulation alone, but 5-LO inhibition by ZM 230487 was barely affected. Thus, it was of interest to study whether ZM 230487 is a competitive or noncompetitive inhibitor in the presence of high concentrations of fatty acid hydroperoxides which are usually generated in crude or purified 5-LO preparations when thiols and/or GSH-peroxidases are absent and at low peroxide levels which are usually found in resting intact cells.

Granulocyte homogenates were incubated with various concentrations of arachidonic acid in the presence or absence of DTT (1 mM) and the indicated concentrations of ZM 230487. The results are given as Lineweaver-Burke plots. As shown in Fig. 6 there is a competitive inhibition of 5-LO by ZM 230487 in the absence of DTT (Fig. 6A). However, under conditions with low levels of fatty acid hydroperoxides (i.e., in the presence of DTT and endogenous glutathione peroxidase), 5-LO was inhibited by ZM 230487 in a noncompetitive

![Graph](image1)

**Fig. 5.** The inhibition of purified human 5-LO by ZM 230487 depends on glutathione peroxidase activity. 5-LO, expressed in E. coli, was purified as described. Aliquots of the 5-LO solution were preincubated for 10 min with the indicated concentrations of ZM 230487 (1 ml, final volume) in the absence or presence of 1 mM GSH with or without glutathione peroxidase (20 milliunits/ml). Then, 5-LO activity was determined as described for cell homogenates. Results are expressed as mean ± standard error of three independent experiments.

![Graph](image2)

**Fig. 6.** Kinetic analysis of 5-LO inhibition by ZM 230487 under reducing and nonreducing conditions. Incubation mixtures consisting of PBS, pH 7.4, 1 mM EDTA, 2 mM CaCl$_2$, 1 mM ATP, and 20 $\mu$g/ml phosphatidylcholine with or without ZM 230487 were prepared at RT with the indicated concentrations of arachidonic acid (1 ml final volume). The 5-LO reaction was started by the addition of aliquots (40 $\mu$l) of the homogenates. After 10 min at 37°, the incubation was stopped with 1 ml methanol and the formed 5-LO products were extracted and analyzed by HPLC as described. Results are given as Lineweaver-Burke plots obtained from 5-LO incubations in the absence (top) or presence (bottom) of 1 mM DTT.
manner and 5-LO inhibition was independent of the arachidonic acid concentration (Fig. 6B).

**Oxidative stress leads to the reduced inhibition of cellular 5-LO activity by ZM 230487.** The data obtained with crude 5-LO preparations and purified enzyme suggested that an increased cellular peroxide tone could lead to a reduced inhibition of 5-LO activity of intact cells by ZM 230487. To test this possibility, granulocytes were stimulated with ionophore A23187 (10 μM) in the presence of the indicated concentrations of ZM 230487 and 13-HpODE (3 μM) or the oxidizing agent diamide (500 μM). As can be seen from Fig. 7, diamide and 13-HpODE shifted the IC$_{50}$ value of ZM 230487 from 50 to 290 and 400 nM, respectively. No such effects of 13-HpODE were observed when the FLAP inhibitor MK-886 was tested (data not shown).

**Discussion**

L-739,010 and methoxytetrahydropyran derivatives like ZM 230487 or ZD 2138 are selective and potent inhibitors of 5-LO in several ex vivo assays with IC$_{50}$ values of 20 to 50 nM in human whole blood and 3 nM in murine macrophages (Crawley et al., 1993). However, 100 to 150-fold higher concentrations were required for similar inhibition of purified 5-LO or 5-LO activity in broken cell preparations (Fig. 1). In a first series of experiments, we found that this shift in 5-LO inhibition was not caused by differences in arachidonic acid availability; rather, it was caused by differences in the peroxide tone generated under the various assay conditions.

Addition of thiols to the homogenates strongly increased the potency of ZM 230487 and L-739,010, and the resulting 5-LO inhibition was comparable with that of intact cells (Fig. 1). In contrast to ZM 230487, no significant shifts of the IC$_{50}$-values by DTT were found for the novel nonredox type inhibitor AKBA and the iron ligand BWA4C (Fig. 2).

In the presence of DTT, 5-LO inhibition by ZM 230487 in broken cell preparations was strongly reduced when selenium-dependent peroxidases were inhibited by iodacetate or when exogenous 13(S)-HpODE was added, whereas 13(S)-HODE, the corresponding alcohol, only slightly affected 5-LO inhibition by ZM 230487 (Figs. 4 and 3). These results and the inability of GSH to potentiate the efficacy of ZM 230487 in homogenates from selenium-deficient BL41-E95-A cells clearly indicate the participation of glutathione peroxidases. These findings were confirmed with the purified enzymes. Inhibition of purified recombinant 5-LO by ZM 230487 was not affected by GSH alone but ZM 230487 was about 20-fold more potent when glutathione peroxidase was included (Fig. 5). The data suggest that strong 5-LO inhibition by ZM 230487 can only be achieved under conditions that allow the efficient reduction of hydroperoxides by glutathione peroxidases. These effects were confirmed with intact cells. Elevation of the cellular peroxide tone either by addition of 13-HpODE or diamide leads to a shift of the IC$_{50}$ value of ZM 230487 from 50 to 400 and 290 nM, respectively (Fig. 7). No such effects were observed with MK-886. The data obtained with compounds belonging to various classes of 5-LO inhibitors suggest that the peroxide dependent 5-LO inhibition could be a unique feature of such nonredox type inhibitors ZM 230487 and L-739,010, because no such effects were observed with BWA4C, AKBA or MK-886.

Kinetic analysis revealed that there is a competitive inhibition of 5-LO activity by ZM 230487 under nonreducing conditions, whereas the compound acts as noncompetitive inhibitor when the hydroperoxide level is low (Fig. 6). The fact that elevated peroxide levels significantly reduce the efficacy and inhibitory properties of ZM 230487 might be of relevance for the in vivo pharmacology of these drugs. Leukotrienes are autocrine and paracrine inflammatory mediators that are released from activated leukocytes at inflammatory sites. Inflammatory reactions are characterized by the release of activated oxygen species which can induce peroxidation of polyunsaturated fatty acids (Comporti, 1985; Chanock et al., 1994). Thus, it is possible that the elevated hydroperoxide levels found at sites of chronic inflammation, after activation of 12- or 15-LO pathways, during reperfusion injury or other pathophysiological processes cause a reduced local efficacy of these compounds. This could explain why ZD 2138, the methyl analogue of ZM 230487, did not prevent pulmonary inflammation and the development of airway hyperresponsiveness although the ex vivo LTB4 levels and the in vivo LTE4 production was significantly reduced (Turner et al., 1996) and although data from FLAP- and 5-LO knock-out mice suggested a significant involvement of the 5-LO pathway in these diseases (Funk, 1996; Griffiths et al., 1997; Irvin et al., 1997). In this context, it is also of interest that ZD 2138 was effective in several cases of acute asthma but failed to inhibit more chronic inflammatory processes (Nasser et al., 1994; Turner et al., 1996).

The determination of LO inhibition in cell homogenates in the presence and absence of GSH or DTT provides a powerful and easy method to study the fatty acid hydroperoxide dependence of LO inhibition by drugs. Because this might be of importance for the in vivo pharmacology of 5- and also 12- or 15-LO inhibitors, it will be of interest to investigate a larger number of compounds belonging to the various classes of LO inhibitors and to characterize LO inhibitors that are under development now.

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**Fig. 7.** Effects of the cellular redox tone on the inhibition of cellular 5-LO activity by ZM 230487. Freshly isolated granulocytes were preincubated at RT for 10 min in absence or presence of 13(S)-HpODE (3 μM) or diamide (500 μM) with the indicated concentrations of ZM 230487. 5-LO activity of intact cells was then determined as described in Experimental Procedures. Results are expressed as mean ± standard error of three independent experiments.