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The Anti-inflammatory Activiy of Metal Complexes of Heterocyclic Thiosemicarbazones, 2-Substituted Pyridine *N*-Oxides and 2-Pyridylthioureas

Iris H. Hall,* S. Y. Chen,* K. G. Rajendran* and D. X. West†
* Division of Medicinal Chemistry and Natural Products, School of Pharmacy, CB 7360, University of North Carolina, Chapel Hill, NC 27599, USA, and † Department of Chemistry, Illinois State University, Norman, IL 61790–4160, USA

The thiosemicarbazones and their related metal complexes were shown to be potent anti-inflammatory agents in rodents at 8 mg kg^{-1}. They were effective in blocking induced edema and endotoxic shock while blocking both local and central pain processes. The ability of the agents to function as anti-inflammatory agents is multifold. First, Tumor Necrosis Factor-alpha (TNFα) and Interleukin-1 (IL-1) release was markedly reduced by the agents. Second, high-affinity receptor binding on fibroblasts of TNFα and IL-1 was significantly inhibited. Third, cellular events, e.g. lysosomal enzymes of specific cells, such as macrophages, were inhibited and prostaglandin cyclo-oxygenase and leukotriene 5′-lipoxygenase enzymic synthetic rates were significantly reduced, which should cause an overall reduction of the inflammatory process.

Keywords: thiosemicarbazones, metal complexes, anti-inflammatory

INTRODUCTION

Thiosemicarbazones and their metal complexes have been shown to have antitumor,[1–3] antiviral,[4] antibacterial,[5] antimalarial,[6] antileprotic[7] and antifungal[8] activities. The amine carboxy- and cyano-boranes have been reported to inhibit induced inflammation in rodents.[9] Their metal complexes of iron, copper, calcium, cobalt and sodium agents were observed to be potent anti-inflammatory agents in rodents, inhibiting induced inflammation, pain and endotoxic shock.[10] The amine–carboxyborane metal complexes were observed to block the release of lysosomal enzymes in mouse liver and polymorphoneutrophils [PMNs]. Oxidative phosphorylation of PMNs was uncoupled and prostaglandin synthetase activity in beef seminal vesicles was inhibited by the agents.[10] Subsequent studies have demonstrated that boron derivatives and their metal complexes regulated these metabolic events by modulating cytokine release and receptor binding to target cells involved in inflammation.[11, 12] The present study concentrates on thiosemicarbazones and their metal complexes to examine their anti-inflammatory actitivities, their mode of action and their toxicity in mice.

MATERIALS AND METHODS

Source of compounds

The thiosemicarbazone, 2-substituted pyridine *N*-oxides and 2-pyridylthioureas [Fig. 1] have been synthesized and their physical and chemical characteristics reported.[2, 3] All other chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, USA. Radioisotopes were obtained from New England Nuclear Corp. DuPont, Boston, MA, USA, and substrates and cofactors were obtained from Sigma Chemical Co., St Louis, MO, USA.

In vivo tests

Anti-inflammatory screen in mice

Male CF_1 mice weighing 28–32 g obtained from Jackson Lab. (Bar Harbor, MA, USA) were used

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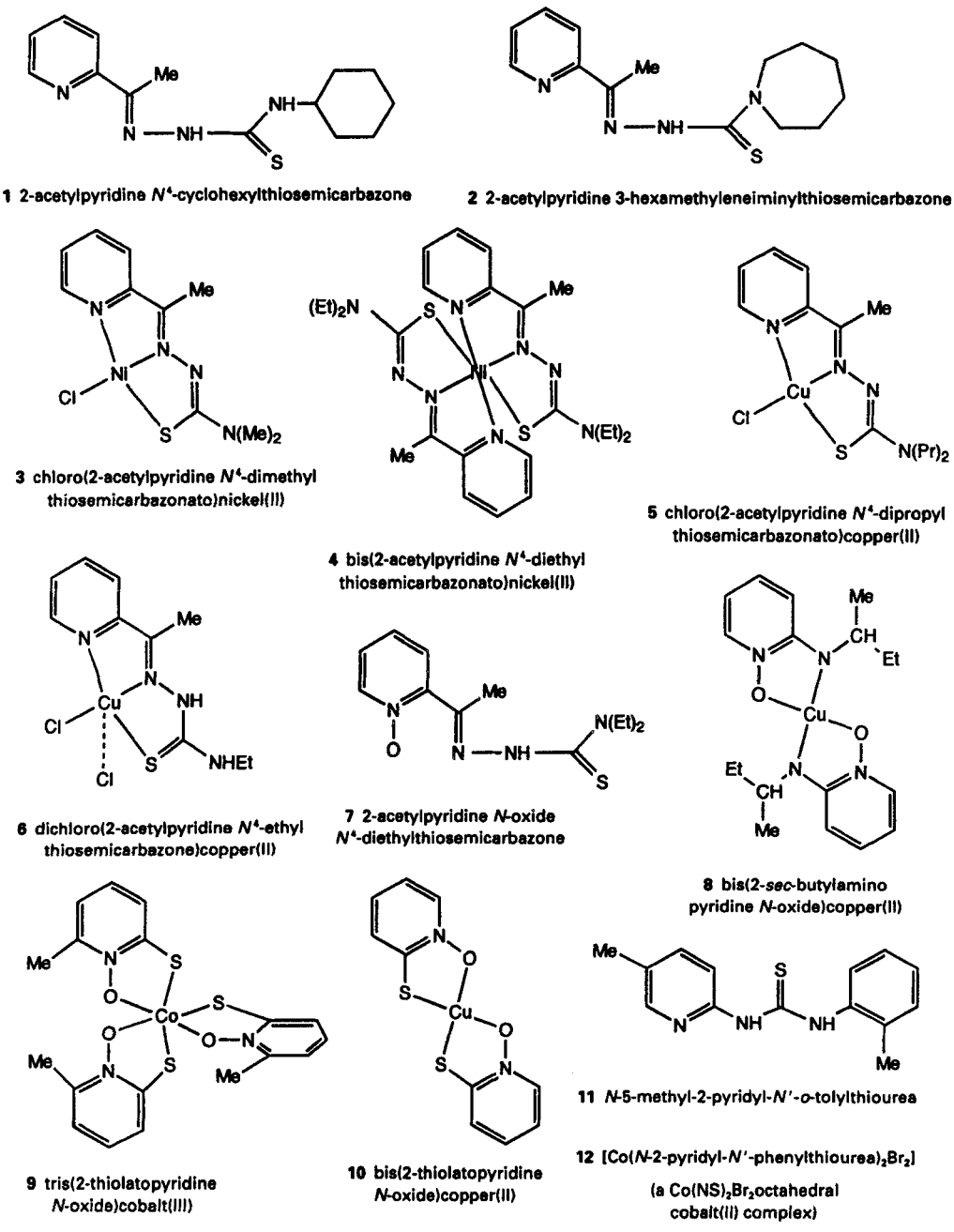

Figure 1 Structural formulae of the thiosemicarbazones, 2-substituted pyridine *N*-oxides and 2-pyridylthioureas included in this study.

to screen agents at 8 mg kg^{-1} intraperitoneal [i.p.]×2 administered 3 h and 30 min prior to administering the irritant, according to Winter's protocol.[13–15] Evaluation of the induced edema was made by injecting 2% carrageenan in 0.9% saline into the plantar region of the foot. The opposite foot injected with 0.9% saline was used as a baseline. The standards indomethacin (10 mg kg^{-1}) and phenylbutazone (50 mg kg^{-1}) were used to compare activity.

Protection against endotoxic shock

CF_1 male mice (29–31 g) were administered *Salmonella abortus equi* (Lot no. 69F4003) lipopolysaccharides (LPS) at 10 mg kg^{-1} i.p. which has an LD_{100} within 48–52 h which was consistent with literature values.[16] Drugs were administered 2 h before and 2 h after injection of the LPS and then subsequently once every 24 h for the rest of the experiment. Deaths were recorded every 12 h and records continued for 96 h. Since animals which had not died within 52 h recovered and were normal after 96 h, the 52 h data were used to calculate the percentage protection from induced endotoxic shock. Indomethacin (8 mg kg^{-1}) and pentoxyfylline (50 mg kg^{-1}) were used as standards.

Local analgesic activity

CF_1 male mice (28.5–32 g) were administered agents at 80 mg kg^{-1} i.p. 20 min before 0.5 mL of 0.6% acetic acid was administered i.p.[17] After 5 min, the number of stretches were counted over the next 10 min. Indomethacin was used as a standard at 8 mg kg^{-1}.

Hotplate tail-flick screen

CF_1 male mice (29–32 g) were administered drugs at 8 mg kg^{-1} i.p. prior to placement on a hotplate maintained at 100°F (37.8 °C). The time elapsed prior to tail-raising was measured using a digital read-out connected to the hotplate.[18] Tail-flick responses of CF_1 mice injected with morphine were used as the standard for this assay.

In vitro TNFα and IL-1 measurements and cellular regulation

IC-21 mouse macrophages were maintained in growth medium RPMI-1640+10% FCS+P/S. After the cells had grown to confluency, *E. coli* LPS at 10 μg mL^{-1} was added to the medium. Agents were incubated at 12.5, 25 and 50 μM final concentration for 18 h. The medium (100 μL) was collected for Tumor Necrosis Factor-alpha [TNFα] determinations. Interleukin-1 [IL-1] release was determined using $P388_{D1}$ cells which were maintained in RPMI-1640+10% FCS+P/S. The L929 bioassay was used to quantify the TNFα and the IL-1 levels. The L929 mouse fibroblasts were grown in Dullbecco's modified Eagle's medium (DMEM)+10% FCS+P/S[19,20] to confluency in 96-well plates and incubated with 100 μl of medium from IC-21 or $P388_{D1}$ cells for 24 h. The cells were stained with 0.2% Crystal Violet in 20% methanol and read at 580 nm using a Molecular Devices scanner (SOFT-max program).

Enzyme activity determinations

Prostaglandin cyclo-oxygenase activity

Mouse J774A macrophages were maintained in DMEM +15% FCS+P/S. Cells (5×10^6) were incubated with agents (12.5, 25 and 50 μM final concentration) and [^{3}H]arachidonic acid (100 Ci mol^{-1}) for 60 min at 37 °C in a CO_2 incubator. The reaction was terminated with 2 M HCl, the mixture extracted twice with ether, and the organic layer evaporated. The residue was dissolved in ethyl acetate and plated on TLC silica-gel plates. These were eluted with chloroform–methanol–water–acetic acid [90:8:1:0.8].[21,22] The plates were developed in iodine vapor, scraped according to the R_f values of standard prostaglandins, and counted in a Packard scintillation beta-counter corrected for quenching.

5′-Lipoxygenase activity

Mouse macrophages J774A (5×10^6 cells) were incubated with agents at 12.5, 25, and 50 μM concentration. The cells were harvested by centrifugation and incubated in a phosphate buffer (pH 7.2), 0.6 mM $CaCl_2$, 1.0 mM $MgCl_2$, the calcium ionophore A23187 and [^{3}H]arachidonic acid. After 20 min incubation at 37 °C, EtOAc–CH_2Cl_2 [2:3] supplemented with 12 mg arachidonic acid was added. The organic phase was extracted and dried. The residue was taken up in ethyl acetate and 100 μL was plated on silica-gel TLC plates eluted with methylene chloride–methanol–acetic acid–water [90:8:1:0.8]. The plates were scraped in the area that corresponds to 5-HETE hydroxylicosatetraenoic acid and counted.[23,24]

Lysosoml hydrolytic enzymic activities

Acid phosphatase, alkaline phosphatase, acidic cathepsin, elastase and trypsin proteolytic activities were measured utilizing mouse macrophages J774A (2×10^6 cells).[13] Mouse macrophage J774A cultures were maintained in DMEM with 10% FCS and P/S. Agents were incubated from 12.5, 25 and 50 μM for 60 min at 37 °C. Acid phosphatase activity at pH 5.0 and alkaline phosphatase activity at pH 8.0 were determined using 0.1 M β-glycerol phosphate in 0.1 M acetate buffer. The reaction was stopped

with 10% trichloroacetic acid (TCA) and centrifuged at 3000 $g \times 6$ min. Inorganic phosphate in the supernatant was determined by the spectrophotometric method of Chen *et al.*[25] The net inorganic phosphate released in 30 min was corrected by subtracting the blank value at time zero. Cathepsin activity was determined using 2% azocasein as the substrate in 0.1 M acetate buffer, pH 5.0, for 30 min at 37 °C. The reaction was terminated with 10% TCA and centrifuged. The supernatant was assayed for acid-soluble peptide fragments at 366 nm and corrected for blank values. *N*-Acetyl glucosaminidase [NAG] activity was determined with *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide. The hydrolysis product *p*-nitrophenol was determined at 400 nm.[26]

In vitro determination of toxicity of complexes

Intestinal mucosa cells

HCT-8 human small-intestinal mucosa cells were grown to confluency in RPMI 1640+10% horse serum+sodium pyruvate+P/S.[27] Agents were incubated with plated cells at 12.5, 25 and 50 μM final concentration. The cells were washed, fixed in 95% methanol, and incubated with 0.2% Crystal Violet. After the dye was washed from the cells, plates were read at 580 nm with a SOFTmax well-counter program.

Enzymic parameters

Enzymes leaking from HCT-8 cells into the supernatant were determined as a measure of agent toxicity. Lactate dehydrogenase activity was determined using pyruvate and the oxidation of NADH at 340 nm.[28] Alkaline phosphatase activity was determined with β-glycerol phosphate in acetate buffer (pH 8.0). The inorganic phosphate released was measured in ammonium molybdate and ascorbic acid at 720 nm.[13] β-Galactosidase activity was determined with lactose in Tris–maleate buffer (pH 7.0). The glucose released was measured with Sigma Kit 520 at 450 nm.[29] Leucine aminopeptidase activity was determined using L-leucine-*p*-nitroaniline in Tris–KCl buffer (pH 8.0). The *p*-nitroaniline released was measured at 405 nm.[30] Trypsin proteolytic activity was determined by the method of Schleuning and Fritz[31] using 2.0 mL of 0.1 M Tris buffer, pH 8.0, and 6 mM of *N*-benzoyl-L-arginine ethyl ester (BAEE) substrate. The hydrolysis of BAEE was determined after 30 min at 253 nm and blank values were subtracted.

In vivo mean survival values

CF_1 male mice (28 g) were grouped so that $n=6$ for each dose employed. Test drugs were prepared in 0.05% Tween-80–water. Dosages were administered i.p. from 5 to 500 mg kg^{-1} as a single dose. The number of deaths was recorded daily. The mean survival dose was determined using a probit method.[1]

Free-radical assays

Free-radical generation was determined by the Fenton reaction, i.e. *o*-phenanthroline, EDTA and ferric chloride with drug concentrations at 25, 50 and 100 μM measured at 510 nm.[32] The superoxide scavenger assay was determined with 2×10^6 J774A macrophages as the reduction of ferricytochrome in phosphate-buffered saline (PBS) buffer, pH 7.2, with drug concentrations at 25, 50 and 100 μM which was determined at 550 nm.[33]

Statistical analysis

Tables 1–6 contain data calculated as the means ± standard deviation expressed as percentages of control. The number of animals in each test group was six. The P values for a significant difference between control and treated groups was determined by the Student's *t*-test.

RESULTS

The thiosemicarbazones and their metal complexes demonstrated good anti-inflammatory activity at 8 mg kg^{-1} in male mice (Table 1). Compounds **1**, **2**, **3**, **5**, **6**, **7**, **10**, **11** and **12** demonstrated greater than 40% reduction of induced edema in mice. Compounds **1**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **11** and **12** reduced the writhing reflex by more than 50% in mice. In the tail-flick assay for central pain compounds **1**, **2**, **3**, **9** and **11** were statistically elevated above the control but none of the compounds was as effective as morphine which demonstrated a two-fold increase at 1 mg kg^{-1}. Compound **1** at 2 mg kg^{-1} day^{-1}, compound **9** at 4 and 8 mg kg^{-1} day^{-1} and compounds **10** and **11** at 4 mg kg^{-1} day^{-1}

afforded 100% protection aganst endotoxic shock induced by LPS *Salmonella* at 52 h. Compounds **1** and **7** were 83% effective at 4 mg kg^{-1} day^{-1}, and compounds **8** and **11** at 8 mg kg^{-1} day^{-1} were effective in preventing death.

TNFα release from IC-21 mouse macrophages was significantly reduced in a

Table 1 *In vivo* anti-inflammatory and analgesic activities and toxicity of thiosemicarbazones and their metal complexes in CF_1 mice at 8 mg kg^{-1} i.p. (n=6)

Compound no.	Percentage of control			Percentage protection[k] to endotoxic shock			Mean survival dose (mg kg^{-1} i.p.)
	Induced edema[h]	Writhing reflex[i]	Tail flick[j]	2 mg kg^{-1}	4 mg kg^{-1}	8 mg kg^{-1}	
1	52±3*	14±2*	178±6*	100	83	67	330
2	58±5*	85±4	169±5*	0	16	33	280
3	51±4*	41±3*	187±6*	66	50	33	142
4	73±5*	11±2*	86±4	16	16	50	>500
5	35±3*	4±1*	123±6*	0	33	16	21
6	46±5*	10±2*	98±6	—	33	0	<10
7	58±5*	54±6*	67±7*	—	83	50	280
8	69±6*	25±3*	113±5	67	67	83	100
9	66±5*	35±4*	169±6*	—	100	100	>500
10	36±4*	13±3*	175±6*	66	100	—	19
11	32±3*	15±2*	98±7	50	100	87	>500
12	24±3*	6±2*	86±5	—	16	50	>500
Control	100±5	100±6	100±6				
Phenylbutazone[a]	52±4*						
Indomethacin[b]	22±3*	43±4*		50	50	33	
Morphine[c]			217±5				
Cortisone[d]						0	
Dexamethasone[e]						67	
Pentoxifylline[f]						67	
LPS[g]						16	

[a] 50 mg kg^{-1} × 2. [b] 8 mg kg^{-1}. [c] 1 mg kg^{-1}. [d] 1.42 mg kg^{-1}. [e] 1 mg kg^{-1}. [f] 50 mg kg^{-1}. [g] 10 mg kg^{-1}. [h] 84 mg increase in paw weight. [i] 61 stretch reflexes/10 min. [j] 12.12 s. [k] Percentage of deaths of six animals at 52 h. * p=0.001 (Student's t-test).

Table 2 Effects of thiosemicarbazones and their metal complexes on TNFα and IL-1 release (n=6)

Compound no.	Percentage of LPS control (x±S.D.)					
	TNFα release from IC-21 macrophages[a]			IL-1 release from $P388_{D1}$[b]		
	25 μM	50 μM	100 μM	25 μM	50 μM	100 μM
LPS control	100±5	100±5	100±5	100±4	100±4	100±4
1	106±6	125±6	109±5	110±5	117±6	109±5
4	75±4*	15±3*	16±4*	111±6	113±4	116±5
5	142±5*	102±4	99±5	6±2*	8±2*	7±1*
9	75±4*	60±4*	59±4*	6±2*	5±1*	5±2*
10	173±5*	101±5	86±4	7±2*	8±3*	8±2*
11	63±3*	63±2*	62±2*	87±5	87±4	100±4
Pentoxifylline			80±4			73±3*

[a] 150 pg (mL medium)$^{-1}$. [b] 26 IL-1 units/mL.* P=0.001.

Table 3 Effects of thiosemicarbazones and their metal complexes on TNFα and IL-1 receptor high-affinity binding of L929 murine fibroblasts ($n=6$)

	Percentage of LPS control ($x\pm$S.D.)					
	^{125}I-TNFα receptor binding[a]			^{125}I IL-1 receptor binding[b]		
Compound no.	25 μM	50 μM	100 μM	25 μM	50 μM	100 μM
Control	100±6	100±6	100±6	100±5	100±5	100±5
1	28±4*	26±5*	30±5*	76±4*	56±5*	42±4*
4	87±4	87±5	77±4*	58±4*	55±3*	44±3*
5	61±5*	50±4*	28±3*	76±3*	48±3*	40±2*
9	102±5	97±4	59±4*	77±4*	75±4*	70±3*
10	34±3*	32±3*	11±2*	20±3*	16±2*	10±2*
11	77±4*	45±4*	30±3*	82±4*	72±4*	70±3*

[a] 12819 cpm (mg protein)$^{-1}$. [b] 2108 cpm (mg protein)$^{-1}$. * $P=0.001$.

concentration-dependent manner by compounds **4**, **9** and **11** over 18 h (Table 2). IL-1 release from $P388_{D1}$ was markedly reduced by compounds **5**, **9** and **10** after 24 h. ^{125}I-TNFα high-affinity receptor binding on L929 fibroblasts after 90 min was inhibited by compounds **1**, **5**, **10** and **11** in a concentration-dependent pattern and compounds **4** and **9** caused significant inhibition at 100 μM (Table 3). ^{125}I-IL-1 high-affinity binding to receptors on L929 cells was inhibited in a concentration-dependent manner with compound **1**, **4**, **5** and **10**; however, compounds **9** and **11** were less effective at 50 and 100 μM.

All of the compounds were effective in inhibiting macrophage acid phosphatase activity in a concentration-dependent manner (Table 4). Compounds **1**, **4**, **5**, **9** and **10** reduced alkaline phosphatase and NAG activities. Compound **11** was essentially inactive in these assays. All of the compounds were inactive in blocking macrophage acid cathepsin activity. None of the compounds was able to reduce free radicals. Compound **10** actually appeared to generate free radicals in the medium. On the other hand, all of these compounds were dual inhibitors of macrophage prostaglandin cyclo-oxygenase and 5′-lipoxygenase activities (Table 5). They appeared to be potent inhibitors of 5′-lipoxygenase activity, with compounds **4**, **9** and **11** causing greater than 75% inhibition at 100 μM.

Human HCT-8 ileum cells were incubated with the compounds at 10^{-4} M concentration (Table 6). Leakage of enzymes from intestinal mucosa villa, which is indicative of tissue damage, measured as elevated lactic acid dehydrogenase, alkaline phosphatase, leucine aminopeptidase, β-glucosidase and α-trypsin in the medium, was negative after 24 h of incubation. In fact the agents actually inhibited some of these enzymic reactions rather than causing elevation or release of enzyme activities.

DISCUSSION

The thiosemicarbazones and their metal complexes demonstrated significant anti-inflammatory activity. All of the compounds at 8 mg kg^{-1} were as active as phenylbutazone at 50 mg kg^{-1}. Selected compounds were comparable with indomethacin at 8 or 10 mg kg^{-1} in reducing induced edema, or phenylbutazone at 50 mg kg^{-1}. More importantly, these compounds were potent with regard to blocking endotoxic shock induced by *Salmonella* LPS, affording 100% protection over 52 h with selected agents. Control animals demonstrated 84% deaths or 16% survival at this time. The thiosemicarbazones and their metal complexes were more potent than pentoxifylline, cortisone, dexamethasone or indomethacin in protecting against induced septic shock. Even a lower dose of 2 or 4 mg^{-1} kg^{-1} day^{-1} was effective in protecting against death. The death rate is probably not due to the toxicity of most of the drugs since 52 h allows ample time for metabolism and elimination of the drug prior to the next dose, i.e. 24 h. Since the mean survival dose was below 25 mg kg^{-1} for compounds **5**, **6** and **10** as a single dose, the fact that these agents offered little protection at 8 mg kg^{-1} day^{-1} in the endo-

toxic screen may well be due in part to the drug's own toxicity. These agents blocked both local pain (writhing reflex) and central pain (tail-flick assay).

The data suggest that the thiosemicarbazone derivatives and their metal complexes were potent inhibitors of lysosomal enzyme release from macrophages invading the inflammation site. Proteolytic cathepsin activity, which functions in the spread of the inflammation, was also inhibited by the agents. Activities of prostaglandin cyclo-oxygenase, which regulates the syntheses of PgH, PgF2a, and PgE and of 5′-lipoxygenase, which regulates leukotrienes C4, D4, E4, and F4, were inhibited significantly by these metal complexes. Leukotrienes are capable of vasoconstriction, increased vascular cell-wall permeability, mucosal secretions, and neutrophil chemotaxis and influx, edema, release of lysosomal enzymes and generation of free oxygen radicals. Both the prostaglandins and leukotrienes are mediators of local inflammation. Thus, these metal complexes can be described as dual inhibitors. The reduction of these mediators may be related directly to the excellent *in vivo* activity in the induced edema and septic shock syndromes. Reduction of TNFα and IL-1 release from invading white blood cells is important in

Table 3 Effects of thiosemicarbazones and their metal complexes on hydrolytic enzyme activities ($n=6$)

	Percentage of control ($x\pm$S.D.)					
	Acid phosphatase activity[a]			Alkaline phosphtase activity[b]		
	25 μM	50 μM	100 μM	25 μM	50 μM	100 μM
Control	100±5	100±5	100±5	100±6	100±6	100±6
1	95±6	92±4	43±3*	64±5*	46±4*	41±5*
4	60±5*	23±3*	15±2*	59±5*	43±4*	26±3*
5	75±4*	43±3*	12±3*	83±5	62±4*	24±3*
9	46±4*	34±3*	10±2*	76±4*	60±5*	45±4*
10	57±5*	26±3*	24±2*	63±4*	50±5*	22±3*
11	142±5*	53±4*	52±3*	151±6*	131±5*	93±5
	NAG activity[c]			Cathepsin activity[d]		
Control	100±4	100±4	100±4	100±6	100±6	100±6
1	71±5*	65±4*	63±4*	120±5	120=5	140±7
4	79±5*	52±4*	50±3*	113±5	103±5	108±5
5	75±5*	63±4*	54±3*	96±5	105±4	115±6
9	64±4*	49±4*	43±3*	97±6	99±5	99±6
10	72±5*	70±4*	67±4*	88±5*	92±6	96±5
11	104±5	102±5	94±5	101±5	97±5	110±6
	Hydroxyl radical generation			Superoxide scavenger assay[f]		
	Fenton reaction[e]			reduction of ferricytochrome *c*		
Control	100±4	100±4	100±4	100±3	100±3	100±3
1	97±5	100±5	99±4	101±5	100±4	100±4
4	95±3	98±4	94±3	101±4	98±4	98±3
5	94±4	97±5	110±5	107±5	107±4	118±6
9	100±5	107±5	113±4	103±5	104±4	104±5
10	160±7	232±5	281±7	115±4	126±5	123±4
11	103±4	96±5	102±5	100±5	98±3	99±3
Zymogen (5 mg mL^{-1})	125±4	167±4*	241±5*			
3% H_2O_2	570±7*	607±5*	626±5*			556±6*

[a] 173 μg. P_i released. [b] 183 μg P_i released. [c] 377 nmol *p*-nitrophenol released. [d] 24 μg didpeptide released. [e] 0.233 O.D. change for iron reduction at 510 nm/10 min. [f] 0.068 O.D. change at 550 nm/15 min. * $P=0.001$.

Table 5 The effects of thiosemicarbazones and their metal complexes on mouse J744 macrophage prostaglandin cyclooxygenase and 5′-lipoxygenase activities ($n=6$)

	Percentage of control ($x\pm$S.D.)					
	Prostaglandin cyclooxygenase[a]			5′-Lipoxygenase[b]		
Compound no.	25 μM	50 μM	100 μM	25 μM	50 μM	100 μM
Control	100±6	100±6	100±6	100±5	100±5	100±5
1	81±5	68±4*	67±5*	74±5	70±6	44±4*
4	91±7	74±5*	62±5*	86±6	33±4	23±3*
5	69±5*	50±5*	37±4*	80±6	75±5*	58±5*
9	57±6*	50±5*	45±5*	47±4*	31±4*	19±3*
10	67±5*	63±5*	63±4*	80±6	44±5*	36±5*
11	91±6	59±5*	55±6*	57±6*	48±5*	21±4*

[a] 2716 dpm. [b] 5560 dpm. * $P=0.001$.

reducing the inflammatory process, because IL-1 is pyrogenic factor, which stimulates synthesis of acute-phase proteins and is chemotaxic to PMNs, and causes release of IL-3 or colony-stimulating factor.[34] TNFα can produce acute-phase responses, i.e. a component of septic shock, and can stimulate the release of chemical mediators, i.e. IL-1, PAF and leukotrienes. The process brings about destruction of tissues, e.g. connective and soft tissue, joints, muscles and synovial membranes. A number of compounds of this series are potent anti-inflammatory agents which would be effective agents *in vivo* to block inflammatory responses. These compounds are representative of heterocyclic thiosemicarbazones (**1**, **2**, **7**), 4- and 6-coordinate copper(II) thiosemicarbazone complexes (**3**, **4**), 4- and 5-coordinate copper(II) thiosemicarbazone complexes (**5**, **6**), copper(II) and cobalt(III) complexes (**8**, **9**, **10**) of 2-substituted pyridine *N*-oxides (**7**), 2-pyridylthioureas (**11**) and a cobalt(II) complex of a 2-pyridylthiourea (**12**). Further investigation is needed to establish the most potent compounds of this chemical series

Table 6 The *in vitro* toxicity of thiosemicarbazones and their metal complexes at 10^{-4} M in human HCT-8 ileum mucosa cells for 24 h ($n=6$)

	Percentage of LPS control ($x\pm$S.D.)[a]					
Compound no.	Lactic dehydogenase	Alkaline phosphatase	Leucine amino peptidase	β-Glucosidase	α-Trypsin	Protein content
Control	100±6	100±7	100±5	100±6	100±5	100±4
1	70±5	98±6	76±6	94±6	125±7	80±5
2	86±5	106±6	127±7	92±5	82±6	92±5
3	23±4*	104±6	107±5	91±6	127±6	91±6
4	25±5*	100±5	105±5	93±6	88±6	89±5
5	18±3*	87±5	81±6	96±5	91±5	98±6
6	1±1*	88±5	77±5	102±6	87±5	92±4
7	1±1*	60±5*	97±6	94±6	138±6*	69±7
8	7±2*	64±4*	131±7*	96±5	101±6	96±6
9	22±4*	79±6*	135±6*	94±5	92±6	89±5
10	94±6	70±5*	67±4*	85±6	110±6	81±5
11	16±3*	74±5*	95±5	90±6	92±6*	88±5
12	5±2*	7±2*	75±5*	89±5	139±7*	97±6

* $P=0.001$.

[a] Data are measured in terms of enzyme activity. Protein content is measured in units of μg/mL of medium.

and to investigate further their mode of action in blocking the inflammatory process.

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