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Biological Evaluation of Compounds with -NCS- Group or Derived from Thiazole and Imidazole. Activity on Prostaglandin Synthetase Complex

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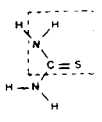
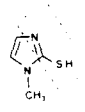
Abstract—The effects of compounds with activity against thyroid peroxidase were tested on the activity of hydroperoxidase and cyclo-oxygenase of the prostaglandin synthetase complex in-vitro. Active compounds were found to inhibit the peroxidase, and the cyclo-oxygenase function. These compounds were also found to have anti-inflammatory activity as demonstrated by the reduction of carrageenan-induced oedema of the hind paw of the rat. Indomethacin and non-steroidal anti-inflammatory drugs tested under the same conditions were shown to have activity towards the cyclo-oxygenase rather than the peroxidase function of the prostaglandin synthetase complex. A common feature of the active compounds was the presence of an -NCS- linkage or free -SH group.

Many nitrogen- and sulphur-containing compounds have an influence on thyroid metabolism either by inhibiting thyroid peroxidase (TPO) (Engler et al 1982a), or by complexing molecular iodine (Jambut-Absil et al 1987; Raby et al 1990). The action on TPO involves the formation of a covalent linkage with haem iron of the enzyme (Engler et al 1982b). The prostaglandin synthetase complex (PGS) which transforms arachidonic acid into prostaglandins comprises a cyclo-oxygenase and a peroxidase of similar haem structure to TPO (Markey et al 1987). This suggested to us that PGS might also be a target for compounds which inhibit TPO. In this study, we investigated the action of aliphatic and heterocyclic compounds containing the -NCS- linkage (Scheme 1) as well as derivatives of imidazole and sulphamide on the cyclo-oxygenase and peroxidase activities of PGS (Table 1).

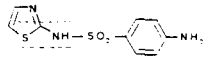


Thiourea

2-Aminothiazole



Methimazole



Sulphathiazole

SCHEME 1

To evaluate potential anti-inflammatory activity, we also determined the activity of these compounds on the carrageenan-induced oedema of the rat hind paw.

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Materials and Methods

Compounds

Hydrogen peroxide solution (9 mM) was prepared from commercial H_2O_2 (Fluka, Switzerland) and was kept at 4°C for several weeks. Sodium arachidonate was made up in 0·5 mL of 0·2 M NaOH from 10 mg ampoules of arachidonic acid (Sigma A 0662). All compounds tested were commercially available, and were dissolved in Tris buffer, pH 8.

2-Methylmercapto 1-methyl imidazole was synthesized in our laboratory, by action of iodomethane on methimazole. Tween 20, glycerol, ethylene diamine tetraacetate (EDTA), *N,N,N'N'*-tetramethylmethylparaphenylene (TMPD) were obtained from Fluka.

Procedure

Preparation of solubilized prostaglandin synthetase. All procedures were carried out between 0 and 4°C. Microsomes of bovine vesicular glands were obtained by ultracentrifugation of cell homogenates in the presence of 5 mM EDTA according to the method of Yamamoto (1982). Microsomal preparations (10–30 mg protein) were resuspended in 20 mM phosphate buffer pH 7·4 containing 0·1 mM EDTA, 50% glycerol, 1% Tween 20 and 5 mM tryptophan. After 30 min homogenization, the preparation was centrifuged at 142 800 *g* for 90 min. The clear supernatant, containing the enzyme activity, was stored at −18°C.

Prostaglandin G_2 (PGG_2) Peroxidase assay. The $PGG_2 \rightarrow PGH_2$ peroxidase activity of the enzyme preparation was determined by measuring the enzyme-catalysed oxidation of TMPD by hydrogen peroxide. The blue reaction product was measured spectrophotometrically at 610 nm, on a Kontron 860 double beam spectrophotometer equipped with a Peltier effect thermostatted sample holder (UVIKON, Saint Quentin en Yvelines, France).

The enzyme was preincubated in the test solution for 10 min before addition of H_2O_2. To 0·1 mL enzyme preparation was added 0·1 M Tris HCl pH 8·0, 80 μM TMPD and

Table 1. Structures.

Thiourea	Thiazole derivatives	Imidazole derivatives	Sulphamides
Thiourea	Thiazole	Imidazole	Sulphadiazine
Monomethylthiourea	2-Aminothiazole	*N*-methyl imidazole	Sulphathiazole
Dimethylthiourea	2-Thiazoline 2-thiol	Methimazole	Sulphamethizole
Tetramethylthiourea		2-Methyl mercapto-3-methylimidazole	Sulphamethoxazole

compound under test 0–1000 μM (final concentration). The reaction was initiated at 25°C by addition of 300 μM H_2O_2. Enzyme activity was evaluated from the change in absorbance over the first 30 s (linear phase of the reaction), recorded against a blank sample of the solution without the enzyme. We checked that the compounds had no direct reductive effects on oxidized TMPD, and did not diminish the rate of TMPD oxidation that occurs in tris-HCl pH 8 solution in contact with air ($=0{\cdot}029$ nm min^{-1} for a 800 μM TMPD solution). For each compound we defined a 50% inhibitory concentration (IC50) as the concentration at which there was a 50% inhibition in enzyme activity with respect to controls (Fig. 1).

Prostaglandin synthetase activity. Overall activity of PGS was determined from the above reaction using 220 μM sodium arachidonate to initiate the reaction (Takeguchi et al 1971). In this case, arachidonate is transformed by the cyclo-oxygenase to hydroperoxide which is then reduced by the PGG_2 peroxidase. The combined activities of the two enzymes was estimated from the oxidation of TMPD (Fig. 2).

Cyclo-oxygenase assay. The cyclo-oxygenase activity was measured at 25°C by monitoring the oxygen consumption in the presence of sodium arachidonate, using a Gilson 5/6 oxygraph in a thermostatted cell fitted with a Clark Yellow

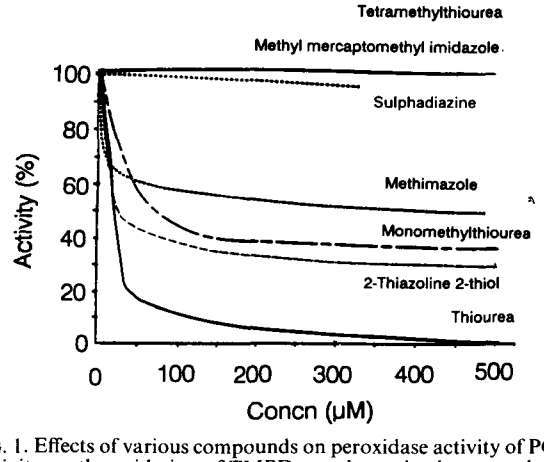


FIG. 1. Effects of various compounds on peroxidase activity of PGS. Activity on the oxidation of TMPD was determined spectrophotometrically at 610 nm.

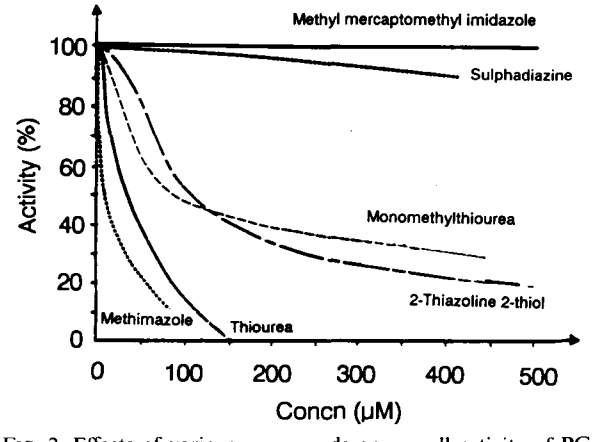


FIG. 2. Effects of various compounds on overall activity of PGS. Activity on the oxidation of TMPD initiated by 220 μM sodium arachidonate was determined spectrophotometrically at 610 nm.

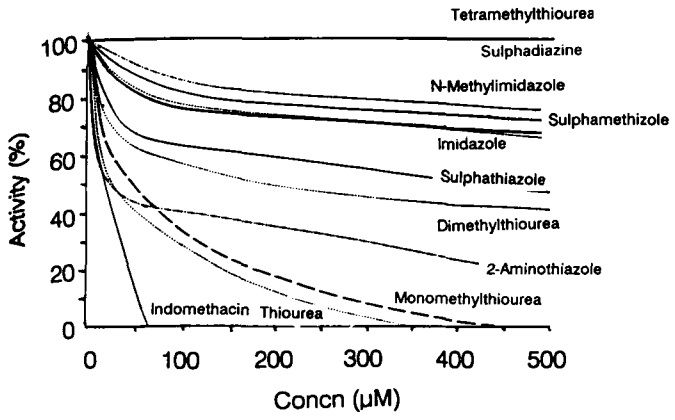


FIG. 3. Effects of various compounds on oxygen consumption by enzyme extract after addition of 110 μM sodium arachidonate, determined in an oxygraph fitted with a Clark electrode.

spring electrode (Gilson instruments, France). To 0·1 mL of enzyme preparation was added 0·1 M Tris HCl pH 8 and 0–1000 μM compound to a final volume of 2 mL. After incubation for 1 min in the cell, when a straight base line was achieved, the reaction was initiated by injecting 10 μL of 22 mM sodium arachidonate (110 μM final concentration). For each compound we defined a 50% inhibitory concentration (IC50) with respect to initial activity of the enzyme estimated from the fall in the oxygen content between 20 and 80 s after addition of arachidonate (Fig. 3).

Anti-inflammatory activity. The method of Winter et al (1962) was used on carrageenan-induced oedema on groups of eight male Wistar rats obtained from Iffa-Credo (France). Compounds were administered orally with a 5% arabic gum suspension of the compound at a dose of 30 mg kg^{-1} 30 min before injection of 0·05 mL carrageenan prepared as a 1% solution in sterile 0·9% NaCl. The volume of the hind paw was measured in a plethysmometer (Apelex, France) immediately after injection and at hourly intervals. We calculated the percentage of swelling inhibition 3 h after carrageenan injection, to coincide with the prostaglandin phase of the reaction (Willis 1969); the swelling observed 2–5 h after carrageenan injection corresponds to prostaglandin liberation (Vinegar et al 1969).

Results

The results of the enzyme studies are summarized in Table 2.

Inhibition of PGG_2 peroxidase

In the thiourea group, methylation reduced activity, as the dimethyl derivative had little activity and the tetramethyl derivative was devoid of activity. This indicated that the action was due to the free -SH group as was observed in the action against thyroid peroxidase (Raby et al 1990). A free -SH is present in thiourea and in one or other tautomeric forms of its mono and dimethyl derivatives, but not in the tetramethyl derivative.

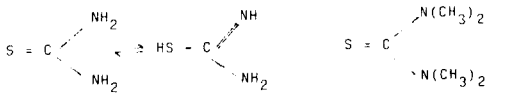
In the thiazole group, thiazole itself was devoid of activity, while the presence of an amino or thiol group conferred significant activity.

Imidazole and *N*-methylimidazole were without action, whereas methimazole with a free -SH group had moderate

Table 2. Effects of compounds on prostaglandin synthetase. For peroxidase assay, the reaction mixture contained: 0·1 M Tris HCl pH 8, 80 μM TMPD, 0–1000 μM compound, 0·1 mL enzyme extract. The reaction was initiated by addition of 300 μM H_2O_2 and monitored at 610 nm. For PGS activity, 220 μM sodium arachidonate was used instead of H_2O_2. To determine cyclo-oxygenase activity, the reaction mixture contained 0·1 mL enzyme, 0–500 or 1000 μM drug, 0·1 M Tris HCl pH 8 for a final volume of 2 mL. Oxygen uptake was measured with an oxygraph.

Compound	Peroxidase IC50[a] (μM)	Cyclo-oxygenase IC50[a] (μM)	Total PGS IC50[a] (μM)
Thiourea	23	32	30
Monomethylthiourea	73	49	100
Dimethylthiourea	935	191	250
Tetramethylthiourea	No inhibition	No inhibition	No inhibition
Thiazole	No inhibition	No inhibition	No inhibition
2-Amino-thiazole	30	27	70
2-Thiazoline 2-thiol	25	1038	80
Imidazole	No inhibition	> 1000	–
N-Methylimidazole	No inhibition	> 1000	– –
Methimazole	330	+ +	10
Methylmercaptomethyli-midazole	No inhibition	No effect	No inhibition
Sulphadiazine	– –	No effect	– –
Sulphathiazole	20	405	175
Sulphamethizole	1190	> 1000	– – –
Sulphamethoxazole	– – –	> 1000	– – –
Reference drugs			
Indomethacin	+	21	1,7
Aspirin	+	1370	168
Flufenamic acid	No inhibition	530	210
Naproxen	No inhibition	43	12
Phenylbutazone	– –		375

[a] IC50: drug concentration leading to a 50% inhibition of enzyme activity under our experimental conditions.
–: inhibition < 20% for 1000 μM; – –: 20–30% inhibition for 1000 μM; – – –: 30–40% inhibition for 1000 μM; +, + +: activation.



Thiourea Tetramethylthiourea

SCHEME 2

activity (Moulard et al unpublished results). In contrast, the methyl derivative of methimazole was without activity. As with the thioureas, it appears that the free -SH group is responsible for the activity (Scheme 2).

Weak activity was observed with all the sulphamides apart from sulphathiazole. The reference compounds had variable, weak activities. Indomethacin and aspirin activated the enzyme, flufenamic acid and naproxen had no action, while phenylbutazone had slight inhibitory activity.

Inhibition of cyclo-oxygenase

The different compounds had different effects on the cyclo-oxygenase. In the thiourea group, the presence of the SH was required for activity. Thiazole had no activity, while the derivatives with a primary amino or -SH group were found to be active.

In the imidazole group, in contrast to the findings on peroxidase activity, both imidazole and its *N*-methyl derivative inhibited the enzyme. Methimazole was found to activate the enzyme, while its methyl derivative was devoid of activity. Apart from sulphadiazine which was inactive, the other sulphamides had significant activity, albeit lower than that of indomethacin.

Table 3. Effects of compounds on carrageenan-induced oedema in the rat paw. Anti-inflammatory activity in-vivo was measured from the inhibition of carrageenan-induced oedema, in the prostaglandin phase of the swelling (3 h after injection). The compounds were given orally at a dose of 30 mg kg^{-1} (300 mg kg^{-1} for aspirin). % inhibition of oedema was calculated with respect to controls who received only arabic gum solution.

Compound	Inhibition of oedema (mean %[a])
Thiourea	45·6
Monomethylthiourea	61·0
Dimethylthiourea	29·7
Tetramethylthiourea	40·8
2-Aminothiazole	42·0
2-Thiazoline 2-thiol	55·2
Imidazole	47·4
N-Methylimidazole	30·3
Methimazole	58·3
Methylmercaptomethylimidazole	53·8
Sulphadiazine	29·2
Sulphathiazole	54·6
Sulphamethizole	53·0
Sulphamethoxazole	58·3
Reference drugs	
Indomethacin	58·5
Aspirin (300 mg kg^{-1})	45·6
Flufenamic acid	60·4
Naproxen	52·5
Phenylbutazone	24·4

[a] Significantly different from control: $P<0·01$ (Student's *t*-test).

Inhibition of prostaglandin synthetase

The PGS test is of interest as it gives an indication of the activity, whether additive or otherwise, of the compound towards the two components of the PGS complex.

For the thioureas, the same behaviour was observed on the complex as on the PGG_2 peroxidase, with a progressive loss of activity with increasing methylation.

Thiazole had no activity, but aminothiazole and 2-thiazoline 2-thiol had a strong action on the whole enzyme complex. Methimazole had marked activity, while its methyl derivative was devoid of activity. Both imidazole, and *N*-methylimidazole had weak activity. Apart from sulphathiazole, the sulphamides had little activity. All the reference compounds had significant activity, comparable with that observed on the cyclo-oxygenase.

Carrageenan-induced oedema

All compounds were found to have activity in the rat hind paw oedema test, although to differing degrees (Table 3). With the thiourea, there was decreasing activity with increase in methylation. The sulphamides had little activity, while indomethacin was the most active of the reference drugs.

Discussion

The activities of peroxidase and cyclo-oxygenase in PGS are structurally inseparable (Yamamoto 1982), although we found that the two activities could be discriminated pharmacologically. Non-steroidal anti-inflammatory drugs act predominantly on the cyclo-oxygenase component and are without activity or activated the PGG_2 peroxidase, whereas the compounds of the classes tested here were found to inhibit the peroxidase and the cyclo-oxygenase function. The compounds with the greatest antithyroid activity (thiourea, monomethylthiourea, 2-aminothiazole, 2-thiazoline-2-thiol and methimazole (Raby et al 1990; Lagorce et al 1991)) also appeared to be the most active on the PGG_2 peroxidase (inhibition at the lowest concentrations). The peroxidase of PGS has the characteristics of a haemeperoxidase including the Fe^{3+} protoporphyrin IX prosthetic group which is involved in electron exchange (Markey et al 1987).

Drugs such as thiourea, its monomethyl derivative, 2-aminothiazole, 2-thiazoline 2-thiol and sulphathiazole with a strong action on the PGG_2 peroxidase and activity on the cyclo-oxygenase also had strong activity on the whole PGS complex as well as in the rat hind paw oedema test.

On the other hand, drugs such as imidazole and *N*-methylimidazole with no action on the PGG_2 peroxidase and little activity on the cyclo-oxygenase, or drugs such as sulphadiazine with no action on the cyclo-oxygenase and little activity on the PGG_2 peroxidase had weak activity on the whole PGS complex and modest activity in the oedema test.

The results from the inhibition of carrageenan-induced oedema of the rat hind paw suggested to us that inhibition of either enzyme (peroxidase or cyclo-oxygenase) produces the same end result. It should be noted that the measurements

were carried out in the prostaglandin phase of the inflammatory response (Vinegar et al 1969).

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