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Benzoselenazolinone Derivatives Designed To Be Glutathione Peroxidase Mimetics Feature Inhibition of Cyclooxygenase/5-Lipoxygenase Pathways and Anti-inflammatory Activity

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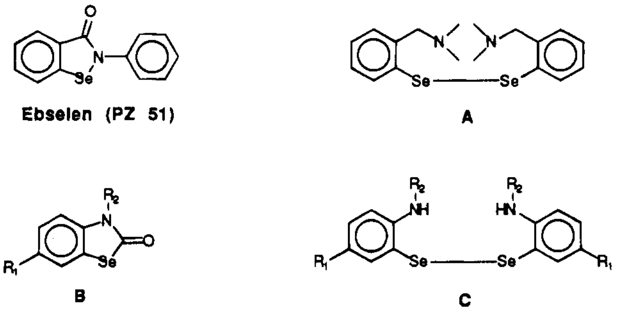
Two series of compounds, substituted benzoselenazolinones and their opened analogs, diselenides, were prepared. The diselenides were designed according to the available SAR about glutathione peroxidase mimics and were expected to have activity. An initial series of tests was performed in order to assess the glutathione peroxidase and antioxidant activity of the diselenides compared to their cyclized analogs. The diselenides were shown to be very potent (up to 3 times the activity of ebselen), whereas the benzoselenazolinones were inactive, thus confirming our hypothesis. A second series of tests was done to determine the anti-inflammatory potency of the two series. Both were found to be potent on cyclooxygenase and 5-lipoxygenase pathways (up to 95% inhibition at 10^{-5} M). Some compounds were selective, and the variations in the activity allowed us to draft some structure−activity relationships. The most interesting compound of each series, 6-benzoylbenzoselenazolinone and bis[(2-amino-5-benzoyl)phenyl] diselenide, was tested *in vivo* on the rat foot edema induced with different phlogistic agents and was shown to have some anti-inflammatory properties.

The rapid growth in the understanding of the implication of reactive oxygen species (ROS) in pathology has attracted considerable attention and brought new ideas for the therapy of a variety of diseases.[1] In particular, ROS, such as superoxide anions or hydroxy radicals, are involved in the initiation, propagation, and maintenance of both acute and chronic inflammatory processes.[2] Thus, the design of novel anti-inflammatory agents according to this concept, as an alternative to the conventional steroid or nonsteroid compounds, was investigated. With this prospect in view, new series of compounds mimicking the natural defenses of the organism against reactive oxygen metabolites were tested, (i) radical scavengers imitating the α-tocopherol/ascorbic acid system,[3] (ii) various copper complexes with the therapeutic use of superoxide dismutase,[3,4] and more recently (iii) organoselenic derivatives with glutathione peroxidase-like activity.[5,6]

The role of glutathione peroxidase is to reduce hydroperoxides via its selenium-containing active center.[7] The enzyme catalytic site includes a selenocysteine residue in which the selenium undergoes a redox cycle involving the selenolate anion as the active form which reduces hydroperoxides. An isoselenazolidin-3-one ring system is then formed as the reaction product.[8,9] Regeneration of the active form of the enzyme is obtained by the nucleophilic attack of glutathione (GSH = γGlu-Cys-Gly) on the heterocycle.

Chart 1. General Formulas of Synthesized Compounds—Analogy with Ebselen



Recently, simple organoselenic compounds were shown to mimic this enzymatic activity *in vitro*. Among them, the most promising drug was ebselen (PZ 51, 2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one; see Chart 1), a heterocyclic compound consistent with the isoselenazole intermediate in the glutathione peroxidase cycle.[10] Ebselen exhibited anti-inflammatory properties, completely inhibiting superoxide anion production by guinea-pig alveolar macrophages,[11] and disclosed GSH-peroxidase-like activity *in vitro* and *in vivo*.[12] Other series have been developed, taking into account other features of the enzyme active site environment.

As determined by X-ray crystal analysis,[13] the active site of glutathione peroxidase is subjected to an electric field due to the dipole moment of helix α_1. For points located near the helix N-terminus, the effect of the dipole is equivalent to the effect of half of a positive unit charge. Hence, the electric charge due to the dipole moment of helix α_1 will stabilize the active site selenolate and enhance its nucleophilic reactivity. This observation prompted Spectror to advance the hypothesis that the inclusion of a strongly basic group proximal to the active selenium atom would be expected to favor the redox catalytic cycle.[6] Within this context, (aminoalkyl)-

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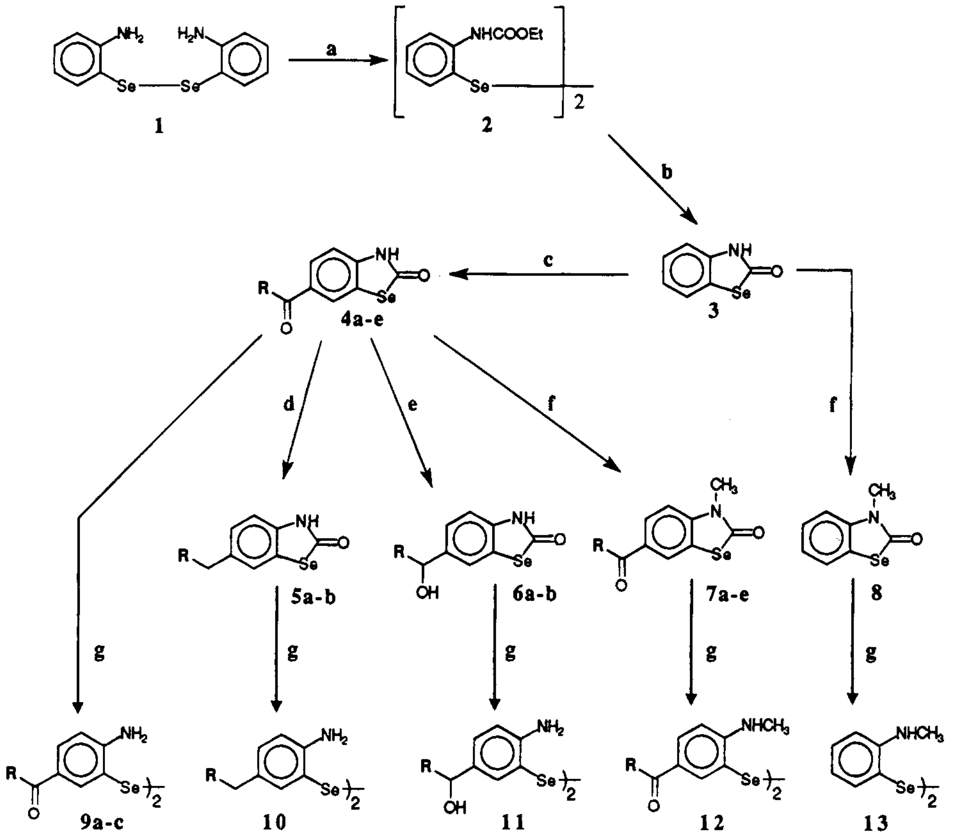
Scheme 1. Synthetic Scheme for Compounds **1**–**13**[a]



[a] Reagent: (a) $ClCOOEt$/pyridine; (b) Sn/HCl; (c) R_1COOH/PPA or $R_1COCl/AlCl_3$/DMF; (d) $(C_2H_5)_3SiH$/TFA; (e) $NaBH_4/NaOH$ 3%; (f) CH_3I/EtONa or $(CH_3)_2SO_4/NaOH$; (g) NaOH 10%/Δ.

diphenyl diselenides (Chart 1, A) were shown to possess GSH-peroxidase activity.

Considering these previous results, we proposed the synthesis of benzoselenazolinones containing a selenium moiety in the heterocycle (Chart 1, B). Such molecules can be considered as prodrugs of the corresponding diselenides (Chart 1, C), since a ring opening can be postulated from previous works on the metabolism of isosteric heterocycles such as benzoxazolinones and benzothiazolinones.[7] Furthermore, the presence of an amino group in the ortho position of the selenium atom and/or the introduction of aminoalkyl side chains on the heterocyclic nitrogen may contribute to the stabilization of various selenium functions and favor the enzymatic activity of the compounds.

The synthesis of 1,3-benzoselenazolin-2-ones as well as the corresponding diselenides has been carried out according to Scheme 1. The main purpose, initially, was to compare the *in vitro* glutathione peroxidase activities of the two series to confirm our hypothesis. Besides the above considerations, we introduced various substituents at the 6-position of the molecule. These substituents can increase the lipophilicity of the molecule, modify the steric and electronic parameters, and influence the selenazole ring stability. The goal was to increase the potential inhibitory activity of 5-lipoxygenase and/or cyclooxygenase by this pharmacomodulation and also to establish structure–activity relationships for the inhibition of each pathway. The substitution of the nitrogen with a methyl group also provided useful information on that purpose. The nitrogen substitution with an aminoalkyl side chain was designed to take away the basic center from the selenium moiety.

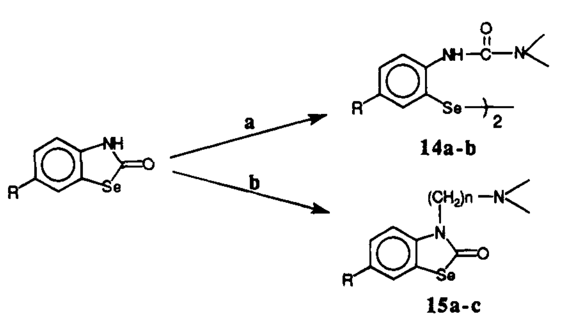
Chemistry

Shown in Scheme 1 are the steps leading to 1,3-benzoselenazolin-2-one (**3**). Bis(*o*-aminophenyl) diselenide (**1**)[14] was treated with ethyl chloroformate to form bis(*o*-urethanophenyl) diselenide. Reductive cyclization by tin and hydrochloric acid afforded **3**. The acylation of **3** to provide 6-acylated 1,3-benzoselenazolin-2-ones **4** was performed by two methods using either acids in the presence of polyphosphoric acid (PPA) or acid chlorides in the presence of $AlCl_3$ in dimethylformamide. The resulting 6-acyl derivatives were submitted to partial or full reduction by two different methods. Aryl or alkyl derivatives **5** were obtained by catalytic reduction of the ketone group using triethylsilane in a trifluoroacetic acid medium, while the ketones **4** were easily converted to secondary alcohols **6** by treatment with sodium borohydride in an alkaline medium. Substitution on the nitrogen atom of the heterocycle was also performed. N-Methylation was carried out either by the action of methyl iodide in the presence of sodium ethylate or by the action of dimethyl sulfate in the presence of sodium hydroxide (compounds **7** and **8**).

Substitution on the nitrogen atom by aminoalkyl side chains was more difficult since, under Mannich conditions, opening of the selenazole ring occurred, leading to the formation of unexpected ureides **14** (Scheme 2).

In contrast, the introduction of an alkylamino side chain was successfully achieved using chloroalkylamines in the presence of potassium carbonate and dimethylformamide.

Scheme 2. Synthetic Scheme for Compounds **14** and **15**[a]



[a] Reagent: (a) 30% aqueous HCHO + amine; (b) $Cl(CH_2)_nNR_1R_2/K_2CO_3$/DMF.

Pharmacology

Antioxidant Activities. *In Vitro* Glutathione Peroxidase Activity. GSH-Px activity was assayed according to the method of Wendel,[15] using hydrogen peroxide or *tert*-butyl hydroperoxide as substrates.

As can be seen in Table 3, all 1,3-benzoselenazolinones were devoid of any glutathione peroxidase-like activity in this test, whereas the diselenides showed very promising activity *in vitro*. Several conclusions can be drawn from these results. On one hand, in contrast with ebselen, the 1,3-benzoselenazole ring was not opened under the nucleophilic attack of GSH. On the other hand, all diselenides exhibited approximatively 3 times the activity of ebselen, which is consistent with previous observations of Spectror.[6] Noteworthy was that *in vivo* 1,3-benzoselenazolin-2-ones could be metabolized into the corresponding diselenides. Compounds **9b**,**c** and **12** were the most active compounds. Thus, partial or total reduction of aroyl substituents in the C-6 position did not increase this biological activity.

In order to confirm the interest of such glutathione peroxidase activity, three tests have been developed to evaluate the antioxidant activity against lipid peroxidation. Actually, oxidative deterioration of polyunsaturated lipids is representative of the particular sensitivity of membrane lipids to oxygen-dependent damages. It has also been proposed that various diseases such as ischemia, inflammation, and atherosclerosis may be initiated, or at least enhanced, by lipid peroxides and other compounds formed during peroxidation of polyunsaturated lipids.[16-18]

Inhibition of Spontaneous Lipid Peroxidation. Rat brain homogenates were assayed for lipid peroxidation in the absence or presence of test compounds (10^{-5} M). Lipid peroxidation was assessed by the thiobarbituric acid-reactive test (malondialdehyde accumulation).[19]

Inhibition of Fe^{2+}/Ascorbate-Induced Lipid Peroxidation. Lipid peroxidation in the above incubation mixture was initiated by the Fe^{2+}/ascorbate system.[19] In both of these peroxidation tests, diselenides exhibited an interesting activity equipotent with that of ebselen (see Table 3). The protective effect of these compounds was consistently effective as compared with that of 1,3-benzoselenazolin-2-ones and also the reference compounds probucol and trolox.

Inhibition of Human Red Blood Cell Hemolysis. The peroxidation of human red blood cells was initiated by AAPH, a water-soluble radical generator, in the presence or absence of test compounds (10^{-5} M). Hemolysis inhibition was estimated spectrophotometrically by the hemoglobin dosage in the supernatant. Tested diselenides and also, interestingly, 1,3-benzoselenazolinones **4b**,**c**, **5b**, **7c**, and **8** inhibited hemolysis to the same level as probucol. It was also noteworthy that in this test the lipophilic character seems to favor the activity (**4b**,**c**, **5b**, and **9b**; see Figure 1).

5-Lipoxygenase and Cyclooxygenase Inhibition. Leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) are metabolites characteristic of arachidonic acid breakdown which are released by stimulated leukocytes. LTB_4 and PGE_2 are known mediators of inflammation. LTB_4 and PGE_2 inhibitors are potential drugs for the treatment of inflammatory diseases. Human granulocytes were stimulated *in vitro* by A 23187, and the formation of LTB_4 and PGE_2 was measured by radioimmunoassay.[20]

Figure 2 shows that many compounds dramatically decreased the formation of LTB_4, representative of the 5-lipoxygenase pathway (5-LO). When comparing the activities of the compounds **9b**,**c**, **10**, and 11, it can be noticed that the increase of the lipophilic character of diselenides by substitution on C-6 with benzoyl, *p*-chlorobenzoyl, phenylcarbinoyl, and benzyl substituents enhanced the inhibition of LTB_4 formation. Moreover, in this test, the 1,3-benzoselenazolinones **4c**, **5b**, **6b**, and **9a** showed consistently good activity values comparable with those of other reference drugs (50–75% inhibition at 10^{-5} M). **4a** selectively blocked the 5-LO pathway.

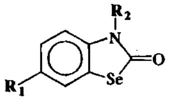
PGE_2 formation is a reflection of the cyclooxygenase pathway (CO). In this series, most of the diselenides which block the 5-LO pathway were equipotent in the inhibition of the cyclooxygenase pathway, but compounds **14a** and **12** exclusively inhibited 5-LO and CO, respectively. 1,3-Benzoselenazolinone **4c** induced an effective decrease of CO pathway with 95% PGE_2 inhibition at 10^{-5} M.

In conclusion, the biological activity in the lipoxygenase pathway as well as in the cyclooxygenase pathway appeared to be very promising for many of the compounds presented here. Some compounds are potent dual CO/LO inhibitors, while others are selective for one arachidonic acid metabolic pathway. Such results were sufficiently encouraging to test the anti-inflammatory activity *in vivo*.

***In Vivo* Anti-inflammatory Properties.** Acute anti-inflammatory activity was tested on two selected compounds, 6-benzoyl-1,3-benzoselenazolinone (**4c**) and 2-amino 5-benzoyl diselenide **9b**, by inhibition of the carrageenan-induced foot edema in rats, according to the method of Winter *et al.* [21] Carrageenan was injected into the subplantar surface of the right hind paw, and the inflammatory process (edema volume) was followed over 4 h. One hour before injection, the test compound or reference compound (ebselen, indomethacin) was administered orally at different concentrations.

As shown in Table 4, both compounds **4c** and **9b** exhibited good anti-inflammatory activity (with a potency = 0.1 × indomethacin). Compound **4c** (100 mg/kg) and indomethacin (10 mg/kg) exhibited maximal inhibition (58.5% and 68.7%, respectively) after 2 h. Compound **9b** (3 mg/kg) and ebselen (3 mg/kg) were effective (45% and 48% inhibition, respectively) after 1 h. It should be noted that the maximum activity is obtained with the lowest administered dose.

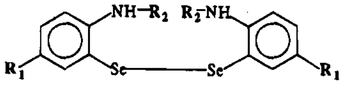
Table 1. 3- and 6-Substituted Benzoselenazolinones



no.	R_1	R_2	recryst solvt	mp (°C)	yield (%)	^1H NMR δ (ppm)
4a[a]	$COCH_3$	H	toluene	185	70−80	(DMSO-d_6) 2.50 (s, 3H, CH_3), 7.20 (d, 1H, H_4 aromatic), 7.90 (dd, 1H, H_5 aromatic), 8.40 (d, 1H, H_7), 12.00 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_9H_7NO_2Se$) C, H, N.
4b[b]	$COCH_2CH_2CH_3$	H	ethanol, 70%	166	48	($CDCl_3$) 1.00 (t, 2H, CH_3), 1.80 (m, 2H, CH_2-CH_2-CH_3), 3.00 (t, 2H, CH_2-CH_2-CH_3), 7.20 (d, 1H, H_4), 7.90 (d, 1H, H_5), 8.15 (s, 1H, H_7), 9.50 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_{11}H_{11}NO_2Se$) H, N; C: calcd, 49.27; found, 48.86.
4c[c]	COC_6H_5	H	ethanol, 95%	204	70−80	($CDCl_3$) 7.20 (d, 1H, H_4), 7.60 (m, 6H, H_5 + H aromatic benzoyl), 8.20 (d, 1H, H_7), 12.00 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_{14}H_9NO_2Se$) C, H, N.
4d[d]	COC_6H_4Cl	H	ethanol, 95%	>260	70	($CDCl_3$) 7.20 (d, 1H, H_4), 7.75 (m, 5H, H_5 + H aromatic *p*-chlorobenzoyl), 8.20 (d, 1H, H_7), 12.00 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_{14}H_8NO_2SeCl$) C, H, N.
4e[e]	COC_6H_4N	H	ethanol, 95%	210	50	(DMSO-d_6) 7.25 (d, 1H, H_4 atomatic), 7.60 (dd, 1H, Hc), 7.75 (d, 1H, H_5 aromatic), 8.20 (s, 1H, H_7), 8.80 (s, 1H, Ha), 8.80 (d, 1H, Hb), 12.10 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_{13}H_8N_2O_2Se$) H, N; C: calcd, 51.50; found, 51.04.
5a	CH_2CH_3	H	cyclohexane	128	80	($CDCl_3$) 1.25 (t, 3H, CH_3), 2.6 (q, 2H, CH_2), 7.10 (s, 2H, H_4 + H_5), 7.45 (s, 1H, H_7), 10.40 (broad peak exchangeable with D_2O, 1H, NH). Anal. (C_9H_8NOSe) C, H, N.
5b	$CH_2C_6H_5$	H	cyclohexane	125−127	95	($CDCl_3$) 4.00 (s, 2H, CH_2), 7.00−7.25 (m, 8H, H aromatic), 9.00 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_{14}H_{11}NOSe$) C, H, N.
6a	$CHOHCH_3$	H	cyclohexane $^2/_3$, ethyl acetate $^1/_3$	171	80	(DMSO-d_6) 1.25 (d, 3H, CH_3), 4.65 (dd, 3H, CH_3), 5.25 (peak exchangeable with D_2O, 1H, OH), 5.65 (s, 1H, CH), 7.1 (dd, 2H, H_4 + H_5 aromatic), 7.6 (s, 1H, H_7 aromatic). Anal. ($C_9H_9NO_2Se$) C, H, N.
6b	$CHOHC_6H_5$	H	cyclohexane $^2/_3$, ethyl acetate $^1/_3$	126	80	(DMSO-d_6) 5.60 (s, 1H, CH), 5.85 (broad peak exchangeable with D_2O, 1H, OH), 7.00−7.50 (m, 8H, H aromatic), 7.60 (s, 1H, H_7 benzoselenazolinone), 11.50 (broad peak exchangeable with D_2O, 1H, NH). Anal. ($C_{14}H_{11}NO_2Se$) C, H, N.
7a	$COCH_3$	CH_3	cyclohexane	132−133	85	($CDCl_3$) 2.5 (s, 3H, $COCH_3$), 3.50 (s, 3H, NCH_3), 7.1 (d, 1H, H_4), 8.00 (dd, 1H, H_5), 8.25 (d, 1H, H_7). Anal. ($C_{10}H_9NO_2Se$) C, H, N.
7b	$COCH_2CH_2CH_3$	CH_3	ethanol 20%	126−127	66	(DMSO-d_6) 3.50 (s, 3H, NCH_3), 1.00 (t, 3H, CH_2-CH_2-CH_3), 1.80 (m, 2H, CH_2-CH_2-CH_3), 3.0 (t, 2H, CH_2-CH_2-CH_3), 7.10 (d, 1H, H_4 aromatic), 8.00 (dd, 1H, H_5 aromatic), 8.20 (d, 1H, H_7). Anal. ($C_{12}H_{13}NO_2Se$) C, H, N.
7c	COC_6H_5	CH_3	ethanol 95%	156	73	($CDCl_3$) 3.50 (s, 3H, CH_3), 7.20 (d, 1H, H_4), 7.60 (m, 6H, H_5 + H aromatic benzoyl), 8.20 (d, 1H, H_7). Anal. ($C_{15}H_{11}NO_2Se$) C, H, N.
7d	COC_6H_4Cl	CH_3	ethanol 95%	180	65	($CDCl_3$) 7.15 (d, 1H, H_4), 7.45 (d, 2H, Hb and Hb′), 7.60 (d, 2H, Ha and Ha′), 7.80 (d, 1H, H_5), 8.00 (d, 1H, H_7). Anal. ($C_{15}H_{10}NO_2SeCl$) C, H, N.
7e	COC_6H_4N	CH_3	ethanol 95%	210	50	($CDCl_3$) 3.5 (s, 3H, CH_3), 7.10 (d, 1H, H_4), 7.50 (dd, 1H, Hc), 7.75 (d, 1H, H_5), 8.10 (d, 1H, H_7), 8.10 (d, 1H, Hd), 8.80 (d, 1H, Hb), 9.00 (s, 1H, Ha). Anal. ($C_{14}H_{10}N_2O_2Se$) H, N; C: calcd, 53.01; found, 52.50.
8	H	CH_3	cyclohexane	57−58	80	(DMSO-d_6) 3.50 (s, 3H, CH_3), 7.40 (m, 4H, H aromatic).
15a	H	(dimethylamino)ethyl	ethanol	232	55	($CDCl_3$) 2.30 (s, 6H, CH_3), 2.60 (t, 2H, CH_2-$N(CH_3)_2$), 4.1 (t, 2H, CH_2-N heterocycle), 7.00−7.60 (m, 4H, H aromatic). Anal. ($C_{11}H_{14}N_2OSe$) C, H, N.
15b	H	morpholinoethyl	dioxane	234	50	($CDCl_3$) 2.50 (m, 6H, CH_2-N morpholine), 3.60 (m, 4H, CH_2-O), 4.1 (t, 2H, N-CH_2-CH_2-N morpholine), 7.00−7.60 (m, 4H, H aromatic). Anal. ($C_{13}H_{16}N_2O_2Se$) C, H, N.
15c	H	(phenylpiperazino)ethyl	diethyl ether	80	85	($CDCl_3$) 2.90−4.00 (m, 10H, CH_2-N piperazine), 4.50 (t, 2H, CH_2-N benzoselenazolinone), 6.70−7.90 (m, 9H, H aromatic), 11.70 (broad peak exchangeable with D_2O, 1H, NH^+). Anal. ($C_{19}H_{22}N_3OSeCl$) C, H, N.

[a] Method B, time 4 h, temperature 80−90 °C. [b] Method A, time 2.5 h, temperature 90−95 °C. [c] Method A, time 3 h, temperature 130 °C. [d] Method A, time 4 h, temperature 130 °C. [e] Method B, time 8 h, temperature 100−110 °C.

Table 2. Opening Derivatives of 6-Acylated and/or 3-Methylated Benzoselenazolinones



no.	R_1	R_2	recryst solvt	mp (°C)	yield (%)	^{1}H NMR δ (ppm)
1	H	H	ethanol 95%	78–80	60	($CDCl_3$) 3.80 (broad peak exchangeable with D_2O, 2H, NH_2), 6.60 (dd, 2H, H_3 aromatic + $H_{3'}$ aromatic), 6.75 (dd, 2H, H_6 aromatic + $H_{6'}$ aromatic), 7.25 (m, 4H, H_4 aromatic + $H_{4'}$ aromatic + H_5 aromatic + $H_{5'}$ aromatic).
9a	$COCH_3$	H	acetone	238	40	(DMSO-d_6) 2.20 (s, 6H, CH_3), 6.40 (s exchangeable with D_2O, 4H, NH_2), 6.75 (d, 2H, H_3), 7.50–7.60 (dd, 2H, H_4), 7.70 (d, 2H, H_6). Anal. ($C_{16}H_{16}N_2O_2Se_2$) C, H, N.
9b	COC_6H_5	H	toluene	190	80	(DMSO-d_6) 3.00 (broad peak exchangeable with D_2O, 1H, NH_2), 6.75 (d, 1H, H_3), 7.50 (m, 5H, H aromatic of the two benzenes), 7.60 (dd, 1H, H_4), 7.75 (d, 1H, H_6). Anal. ($C_{26}H_{20}N_2O_2Se_2$) C, H, N.
9c	COC_6H_4Cl	H	isopropyl alcohol	191	60	(DMSO-d_6) 3.62 (unperfect signal exchangeable with D_2O, 4H, NH_2), 3.75 (s, 4H, CH_2), 6.57 (d, 2H, H_3), 6.84–7.25 (m, 14 H, H_4 + H aromatic). Anal. ($C_{26}H_{18}N_2O_2Se_2Cl_2$) C, H, N.
10	$CH_2C_6H_5$	H	ethanol 95%	101	70	(DMSO-d_6) 6.57 (s, 4H, broad peak exchangeable with D_2O, NH_2), 6.83 (d, 2H, H_3), 7.26–7.71 (m, 12 H, H_4 and H aromatic). Anal. ($C_{26}H_{24}N_2Se_2$) C, H, N.
11	$CHOHC_6H_5$	H	toluene	>270	50	(DMSO-d_6) 5.16 (broad peak exchangeable with D_2O, 4H, NH_2), 5.50 (d, 2H, *CH*OH), 5.65 (d exchangeable with D_2O, 2H, OH), 6.67 (d, 2H, H_3), 6.50–7.90 (m, 14 H, H aromatic). Anal. ($C_{26}H_{24}N_2O_2Se_2$) C, H, N.
12	COC_6H_5	CH_3	ethanol	199–200	60	(DMSO-d_6) 2.85 (d, 6H, CH_3), 5.30 (d exchangeable with D_2O, 2H, *NH*-CH_3), 6.60 (d, 2H, H_3), 7.35–7.95 (m, 14 H, H aromatic). Anal. ($C_{28}H_{24}N_2O_2Se_2$) C, H, N.
13	H	CH_3	water	87–88	70	($CDCl_3$) 2.70 (s, 3H, CH_3), 4.50 (broad peak exchangeable with D_2O, 1H, NH), 6.50 (d, 2H, H_6 aromatic + H_3 aromatic), 7.30 (ddd, 2H, H_4 aromatic + H_5 aromatic). Anal. ($C_{14}H_{16}N_2Se_2$) H, N; C: calcd, 45.42; found, 44.56.
14a	H	$CON(CH_3)_2$	chromatography[a]	116	50	($CDCl_3$) 2.75 (s, 6H, CH_3), 6.90 (ddd, 1H, H_6), 7.40 (m, 2H, H_4 + H_5), 8.20 (dd, 1H, H_7). Anal. ($C_{18}H_{22}N_4O_2Se_2$) C, H, N.
14b	COC_6H_5	$CON(CH_3)_2$	chromatography[a]	194–195	50	(DMSO-d_6) 2.85 (s, 12H, CH_3), 7.50–7.95 (m, 12H, H_4 + H aromatic), 8.05 (d, 2H, H_6), 8.40 (d, 2H, H_3). Anal. ($C_{32}H_{30}N_4O_4Se_2$) C, H, N.

[a] Purification by silica gel chromatography using $CHCl_3$(2)/ethyl acetate(1) as eluent.

A passive foot anaphylaxis model in rats was also used as the primary test for anti-allergic activity. Rats were injected in the right hind paw with 0.1 mL of rabbit antirat serum at a dilution previously shown to produce edema upon antigen challenge. For 4 h after injection, the foot volume of each rat was determined plethysmographically for the treated and untreated animals.

One hour before injection, test compounds and references were administered orally at different concentrations. In this test, only compound **4c** revealed significant anti-inflammatory activity, equivalent to that of ebselen but inferior to that of indomethacin (see Table 5).

Discussion

The biological activities of various compounds presented here are of great interest. All the diselenides exhibited high glutathione peroxidase activity. The basic backbone for such an activity was bis(*o*-aminophenyl) diselenide (**1**). Different substitutions in the 6-position did not seem to significantly modify this activity. Nevertheless, in both glutathione peroxidase tests, compound **9b** (6-benzoyl derivative) was the most efficient. Inhibition of lipid peroxidation may reflect glutathione peroxidase activity or radical scavenger activity. The results obtained in this test were in accordance with glutathione peroxidase activity (probucol which is a radical scavenger shows a weak activity). Indeed, benzoselenazolinones were significantly less active than their related diselenides. This is due to the fact that these cyclic derivatives can not open *in vitro* and therefore can not exert any catalytic activity, whereas the opened derivatives (including ebselen which is opened by the nucleophilic attack of glutathione) can react with peroxides. High values were obtained for all diselenides independently of their various substitutions, and we can assume that part of the potency is due to the basic center that we introduced according to the rules established by Spectror *et al.*[6] This corroborates our first conclusion concerning the structure–activity relationships in the glutathione peroxidase test.

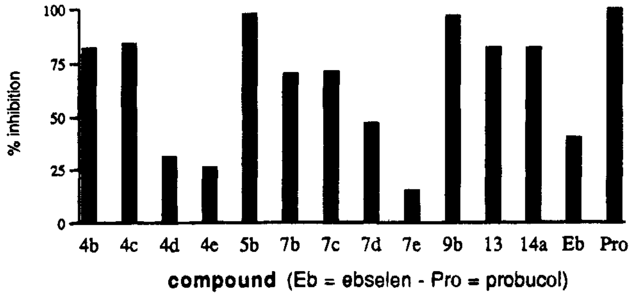
Surprisingly, in the AAPH test, all compounds protected the red cells from hemolysis. The observed effect on AAPH-induced hemolysis inhibition is in favor of radical scavenger activity. This effect could also result from a membrane stabilizing effect or a membrane fluidity increase. Ebselen showed a moderate effect in this test, whereas inhibition by probucol was more effective. Hemolysis inhibition also reflects the lack of cellular toxicity, since cytotoxic compounds induce hemolysis.

The results of the lipoxygenase pathway (LTB_4) and the cyclooxygenase (PGE_2) pathway inhibition studies were consistent with those of the AAPH hemolysis. Benzoselenazolinones as well as diselenides were found to be active in both tests, with high values comparable with those of the reference compounds (NDGA for 5-LO

Table 3. GSH-Px Activity and Lipid Peroxidation Inhibition of Tested Compounds[a]

	GSH-Px activity		lipid perox (% inh)	
compound	method 1a	method 1b	spontan	induced
4a	12	0	1	6
4b	0	0	−3	−2
4c	0	0	0	−3
4d	0	0	−5	5
4e	0	0	−5	−8
5b	−1	0.7	−6	−7
6b	10	0	−1	−1
7b	0	0	2	−3
7c	0	0	−1	−3
7d	0	0	−3	2
7e	0	0	4	1
9a	238[b]	247[b]	100[b]	99[b]
9b	300[b]	261[b]	87[b]	95[b]
9c	279[b]	116[b]	100[b]	100[b]
10	242[b]	227[b]	91[b]	97[b]
11	245[b]	54[b]	92[b]	97[b]
12	298[b]	68[b]	100[b]	100[b]
13	267[b]	325[b]	52[b]	37[b]
14a	178[b]	236[b]	96[b]	97[b]
14b	212[b]	212[b]	98[b]	99[b]
ebselen	100[b]	100	95[b]	97[b]
prubucol	0	ND	37[b]	27[b]
trolox	0	ND	15	13

[a] *tert*-Butyl hydroperoxide was used in method 1, and hydrogen peroxide was used in method 2. Compounds are 2×10^{-7} M in the GSH-Px test and 10^{-5} M in the lipid peroxidation test. GSH-Px activities are given in percentage of ebselen's activity; lipid peroxidation results are percentage of lipid peroxidation inhibition. [b] $p < 0.05$ (difference with the reference is significant).



All measures are ±2% (variation from average)

Figure 1. AAPH-induced hemolysis percentage inhibition at 10^{-5} M.

and ibuprofen for CO). In general, diselenides with 6-substitution consistently showed good activity in both tests, with the best results for benzoyl, *p*-chlorobenzoyl, hydroxybenzyl, or benzyl substituents (see **9b**,**c**, **10**, and **11**). In the benzoselenazolinone series, electronic or steric parameters on the aromatic group seem to be important since a 6-substitution with a nicotinoyl or *p*-chlorobenzoyl group (**4d**,**e** and **7d**,**e**) inhibits the activity on LTB_4. These features may be correlated to a hydrophobic cavity in the enzyme with a fixed size. This rule is not true with opened derivatives since all compounds are very potent but one can notice that nitrogen substitution decreased LTB_4 inhibition (see **9b** and **12**). Also noteworthy was that **4a**,**b**, **6b**, and **14a** inhibited exclusively the 5-LO pathway.

For the PGE_2 inhibition, the positive influence of a benzoyl group at the 6-position is evident (see **9b** and **14b** compared with **4b**, **7b**, and **14a**) and strongly suggests priority selection of such aromatic substitution in approaches to anti-inflammatory compounds. In contrast with the LO pathway, nitrogen substitution did not affect the activity (**12**).

Two types of compounds with two sets of structure–activity relationships can be distinguished. 6-Substituted benzoselenazolinones with a benzoyl group are inhibitors of the CO pathway, while all 6-substituted compounds with either acetyl, benzoyl, hydroxybenzyl, or benzyl (hydrophobic) groups are 5-LO inhibitors *with the condition that the nitrogen atom is not substituted.* For example, **4a** is selective for the 5-LO pathway, and **12** is selective for the CO pathway, whereas **4c** and **9b** are found active in both tests.

With this in mind, two selected compounds, **4c** and **9b**, were scheduled for *in vivo* anti-inflammatory assays. Inhibition of carrageenan-induced hind paw edema is effective for prostaglandin inhibitors, while passive foot anaphylaxis is useful as a primary screen for detecting compounds having anti-allergic activity. Compounds **4c** and **9b** revealed interesting anti-inflammatory properties with a therapeutic index comparable to that of indomethacin (higher administered dose but much lower toxicity for the seleno compounds, *e.g.*, no major trouble observed at a dose of 512 mg/kg per os[22]). In the second test, only compound **4c** was found effective. The anti-inflammatory effect can be attributed to an activity on arachidonate metabolites as well as to the glutathione peroxidase activity previously observed *in vitro*. Both can decrease the severity of the inflammation. It is not possible to assess the ratio between the two effects without further investigation. The lack of correlation between *in vitro* and *in vivo* experiments may be due to either a lack of oral absorption or a very fast metabolization. This could explain the poor dose–effect relationship, the fast activity of **9b** after 1 h, and the important interindividual variation, not allowing to establish statistical significance. It should be noted that compounds with an aminoalkyl side chain appeared to be toxic.

Experimental Section

Melting points were taken in a capillary tube using a Büchi SMP 20 melting point apparatus and are uncorrected. IR spectra were obtained in potassium bromide pellets using a Perkin-Elmer spectrophotometer Model 297. NMR spectra were determined with a Bruker Model WP 20 SY spectrophotometer using TMS as the internal standard. The mass spectra were performed by the analytical center of the Lille II University. Elemental analyses were carried out by the analysis department of CNRS, at Solaize Vernaison, France. TLC was performed on alumina 60 F 254 precoated plates (Merck) using a 2:3:5 mixture of acetone, toluene, cyclohexane as the eluent.

Acylation of Benzoselenazolinone: Method A (4b–d, 5b, 6b, and 7b–d; see Table 1). A stirred mixture of benzoselenazolinone (2 g, 0.01 mol), polyphosphoric acid (50 g), and the corresponding acid (0.01 mol) was heated in an oil bath under reflux for the indicated time, at the appropriate temperature (Table 1), and then poured into cold water. The precipitate was collected, washed with water, dried, and recrystallized from an appropriate solvent.

Acylation of Benzoselenazolinone: Method B (4a,e, 5a, 6a, and 7a,e; see Table 1). In a round-bottomed flask containing aluminum chloride (12 g, 0.09 mol) was added dimethylformamide (3.1 mL, 0.04 mol) dropwise followed by benzoselenazolinone (2 g, 0.04 mol). The mixture was heated to 70–80 °C and homogenized. The acid chloride (0.011 mol) was added slowly, and a condenser was attached. The temperature was raised as indicated (in Table 1) and maintained for the appropriate time. The mixture was poured onto crushed ice. The precipitate was collected, washed with water, dried, and recrystallized from the appropriate solvent.

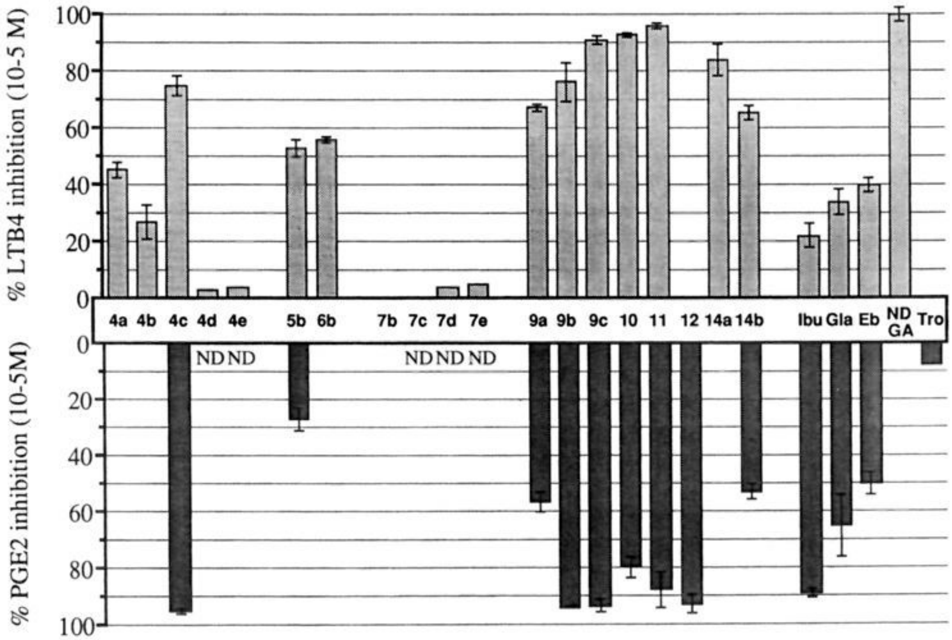


Figure 2. PGE_2 and LTB_4 percentage inhibition at 10^{-5} M.

Table 4. Inhibition Percentages of Carrageenan-Induced Edema

compound	dose (mg/kg)	T0.5H	T1H	T2H	T3H	T4H
4c	3	−42.9	−7.8	27.8	27.5	9.6
	30	−22.2	14.2	31.7	35.6[a]	26.1
	100	−76.2	9.9	51.6[a]	58.5[a]	55.5[a]
9b	3	−33.3	39.0	45.0	32.9	18.2
	30	−20.6	43.3	18.7	13.7	8.5
	100	−27.0	40.4	29.2	9.2	5.0
ebselen	3	−63.5	29.1	30.3	21.4	13.5
	30	−53.97	47.5	10.2	−4.1	−7.5
	100	−93.6	26.9	18.4	13.7	0.6
indomethacin	0.1	−123.8	11.3	1.7	4.0	−1.7
	1	−22.2	41.8	40.2[a]	40.0[a]	29.5
	10	−57.1	26.2	55.5[a]	68.7[a]	65.2[a]

[a] $p < 0.05$ (difference with the reference is significant).

Table 5. Inhibition Percentages of Antirat Serum-Induced Edema

compound	dose (mg/kg)	T0.5H	T1H	T2H	T3H	T4H
4c	3	−41.8	−16.7	−10.7	−14.1	−1.4
	30	−9.8	5.3	25.6	19.7	25.2[a]
	100	−0.5	6.0	12.7	18.0[a]	19.3
9b	3	−16.8	−7.9	−3.3	0.2	4.8
	30	−23.4	−18.2	0.7	−3.7	5.5
	100	−37.5	7.2	4.9	−3.9	2.0
ebselen	3	−9.8	2.3	−1.1	−6.6	−2.6
	30	−19.6	−3.8	8.2	3.7	7.9
	100	4.9	15.9	22.0	16.7	16.3
indomethacin	0.1	−28.3	−7.9	4.4	4.4	16.9
	1	−8.1	−3.0	10.0	1.6	7.5
	10	−21.2	−1.5	22.5	25.5	24.5

[a] $p < 0.05$ (difference with the reference is significant).

Example 1. 6-Benzoylbenzoselenazolinone (4c). Benzoselenazolinone (2 g, 0.01 mol), polyphosphoric acid (50 g), and benzoic acid (1.35 g, 0.011 mol) were introduced in a round-bottomed flask. The mixture was heated at 130 °C for 4 h with a mechanical stirrer and then poured into cold water. The precipitate was collected, dried, and recrystallized from 95% ethanol.

Example 2. 6-Acetylbenzoselenazolinone (4a). In a round-bottomed flask containing aluminum chloride (12 g, 0.09 mol) was added dropwise dimethylformamide (3.1 mL, 0.04 mol) followed by benzoselenazolinone (2 g, 0.04 mol). The mixture was heated to 70−80 °C and stirred. Acetyl chloride (1 mL, 0.012 mol) was added slowly. After attaching a condenser, the mixture was heated at 80−90 °C for 4.5 h and then cooled to 40−50 °C and poured into crushed ice. The precipitate was collected, washed with water, dried, and recrystallized from toluene.

Reduction of 6-Acylbenzoselenazolinones into 6-Alkyl- or 6-(Arylmethyl)benzoselenazolinones: Method C (5a,b; see Table 1). To a stirred slurry containing 6-acylbenzoselenazolinone (0.0125 mol) and trifluoroacetic acid (20 mL, 0.27 mol) cooled in an ice bath was added triethylsilane (4.5 mL, 0.028 mol) dropwise via an addition funnel. A calcium chloride trap was then attached. The stirring was continued over the indicated period. The mixture was poured into cold water, and the precipitate was collected, washed with water, dried, and recrystallized from an appropriate solvent.

Example 3. 6-Benzylbenzoselenazolinone (5b). In a round-bottomed flask, 6-benzoylbenzoselenazolinone (3 g, 0.01 mol) and trifluoroacetic acid (20 mL, 0.27 mol) were introduced. The mixture was cooled in an ice bath. Triethylsilane (4.5 mL, 0.028 mol) was added dropwise via an addition funnel. A calcium chloride trap was attached. The stirring was continued for 24 h at room temperature, and then the solution was poured into cold water (1 L). The precipitate was collected, washed with water, dried, and recrystallized from cyclohexane.

Reduction of 6-Acylbenzoselenazolinones into 6-(Hydroxyalkyl)- or 6-(Arylhydroxymethyl)benzoselenazolinones: Method D (6a,b; see Table 1). In a round-bottomed flask cooled to 0 °C, 6-acylbenzoselenazolinone (0.01 mol) and 15 mL of 3% sodium hydroxide were introduced. Sodium borohydride (0.75 g, 0.02 mol) was then added slowly. The mixture was stirred at room temperature for the specified time and acidified with 10% hydrochloric acid. The precipitate was collected, washed with water, dried, and recrystallized from an appropriate solvent.

Example 4. 6-(Hydroxybenzyl)benzoselenazolinone (6b). In a round-bottomed flask cooled to 0 °C, 6-benzoylbenzoselenazolinone (3 g, 0.01 mol) and 3% sodium hydroxide (15 mL) were introduced. Sodium borohydride (0.75 g, 0.02 mol) was then added slowly. The mixture was stirred at room temperature for 6 h and then acidified with 10% hydrochloric acid. The precipitate was collected, washed with water, dried, and recrystallized from 33% ethyl acetate/cyclohexane.

N-Methylation: Method E (7a−e and 8; see Table 1). To a stirred mixture containing sodium (0.35 g, 0.015 atom/g) and ethanol was added benzoselenazolinone or 6-acylbenzoselenazolinone (0.01 mol). After 15 min, methyl iodide (3.1 mL, 0.05 mol) was added dropwise. The mixture was stirred overnight, and the solvent was removed under reduced pressure. The resulting solid was washed with water, dried, and recrystallized from an appropriate solvent.

Example 5. 3-Methylbenzoselenazolinone (8). In a round-bottomed flask containing sodium (0.35 g, 0.015 atom/g) dissolved in 50 mL of ethanol was added benzoselenazolinone (2 g, 0.01 mol). After 15 min, methyl iodide (3.1 mL, 0.05 mol) was added dropwise. The mixture was stirred overnight, and the solvent was removed under reduced pressure. The resulting solid was washed with water, dried, and recrystallized from cyclohexane.

Diselenides (1, 9a–c, and 10–13; see Table 2). A stirred mixture of benzoselenazolinone or substituted benzoselenazolinone (0.01 mol) and 10% aqueous sodium hydroxide (50 mL) was refluxed for 15 h under nitrogen, cooled to room temperature, and neutralized with hydrochloric acid. The mixture was stirred for 24 h, and the resulting yellow or orange precipitate was collected, washed with water, dried, and recrystallized from an appropriate solvent.

Example 6. Bis(2-amino-5-benzoylphenyl) Diselenide (9b). A stirred mixture of 6-benzoylbenzoselenazolinone (3 g, 0.01 mol) and 10% aqueous sodium hydroxide (50 mL) was refluxed for 15 h under nitrogen, cooled to room temperature, and neutralized with hydrochloric acid. The mixture was stirred for 24 h, and the resulting yellow or orange precipitate was collected, washed with water, dried, and recrystallized from toluene.

Bis[2-(*N'*,*N'*-dimethylureido)phenyl] Diselenide (14a). To a stirred slurry containing benzoselenazolinone (2 g, 0.01 mol) and 40% aqueous dimethylamine (1.8 mL, 0.015 mol) was added 37% aqueous formaldehyde (0.9 mL, 0.011 mol). The mixture was stirred for 2 h, 95% ethanol (1 mL) was then added, and the mixture was refluxed for 12 h.

Bis[2-(*N'*,*N'*-dimethylureido)-5-benzoylphenyl] Diselenide (14b). A stirred slurry containing 6-benzoylbenzoselenazolinone (3 g, 0.01 mol), 40% aqueous dimethylamine (1.8 mL, 0.015 mol), and 95% ethanol (5 mL) was heated at 80 °C for 15 h and then cooled to room temperature. The precipitate was collected. To the liquid layer was added 40% aqueous dimethylamine (1.2 mL, 0.01 mol). The mixture was heated under reflux for 9 h. After the mixture had cooled to room temperature, the solid was collected and combined with the first precipitate.

3-[(Dimethylamino)ethyl]benzoselenazolinone Hydrochloride (15a). A stirred slurry containing benzoselenazolinone (2 g, 0.01 mol), (dimethylamino)ethyl chloride hydrochloride (1.44 g, 0.01 mol), potassium carbonate (5.52 g, 0.04 mol), and dimethylformamide (30 mL) was heated at 50 °C for 7.5 h and then filtered. The solvent was evaporated under reduced pressure. Water was added, and the aqueous layer was extracted with chloroform. The organic layer was dried, and hydrogen chloride (0.36 g, 0.01 mol) was bubbled into the solution. The solvent was evaporated under reduced pressure, and the solid was recrystallized from ethanol.

3-(Morpholinoethyl)benzoselenazolinone (15b). A stirred slurry containing benzoselenazolinone (2 g, 0.01 mol), 2-(chloroethyl)morpholine (1.86 g, 0.01 mol), potassium carbonate (5.52 g, 0.04 mol), and dimethylformamide (30 mL) was heated at 50 °C for 7 h and then filtered. The solvent was evaporated under reduced pressure, and diethyl ether (150 mL) was added. The solution was dried, filtered, and concentrated. The compound recrystallized spontaneously after 15 h.

3-[2-(4-Phenyl-1-piperazinyl)ethyl]benzoselenazolinone Hydrochloride (15c). A stirred slurry containing benzoselenazolinone (2 g, 0.01 mol), 1-(2-chloroethyl)-4-phenyl piperazine hydrochloride (2.62 g, 0.01 mol), potassium carbonate (5.52 g, 0.04 mol), and dimethylformamide (30 mL) was heated at 50 °C for 20 h and then filtered. The solvent was evaporated under reduced pressure. Water was added, and the aqueous layer was extracted by chloroform. The organic layer was dried, and hydrogen chloride (0.36 g, 0.01 mol) was bubbled into the solution. The solvent was evaporated under reduced pressure. The solid was filtered, dried, and recrystallized from ethanol.

Pharmacological Tests. Glutathione Peroxidase Activity. Test compounds were dissolved in DMSO to obtain a 2 mM concentration. Other reagents were dissolved in 50 mM Tris-HCl buffer containing 0.1 mM Na_2 EDTA, $2H_2O$, and 1 mM sodium azide. Glutathione reductase (100 UI, 100 μL), glutathione (1 mM, 10 μL), NADPH (0.14 mM, 10 μL), test compound (20 μM, 10 μL of a 2 mM solution), and buffer (865 μL) were introduced in a 1 mL quartz cell. The mixture was incubated for 2 min at 37 °C. Subsequently, 0.5 mM, 5 μL, hydroperoxide (H_2O_2 or *tert*-butyl hydroperoxide) was added. The solution was stirred, and absorption at 340 nm was recorded for 1.5 min using a Uvikon 930 double-beam spectrophotometer. A blank of sample without adding peroxide was prepared. Each measure was taken three times. The spontaneous nonenzymatic reaction was measured with a sample containing no compound, and its value was subtracted from the other measures. The glutathione peroxidase activity was represented by the speed of NADPH decrease at the beginning of the reaction.

Lipid Peroxidation Inhibition. After decapitation, the cerebral hemispheres of Wistar male rats (250–350 g) were removed and placed on a refrigerating plate. Homogenization with deoxygenated 0.05 M phosphate buffer, pH 7 (Na^+ 15 mM, K^+ 145 mM) was carried out using nine volumes of buffer at 0 °C; 1 mL samples of brain homogenate were then distributed into 2 cm diameter glass flasks which were kept in ice.

For spontaneous peroxidation inhibition, the flasks were incubated at 37 °C for 30 min in a water bath under stirring to give a good homogenate oxygenation.

For induced peroxidation, the procedure was similar, but 50 μL of a free radical generator system (10 μM iron/250 μM ascorbate) was added to the homogenate before incubating.

Compounds to test were dissolved in 40% or 100% DMSO (final volume 25 μL). The same final DMSO concentration was present in reference flasks. After 30 min, a 25 μL sample was taken and placed in a 0 °C refrigerated tube and deoxygenated buffer was added up to 1 mL. Lipid peroxidation intensity was measured by determining the rate of substances reacting with thiobarbituric acid (SRBTA) expressed in nmol of MDA, by a method derived from that of Yagi.[15] To 1 mL samples was added 1.5mL of deoxygenated reagent. The reagent was TBA (thiobarbituric acid):0.33% TCA (trichloracetic acid):13.5% HCl (hydrochloric acid), 0.85 N. The tubes were also deoxygenated by a nitrogen flow, heated for 15 min at 100 °C in a water bath, and cooled for 5 min in cold water. After 1 mL of 70% TCA had been added, the tubes were centrifuged for 10 min at 2500 rpm. The fluorescence intensity of the upper phase was then measured at the following wavelengths: excitation 515 nm/emission 553 nm. At the same time, a calibration range was made with a solution of malonaldehyde bis(dimethylacetal) (Aldrich). Each result was the average of five tests.

AAPH-Induced Hemolysis Inhibition. After centrifugation of a blood sample, red blood cells were washed three times with 9% NaCl; 250 μL of the contents was suspended in 50 mL of 9% NaCl. 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH, 75 mM, 750 μL), the red cell suspension (1 mL), and test compound (10^{-5} M, 200 μL) were brought into contact. At the same time, a blank without AAPH was made for each tested compound. A reference with only red cells and a control without compound were also carried out.

After 30 min of incubation in a water bath at 37 °C with stirring, the red blood cell suspension was centrifuged (3000 rpm) for 10 min at 4 °C and the upper phase optical density, at 403 nm, was measured in comparison to the reference without AAPH. The hemolysis inhibition rate was calculated in comparison with the AAPH reference which represents 100% hemolysis.

LTB_4 and PGE_2 Macrophage Production Inhibition. The macrophage isolation was performed according to the method described by Trush and Coll.[19] Human venous blood, coming from healthy donors who were not under any treatment for at least 2 weeks, was taken in poly(propylene) tubes containing 1 volume of anticoagulant substance (2.73% citric acid, 4.48% sodium citrate, 2% glucose) for 10 volumes of blood. During the following hour, dextran 6% was added to the blood

(0.3 mL of dextran/1 mL of blood). After 30 min of incubation at 37 °C, the plasma rich in white cells was cooled to 4 °C and centrifuged at 100*g* for 5 min. The dottle was suspended in 3 mL of 0.83% NH_4Cl and centrifuged at 100*g* for 5 min at 4 °C.

The dottle rich in mono- and polynucleated white cells was collected in 5 mL of phosphate buffer, pH 7.4, with the following composition: 137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 0.9 mM $CaCl_2$, and 0.5 mM $MgCl_2$ and laid down on 3 mL of ficoll Histopaque-1077 solution. After centrifuging at 420*g* for 30 min at 4 °C, the dottle rich in granulocytes was resuspended in 5 mL of phosphate buffer and centrifuged again at 100*g* for 5 min at 4 °C. Finally, the granulocytes were counted, and the density was adjusted to 3×10^6 cells/mL of phosphate buffer.

The cells (3×10^6 cells/mL) were preincubated at 37 °C for 15 min in the presence or absence of the compounds (the final concentration of the compounds was 10^{-5} M). Then, the cells were stimulated for 15 min at 37 °C by A 23187 (5×10^{-6} M) (Calbiochem) (mother solution 10^{-2} M in DMSO). The basic rate was measured from cells receiving neither compounds nor A 23187.

The produced PGE_2 or LTB_4 amounts were measured by specifics radioimmuno assay kits (DuPont Nem). A calibration range was made in the same conditions with known amounts of LTB_4 and PGE_2. Results processing and calculation were carried out with the Immunofi EIA/RIA software (Beckman). For each compound, measurements were taken twice on three different donors.

Rat Hind Paw Edema. A procedure adapted from Winter *et al.*[20] was employed to measure the inhibition of carrageenan- or antirat serum-induced foot edema in rats. The drugs were tested at 3, 30, and 100 mg/kg. A 0.5 mL volume of drug incorporated in 2% gelatin was administered orally to fasted Wistar rats (200−240 g body weight) in groups of six. One hour later, 0.1 mL of either carrageenan suspension (2% in 9% NaCl) or rabbit antirat serum was administered by subplantar injection into the hind paw. Paw volume was measured using water plethysmometer displacement immediately after injection and 0.5, 1, 2, 3, and 4 h after injection of the phlogistic agent. The average foot swelling in a group of drug-treated animals was compared to that of a group of vehicle-treated animals and expressed as the percent inhibition.

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