Structure−Cytoprotective Activity Relationship of Simple Molecules Containing an α,β-Unsaturated Carbonyl System

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In previous reports we attributed the cytoprotective activity of several sesquiterpene lactones to the presence of a nonhindered electrophilic acceptor in their structure. We suggested that the mechanism of protection would be, at least in part, mediated through a reaction between the electrophilic acceptor and the sulfhydryl-containing groups of the mucosa. We report here the gastric cytoprotective effect of simple molecules containing an α,β-unsaturated carbonyl group. In the present paper, we undertake the study of molecular accessibility and molecular shape, in addition to conformational, electronic, and steric factors. Our results helped to establish two important facts connecting chemical structure with cytoprotective effect. Firstly, an adequate molecular accessibility appears to be necessary to produce the biological response, and secondly, the α,β-unsaturated carbonyl system has to be included in a cyclic structure or, at least, in the proximity of a cyclic system.

Introduction

According to Szabo[1] gastric cytoprotection might be mediated by at least two different mechanisms, one concerning prostaglandins (PG) and the other involving sulfhydryl (SH)-containing compounds of the mucosa.[2] PG and PG derivatives have been shown to prevent the formation of ulcers by a mechanism independent of their antisecretory activity and to protect the gastric mucosa against lesions induced by various necrotizing agents.[3,4]

We have elsewhere demonstrated that the sesquiterpene lactones helenalin (**1**) and dehydroleucodin (**2**) (Figure 1) significantly prevent the formation of gastric lesions induced by various necrotizing agents.[5,6] The cytoprotective activity was also demonstrated in other sesquiterpene lactones having a common feature: an α,β-unsaturated carbonyl group in their structure.[6,7] In previous papers[6−8] we demonstrated that the mechanism of the protective action of sesquiterpene lactones is related to the above-mentioned mechanisms.

The precise action mechanism of these compounds at the molecular level is still unknown. However, we attributed the cytoprotective activity to the presence of a nonhindered electrophilic acceptor in the molecules under study, and we suggested that the mechanism of protection might be mediated, at least in part, by a reaction between the electrophilic acceptor and the sulfhydryl-containing groups of the mucosa.

Our previous reports[6,7] indicate that the α-methylene-γ-lactone and cyclopentenone are among the most active functional groups. From these results, we focused our attention on the potentially active sites of these natural products. We now evaluate the isolated reactive centers and other structurally related compounds.

We report here the gastric cytoprotective effect of simple molecules containing an α,β-unsaturated carbonyl group (Table 1). In this paper we undertake the study of molecular accessibility and molecular shape in addition to the conformational, electronic, and steric factors.

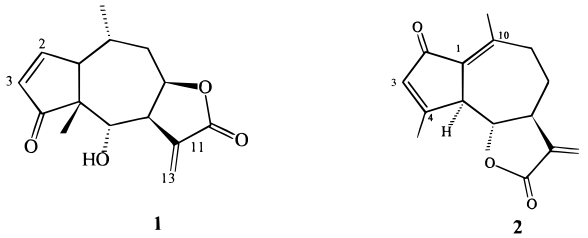

Figure 1. Cytoprotective sesquiterpene lactones.

Results and Discussion

Compounds **3** and **4** (Table 1) showed an important cytoprotective activity. This activity is comparable to those previously reported for the most active sesquiterpene lactones.[6,7] These results might confirm that the presence of a nonhindered α,β-unsaturated carbonyl group is an essential structural requirement for this activity. On the other hand, in view of the results obtained, it might be said that the activity is not related to the guaianolide or pseudoguaianolide structure (skeletal carbon framework) in the sesquiterpene lactone molecules. Other active compounds were molecules **5**−**7** and **25**. It should be noted that all the active compounds have an electrophilic unsaturated bond conjugated with a carbonyl group.

In this paper, we introduce the analysis of the sizes of van der Waals molecular surfaces (VWMS) of the potentially reactive centers whose values can be used as an indication of molecular accessibility. The analysis of this parameter at C_β for the active compounds shows high values of VWMS (Table 1), indicating an excellent molecular accessibility.

The VWMS results are in agreement with those obtained from molecular interaction calculations. These results are shown in Figure 2 in which the curves of molecular interaction between **3** and **4** with a blockable cysteine residue are described. These curves are similar to those previously reported for the α-methylene-γ-butyrolactone and the α,β-unsaturated cyclopentenone

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Table 1. Cytoprotective Effect, van der Waals Molecular Surface (VWMS), and Molecular Interaction (MI) Results

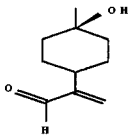
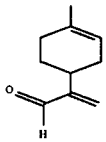
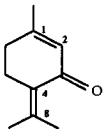
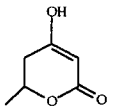
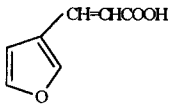
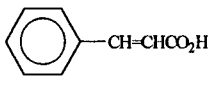
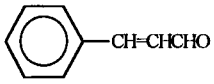
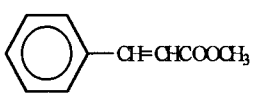
Compound			Cytoprotective effect [a]	VWMS [b] (Å^2)	MI [c]
No.	Name	Structure			
3	α-methylen-γ-butyrolactone		0.25 ± 0.001	7.37	2.0
4	2-cyclopenten-1-one		1.30 ± 0.40	5.40	2.0
5	5,6-dihydro-2H-piran-2-one		0.55 ± 0.05	5.40	2.0
6	aldehyde I		0.70 ± 0.16	7.75	2.0
7	aldehyde II		0.90 ± 0.17	7.73	2.0
8	3-methyl-2-cyclopenten-1-one		4.30 ± 0.50	3.07	0.5
9	piperitenone		4.30 ± 0.80	C_1-C_2 2.98 C_4-C_8 3.02	0.4 0.2
10	5,6-dihydro-4-hydroxy-6-methyl-2H-piran-2-one		4.66 ± 0.57	4.06	1.2
11	3-furan-acrylic acid		4.60 ± 0.30	4.09	1.2
12	cinnamic acid		5.0 ± 0.01	4.84	1.0
13	cinnamaldehyde		4.5 ± 0.01	4.85	1.0
14	methylcinnamate		2.90 ± 0.01	4.90	1.2

Table 1 (Continued)

Compound			Cytoprotective effect [a]	VWMS [b] (Å²)	MI [c]
No.	Name	Structure			
15	methyl-3,3-dimethylacrylate	$(CH_3)_2C=CHCOOCH_3$	4.50 ± 0.80	3.08	0.5
16	tyglic acid	$CH_3CH=C(CH_3)COOH$	5.0 ± 0.001	5.06	1.6
17	angelic acid	$CH_3CH=C(CH_3)COOH$	3.5 ± 0.50	5.00	1.4
18	methyltyglate	$CH_3CH=C(CH_3)COOCH_3$	5.0 ± 0.001	5.07	1.6
19	methylangelate	$CH_3CH=C(CH_3)COOCH_3$	5.0 ± 0.001	4.99	1.4
20	ethyltyglate	$CH_3CH=C(CH_3)COOC_2H_5$	4.80 ± 0.30	5.03	1.5
21	tyglicaldehyde	$CH_3CH=C(CH_3)CHO$	4.60 ± 0.50	5.05	1.5
22	ethylacrylate	$CH_2=CHCOOCH_2CH_3$	4.30 ± 0.40	7.39	3.0
23	propenenitrile	$CH_2=CH-C\equiv N$	3.50 ± 0.50	7.39	3.0
24	methylmethacrylate	$CH_2=C(CH_3)COOCH_3$	4.80 ± 0.30	7.39	3.0
25	ethylpropiolate	$CH\equiv CCOOCH_2CH_3$	0.43 ± 0.01	9.66	3.0

[a] See the Experimental Section. Each data point represents the mean ± SD for 5 rats. [b] VWMS values were calculated at C_β; for more details see Calculational Methods. [c] See Calculational Methods.

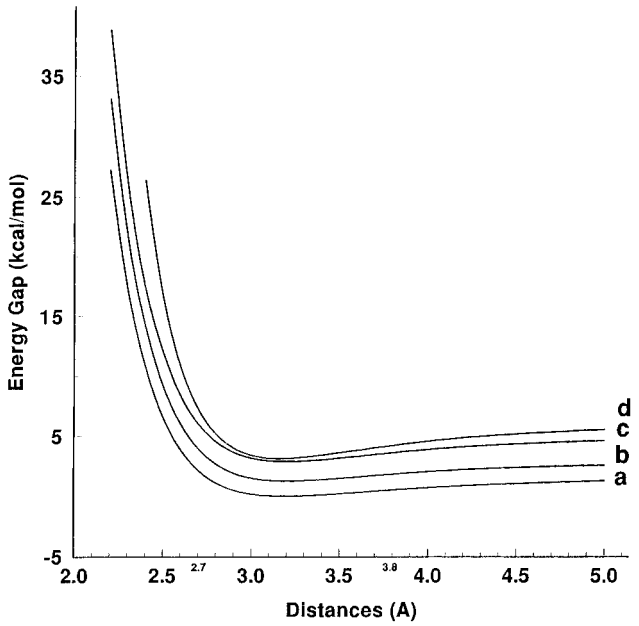


Figure 2. Energy profiles for the intermolecular interactions between (a) compound **3** and a blockable cysteine residue, (b) compound **4** and a blockable cysteine residue, (c) $C_{11}-C_{13}$ double bond of helenalin (**1**) and a blockable cysteine residue, and (d) C_2-C_3 double bond of helenalin (**1**) and a blockable cysteine residue.

rings of helenalin (**1**).[7] As can be seen from Figure 2, the minimum energy was reached at an interaction distance of 3.2 Å, with a ΔE < 2 kcal/mol between 3.2 and 2.7 Å. This energetic increment would be overcome by the energy delivered in the process of bond formation, which has been evaluated from *ab initio* 6-31G* calculations.[9]

Compound **8** is inactive while 2-cyclopenten-1-one (**4**) was active, in spite of the similarity of their structures (Table 1). It is interesting to note that the VWMS value obtained for **8** is lower with respect to that attained for the active molecule **4**. This result is a consequence of a significant steric hindrance produced by the methyl group at C_3 of the cyclopentenone ring. Figure 3 shows the energy profile of molecular interactions obtained for compound **8** which is comparable with those previously reported for the inactive functional groups present in dehydroleucodin (**2**).[6] The interaction curves for the inactive systems show that the minimum energy was reached at an interaction distance of about 3.8 Å (larger than the sum of the van der Waals radii of carbon and sulfur). Also, a strong energetic increment is observed when the interaction distance is reduced from 3.8 to 2.7 Å (more than 20 kcal/mol). This energetic increment might also prevent the approximation of the interactive atoms (C and S) to an adequate interaction distance, close enough to form the new bond (transition state distance). The energetic difference of about 20 kcal/mol between the active and nonactive centers is large enough to explain the difficulty in the approach of the interacting molecules in the latter.

Figure 4 shows a spatial view of the molecular interactions between compounds **4** and **8** with a block-

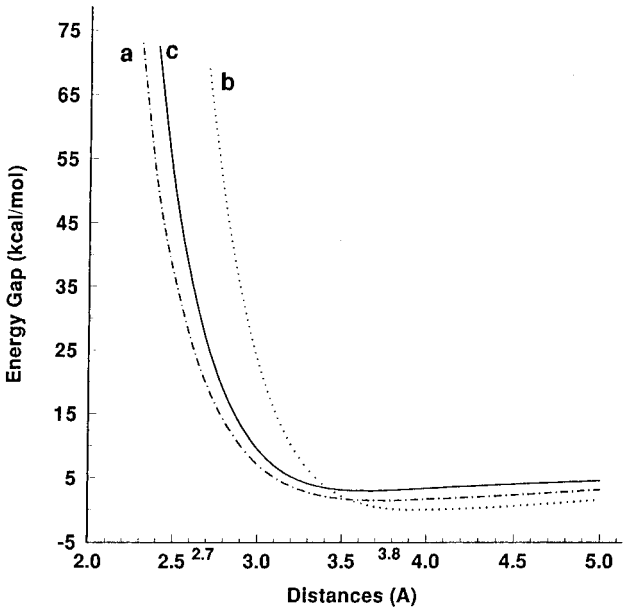


Figure 3. Energy profiles for the intermolecular interactions between (a) compound **8** and a blockable cysteine residue, (b) C_3-C_4 double bond of dehydroleucodin (**2**) and a blockable cysteine residue, and (c) $C_{10}-C_1$ double bond of dehydroleucodin (**2**) and a blockable cysteine residue.

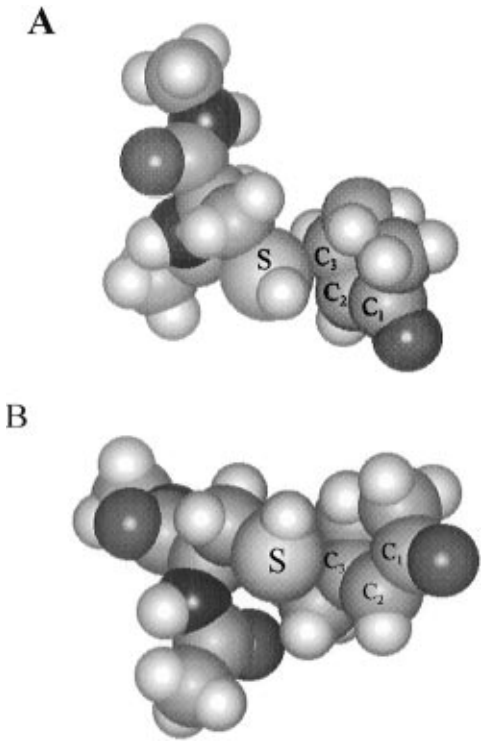


Figure 4. Spatial view of the intermolecular interaction between (A) compound **4** and a blockable cysteine residue and (B) compound **8** and a blockable cysteine residue. In this figure it is possible to appreciate the different degree of difficulty for the approaching process of the electrophilic acceptors ($C_3-C_2-C_1$) of compounds **4** and **8** to the sulfhydryl group (S).

able cysteine residue of the gastric mucosa. The different degree of difficulty for the approaching process of the electrophilic acceptor to the sulfhydryl group can be appreciated in this figure. The methyl group at C_3 of molecule **8** plays an important shielding effect in this event making the approaching process more difficult. These results were confirmed when the electronic aspects of the molecules were analyzed. The molecular

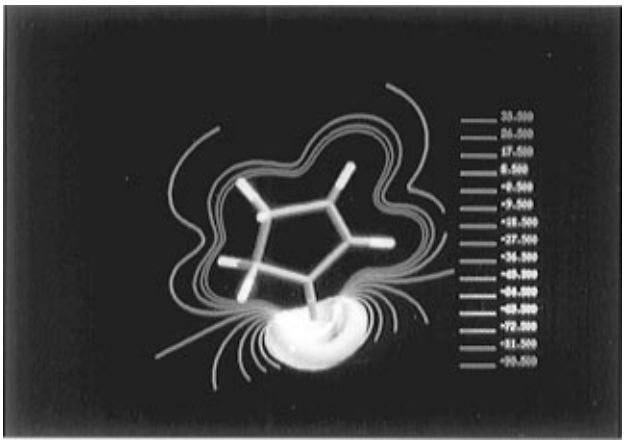


Figure 5. Molecular electrostatic potential map obtained for compound **4**.

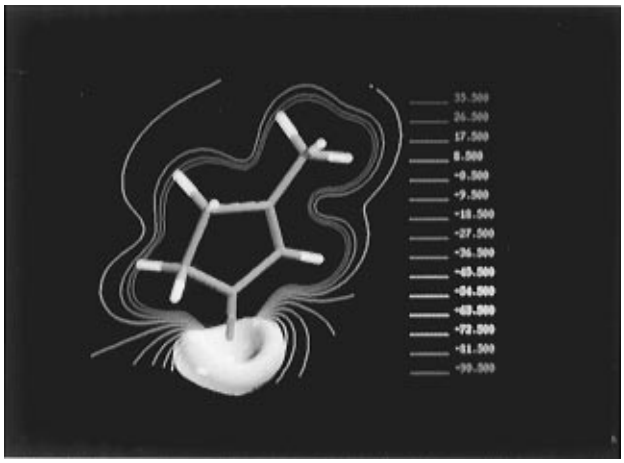


Figure 6. Molecular electrostatic potential map obtained for compound **8**.

electrostatic potential maps (MEPs) obtained for compounds **4** and **8** (Figures 5 and 6) emphasize the importance of steric factors rather than electronic effects to explain the different cytoprotective activity obtained for these molecules. These results are evident because these maps do not illuminate any particularly significant electronic difference between the two compounds. Thus, the lack of activity of compound **8** might be the result of a severe steric hindrance.

The lack of cytoprotective activity shown by the compounds **9**−**21** may be explained in terms of their VWMS values. The difference in activity may be due to specific steric and/or conformational effects. Thus, the molecular accessibility appears to play an important role in the activation mechanism of the cytoprotection process. From these results it is clear that a significant molecular accessibility (a high VWMS value) seems to be essential for activation, since the molecules showing VWMS values lower than 5 resulted to be less active or even deprived of any cytoprotective activity. The results obtained from molecular interaction (MI) calculations are in complete agreement with this assumption.

However, on the basis of steric factors, we can not explain why α-methylene-γ-butyrolactone (**3**) was active while ethyl acrylate (**22**) was not active, in spite of the similarity of their structures. The steric parameters are not applicable to those cases in which both VWMS and molecular interaction calculations indicate an adequate molecular accessibility (i.e., compounds **22**−**24**).

We performed an exhaustive conformational study for the molecules reported here using theoretical calculations (AM1 and *ab initio*). These calculations predict that the preferred spatial orderings for the noncyclic compounds are extended or half-extended conformations. We considered the low-energy conformations as the preferred forms of the molecules. These extended conformations obtained for the noncyclic molecules have a spatial arrangement and molecular shape which are different from those adopted by the active cyclic molecules. An interesting observation is that all the noncyclic compounds (**15**−**24**) were inactive. An exception was the molecule **25**. From these results, we can suggest a new structural requirement for the cytoprotective activity of these compounds, that is, the electrophilic acceptor has to be included in a cyclic structure or, at least, in the proximity of a cyclic system (probably for hydrophobic reasons).

Particularly noteworthy was the finding that ethyl propiolate (**25**) shows a moderate but significant cytoprotective activity. This result was unexpected, because compound **25** was the only noncyclic compound in this series which showed cytoprotective activity. However, it should be noted that **25** has the highest value of VWMS, and therefore the cytoprotective effect may be explained in terms of the excellent molecular accessibility.

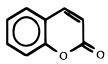
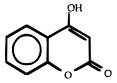
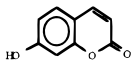
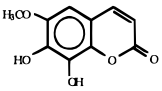
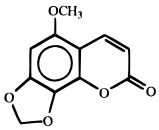
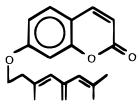
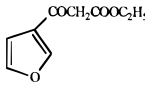
Finally, we can say that the electronic aspects appear to play a determinant role in those molecules in which the α,β-unsaturated carbonyl function is localized in the vicinity of an aromatic system. It is evident that the lack of activity of compounds **26**−**32** could be related to their partial aromaticity which lowers the electrophilicity of the α,β-unsaturated carbonyl function. These results are an additional support to our hypothesis suggesting a Michael addition as the molecular mechanism for this series. Based on the above concepts, it is possible to explain the observed lack of cytoprotective activity of compounds shown in Table 2.

In summary, from our results it can be concluded that the presence of an α,β-unsaturated carbonyl system appears to be a structural requirement necessary but not sufficient for cytoprotection. On the other hand, the results presented here support the establishment of important facts connecting chemical structure with a cytoprotective effect. Firstly, an adequate molecular accessibility appears to be necessary to produce the biological response, and secondly, inclusion of the α,β-unsaturated carbonyl system in a cyclic structure led to the most potent cytoprotective agents. This result is not unexpected because it is well known that in general cyclic derivatives, e.g., cycloalkenones, are more reactive that their acyclic counterparts, e.g., acyclic alkenones.[10]

The discriminating properties discussed above can thus become the basis for structure−activity correlations in substituted double bonds with respect to a nucleophilic addition. Furthermore, this type of discriminant reactivity criterion can be correlated with experimental measurements of cytoprotective activity.

Our results support the hypothesis that nucleophilic addition is a possible molecular mechanism for the cytoprotective activity of molecules containing an α,β-unsaturated carbonyl system and reaffirm the discriminating reactivity criteria that are anchored in the properties of the molecules and should provide useful tools for predicting the ability of untested compounds in this series to exhibit similar biological activities.

Table 2. Cytoprotective Effect and van der Waals Molecular Surface (VWMS) Results

Compound			Cytoprotective Effect	VWMS (Å)
No.	Name	Structure		
26	coumarin		4.70 ± 0.28	5.06
27	4-hydroxy-coumarin		4.62 ± 0.32	4.13
28	umbelliferone		3.80 ± 0.24	5.08
29	fraxetin		4.75 ± 0.18	5.07
30	sabandine		4.20 ± 0.35	5.06
31	5'-oxo-Δ^3-auraptene		4.30 ± 0.37	5.07
32	ethyl-β-oxo-3-furan-propionate		4.75 ± 0.50	6.72

Experimental Section

General Procedures. Analytical thin-layer chromatography (TLC) was performed on 0.25 mm silica gel precoated plates with fluorescent indicator UV 254. Column chromatography purification was performed on a medium pressure chromatography system using Merck lobar (A, B, or C) silica gel columns with the help of a Fluid Metering Inc. pump. Different mixtures of EtOAc:hexanes were used as eluent.

^{13}C NMR spectra were recorded in a Bruker WP-80 spectrometer in $CDCl_3$; the ^1H NMR spectra were recorded in a Bruker WP-80 or in a Varian EM 360 A spectrometer in $CDCl_3$. Chemical shifts are reported in parts per million downfield from tetramethylsilane as internal standard. IR spectra were recorded in a Beckmann IR 10 spectrometer. Mass spectra were recorded on a Varian MAT 112 S spectrometer at 70 eV and 0.7 mA. For high-resolution measurements peak matching was used with an error smaller than 20 ppm and a resolution better than 6000 in the 10% valley definition.

Natural Products. The piperitenone (**9**) sample used in the present study showed physical and spectral properties identical with those previously reported.[11] Umbelliferone (**28**) was from Baccharis Darwinii Hook. Arm. Fraxetin (**29**) was from Gochnatia Argentina (Cabr.) Cabr. Sabandine (**30**) was

from Pterocaulon De Candolle Virgatum. 5′-Oxo-Δ^3-auraptene (**31**) was from Baccharis Darwinii Hook. Arm.

Chemistry. α-Methylene-γ-butyrolactone (**3**), 2-cyclopenten-1-one (**4**), 5,6-dihydro-2*H*-pyran-2-one (**5**), 3-methyl-2-cyclopenten-1-one (**8**), 5,6-dihydro-4-hydroxy-6-methyl-2*H*-pyran-2-one (**10**), 3-furanacrylic acid (**11**), tyglic acid (**16**), propenenitrile (**23**), methyl methacrylate (**24**), ethyl propiolate (**25**), coumarin (**26**), and ethyl β-oxo-3-furanpropionate (**32**) were purchased from Aldrich Co. Cinnamic acid (**12**) was purchased from Merck Co.

Aldehyde I (6) and Aldehyde II (7). **6** and **7** were prepared according to the procedure previously reported.[11]

Cinnamaldehyde (13). Cinnamic acid (296 mg, 2 mmol) was disolved in 15 mL of dry THF. The solution was cooled to 0 °C, and then BH_3·THF (1 M, 2.2 mL, 2.2 mmol) was added dropwise. The solution was kept with stirring for 2 h. Then the solution was poured on brine and extracted with CH_2Cl_2 (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated. The oily residue was chromatographed in a MPC system using a 5:95 mixture of EtOAc: hexanes as eluent. Compound **13**: 185 mg, 70%; spectral and physical properties were identical with those previously reported.[12]

Methyl Cinnamate (14). Diazomethane solution in THF was added dropwise to a stirred solution of cinnamic acid (500 mg) in dry THF (30 mL) at 0 °C until the complete conversion to the methyl ester. The solvent was removed in vacuo and the sample purified by chromatography. Compound **14** (530 mg) was isolated as an oil. ^{1}H and ^{13}C NMR were identical with those previously reported.[13]

Methyl 3,3-Dimethylacrylate (15). Diazomethane solution in THF was added dropwise to a stirred solution of 3,3-dimethylacrylic acid (500 mg) in dry THF (30 mL) at 0 °C until the complete conversion to the methyl ester. The solvent was removed in vacuo and the sample purified by chromatography. Compound **15** (505 mg) was isolated as an oil. ^{1}H and ^{13}C NMR were identical with those previously reported.[14]

Angelic Acid (17). Tyglic acid (500 mg, 5 mmol) was dissolved in dry benzene (100 mL), and diphenyl disulfide (1092 mg, 5 mmol) was added. The solution was irradiated with a UV lamp of 1000 W for 24 h. The solvent was removed in vacuo and the only residue purified using a medium pressure chromatography system (MPC) and mixtures of EtOAc:hexanes as eluent. Angelic acid (115 mg, 23%) was isolated together with 300 mg of tyglic acid. Compound **17** was identified by comparison with previously reported data.[15]

Methyl Tyglate (18). Diazomethane solution in THF was added dropwise to a stirred solution of tyglic acid (500 mg) in dry THF (30 mL) at 0 °C until the complete conversion to the methyl ester. The solvent was removed in vacuo and the sample purified by chromatography. Compound **18** (498 mg) was isolated as an oil. ^{1}H and ^{13}C NMR were identical with those previously reported.[15]

Methyl Angelate (19). **19** was prepared according to the procedure described previously for the preparation of methyl tyglate. The spectral and physical properties of **19** were identical with those previously reported.[15]

Ethyl Tyglate (20). Tyglic acid (200 mg, 2 mmol) was dissolved in absolute ethanol (10 mL) and sulfuric acid (2 drops). The solution was refluxed for 3 h in the presence of molecular sieves. The solution was poured on 5% $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2. Ethyl tyglate was purified using a MPC and mixtures of EtOAc:hexanes as eluent. Compound **20** (130 mg) was isolated together with 50 mg of tyglic acid. Compound **20** was identified by comparisson with previously reported data.[15]

Tyglic Aldehyde (21). Tyglic acid (200 mg, 2 mmol) was disolved in 15 mL of dry THF. The solution was cooled to 0 °C, and then BH_3·THF (1 M, 2.2 mL, 2.2 mmol) was added dropwise. The solution was kept with stirring for 2 h. Then the solution was poured on brine and extracted with CH_2Cl_2 (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated. The oily residue was chromatographed in a MPC system using a 5:95 mixture of EtOAc: hexanes as eluent. Compound **21**: 137 mg, 81%; spectral and physical properties were identical with those previously reported.[16]

Ethyl Acrylate (22). Acrylic acid (144 mg, 2 mmol) was dissolved in absolute ethanol (10 mL) and sulfuric acid (2 drops). The solution was refluxed for 3 h in the presence of molecular sieves. The solution was poured on 5% $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2. Ethyl acrylate was purified using a MPC and mixtures of EtOAc:hexanes as eluent. Compound **22** (85 mg) was isolated together with 50 mg of acrylic acid. Compound **22** was identified by comparison with previously reported data.[17]

Calculational Methods. The calculations reported here were performed using the semiempirical AM1 method[18] as well as *ab initio* computations using the split valence 3-21G basis set. All the parameters defining each molecular structure were optimized at both semiempirical and *ab initio* levels. Semiempirical calculations were performed with the MOPAC 6.0 package.[19] *Ab initio* calculations were performed with the MONSTERGAUSS computer program.[20]

Also, molecular electrostatic potential maps (MEPs) were calculated for the compounds under study in order to complete the electronic analysis. The MEPs[21,22] can be a useful descriptor of the recognition process between the ligand and the drug-active site; this is due to the long-range nature of the electrostatic force, where the Coulombic attenuation factor is related only to the inverse of the distance. The molecular electrostatic potential of the molecule was calculated at the AM1 level using the SPARTAN package.[23] MEPs were calculated at 2 Å above the plane of the molecule according to Pullman's suggestions.[24, 25] As this author indicates, useful information concerning nucleophilic attack can be obtained from MEPs only if they are calculated beyond the van der Waals radius of the molecule.

In order to describe more accurately the conformational properties and molecular shapes of the compounds under study, two different geometrical aspects were considered as follows: (a) a hypothetical molecular interaction between the compounds under study and a cysteine blockable residue as the active portion of reduced glutathione present in the gastric mucosa and (b) the van der Waals molecular surface (VWMS) of the nucleophilic acceptors which would be involved in the reaction.

The molecular interactions were simulated under molecular mechanics calculations. In this respect, as we were particularly interested in the comparison of the present results with those previously reported for sesquiterpene lactones,[6,7] the same calculational method as the one previously used was applied.

In order to estimate the molecular interactions we used the following equation: MI = AMI/UMI, where MI = parameter evaluating the molecular interactions, AMI = amount of available molecular interactions, and UMI = amount of unavailable molecular interactions. This relation was obtained taking into account different spatial orientations in order to simulate the attack process on the cysteine residue. For all the molecules studied, 24 different spatial orientations were calculated. For available molecular interactions (AMI), we considered those which showed an energy profile characteristic for active molecules (see Results and Discussion section). All the other molecular interactions were considered unavailable (UMI).

The VWMSs of the potentially reactive groups were evaluated using an analytical approximation designed by our group.[26] A summary of this method is presented here, but the description in detail of this calculational method has been reported in ref 26.

van der Waals Molecular Surface (VWMS) Calculations. 1. Calculating Areas. The characteristic van der Waals radii of the elements were assigned to the different atoms of the molecules. The helmets (spherical sectors) were calculated from the Lee and Richards[27] equation:

$$b_{01} = \pi R_0(R_0 + R_1 - D)[1 + (R_1 - R_0)/D]$$

where D is the distance between the centers S_0 and S_1. In

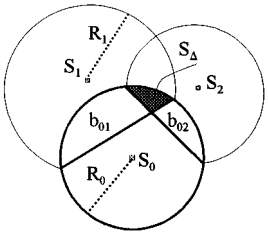


Figure 7. Schematic view of a triple overlap among three coplanar spheres. This figure shows the intersection area S_Δ.

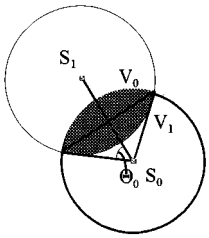


Figure 8. Schematic view showing the overlap between two spheres. θ_0 is the angle determined by the segment S_0S_1 and the S_0 center with a point of the intersection circumference.

cases in which two helmets overlap (like, for example, b_{01} and b_{02} in Figure 7) the calculation was corrected, evaluating the intersection area S_Δ from the equation previously reported by our group.[26] Thus, the total accessible area was obtained from:

$$S_0(\text{acc}) = S_0 - b_{01} - b_{02} + S_\Delta$$

2. Calculating Volumes. In order to evaluate the free volumes related with a sphere, it is necessary to subtract the overlapping volumes of bonded atoms which have significant Mayer indices. In order to calculate the intersection volume, the average of the penetrating volumes was considered:

$$V_{\text{bound}} = (V_0 + V_1)/2$$

where each volume is evaluated with the equation

$$V_0 = 4\pi {R_0}^3/3[{}^1/_2 + {}^1/_4\theta \cos^3\theta_0 - {}^3/_4 \cos\theta_0]$$

θ_0 is the angle shown in Figure 8. The free volumes of the spheres S_0 and S_1 can be obtained from:

$$V_0(\text{free}) = {}^4/_3\pi {R_0}^3 - V_{\text{bound}}$$

$$V_1(\text{free}) = {}^4/_3\pi {R_1}^3 - V_{\text{bound}}$$

Induction of Gastric Lesions. Gastric lesions were produced according to the method of Robert et al.[4] Male Wistar rats weighing ca. 180 g were fasted for 24 h and deprived of water for 19 h prior to the experiments. All rats were housed in wire mesh-bottom cages throughout the study to prevent coprophagy. Absolute EtOH (1 mL) administered orally was employed as the necrotizing agent, and 1 h later the animals were decapited. The stomachs were removed, opened along the greater curvature, and washed gently with ice-cold saline solution. The degree of erosion in the glandular part of the stomach was assessed from a scoring system designed by Marazzi-Uberti and Turba[28] as follows: 0, no erosions; 1, 1−3 small erosions (4 mm diameter or smaller); 2, more than 3 small erosions or 1 large erosion; 3, 1 large erosion and more than 3 small erosions; 4, 3−4 large erosions; 5, any very large erosion or ulcer perforation.

The results are expressed in terms of an ulcer factor which is the average severity of erosions per rat for each group on the scale from 0 to 5. The sum of these values was divided by the number of animals. The control rats treated with absolute EtOH showed an average score of 4.8. The control rats without treatment showed an average score of 0.0. The drugs tested in this study were prepared just before the experiment as follows: Compounds **1**−**23** were suspended in water. The control rats (group 1) were given 1 mL of absolute EtOH. Compounds **1**−**23** (40 mg/kg) were given 1 h before oral administration of EtOH (group 2).

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Supporting Information Available: VWMS calculational method (13 pages). Ordering information is given on any current masthead page.

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