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ARTICLE

Hypochlorous Acid Scavenging Activities of Thioallyl Compounds from Garlic

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The hypochlorous acid (HOCl) scavenging capacities of 10 garlic compounds containing modifications in the thioallyl group (−S—CH_2CH=CH_2) were determined by a catalase protection assay, and the corresponding structure−activity relationships using molecular descriptors were calculated. This scavenging activity was enhanced by increasing the number of S atoms or by the alanyl group (−CH_2CH—NH_2—COOH) and decreased in the absence of the C=C bond or in the presence of a sulfoxide group in the thioallyl group. Interestingly, *S*-allylcysteine and its corresponding sulfoxide (alliin) showed the highest and lowest HOCl-scavenging capacities, respectively. Quantitative modeling by multiple regression analysis and partial least-squares projections showed that the topological descriptor polar surface area and two electronic properties, namely, highest occupied molecular orbital and total energy, contributed mainly to variations in the HOCl scavenging activity of thioallyl compounds. These observations provide new insights on the antioxidant mechanism of garlic derivatives in processes involving HOCl production.

KEYWORDS: Garlic; thioallyl compounds; hypochlorous acid; ROS scavenging; catalase protection assay; molecular descriptors; structure−activity relationship

INTRODUCTION

Oxidative damage is an important trait in the pathophysiology of several conditions such as aging, cancer, and diseases involving neurodegenerative, cardiovascular, or inflammatory processes (*1*). Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$), peroxyl ($HOO^{\bullet}$), peroxynitrite ($ONOO^-$), or hypohalous acids (HOX, X = Cl, Br) are widely recognized effectors in the oxidative transformation of cellular membranes and DNA. ROS may be exogenously derived from the environment or endogenously produced in metabolic pathways during the transformation of normal or xenobiotic substrates (*2*). Among these strong oxidants, HOX have potent antibacterial activities under normal function of the mammalian immune system. These are produced in vivo by the heme−peroxidase enzyme myeloperoxidase from either activated neutrophils, monocytes, and macrophages (MPO, HOCl-forming) or eosinophils (EPO, HOBr-forming), and the predominant species corresponds to HOCl (*3*). These HOX species may be controlled by endogenous antioxidant systems such as superoxide dismutase, catalase, uric acid, glutathione peroxidase, and glutatione *S*-transferase among others (*1*). However, if HOX levels are excessive or misplaced exceeding these systems, HOX react directly with amino acids and proteins as major targets, but carbohydrates, thiol-containing antioxidants, membrane lipids, and DNA are also affected (*4*). These processes are related to host tissue damage and to the establishment of inflammatory diseases such as arthritis, cystic fibrosis, asthma, heart disease, and some types of cancer (*5*−*7*). The use of exogenous antioxidants from synthetic and natural sources is an ongoing strategy for treating these oxidative stress-mediated abnormalities.

Garlic (*Allium sativum* L.) is a perennial crop used worldwide since ancient times as a food spice with a variety of medicinal qualities. These include antimicrobial as well as health care properties such as anticancer, antihypertensive, antithrombotic, hepato- and cardioprotective, and hypolipidemic activities (reviewed in ref *8*). Substantial evidence suggests that these biological activities are due to the unique organosulfur compounds (OSCs) of garlic cloves, which have diverse molecular effects including enzyme modulation and potent antioxidant activities mediated by radical scavenging (*9*). OSCs commonly found in garlic include four types of thioallyl compounds (TAC): *S*-allylcysteine sulfoxide (alliin, the most abundant sulfur compound contained in mesophyll storage cells), *S*-allylcysteine (obtained by long-time organic fermentation of whole bulbs in ethanolic solutions), allyl thiosulfinates (e.g., allicin, obtained by the transformation of alliin by the vascular sheath cell enzyme allinase when bulbs are cut, crushed, or chopped in water), and some products of allicin decomposition (allyl sulfides and ajoenes) (*10*). Interestingly, aqueous extracts from raw or powdered garlic

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are able to scavenge $O_2^{\bullet-}$, $HO^{\bullet}$, $HOO^{\bullet}$, $ONOO^-$, and HOCl species (*11*−*13*). However, these abilities may be attributed to multiple TAC from garlic as these exhibit different patterns of radical scavenging as previously reported, that is, alliin scavenged $O_2^{\bullet-}$ and $HO^{\bullet}$, allyl disulfide scavenged $HO^{\bullet}$ but not $O_2^{\bullet-}$, and allicin was unable to scavenge $O_2^{\bullet-}$ (*14*) and its $HO^{\bullet}$ and $HOO^{\bullet}$ scavenging abilities are controversial (*14*−*16*). *S*-Allylcysteine is able to scavenge $O_2^{\bullet-}$, $HO^{\bullet}$, $HOO^{\bullet}$, $ONOO^-$, and HOCl (*14*, *17*, *18*). With regard to the HOCl scavenging activity of garlic aqueous extracts, it is eliminated if intact cloves are microwave-treated but not if these are heated before or after cutting, suggesting that allinase activity (i.e., allicin formation) may not be critical (*13*).

In the present work we have analyzed the ability of eight TAC from garlic and two propyl sulfide analogues from onion to scavenge HOCl using the absorption spectrum of the antioxidant, heme-containing enzyme catalase as a model that tests the disrupting action of hypochlorite over the prosthetic group (*13*, *19*). In addition, the structures of the 10 compounds under study were characterized by calculating several nonempirical quantum descriptors, especially those related to electronic and molecular transport properties, and a quantitative modeling study was carried out using multiple regression analysis (MLR) and partial least-squares (PLS) procedures. Thus, the main purpose of these analyses was to obtain structure−activity parameters from TAC of garlic to understand their different HOCl scavenging capacities, which can provide insights for further molecular design of ROS-specific scavengers.

MATERIALS AND METHODS

Reagents. Bovine liver catalase, ascorbic acid (AA), and *N*-acetylcysteine (NAC) were from Sigma Chemical Co. (St. Louis, MO), and sodium hypochlorite (NaOCl), sulfuric acid, and acetone were from J. T. Baker (Xalostoc, Edo. México, Mexico). The following TAC were used: alliin (*S*-allylcysteine sulfoxide, ALI), diallyl sulfide (DAS), and dipropyl sulfide (DPS) from Fluka Chemika AG (Buchs, Switzerland); diallyl disulfide (DADS), dipropyl disulfide (DPDS), allyl methyl sulfide (AMS), and allyl mercaptan (AM) from Aldrich Chemical Co. (Milwaukee, WI); *S*-allyl-L-cysteine (SAC) from TCI (Tokyo Kasei Kogyo Co., Tokyo, Japan); and diallyl trisulfide (DATS) from MP Biomedicals Inc. (Solon, OH). Allicin (diallyl thiosulfinate, ALC) was produced by oxidation of DADS using the protocol reported by Lawson (*17*). In this, 1 g of DADS was dissolved in 5 mL of acetic acid under stirring in an ice bath. Hydrogen peroxide (1.5 mL, 30% v/v) was added stepwise, and the reaction was allowed to proceed for 30 min. After this step, the reaction was kept at 13 °C for 20 min, and it was placed on an ice bath again for 5 h to minimize the content of remaining DADS. Reaction was stopped by adding 15 mL of distilled water at pH 6.5, and it was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with 5% of sodium bicarbonate (3 × 20 mL) and distilled water (2 × 20 mL) and dried over Na_2SO_4. The solvent was left to evaporate until yellowish oil (ALC, yield of approximately 47% per assay) was obtained. For stabilization and storage of ALC, the oily product was resuspended in water at 1.5% (w/v) and kept in 1 mL aliquots at −70 °C until analyzed and used. Under these conditions, ALC retained its full activity for up to 6 months. The identification of ALC was performed by nuclear magnetic resonance (^{13}C and ^1H spectra) using a JEOL Eclipse spectrometer with $CDCl_3$ as solvent and tetramethylsilane as reference. The purity of ALC was determined by HPLC (Waters model 996 coupled to a Waters model 600 pump) using 4.6 × 250 mm Spherisorb columns (10 μM particle size), mobile phase 30% methanol/70% water, flow rate = 0.4 mL/min, and UV detection at 254 nm, rendering an average time retention of 3.61 min and a purity of 90−92% for synthetic allicin.

Preparation of HOCl and TAC. HOCl was obtained immediately before use by mixing a 1% (v/v) solution of NaOCl with 0.6 M sulfuric acid at pH 6.2. The HOCl concentration was further determined spectrophotometrically at 235 nm using the molar extinction coefficient of 100 M^{-1} cm^{-1}. Solutions of ALC, SAC, and ALI were prepared in 50 mM phosphate buffer, pH 7.4; all other compounds were dissolved in acetone diluted 1:2 with water. AA was disolved in 50 mM phosphate buffer, pH 7.4.

HOCl Scavenging Assay. The elimination of the catalase peak as a consequence of the destruction of the heme prosthetic group by HOCl was determined spectrophotometrically at 404 nm (*13*). The HOCl scavenging capacity of garlic TAC or the reference compounds (AA and NAC) was made evident by the inability of HOCl to eliminate/decrease the peak in a concentration-dependent way. Experiments were carried out essentially as described (*19*), and data were registered in a DU-640 series Beckman spectrophotometer (Beckman Coulter, Fullerton, CA). A solution of 49.8 μM bovine liver catalase (16.6 μM, final concentration) was mixed with 18 mM HOCl (6 mM, final concentration) with increasing concentrations of organosulfur compounds (**Table 1**). AA and NAC were used as reference compounds (*13*).

Determination of HOCl Scavenging Capacity. Scavenging capacity was expressed as 50% of the inhibitory concentration (IC_{50}) value, which represents the concentration of organosulfur test or reference compounds required to give a 50% reduction in catalase destruction relative to the one obtained in the tube that did not contain organosulfur compound or reference compounds. The IC_{50} of each test compound was calculated by the least-squares method. The lower the IC_{50} value, the higher the scavenging capacity of the compound (*13*).

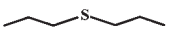
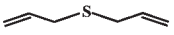
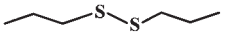
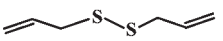
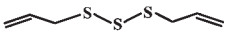
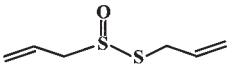
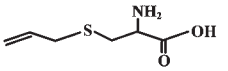
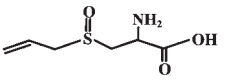
Structure−Activity Relationship Analyses. The three-dimensional structures of the studied compounds were geometrically optimized using the SPARTAN '04 package from Wave function (Irvine, CA). The dipole moment (μ) and total energy (E_{TOTAL}) and the corresponding energies for highest occupied molecular orbitals (E_{HOMO}) and lowest unoccupied molecular orbitals (E_{LUMO}) were calculated by the semiempirical quantum-chemical method AM1. For each molecule, the conformer with the major contribution to Boltzman population as found by Monte Carlo search was considered. In addition, two properties related to molecular transport (absorption prediction) were included. One was the octanol/water partition coefficient (log P_{oct}), the values of which were taken as reported in previous studies for AMS, DPS, DPDS, and ALC (*20*), for DAS, DADS, and DATS (*21*), and for SAC and ALI (*22*), whereas for AM this value was obtained from the Good Scents Co. database. The other molecular transport property analyzed was the molecular polar surface area (PSA). This was calculated for all compounds following the fragment-based contribution procedure (*23*). The relationship between the values of each of these molecular descriptors and their HOCl scavenging activities (expressed as IC_{50} values) was determined by multiple regression analysis (MLR), and the relative contributions of each descriptor to simplified models were cross-validated by the partial least-squares (PLS) procedure, both tools contained in the 16.0.18 version of Statgraphics Centurion XVI software.

RESULTS

HOCl Scavenging Activities of Garlic TAC. The 10 TAC tested at different concentrations were able to re-establish at different degrees the absorption peak of catalase at 404 nm that was abolished by HOCl attack when TAC were not present (compare curves 1 and 2 in each spectrophotometric pattern shown in **Figure 1**). This protective effect was performed in a dose-dependent manner and was compound-specific because none of the vehicles used (acetone and water) affected the catalase peak. The two reference compounds (AA and NAC) also protected catalase (not shown), although the latter was more efficient; indeed, these compounds served as reference scavengers of low and high efficiency (IC_{50} = 3.24 ± 0.31 and 0.99 ± 0.07 mM, respectively).

On the basis of the concentration−response data, the IC_{50} values were calculated for all test compounds (**Table 1**). The activities ranged from 0.56 to 3.57 mM. The simplest TAC tested (AM) had an intermediate efficacy (1.45 mM) that was decreased by substituting the H atom by a methyl group as in AMS (1.88 mM). If the H atom was substituted by another allyl group as in DAS, the IC_{50} value increased to a lower extent (1.68 mM). When the two C=C double bonds of DAS were saturated, as in the onion derivative DPS, the scavenging capacity dramatically decreased

Table 1. Chemical Structures and HOCl Scavenging Activity of Eight Thioallyl Compounds from Garlic and Two Related Propyl Sulfides from Onion[a]

Compound name	Structure	IC_{50} (mM) ± S.D.
Allyl Mercaptan		1.455 ± 0.258
Allyl Methyl Sulfide (AMS)		1.884 ± 0.370
Dipropyl Sulfide (DPS)		3.163 ± 0.978
Diallyl Sulfide (DAS)		1.680 ± 0.256
Dipropyl Disulfide (DPDS)		1.374 ± 0.037
Diallyl Disulfide (DADS)		1.023 ± 0.449
Diallyl Trisulfide (DATS)		0.896 ± 0.022
Diallyl Thiosulfinate, Allicin (ALC)		1.175 ± 0.079
S-Allyl Cysteine (SAC)		0.565 ± 0.157
S-Allyl Cysteine Sulphoxide, Alliin (ALI)		3.575 ± 0.638

[a] IC_{50} = millimolar concentration of compound required to achieve 50% of HOCl scavenging capacity. Data are the mean ± standard deviation (S.D.) of three separate experiments.

to 3.16 mM. In fact, DPS was one of the two compounds (along with ALI) exhibiting the lowest HOCl scavenging activity. Otherwise, a significant enhancing effect of scavenging capacity was observed when one additional S atom was present as in DADS (1.02 mM), whereas the corresponding onion derivative with saturated double bounds, DPDS, displayed lower activity (1.37 mM). Two other modifications of the structure of DADS rendered a differential trend in scavenger efficacy: when one additional S atom was present as in DATS, the scavenging activity was even better (0.89 mM); however, the addition of one oxo group (O═) to one of the two S atoms, as was the case of diallyl thiosulfinate (ALC), resulted in a lower scavenger efficacy (1.17 mM). By substituting the thioallyl group in ALC by an alanyl group ($-CH_2CH(NH_2)-COOH$), which gave rise to alliin (ALI), a further dramatic decrease in scavenging ability was observed (3.57 mM), and this compound was the lowest HOCl scavenger tested. Interestingly, the simple removal of the oxo group from ALI to became SAC gave rise to the more dramatic enhancing effect on scavenging capacity that indeed converted SAC to be the most efficient scavenger tested (IC_{50} = 0.56 mM).

As a next step, it was of interest to compare the HOCl scavenging activity of the TAC tested with that observed in two reference scavengers and antioxidants of physiological importance as are AA and NAC. For statistical estimations, Dunnett's multiple-comparisons test was used. In this case, AA behaved as a scavenger of low efficiency (IC_{50} = 3.49 ± 0.31 mM), and all compounds tested except for DPS and ALI were more effective HOCl scavengers than AA ($P < 0.05$; **Figure 2**). On the contrary, when NAC was used as a scavenger of high efficiency (IC_{50} = 0.99 ± 0.07 mM), only DATS and SAC were more effective in scavenging HOCl ($P < 0.05$; **Figure 2**).

Quantitative Estimations of the Structure−HOCl Scavenging Activity Relationship of TAC. The values of 4 electronic and 2 molecular transport descriptors were retrieved or calculated for the 10 TAC tested and these are listed in **Table 2**. With regard to the electronic descriptors, some trends were preliminarily observed. The E_{TOTAL} parameter ranged from ≈+30 kcal/mol (DAS, DADS, and DATS, good HOCl scavengers) to negative values of ≈−111 kcal/mol (ALI, the lowest HOCl scavenger), albeit SAC, the best scavenger tested, had a rather negative value (−87 kcal/mol). The E_{HOMO} values were always negative in a narrow range where the lowest scavenger (ALI) exhibited the highest negative value (−230 kcal/mol) and SAC the lowest negative one (−191 kcal/mol). Otherwise, the E_{LUMO} descriptor exhibited a range from −51 kcal/mol (DATS) to +20 kcal/mol (DPS) without a suggestive trend. This was also the case for the absolute values of the dipole moment, which ranged from 1.40 (DATS) to 3.49 D (ALI), the latter being the unique compound with μ higher than SAC (2.30 D). With rgard to the molecular transport descriptors, neither the lipophilicity (as depicted by log P_{oct}) nor the topological PSA seemed to keep a significant relationship to HOCl scavenging capacity because the more hydrophilic and polar compounds (SAC and ALI) exhibited opposite scavenging activities. From these observations, it was very likely that more than a single property should explain the differential scavenging activities of TAC tested.

As a statistical approach, the MLR method was used for the 10 TAC to assess possible relationships between their HOCl scavenging activity and the 6 electronic/transport descriptors in a quantitative manner. Some considerations were decisive for the inclusion or rejection of descriptors (predictor variables) in the screened and simplified models. In particular, pairs of variables

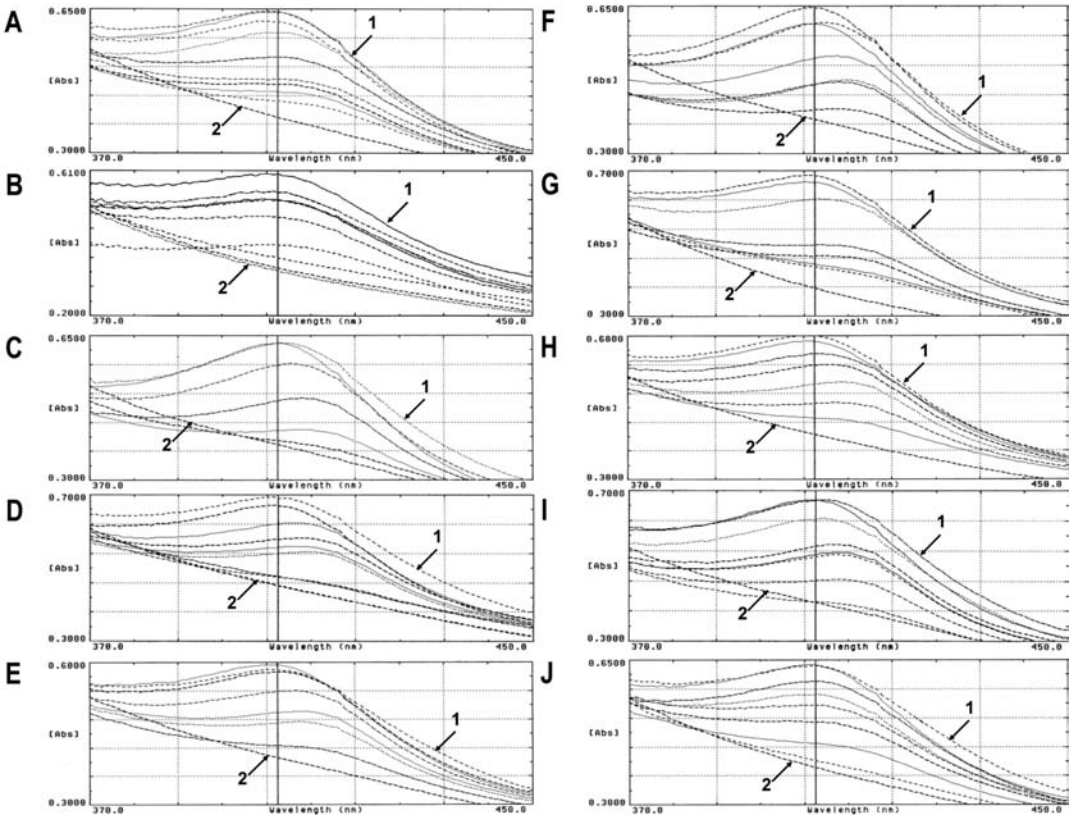


Figure 1. Spectrophotometric patterns of concentration−response data in the assay system for HOCl using eight TAC from garlic and two onion derivatives: (**A**) allicin; (**B**) alliin; (**C**) allyl mercaptan; (**D**) allyl methyl sulfide; (**E**) diallyl disulfide; (**F**) diallyl sulfide; (**G**) dipropyl disulfide; (**H**) dipropyl sulfide; (**I**) diallyl trisulfide; (**J**) *S*-allylcysteine. Arrows numbered 1 indicate the catalase peak without HOCl, and arrows numbered 2 indicate the abolished catalase peak by 6 mM HOCl.

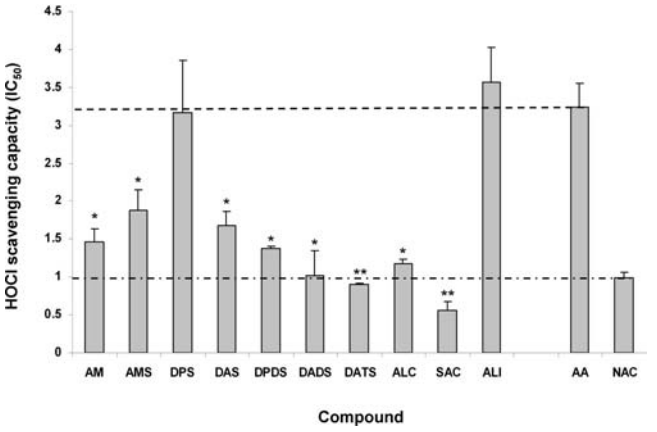


Figure 2. HOCl scavenging capacity (IC_{50} values, in mM) of garlic TAC and reference compounds (AA, ascorbic acid; ALC, allicin; ALI, alliin; AM, allyl mercaptan; AMS, allyl methyl sulfide; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DPDS, dipropyl disulfide; DPS, dipropyl sulfide; NAC, *N*-acetylcysteine; SAC, *S*-allylcysteine). ∗, IC_{50} lower than AA (- - -) ($P < 0.05$); ∗∗, IC_{50} lower than NAC (· · · ·) ($P < 0.05$).

with an $r \geq 0.75$ were considered as being intercorrelated, and that with the lower R^2 statistics versus HOCl scavenging ability was discarded in the simplified models. Also, only regression models without serious multicollinearity (i.e., absolute values of coefficient correlation < 0.5) and a $P < 0.05$ in ANOVA among all predictor variables were considered as statistically valid. Thus, the possible 60 combinations of predicted versus observed activities were screened. From these, only one simplified and validated equation could be obtained. In an initial approach using all six descriptors, the maximal fit observed was as follows:

$$\log IC_{50} = \underset{}{4.6994} \underset{(1.557)}{} + \underset{(0.056)}{0.0303} \underset{(0.008)}{} E_{HOMO} \underset{(0.039)}{} + \underset{(0.965)}{0.0004} \underset{(0.010)}{} E_{LUMO} + \underset{(0.224)}{0.0053} \underset{(0.003)}{} E_{TOTAL} + \underset{(0.383)}{0.308} \underset{(0.302)}{} \mu + \underset{(0.331)}{0.0135} \underset{(0.011)}{} PSA + \underset{(0.729)}{0.0515} \underset{(0.135)}{} \log P_{oct} \quad (1)$$

$$R^2 = 0.905; R^2(cv) = 0.715; s = 0.131; n = 10; DW = 1.746$$

In this and the following equation, the numbers in parentheses indicate the standard errors and *P* values (below) of the respective coefficients, *n* is the number of compounds tested, *s* is the standard error, R^2 is the squared correlation coefficient, R^2(cv) is the squared cross-validation coefficient, and DW is the Durbin−Watson statistic for possible correlations among residuals. Despite the high statistical significance determined, this equation was not suitable for interpretations because there were three pairs of variables strongly intercorrelated (r values of E_{TOTAL}/μ, −0.768; $E_{TOTAL}/\log P_{oct}$, 0.816; and $\mu/\log P_{oct}$, −0.779). Instead, the equation that proved useful for statistically valid interpretations was based on three descriptors as follows:

$$\log IC_{50} = \underset{}{3.4367} \underset{(0.915)}{} + \underset{(0.009)}{0.0203} \underset{(0.004)}{} E_{HOMO} \underset{(0.005)}{} + \underset{(0.009)}{0.0041} \underset{(0.001)}{} E_{TOTAL} + \underset{(0.003)}{0.0115} \underset{(0.002)}{} PSA \quad (2)$$

$$R^2 = 0.824; R^2(cv) = 0.736; s = 0.126; n = 10; DW = 2.176$$

Table 2. Values of Molecular Descriptors for TAC Shown in **Table 1**

compd[a]	log P_{oct}	E_{total} (kcal/mol)	E_{HOMO} (kcal/mol)	E_{LUMO} (kcal/mol)	dipole (D)	polar surface area (Å^2)
AM	1.51	13.71	−205.72	9.74	1.78	39.80
AMS	1.76	8.62	−195.63	11.54	1.57	25.30
DPS	2.88	−35.56	−195.48	20.50	1.60	25.30
DAS	2.75	26.38	−196.45	8.12	1.54	25.30
DPDS	3.84	−29.49	−206.77	−33.94	2.16	50.60
DADS	2.91	31.57	−206.96	−36.87	2.14	50.60
DATS	3.01	30.46	−209.33	−51.57	1.40	75.90
ALC	1.87	−0.78	−212.71	−33.70	2.25	61.58
SAC	−2.15	−87.10	−191.66	12.31	2.30	88.62
ALI	−3.87	−111.47	−230.07	−1.40	3.68	105.69

[a] ALC, allicin; ALI, alliin; AM, allyl mercaptan; AMS, allyl methyl sulfide; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DPDS, dipropyl disulfide; DPS, dipropyl sulfide; SAC, *S*-allylcysteine.

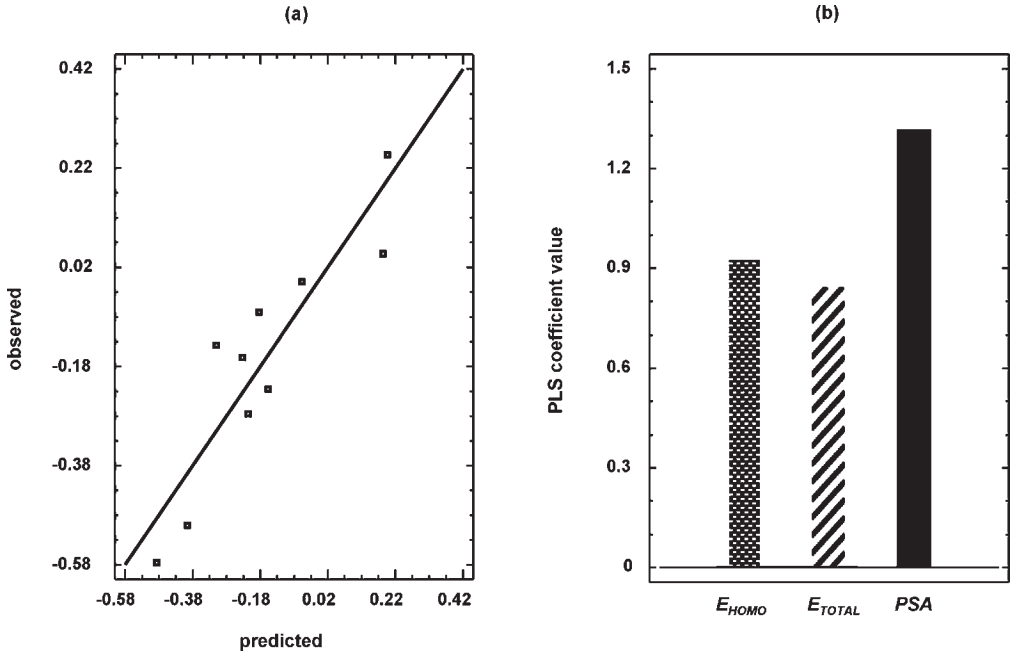


Figure 3. Relationships between the experimental and calculated HOCl scavenging activities of garlic TAC shown in **Table 1** using MLR analyses corresponding to eq 2 (**a**) and comparison of PLS regression coefficients assessing the contribution of each molecular descriptor listed in **Table 2** for eq 2 (**b**).

This equation is highly significant, and there were no intercorrrelations among the variables (r values of E_{HOMO}/E_{TOTAL}, 0.283; E_{HOMO}/PSA, −0.229; E_{TOTAL}/PSA, −0.644). Indeed, the inclusion of two electronic descriptors (E_{HOMO} and E_{TOTAL}) and one transport descriptor (PSA) yielded good-fitting and predictive capabilities to the simplified model as assessed by a low s value and high R^2 and R^2(cv) values that led to a very satisfactory agreement between the observed and predicted IC_{50} values (**Figure 3a**).

To define the relative contribution of each descriptor included in the simplified model shown in eq 2, this was cross-validated by the PLS procedure using the leave-one-out method with unmodified data. The resulting model was statistically significant by ANOVA test (P value = 0.011). By analyzing the relationship of the selected descriptors and the corresponding PLS standardized coefficients, it could be observed that in this three-component model the topological descriptor PSA was the main contributor to variations in scavenging capacity of TAC (PLS coefficient = 1.31, **Figure 3b**), whereas both electronic descriptors (E_{LUMO} and E_{TOTAL}) had a lesser contribution to IC_{50} variations (PLS coefficients = 0.93 and 0.85, respectively; **Figure 3b**). Therefore, the electronic densities, primarily at the topological and secondarily at the molecular orbital levels, mostly explained the variations in HOCl scavenging capacity of the compounds tested.

DISCUSSION

Scavenging of ROS is one of the mechanisms whereby garlic derivatives may confer important health care benefits. HOCl is a strong oxidant endogenously and excessively produced in inflammatory, degenerative, and neoplastic disorders; thus, effective therapies and/or prophylaxes using exogenous scavengers of high specificity are required. From our previous studies, it was observed that aqueous extracts from raw garlic and unpeeled cloves treated with distinct thermal processes were able to differentially scavenge HOCl, and SAC was the only garlic compound previously studied in this context (*13*, *18*). In this work, eight derivatives of garlic displaying systematic modifications of the basic thioallyl structure (TAC) and two propyl sulfide analogues from onion were tested using the catalase protection assay to explore their ability to scavenge HOCl. The thioallyl group was studied on the basis of its natural abundance in garlic compounds and on its bona fide characteristics as pharmacophore. This group has been identified as the moiety having trophic and protective effects at organ and cellular levels upon drug toxicity and carcinogenesis in experimental models (*24*, *25*). Synthetic, nongarlic TAC have been further obtained and evaluated (*26*) providing a direct precedent for rational drug design with this pharmacophore.

As has been observed for other ROS, the HOCl scavenging capacity was distinct among garlic TAC but relied on certain structural features of the compounds. On the one hand, the HOCl scavenging capacity of garlic extracts was not dependent on ALC formation (*13*), and the present data support that although ALC had a good scavenging activity, several other compounds contained in fresh, heated/boiled garlic extracts (including ALI, a scavenger of low capacity) are still able to trap HOCl. On the other hand, the concentration−activity data for all TAC tested showed that their HOCl trapping ability was *enhanced* by substituting the former H atom from the thiol group by an increasing number of S atoms or by the alanyl group; however, scavenging was *decreased* by the absence of the C=C bond or the presence of sulfoxide groups in the thioallyl moiety. This latter observation was supported by the higher HOCl trapping activity of DADS as compared to ALC and that of SAC versus ALI.

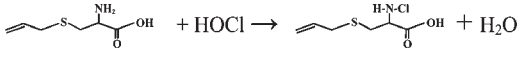
These data seem to be consistent with several types of reaction mechanisms between TAC and HOCl where the $-S-CH_2-CH=CH_2$ moiety is likely delivered as a modified product from primary reactions. One such reaction might be the oxidation of sulfhydryl groups to form disulfides (*27*) as with AM:

$$H-S-CH_2-CH=CH_2 + HOCl \rightarrow CH_2=CH-CH_2-S-S-CH_2-CH=CH_2 + H_2O + Cl^- \quad (3)$$

Another likely reaction may occur with the di- or trisulfides to produce sulfinic acids (*27*) as follows:

$$R-S_n-S-CH_2-CH=CH_2 + HOCl \rightarrow R-S_nO_2H + HO_2-S-CH_2-CH=CH_2 + HCl \quad (4)$$

In this case, R corresponds to an allyl (as in DADS and DATS), propyl (as is DPDS), or allyl sulfinate group (as in ALC). In this reaction, the formation of HCl contributes to further HOCl dissipation. Third, if α-amino acids (such as SAC and ALI) are exposed to HOCl, a chlorination reaction likely occurs at the α-amino group (*28*):



(5)

Because this group is attached to an amino acid, this chloramine product readily decomposes to the corresponding amide, releasing ammonia and HCl that in turn help to dissipate HOCl. Nevertheless, the mechanism for the split of the (allyl)S−C bond in AMS, DPS, DAS, ALC, SAC, and ALI by HOCl seems to be much more complex and likely needs multiple electron transfers and reaction intermediates to take place. Furthermore, there is a possibility that some other reaction products might also dissipate HOCl as aforementioned. One such example was shown with 2-propenesulfenic acid, a Cope elimination product of ALC that was shown to be the responsible for $HOO^{\bullet}$ scavenging instead of ALC itself (*16*). The HOCl trapping by TAC regarding this specific issue deserves future research.

Other implications from the structure−activity data obtained in this study were derived from the comparison of the HOCl scavenging activity of tested TAC with that of two scavengers of low and high efficiency that in humans are derived from exogenous and endogenous sources (AA and NAC, respectively). The different ability to trap HOCl by these compounds is explained by the underlying mechanism: AA has two hydroxyl groups that are oxidized by two HOCl to form dehydroascorbic acid (*29*), whereas NAC is able to scavenge up to four HOCl molecules as its sulfhydryl group may be oxidized three times by three HOCl molecules and the fourth reacts with the α-amino group (*30*), which renders it a much more efficient scavenger. This is consistent with the strong relationship of the acetylalanyl moiety of NAC to the alanyl group of SAC and the thiol group of NAC that was functionally improved by the increasing number of S atoms present in DATS, two structural traits that combined to HOCl dissipation by the HCl product could reinforce the high scavenging efficacy of SAC and DATS. Otherwise, DPS and ALI have structural traits (absence of C=C bond and presence of the oxo group, respectively) that must hinder the reactivity of HOCl on R—SH or R—S—S—R moieties of TAC. On the basis of these observations, it is tempting to speculate that other TAC exhibiting disulfide bonds and alanyl groups (e.g., *S*-allylmercaptocysteine) might be predicted as very efficient HOCl scavengers (even better than SAC), whereas others with disulfide bonds and oxo groups (e.g., ajoenes) would have a more limited ability to scavenge HOCl.

In further analyses, molecular descriptors of TAC were used as quantitative resources to model their interaction with HOCl as the biological target. In this context, it is well-known that further to their antioxidant properties, TAC from garlic are also able to modulate a broad spectrum of enzymes involved in a plethora of biological responses (*31*−*33*). The few previous studies that considered molecular descriptors of organosulfur compounds (OSCs) from garlic, including some TAC, assessed structure−enzyme inhibition (*20*, *21*) and neuroprotective activity−ROS scavenging relationships (*22*). Although the enhancing effect of the thioallyl moiety on the inhibition of carcinogen (benzo[*a*]pyrene) activation mediated by cytochrome P450 1 (*20*) and the effect of the alanyl group conferring higher neuroprotective activity (*22*) were determined, data concerning quantitative structure−activity relationships (QSAR) for OSCs were reported only in a model of inhibition of soybean 15-lipoxygenase by a series of 28 OSCs from garlic essential oil comprising mono-, di-, and trisulfides (*21*). Of interest, from a large number of descriptors evaluated by MLR and PLS analyses, only descriptors related to molecular size (solvent-accessible surface area and average distance/distance degree) and electron-acceptor nature (E_{LUMO}) of OSCs contributed significantly to variations in their inhibitory effect on lipoxygenase activity (*32*). Nevertheless, modeling TAC−enzyme interactions should be more difficult than for TAC−HOCl interactions as enzymes are much more complex molecules with intricate shapes and sizes within and neighboring catalytic sites when compared to the very reactive and small HOX molecule. In the present work this latter observation would contribute to retrieve statistically valid models even using a smaller set of 10 compounds tested. Prompted by this rationale, the present study systematically included electronic/quantum-chemical (E_{TOTAL}, E_{HOMO}, E_{LUMO}, and dipole) and transport/permeability (log P_{oct} and PSA) indices in MLR and PLS analyses to identify the properties of 10 TAC by determining their HOCl scavenging capacity and the relative contributions of each descriptor. To our knowledge, this is the first time that quantitative structure−antioxidant activity relationships for garlic compounds are determined.

Among electronic properties, E_{HOMO} and E_{TOTAL}, in that order, contributed to variations in IC_{50} values (eq 2). This is in good agreement with the high reactivity and electron-donor nature of a TAC antioxidant that has to be oxidized upon interaction with HOCl (eqs 3−5). In other terms, the less negative (i.e., less stable) is the electronic charge level on the more external electron orbitals, the higher is the HOCl scavenging ability of TAC. Furthermore, the improved model fitting TAC−HOCl interactions (eq 2) included the topological PSA descriptor as a primary contributor. First, it is well-known that this index, at

values of 50−90 Å^2, predicts good cellular permeability, and it was the case for some TAC exhibiting high HOCl scavenging activity (e.g., ALC, DADS, DATS, and SAC). This would imply an increased bioavailability of these compounds (by crossing the blood−brain barrier) and helps to explain their neuroprotective effect that in turn is related to ROS scavenging (*22*). Second, the importance of the broader size of the polar area on the R—NH_2 (26.02 Å^2), R—SH (38.8 Å^2), R—S—S—R (50.6 Å^2), and R—S—S—S—R (75.9 Å^2) moieties that interact with the HOCl oxidant (eqs 3−5) as compared to other groups with lower or negligible reactivity with HOCl (e.g., R═O, 17.07 Å, and R—OH, 20.23 Å^2, both present in AA). Despite the fact that ALI had the highest PSA value (105.69 Å^2), the presence of the oxo group surpasses the influence of PSA by hindering the reactivity of the adjacent S atom that likewise displays an apparently favorable PSA (32.09 Å^2). A likely explanation for this effect is that the electron density of the S atom is enhanced by the adjacent allyl group but drastically decreased by the O atom, which is less polarizable, indeed impairing the nucleophilic nature of the adjacent S atom.

The rational design of HOCl scavengers with higher efficacy still deserves further analyses for several reasons. One relates to the fact that some compounds structurally related to TAC, as are the 4-mercaptoimidazoles (*32*), or structurally distinct, as are the nonsteroid anti-inflammatory pyrazolones (*33*) or the β-lactam antibiotics cephalosporins (*34*), are able to scavenge HOCl at the micromolar range in vitro. However, these compounds are actually intended for other therapeutic purposes and may have severe secondary effects. Another relates to the fact that TAC from garlic are able to scavenge other ROS, and particularly SAC, the most efficient HOCl scavenger probed in the present work, is also an efficient $O_2^{\bullet-}$, $HO^{\bullet}$, $HOO^{\bullet}$, and $ONOO^-$ scavenger at the submillimolar range in vitro (*14*, *17*); moreover, SAC itself has been reported as either an inefficient (*20*) or highly active (*32*, *35*) enzyme modulator. However, SAC exhibits other important several advantages including easy gastrointestinal absorption and bioavailability, low toxicity, and cost-effective synthesis (*22*). Ultimately, garlic derivatives have important advantages over most synthetic compounds as they have proven benefits in chemoprevention besides their multiple therapeutic applications (*8*, *9*, *24*).

Taken together, the observations derived from this work may have important implications in modeling HOCl-mediated oxidative stress because catalase is an antioxidant, protective enzyme that may be located at inflammation sites and is able to be inactivated by HOCl at the physiologically feasible concentrations (*29*) mimicked in this study. Also, the presented data might provide a platform for future design of HOCl scavengers with improved action in processes associated with overproduction and/or misplacing of this oxidant. In addition, more studies concerning the structure−trapping relationship with other ROS by the TAC tested herein will help to unravel the differential requirements and mechanisms underlying the multiple health care benefits associated with consumption of garlic nutraceuticals.

ABBREVIATIONS USED

TAC, thioallyl compounds; PSA, topological polar surface area; E_{TOTAL}, total energy; E_{HOMO}, energy of the higher occupied molecular orbital; E_{LUMO}, energy of the lower unoccupied molecular orbital; μ, dipole moment; log P_{oct}, logarithm of the octanol/water partition coefficient; ROS, reactive oxygen species; $O_2^{\bullet-}$, superoxide radical; $HO^{\bullet}$, hydroxyl radical; $HOO^{\bullet}$, peroxyl radical; $ONOO^-$, peroxynitrite; AA, ascorbic acid; ALC, allicin; ALI, alliin; AM, allyl mercaptan; AMS, allyl methyl sulfide; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DPDS, dipropyl disulfide; DPS, dipropyl sulfide; NAC, *N*-acetylcysteine; SAC, *S*-allylcysteine; IC_{50}, half-maximal inhibitory concentration; MLR, multilinear regression analysis; PLS, partial least-squares analysis.

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