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Sulfenic acid – derived glycoconjugated disulfides and sulfoxides: a biological evaluation on human red blood cells

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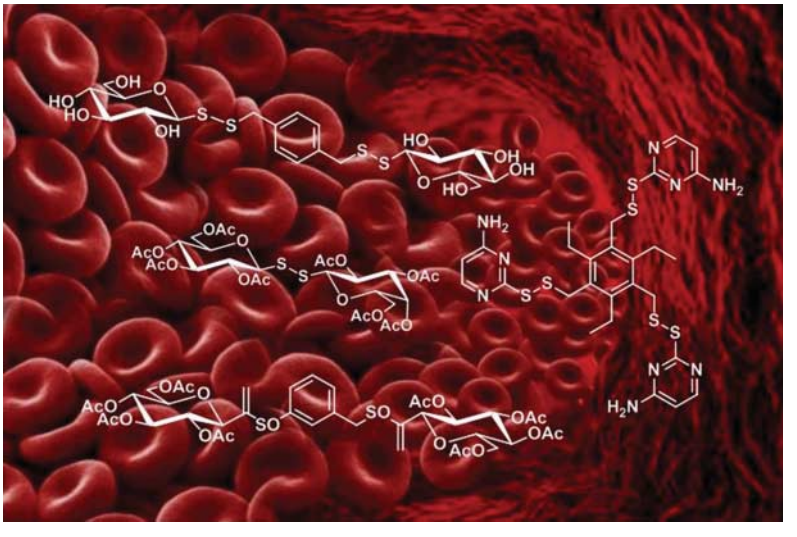
This article examines the effects of some glycoconjugated disulfides and sulfoxides on red blood cells (RBCs). Compounds under study show sugar units connected directly or through a benzene platform, and have been obtained following synthetic pathways based on the sulfenic acid chemistry. In order to evaluate the relationship between the structural features of the thioglycoconjugates under investigation and their potential biological activity, we were interested in assessing both the cytotoxicity and hemolytic activity produced on human RBCs by overnight exposition to the thioglycoconjugates. The absence of both cytotoxic effect and hemolysis produced by the tested compounds on erythrocytes supports the rational design of hemocompatible molecules for biomedical applications.



Keywords: sulfenic acids; disulfides; sulfoxides; cytotoxicity; hemolytic activity

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1. Introduction

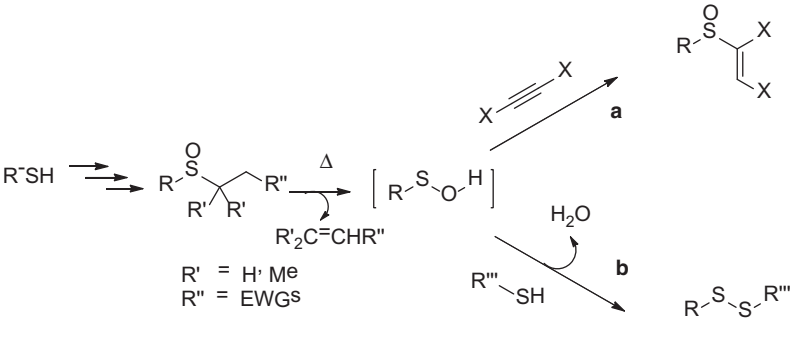
Sulfenic acids are frequently occurring intermediates in biological and synthetic chemistry. Among the numerous examples, cysteine-derived sulfenic acids are recognized as key intermediates in signal transduction (*1*), transcriptional regulation events (*2*), oxidative stress response (*3*). They also play catalytic and structural roles in enzymes (*4*). Furthermore, the sulfenic function is involved in the biosynthesis of thiosulfinates that give the characteristic odor and flavor to the *Allium* species, and in the lachrymatory process of cut onion (*5*). Recent studies have been addressed to the comprehension of the redox chemistry of sulfenic acids, not yet understood exhaustively, and model systems have been used to mimic the destiny of these transient species in nature (*6*). On the other hand, the electronic nature of their S−O bond and its involvement in various, often stereospecific, reactions, has prompted many applications of sulfenic acids in organic synthesis, such as their use as key intermediates in the preparation of peculiar sulfoxides (*7*) and disulfides.

One of the straightforward ways of generation of transient sulfenic acids is represented by their *syn*-elimination from proper sulfoxide precursors, in neutral conditions, through thermolysis (*8*), as shown in Scheme 1. The intermolecular *syn*-addition of sulfenic acids to triple bonds (pathway **a**) gives vinyl sulfoxides, while their condensation with thiols (pathway **b**) leads to the formation of unsymmetrically substituted disulfides.

The reaction of sulfenic acid/unsaturated moiety is a stereospecific and, in most of the cases, highly regioselective process that offers significant chances of modulating the synthesis of unsaturated sulfoxides due to the possibility of changing both the nature of the unsaturated acceptors and the sulfenic acid skeleton (*9*).

The condensation process that involves sulfenic acids and thiols allows an easy entry into the chemistry of the disulfide bond. This reaction can be regarded as an efficient and smooth general process for the construction of libraries of unsymmetrically substituted disulfides. It allows the presence of base/acid and/or thermolabile functional groups in both the sulfenic acid and the thiol on the basis of the choice of suitable precursors of the sulfenic acids, and offers wide chances of building the disulfide bond between different structural skeletons such as homo- and hetero-aromatic, amino acidic and sugar residues (*10*).

As a part of a program centered on the study of sulfenic acid reactivity in both the directions above mentioned, thioglycoconjugates with two or three sugar moieties separated by arene spacers have been synthesized (*10a,c*). The relationship between their molecular structure and biological activity was investigated with respect to their ability to induce apoptotic cell death, taking into account the pivotal role of apoptosis regulation in both the controlled expansion and removal of immune cells and cancer progression and therapy (*11*).



Scheme 1. *In situ* generation of sulfenic acids and correlated reactions.

Herein, a collection of thioglycoconjugated sulfoxides and disulfides is presented that was exposed to human red blood cells (RBCs). The aim of this study was the evaluation of the cytotoxic effects and hemolytic activity of such thioglycoconjugates on human erythrocytes and comparison of the collected results with those already present in the literature (*12*). The predictive value of *in vitro* cytotoxicity tests is based on the idea of 'basal' cytotoxicity, namely that toxic molecules or pharmaceutical preparations affect basic functions of cells which are common to all cells, and that the toxicity can be measured by assessing cellular damage (*13*). Before any investigation on mechanisms of action of different molecules or pharmaceutical preparations, it is important to perform cytotoxicity assays. The development of *in vitro* cytotoxicity assays has been driven by the need of rapidly evaluating the potential toxicity of large numbers of molecules, limiting animal experimentation whenever possible, and carrying out tests with small quantities of each compound.

2. Results and discussion

Polythiols **1**–**4** and 1-thio-β-D-glucopyranose 2,3,4,6-tetraacetate (**5**; Figure 1) were used as starting products for the preparation of suitable sulfoxide precursors of the corresponding sulfenic acids (Scheme 1), (*9j*, *10a–c*) that are generated *in situ* and represent the key intermediates in the syntheses of glycoconjugated sulfoxides and disulfides **8**–**15** (Figure 2). 1-Thio-β-D-glucopyranose 2,3,4,6-tetraacetate (**5**) was also employed as partner (R‴-SH of pathway **b** in Scheme 1) in the condensation reaction of sulfenic acids with thiols, leading to the formation of compounds **9**–**14** (*10a,b*). 1-Thio-β-D-galactopyranose 2,3,4,6-tetraacetate (**6**) was used as sulfenic acid trapper for obtaining compound **15** (*10a*) and, finally, the commercially available 2-thiocytosine (**7**) was involved in the sulfenic acid/thiol condensation reaction for preparing compound **16** (*10b*).

Noteworthy, the arene framework of compound **10** is functionalized with two different arms, one showing a disulfide function linked to the monosaccharide moiety and the other showing a 2-(methoxycarbonyl)ethylsulfinyl function that possesses the structural characteristics for generating the corresponding sulfenic function by thermolysis. The isolation of compound **10** opened a new scenery in the way of thinking the reaction pathway of the sulfenic acid/thiol condensation when bis-functionalized substrates are involved. The synthetic route to compound **11** is outlined in Scheme 2, with a stepwise formation of the two sulfenic functions and therefore a more complex pathway than expected before (*10c*).

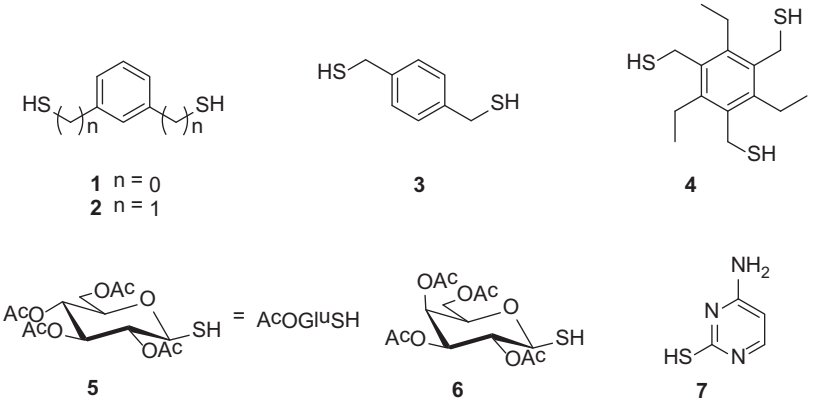


Figure 1. Thiols involved in the synthesis of bioinvestigated disulfides and sulfoxides.

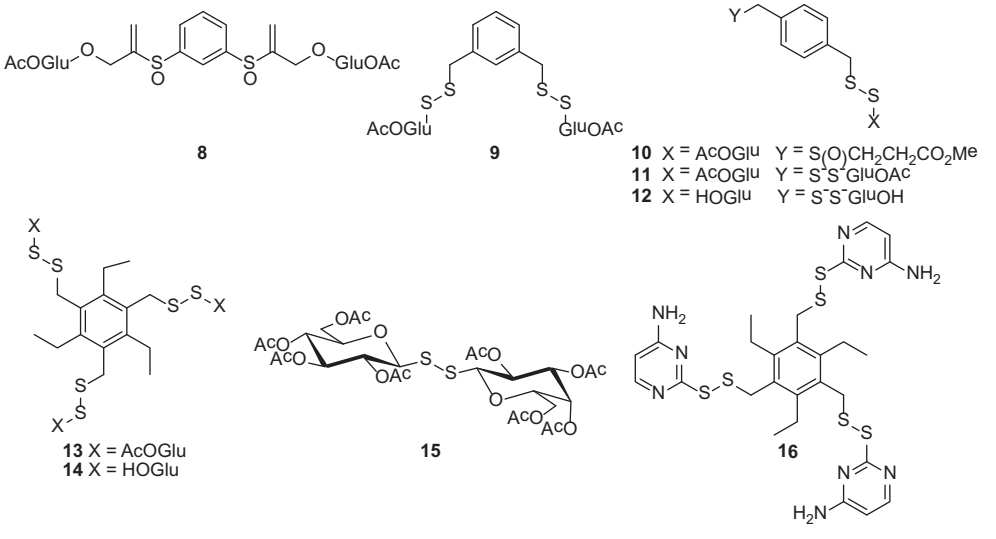
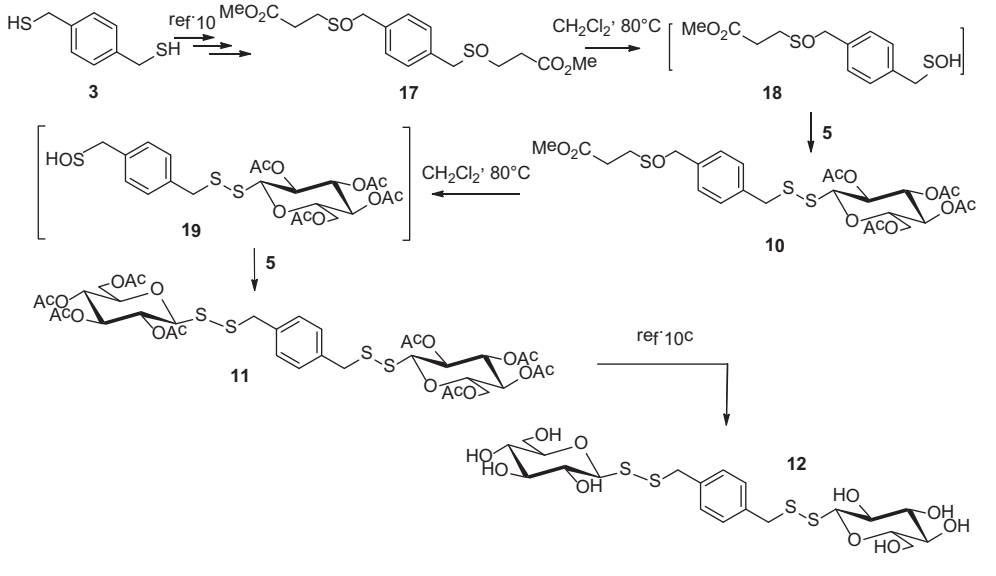


Figure 2. Bioinvestigated disulfides and sulfoxides.



Scheme 2. The synthetic route to compounds **11** and **12**.

It appears that the symmetrical bis(sulfoxide) **17** (*10c*) allows the generation *in situ* of just one sulfenic function that, in turn, condenses with the monosaccharide **5**. A second *syn*-elimination and a second condensation with thiol **5** is necessary to obtain the bis(disulfide) **11** from **10**, through the sulfenic acid **19**. The final deprotection converts **11** into the water-soluble bis(disulfide) **12** (*10c*).

We did not isolate any analogous intermediate in the synthesis of compounds **13** and **16**, (*10b*) starting from thiol **4** and following pathway **b** of Scheme 1. However, the appearance and disappearance of spots on the TLC plates, before ending of the reactions for preparing the tris(disulfides) **13** and **16**, support the stepwise formation of the sulfenic functions and condensation with thiols.

These mechanistic considerations, in relation to the isolation of intermediates such as **10**, can also provide new explanations for stereochemical outcomes previously observed (*9i*).

Compound **8** is the result of the generation *in situ* of the corresponding sulfenic functions from 3,3′-[1,3-phenylenebis(sulfinyl)]bispropanoic acid 1,1′-dimethyl ester and their completely regioselective *syn*-addition to the triple bond of 2-propyn-1-yl β-D-glucopyranoside 2,3,4,6-tetraacetate (*9j*).

Some of the compounds reported in this paper have been subjected to biological studies proving that they induce detectable levels of cell death toward several cancer cell lines (*9j*,*10c*). In the aim of finding additional therapeutic value to our preliminary results and examining the level of toxicity on human cells, for instance RBCs, we have performed with compounds **8**–**15**, a common cytotoxicity test, the trypan blue assay that measures cytotoxicity based on alterations in plasma membrane permeability and consequent dye uptake, normally excluded by viable cells. This method involves direct counting of viable cells (*13*). Analogous biotests were performed on 2-thiocytosine derivative **16** owing to the presence of the biorelevant pyrimidine residues. Human whole blood samples collected from four healthy donors were used within 24 h after bleeding. Each of compounds **8**–**16** was dissolved (0.1%) in dimethyl sulfoxide (DMSO) or dichloromethane (DCM). Stock solutions were diluted in a saline solution at pH 7.4. As a control, the same cells were exposed to the vehicle alone (DMSO or DCM), in amounts corresponding to those employed for dissolving the compounds under study. In order to define cell viability, the samples were incubated in the saline solution for 1, 3 h, and overnight. Trypan blue stock solution was then added to the RBCs, hence they were loaded on a hemocytometer and examined immediately under a microscope at low magnification. The numbers of blue staining and total cells were counted. The trypan blue assay showed a viability of 92–100% in all experiments (data not shown). There were no statistical differences in the viability depending on exposure durations at 10^{-4} M concentrations of each compound.

Furthermore, we decided to subject compounds **8**–**16** to the hemolysis assay, a sensitive and accurate tool used as a guide to assess the safety and utility of a molecule or pharmaceutical preparations (*14*). This test evaluates hemoglobin (Hb) release in the plasma (as an indicator of RBC lysis) following molecule exposure (*12*). Human whole blood samples collected from four healthy donors were used within 24 h after bleeding and were incubated in a saline solution. After overnight incubation at 37°C, the samples were centrifuged and the supernatants were harvested. As a measure of hemolysis, Hb concentration of the supernatants was determined photometrically. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% hemolysis. In the treatment of RBCs with each of compounds **8**–**16**, hemolysis did not occur at 300 and 200 mOsm/kg, while became clearly visible at 100 mOsm/kg to accomplish itself

Table 1. Percentage of hemolysis by compounds **8**–**16** (10^{-4} M).

	Osmolarity (mOsm/kg)				
Compounds	300	200	150	100	0
Control	*0%*	*0%*	*4%*	*100%*	*100%*
8	0%	0%	4%	98%	100%
9	0%	0%	4%	97%	100%
10	0%	0%	4%	95%	100%
11	0%	0%	4%	98%	100%
12	0%	0%	4%	98%	100%
13	0%	0%	4%	100%	100%
14	0%	0%	4%	100%	100%
15	0%	0%	4%	100%	100%
16	0%	0%	4%	96%	100%

at 0 mOsm/kg, as in the control conditions (Table 1). The maintenance of integrity of RBC membrane up to 150 mOsm/kg and the hemolysis occurrence at identical concentrations of the control conditions suggest that none of the compounds under study shows toxicity effects in human erythrocytes at the concentration tested and overnight.

3. Conclusions

The collection of compounds **8**–**16**, all showing sulfoxide and/or disulfide functions connected to biorelevant residues, have been obtained by the stepwise generation *in situ* of more than one sulfenic function from suitable precursors and the reactions of such transient species with substituted propynyl derivatives or thiols. Some of the mechanistic features of such reactions have been discussed. Human erythrocytes from various subjects were used to assess both the cytotoxicity and hemolytic activities of compounds **8**–**16**. Results obtained *in vitro* demonstrated that all the molecules under study show neither cytotoxicity nor hemolytic effect. On the basis of previously reported biological results (*10*) and the actual performed tests, this family of compounds could represent a new frontier in biomedical applications.

4. Experimental

4.1. *Chemistry*

Solvents were purified according to standard procedures. Reactions were monitored by TLC on commercially available precoated plates (Aldrich silica gel 60 F254) eluted with EtOAc/MeOH, and the products were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. H_2SO_4 (0.6 mL)]. Silica gel used for column chromatography was Aldrich 60. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Mercury 300 spectrometer at 300 and 75 MHz, respectively, in $CDCl_3$ solutions; the assignments are supported by Attached Proton Test and COrrelation SpectroscopY and Heteronuclear Single Quantum Coherence experiments; proton and carbon nuclei identified by apex pertain to the glucose residue of compound **10**. Glycoconjugated disulfides and sulfoxides **8**, **9**, **11**–**16** have been already described (*9j*, *10a–c*).

1-{[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)dithio]methyl}-4-{[(2-methoxycarbonylethyl) sulfinyl]methyl}benzene (**10**)

A solution of bis(sulfoxide) **17** (0.37 g, 1 mmol) and 1.46 g (4 mmol) of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (2 mmol for each sulfinyl function) in 20 mL of 1,2-dichloroethane was maintained at reflux temperature (83°C) under stirring. The reaction, monitored via TLC and ^{1}H NMR, was stopped when the starting compound **17** disappeared. The reaction mixture was purified by flash chromatography. The excess of glucosylthiol was recovered from the column, together with 10% maximum of bis(disulfide) **11**. 1-{[(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)dithio]methyl}-4-{[(2-methoxycarbonylethyl)sulfinyl]methyl}benzene (**10**) was obtained as a 1:1 diastereomeric mixture in 52 % yield. TLC: *Rf* 0.35 (eluant EtOAc/MeOH 90:10). Low melting solid. ^{1}H NMR: δ7.30 (m, 4H, ArH), 5.4–5.0 (m, 3H, H-2′, 3′, 4′), 4.54 (d, $J_{1',2'} = 10.0$ Hz, 0.5H, H-1′) of one diastereoisomer, 4.50 (d, $J_{1',2'} = 10.0$ Hz, 0.5H, H-1′) of the other diastereoisomer, 4.3–4.1 (m, 2H, H_2-6′), 4.0–3.9 (m, 4H, 2×ArC$\underline{H}_2$), 3.8–3.7 (m, 1H, H-5′), 3.71 (s, 3H, OCH_3), 3.0–2.8 (m, 4H, CH_2CH_2), 2.09, 2.05, 2.04, and 2.02 (4s, 12H, $4^{\times}COCH_3$). ^{13}C NMR: δ 171.73, 170.47, 170.18, 169.38, and 169.10 (5 x CO), 137.23 (C-4), 130.37, 130.23, 130.13, and 129.84 (C-2,3,5,6), 128.80 (C-1), 87.68 (C-1′), 76.13, 73.77, 69.07, and 68.02 (C-2′-5′), 62.05 (C-6′), 61.86 (Ar$\underline{C}H_2$SO), 52.18 (OCH_3), 43.84 (CH_2SS and $\underline{C}H_2CH_2CO_2CH_3$),

26.73 ($\underline{C}H_2CO_2CH_3$), 20.74, 20.69, 20.64, and 20.55 ($4\times CO\underline{C}H_3$). Anal. Calcd for $C_{26}H_{34}O_{12}S_3$ (634.74): C, 49.20; H, 5.40. Found: C, 49.37; H, 5.47.

4.2. *Biology*

Molecules were dissolved (0.1%) in DMSO or DCM. Stock solutions were diluted in saline solution having the following composition (mM): 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 D-glucose, 1 $CaCl_2$; pH 7.4. As a control, the same cells were exposed to the vehicle alone (DMSO or DCM), in amounts corresponding to those employed for dissolving the compounds.

4.2.1. *Trypan blue assay*

Human whole blood samples collected from four healthy donors were used within 24 h after bleeding. In order to define cell viability, the samples were incubated in the saline solution described above containing each molecule under study at 10^{-4} M concentration for 1, 3 h, and overnight. Afterward, 0.1 mL of trypan blue stock solution was added to 0.1 mL of RBCs, hence they were loaded on a hemocytometer and examined immediately under a microscope at low magnification. The numbers of blue staining and total cells were counted. The percentage cell viability was calculated by the formula:

$$\text{Cell viability (\%)} = \frac{\text{Number of viable cells (unstained cells)} \times 100}{\text{Total number of cells (stained and unstained)}}.$$

4.2.2. *Hemolysis test*

Human whole blood samples collected from four healthy donors were used within 24 h after bleeding and were incubated in saline solution containing each molecule under study at 10^{-4} M concentration. After overnight incubation at 37°C, the samples were centrifuged (5 min at 1500 rpm), and the supernatants were harvested. As a measure of hemolysis, hemoglobin (Hb) concentration of the supernatants was determined photometrically at 540 nm. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% hemolysis.

Acknowledgements

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References

(1) Paulsen, C.E.; Carroll, K.S. *ACS Chem. Biol*. **2010,** *5,* 47–62.
(2) Poole, L.B.; Nelson, K.J. *Curr. Opin. Chem. Biol.* **2008,** *12,* 18–24.
(3) Griffiths, S.W.; King, J.; Cooney, C.L. *J. Biol. Chem.* **2002,** *277,* 25486–25492.
(4) (a) Hall, A.; Karplus, P.A.; Poole, L.B. *FEBS J.* **2009,** *276,* 2469–2477; (b) Poole, L.B.; Karplus, P.A.; Claiborne, A. *Annu. Rev. Pharmacol. Toxicol.* **2004,** *44,* 325–347.
(5) (a) Block, E. *Angew. Chem., Int. Ed. Engl.* **1992,** *31,* 1135–1178. (b) Vaidya, V.; Ingold, K.U.; Pratt, D.A. *Angew. Chem., Int. Ed.* **2009,** *48,* 157–160. (c) Block, E. *Garlic and Other Alliums: the Lore and the Science*; RSC Publishing: Cambridge, 2009.
(6) McGrath, A.J.; Garrett, G.E.; Valgimigli, L.; Pratt, D.A. *J. Am. Chem. Soc.* **2010,** *132,* 16759–16761.
(7) Hogg, D.R. In *The Chemistry of Sulphenic Acids and their Derivatives*; Patai, S., Ed.; Wiley-Interscience: Toronto, 1990; pp 361–402.

(8) Aversa, M.C.; Bonaccorsi, P.; Madec, D.; Prestat, G.; Poli, G. In *Innovative Catalysis in Organic Synthesis: Oxidation, Hydrogenation and C-X Bond Forming Reactions*; Andersson, P.G., Ed.; Wiley-VCH: Weinheim, 2012; pp 47–76.
(9) (a) Aversa, M.C.; Bonaccorsi, P.; Giannetto, P.; Jafari, S.M.A.; Jones, D.N. *Tetrahedron: Asymmetry* **1992,** *3,* 701–704. (b) Jones, D.N.; Aversa, M.C.; Bonaccorsi, P.; Giannetto, P. *Tetrahedron: Asymmetry* **1994,** *5,* 805–808. (c) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P.; Giannetto, P.; Jones, D.N. *J. Org. Chem.* **1997,** *62,* 4376–4384. (d) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P.; Giannetto, P.; Nicolò, F. *J. Org. Chem.* **1999,** *64,* 2114–118. (e) Aversa, M.C.; Barattucci, A., Bonaccorsi, P.; Giannetto, P.; Policicchio, M. *J. Org. Chem.* **2001,** *66,* 4845–4851. (f) Aucagne, V.; Aversa, M.C.; Barattucci, A.; Bonaccorsi, P.; Giannetto, P.; Rollin, P.; Tatibouët, A. *J. Org. Chem.* **2002,** *67,* 6925–6930. (g) Aversa, M.C.; Barattucci, A., Bonaccorsi, P., Giannetto, P. *J. Org. Chem.* **2005,** *70,* 1986–1992. (h) Aversa, M.C.; Barattucci, A.; Bilardo, M.C.; Bonaccorsi, P.; Giannetto, P.; Rollin, P.; Tatibouët, A. *J. Org. Chem.* **2005,** *70,* 7389–7396. (i) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P.; Faggi, C.; Papalia, T. *J. Org. Chem.* **2007,** *72,* 4486–4496. (j) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P.; Marino-Merlo, F.; Mastino, A.; Sciortino, M.T. *Bioorg. Med. Chem.* **2009,** *17,* 1456–1463. (k) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P. *Eur. J. Org. Chem.* **2011,** 5668–5673.
(10) (a) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P. *Eur. J. Org. Chem.* **2009,** 6355–6359. (b) Aversa, M.C.; Barattucci, A.; Bonaccorsi, P. *Synlett*. **2011,** 254–258. (c) Bonaccorsi, P.; Marino-Merlo, F.; Barattucci, A.; Battaglia, G.; Papaianni, E.; Papalia, T.; Aversa, M.C.; Mastino, A. *Bioorg. Med. Chem.* **2012,** *20,* 3186–3195.
(11) Opferman, J.T. *Cell Death Differ.* **2008,** *15,* 234–242.
(12) (a) Adhikari, D.; Samanta, S.K.; Dutta, A.; Roy, A.; Vedasiromoni, J.R.; Sen, T. *Indian J. Pharmacol.* **2007,** *39,* 155–159; (b) Eberhard, M.; Ferlinz, K.; Alizzi, K.; Cacciato, P.M.; Faggio, C.; Föller, M.; Lang, F. *Cell. Physiol. Biochem.* **2010,** *26,* 761–766. (c) Zelenak, C.; Pasham, V.; Jilani, K.; Tripodi, P.M.; Rosaclerio, L.; Pathare, G.; Lupescu, A.; Faggio, C.; Qadri, S.M.; Lang, F. *Cell. Physiol. Biochem*. **2012,** *30,* 282–294.
(13) (a) Hoskins, C.; Wang, L.; Cheng, W.P.; Cuschieri, A. *Nanoscale Res. Lett.* **2012,** *7,* 77/1–77/12. (b) Grillo, C.A.; Reigosa, M.A.; de Mele, L.; Fernandez, M.A. *Contraception* **2010,** *81,* 343–349.
(14) Amin, K.; Dannenfelser, R.-M. *J. Pharm. Sci.-US* **2006,** *95,* 1173–1176.