SHORT COMMUNICATION

The reaction of cinnamaldehyde and cinnam(o)yl derivatives with thiols

Alessandro Autelitano\textsuperscript{a}, Alberto Minassi\textsuperscript{a}, Alberto Pagani\textsuperscript{a}, Orazio Taglialatela-Scafati\textsuperscript{b,*}, Giovanni Appendino\textsuperscript{a,†}

\textsuperscript{a}Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Largo Donegani 2, Novara 28100, Italy
\textsuperscript{b}Dipartimento di Farmacia, Università di Napoli Federico II, Via Montesano 49, Napoli 80131, Italy

Received 18 April 2017; revised 1 June 2017; accepted 2 June 2017

KEY WORDS
Cinnmaldehyde; Michael addition; Electrophiles; Conjugation; Cysteamine; Chalcones

Abstract Spurred by the alleged relevance of the thia-Michael reaction in the bioactivity of various classes of cinnam(o)yl natural products and by the development of a quick NMR assay to study this reaction, we have carried out a systematic study of the “native” reactivity of these compounds with dodecanethiol and cysteamine as models, respectively, of simple thiols and reactive protein thiols that can benefit from iminium ion catalysis in Michael reactions. Cinnamoyl esters and amides, as well as cinnamyl ketones and oximes, did not show any reactivity with the two probe thiols, while cinnamaldehyde (1a) reacted with cysteamine to afford a mixture of a thiazoline derivative and compounds of multiple addition, and with aliphatic thiols to give a single bis-dithioacetal (6). Chalcones and their vinylogous C5-curcuminoid derivatives were the only cinnamoyl derivatives that gave a thia-Michael reaction. From a mechanistic standpoint, loss of conjugation in the adduct might underlie the lack of a native Michael reactivity. This property is restored by the presence of another conjugating group on the carbonyl, as in chalcones and C5-curcuminoids. A critical mechanistic revision of the chemical and biomedical literature on cinnamaldehyde and related compounds seems therefore required.

© 2017 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Cinnamon (*Cinnamomum verum* J. S. Presl. and *C. aromaticum* Nees.) is one of the oldest spices, being already mentioned in the early Chinese medical treatises and in the old Sanskrit texts. The trade of cinnamon from India to the Mediterranean area is documented from the beginning of the Egyptian civilization, and cinnamon quills and cinnamon oil are nowadays extensively used in flavoring, perfumery, beverages, and medicines. The main constituent of cinnamon oil (up to over 80%) is cinnamaldehyde (*1a*, Fig. 1), a pleiotropic bioactive agent of current interest as anti-diabetic and antifungal agent. Cinnamaldehyde is a reactive compound, whose chemical “exuberance” is responsible not only for the many beneficial effects associated to cinnamon, but also for allergic reactions to cinnamon-containing perfumes, cosmetics, and sweets (cinnamon buns, cinnamon cereals and apple cakes). Severe allergic reactions of the skin and mucous membranes have also been observed in baking personnel and in workers processing cinnamon, and because of this allergic potential, the acceptable daily intake (ADI) of *1a* has been set at the rather low value of 1.25 mg/kg. Cinnamaldehyde is also extensively used in detergents and household cleaner, and is, in an industrial perspective, a high production volume (HPV) material, with an estimated consumption of 1000 metric tons per year.

The allergic reactions to cinnamaldehyde have been related to its Michael reactivity and its ability to form stable adducts with proteins. A similar mechanism has been proposed to explain the capacity of *1a* to activate TRPA1, the mustard oil receptor, by alkylation of the thiol-rich ankyrin moiety of this ion channel. There is little doubt that cinnamaldehyde is capable to react with thiol groups, and this compound has, indeed, been extensively used in biochemical studies as a thiol-active agent, just like iodoacetamide or phenylarsine oxide. However, the precise mechanism by which cinnamaldehyde traps thiols is unclear. The reaction of cinnamaldehyde with thiols has been considered the archetypal conjugated addition reaction that does not take place to any significant extent in the absence of secondary or primary amine, establishing itself as a benchmark reaction to evaluate the performance of organocatalysts. In accordance with this view, mutation studies with biological targets of *1a*, including TRPA1, have highlighted the relevance of the formation of an imine with an arginine residue as a prelude to the Michael addition. On the other hand, formation of hemithioacetals and not of Michael adducts was observed in the reaction of cinnamaldehyde and thiols in ionic liquids, and spontaneous Michael reaction with thiols was also highlighted. On the other hand, formation of unstable Michael adducts, quickly reverting to the starting enones, has also been reported.

Cinnamoyl derivatives might as well give Michael adducts under a judicious selection of catalysts, promoters, and pHs, but there is a surprising lack of information on the “native”, uncatalyzed reactivity of these compounds with thiols. Given the biomedical relevance of cinnamoyl derivatives in drug discovery and nutrition, we have investigated the behavior of this class of compounds with two probe thiols, the odorless dodecanethiol as a model of a simple thiol, and cysteamine as a model of reactive thiol in a protein that can benefit from imine catalysis.

2. Results and discussion

Cinnamaldehyde is an ambident electrophile, in principle capable to react with thiols both at the carbonyl and at the β-carbon. In practice, neither of these reactions is supposed to take place without Lewis acid catalysis (attack to the carbonyl and formation of the diithiocetal) or organocatalysis (preformation of an iminium ion followed by Michael addition). In terms of frontier molecular orbitals, these maneuvers decrease the LUMO energy and remodulate its coefficients, increasing its value on the carbonyl (ipso) carbon (Lewis acid catalysis) or on the β-carbon (organocatalysis). In accordance with this view, reaction of cinnamaldehyde with cysteamine in DMSO (cysteamine assay, Fig. 2) gave as a major compound the thiazoline adduct (Fig. 3), resulting from imine formation at the carbonyl group and Michael addition at the conjugated double bond. Several other compounds were also formed, resulting from the multidentate reactivity of cinnamaldehyde, and dilution with CDCl3 did not revert their formation. Compound *4a* (Fig. 3) was the result of the intermolecular version of the process generating the thiazoline, with one molecule of cysteamine (or its disulfide oxidation product) trapping the carbonyl and the other one the olefinic double bond, while compounds *5a* and *5b* (Fig. 3) resulted from the reaction of two molecules of cinnamaldehyde with three molecule of cysteamine. The formation of these compounds shows that cinnamaldehyde acts as a multiple trap for “active” thiol groups, being capable...
with cysteamine in DMSO, despite being claimed to be Michael chalcone with thiols, we also investigated the reaction of under the conditions of the assay. Tendency to reversion was observed, and the adducts were stable (with aliphatic thiols. Thiol adducts of chalcone have been reported quickly with cysteamine also in CDCl3, and were even reactive and (B’) Adducts from chalcones. (A) Adduct from a generic substrate; (B) and (B’) Adducts from chalcones.

Figure 4 Effect of resonance on the structure of the Michael adducts of cinnam(o)yl derivatives. (A) Adduct from a generic substrate; (B) and (B’) Adducts from chalcones.

cinnamaldehyde with dodecanethiol. Much to our surprise, we observed formation of the bis-dithioacetal (6, (Fig. 5) contaminated by its corresponding thiosemicarbazone when the two reagent were mixed in a 1:2 ratio in a variety of NMR solvents. The reaction has preparative value, and when carried out in CH2Cl2 with 4 equivalents of dodecanethiol, it afforded 6 in ca. 70% yield. No reaction occurred with dihydrocinnamaldehyde (7, (Fig. 5), in accordance with the textbook notion that formation of dithioacetals from aldehyde requires the presence of strong Lewis acids to activate the carbonyl group18. An ethyl group is less encumbered than an ethynyl, but the smooth reaction of cinnamaldehyde with thiols and the complete lack of reactivity of its dihydroderivative are difficult to explain on purely steric basis, and the observation is in sharp contrast to the notion that an α-unsaturation decreases the reactivity of a carbonyl toward nucleophilic addition. Since no reaction occurred when cinnamaldehyde was treated with alcohols, a possible explanation could be that conjugation to a phenyl reduces the hardness of the carbonyl to the point of making it possible for a weak and soft nucleophile like sulfur to attack it without any previous catalysis. On the other hand, no reaction occurred with simple amines like 2-phenylethylamine.

Taken together, the results of this comparative study show that cinnamyl derivatives can trap thiols in a Michael fashion only when loss of conjugation is compensated by the presence of another phenyl ring bound to the carbonyl, so that there is no overall loss of conjugation associated to the reaction. Cinnamaldehyde itself can trap thiols only after imine formation with cysteamine, or, with simple thiols, by attacking to the carbonyl. While our observations do not dismiss the potential for a mainstream Michael addition to take place with the other cinnam(o)yl derivatives under cellular conditions, they suggest that this would require a special chemical milieu or a special promotion/catalysis to take place.

3. Experimental

3.1. General experimental procedures

1H NMR (500 MHz) and 13C NMR (125 MHz) spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal (CDCl3: δH = 7.26, δC = 77.0; DMSO-d6: δH = 2.50). Low- and high-resolution ESI-MS spectra were obtained on an LTQ OrbitrapXL (Thermo Scientific) mass spectrometer. All compounds investigated are commercial (Aldrich) except the oxime 2a16, its acetyl derivative 2b16 and the cinnamamide 1d18 that were prepared according to literature.

3.2. Cysteamine assay

In an NMR tube, an exact amount of substrate (ca. 5 mg) was dissolved in 500 μL dry DMSO-d6, and the 1H NMR spectrum was recorded. Two equivalents of cysteamine or dodecanethiol were then added, and the spectrum was immediately recorded, with acquisition finishing within 5 min from the addition. The
reaction was typically monitored by observing the changes in the olefin region of the spectrum. To test the reversibility of the addition, the sample was diluted to 1:10 with CDCl₃, and the spectrum was recorded again. Reversion was evaluated by comparing this spectrum with an original CDCl₃ spectrum of the product under investigation.

### 3.3. Chemistry

#### 3.3.1. 7-Phenyl-2,3,6,7-tetrahydro-1,4-thiazepine (3)

1H NMR (CDCl₃): δ 7.30–7.20 (6H, m), 3.80 (1H, m), 2.92 (1H, m), 2.73 (1H, m), 2.53 (2H, m), 1.75 (2H, m). ESI-MS: m/z 192 [M + H]⁺; HR-ESI-MS: m/z 192.0842; Calcd. for C₁₉H₁₉NS₂ 192.0847.

#### 3.3.2. E-2-(3-(Aminooethyl)thio)-3-phenylpropylidene)amino) ethane-1-thiol (4a)

1H NMR (CDCl₃): δ 8.10 (1H, d, J = 6.5 Hz), 7.55 (2H, d, J = 6.5 Hz), 7.38–7.20 (8H, m), 7.15 (1H, d, J = 15.5 Hz), 6.85 (1H, dd, J = 15.5, 6.5 Hz), 4.45 (1H, m), 3.70 (1H, m), 3.0 (4H, m), 2.65–2.55 (8H, overlapped), 1.75 (2H, m). ESI-MS: m/z 460 [M + H]⁺; HR-ESI-MS: m/z 460.1911; Calcd. for C₁₂H₂₁N₂S₂ 460.1914. The disulfide 4b was only detected by ESI-MS: m/z 349 [M + H]⁺.

#### 3.3.3. Compounds 5a/5b

1H NMR (CDCl₃): δ 8.10 (1H, d, J = 6.5 Hz), 7.55 (2H, d, J = 6.5 Hz), 7.38–7.20 (8H, m), 7.15 (1H, d, J = 15.5 Hz), 6.85 (1H, dd, J = 15.5, 6.5 Hz), 4.45 (1H, m), 3.70 (1H, m), 3.0 (4H, m), 2.65–2.55 (8H, overlapped), 1.75 (2H, m). ESI-MS: m/z 460 [M + H]⁺; HR-ESI-MS: m/z 460.1911; Calcd. for C₁₂H₂₁N₂S₂ 460.1915.

#### 3.3.4. Reaction of cinnamaldehyde with dodecanethiol

To a stirred solution of 1a (330 mg, 2 mmol) in CHCl₃ (5 mL), dodecanethiol (1.610 g, 8 mmol, 4 mol equiv.) was added. After stirring overnight at room temperature, the reaction was worked up by washing with 2% NaOH to remove the excess dodecanethiol and then with brine. The residue was purified by gravity column chromatography on silica gel (petroleum ether) to afford 832 mg 6 (80%) as a colorless and odorless oil.

#### 3.3.5. E-(3-Phenylprop-2-ene-1,1-diyl)bis(dodecylsulfane) (6)

IR νmax (KBr): 1645, 1620, 1480, 1290, 987, 896 cm⁻¹.

1H NMR (CDCl₃): 7.35 (5H, m), 6.54 (1H, d, J = 15.6 Hz), 6.18 (1H, dd, J = 15.6, 9.5 Hz), 4.45 (1H, d, J = 9.5 Hz), 2.60 (4H, m), 1.60 (4H, m), 1.52 (4H, m), 1.30 (32H, m), 0.85 (6H, t, J = 6.7 Hz). ESI-MS: m/z 519 [M + H]⁺; HR-ESI-MS: m/z 519.4049; Calcd. for C₃₈H₇₅N₂S₂ 519.4058.

### References

1. Gray EW, Miller JL. The spice trade of the Roman Empire 29 B.C.—A.D. 641. J Rom Stud 1970;60:222–4.
2. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Altern Med 2014;2014:2014:642942.
3. Cocchiara J, Letizia CS, Laiko J, Lapičanský A, Api AM. Fragrance material review on cinnamaldehyde. Food Chem Toxicol 2005;43:967–923.
4. Council of Europe. Natural flavouring substances, their sources and added artifical flavouring substances. In: Matieres aromatisantes naturelles, leurs sources, et matieres aromatisantes artificielles ajoutees. Strasbourg, France: Council of Europe; 1974. p.145.
5. Kumar H, Kim IS, More SV, Kim BW, Choi DK. Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic diseases. Nat Prod Rep 2014;31:109–39.
6. Kuipers DP, Scripture JP, Gunnink SM, Salie MJ, Schotanus MP, Ubels JL, et al. Differential regulation of GLUT1 activity in human corneal limbal epithelial cells and fibroblasts. Biochimie 2013;95:258–63.
7. Marigo M, Schulte T, Franžen J, Jørgensen KA. Asymmetric multi-component domino reactions and highly enantioselective conjugated addition of thiols to α,β-unsaturated aldehydes. J Am Chem Soc 2005;127:15710–1.
8. Blanco V, Carlone A, Hänni KD, Leigh DA, Lewandowski B. A rotaxane-based switchable organocatalyst. Angew Chem Int Ed 2012;51:5166–9.
9. Buey RM, Calvo E, Barasoaín I, Pineda O, Edler MC, Matesanz R, et al. Cyclosporin binds covalently to microtubule proteins and heme taxoid binding sites. Nat Chem Biol 2007;3:117–25.
10. Yadav JS, Reddy BV, Kondaji G. Eco-friendly and highly chemoselective 1,3-oxathio- and 1,3-dithioacetalization of aldehydes using ionic liquids. Org Biomol Chem 2003;1:15710–15715.
11. Sirotanovic KD, Bajon-Pastor MM. Addition of mercaptans to unsaturated aldehydes. II. Addition of ethylmercaptan, amylmercaptan, and benzymercaptan to unsaturated aromatic aldehydes. Glas Ham Drahtova Beogr 1966;31:329–37.
12. Gersch M, Kreuzer J, Sieber SA. Electrophilic natural products and their biological targets. Nat Prod Rep 2012;29:659–82.
13. Amslinger S, Al-Rifai N, Winter K, Wörmann K, Scholz R, Baumeister P, et al. Reactivity assessment of chalcones by a kinetic thiol assay. Org Biomol Chem 2013;11:549–54.
14. Zhu R, Liu H, Liu C, Wang L, Ma R, Chen B, et al. Cinnamaldehyde in diabetes: a review of pharmacology, pharmacokinetics and safety. Pharamcol Res 2017;122:78–89.
15. Avonto C, Tagliatela-Scafati O, Pollastro F, Minassi A, Di Marzo V, De Petrocellis L, et al. An NMR spectroscopic method to identify and classify thiol-trapping agents: revival of Michael acceptors for drug discovery?. Angew Chem Int Ed 2011;50:467–71.
16. DeFalco J, Steiger D, Gustafson A, Emerling DE, Kelly MG, Duncton MA. Oxime derivatives related to AP18: agonists and antagonists of the TRPA1 receptor. Bioorg Med Chem Lett 2010;20:276–9.
17. Allen CF, Fournier JO, Humphlett WJ. The thermal reversibility of the Michael reaction: IV. Thiol adducts. Can J Chem 1964;42:2616–20.
18. Smith MB, March J. In: March’s advanced organic chemistry: reactions, mechanisms, and structure. 6th ed. Hoboken: Wiley; 2007. p. 1227–80.
19. Gowda RR, Chakraborty D. Fe⁺⁺-catalyzed synthesis of primary amides from aldehydes. Eur J Org Chem 2011;2011:2226–9.