



# Cinnamaldehydes: Synthesis, antibacterial evaluation, and the effect of molecular structure on antibacterial activity

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## ABSTRACT

*trans*-Cinnamaldehyde is a major component of *Cinnamomum cassia* and has been reported to give rise to antimicrobial properties in cinnamon spice. In order to better understand the relationship between the structure of *trans*-cinnamaldehyde and its antimicrobial role, fifteen structurally different *trans*-cinnamaldehyde derivatives were selected for study based on their predicted electrophilicities. Both synthesized and commercial *trans*-cinnamaldehyde derivatives were evaluated for their antibacterial activity, with modest mM activity levels found against both *E. coli* and *S. aureus*. An initial mechanism of action study, suggesting that the electrophilicity of the *trans*-cinnamaldehydes affects the antibacterial activity, was conducted as well as an exploration of their ability to alter bacterial cell wall integrity. In addition, *in vivo* toxicity levels were determined using the larvae of the greater wax moth, *Galleria mellonella*, with all derivatives tested showing low toxicity.

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## 1. Introduction

The use of natural substances as food additives, rather than synthetic substances or traditional preservation techniques, has gained consumer popularity in recent years due to perceived nutritional and safety qualities [1–3]. *trans*-Cinnamaldehyde (TC) (Fig. 1, structure 1) is a major component of *Cinnamomum cassia* and gives rise to much of the reported antimicrobial properties of this spice [4]. Isolated TC has been shown to effectively inhibit the growth of an array of microorganisms such as bacteria, moulds, and yeasts [2–5]. In human dermatological studies, the No-Observed-Adverse-Effect-Level (NOAEL) for cinnamaldehyde sensitization has been set at 0.5% [6], with the mechanism of skin sensitization of cinnamaldehyde having been attributed to its Michael acceptor properties [7]. However, there are no restrictions on the use of TC as a flavour additive [7], and is Generally Recognized as Safe (GRAS) by the FDA [8]. Related cinnamaldehyde derivatives have been reported as having potential to be cost-effective, food-compatible, broad-spectrum antimicrobial additives that could be used against an array of pathogenic micro-organisms [8]. Other reports describe their potential as antitumour, anticancer, and anti-

inflammatory agents [6,9–15]. We are interested in the study of antimicrobial agents and microbial metabolite biosynthesis [16–19], with a particular focus in naturally occurring compounds and their derivatives with respect to their antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) [20]. Staphylococci can be found in commercial food products of animal origin or those that are handled by humans [21], is attributed to medically-relevant biofilm formation [22,23], and can also develop multidrug resistance [22,24]. *E. coli* is also a major food-borne pathogen, whose sources are extensive [25], and where infection can lead to severe symptoms and may even be followed by life-threatening complications [26].

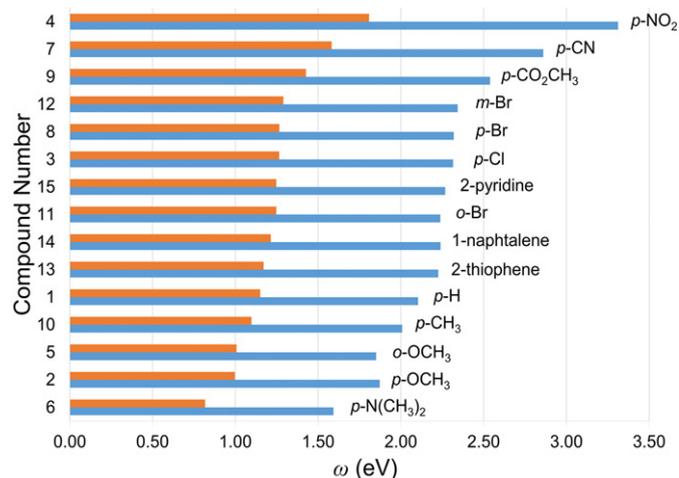
## 2. Results and discussion

### 2.1. Synthesis

TC has been described in the literature as both an antimicrobial agent and as a Michael acceptor [2–5,8,10,27–34], although, to the best of our knowledge, a systematic study exploring how chemical structure and electrophilicity relates to antimicrobial activity has not been reported. Herein, we report the synthesis, antibacterial evaluation, and an initial mechanism of action study for a family of *trans*-cinnamaldehydes. The *trans*-cinnamaldehydes were selected based on their predicted electrophilicities in order to better understand how

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**Fig. 1.** Global electrophilicities for various cinnamaldehyde derivatives computed by the IP/EA method (orange bars) or by frontier orbital method (blue bars).

this might affect their antimicrobial activities. Several synthetic methods have been reported for the generation of cinnamaldehydes, including oxidative enamine catalysis [35,36], the use of a palladium-catalyzed Heck-Saegusa reaction [37], and Dess–Martin oxidation of alcohols [38]. For our study, the majority of the 15 cinnamaldehydes were synthesized, and in all but one case this was achieved using a modified Wittig procedure, Scheme 1 and Scheme 2 [39]. The remaining cinnamaldehydes were purchased from commercial sources.

When the Wittig procedure was applied to the synthesis of *p*-methylcinnamaldehyde (Fig. 1, structure 10), a complex mixture resulted. An alternative synthetic strategy was therefore required, Scheme 2 [40,41]. This involved Fischer esterification of the starting carboxylic acid, followed by reduction using DIBAL-H. The final oxidation by manganese dioxide furnished the desired *p*-methylcinnamaldehyde in an overall yield of 79% over the three steps.

## 2.2. Calculated electrophilicities

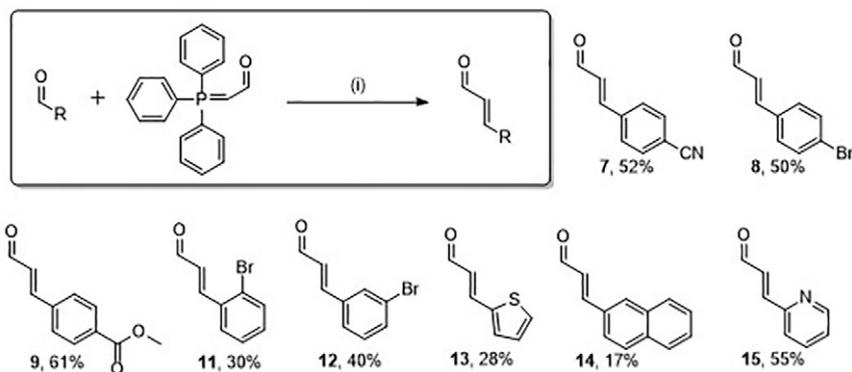
The quantification of electrophilic and nucleophilic organic processes by means of electronic structure calculations has been greatly facilitated by the introduction of global and local reactivity scales. The global electrophilicity index  $\omega$  introduced by Parr et al. has been extensively employed on a wide range of organic compounds, including dienes, dienophiles [42], carbenes [43],  $\alpha,\beta$ -unsaturated carbonyls [44], and reactants involved in 1,3-dipolar cycloadditions [45], returning information about their reactivity towards nucleophiles and expected regio- and chemoselectivities. The global electrophilicity index  $\omega$

provides a measure for the ability of a molecule to accept electrons from its environment. Information about the intramolecular selectivity within the same molecule is obtained by invoking a local electrophilicity index  $\omega_{\text{R}}^+$ , which allows for identification of the most electrophilic site in a molecule. The global electrophilicity indices for the *trans*-cinnamaldehyde derivatives considered in this study were evaluated in terms of the frontier orbital energies and also the IPs/EAs at the ground state of the molecules using the B3LYP/6-31G(d,p) level of theory (see ESI). Qualitatively similar results were obtained with either approach (Fig. 1). The ranking of the molecules according to their electrophilicity (top to bottom) can be rationalized in terms of substituent effects induced by electron-withdrawing and electron-donating substituents that result in electrophilic activation or deactivation of, respectively. Taking TC as the reference, the introduction of electron-donating groups such as ( $-\text{OCH}_3$ ,  $-\text{CH}_3$ ,  $-\text{N}(\text{CH}_3)_2$ ) (Fig. 1, structures 2, 5, 10 and 6) into the *para*- or *ortho*-position of the phenyl ring causes  $\omega$  and therefore the electrophilicity to decrease. In contrast, halide or electron-withdrawing residues attached to the phenyl ring cause moderate ( $-\text{Br}$ ,  $-\text{Cl}$ ) (Fig. 1, structures 8, 11, 12 and 3) or large ( $-\text{NO}_2$ ,  $-\text{CN}$ ,  $-\text{CO}_2\text{CH}_3$ ) (Fig. 1, structures 4, 7 and 9) electrophilic activation, as seen from the increase in  $\omega$ . For the series of halide derivatives the exact location of the  $-\text{X}$  substituent at either the *o*-, *p*- or *m*-position of the phenyl ring only has a very small influence on the degree of activation, and these compounds share very similar  $\omega$  values. Replacement of the phenyl ring in the parent TC compound by polycyclic or heterocyclic substituents leads to relatively moderate electrophilic activation in all cases.

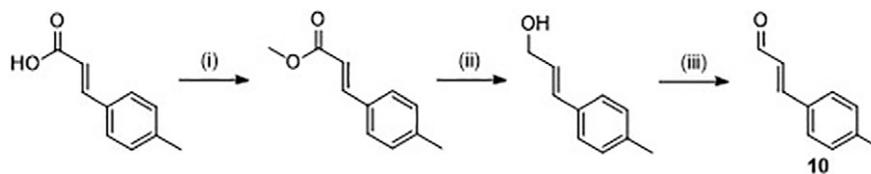
## 2.3. Biological evaluation

TC and the cinnamaldehyde derivatives were assessed for their *in vitro* antibacterial activity using a standard broth microdilution method (see ESI) [46]. The bacterial strains tested were *S. aureus* and *E. coli* (both clinical isolates, see ESI). The antibacterial results have been separated into three tables. Table 1: *para*-substituted *trans*-cinnamaldehydes; Table 2: where the importance of substituent position on the phenyl ring is explored; Table 3: where the affect replacement of the phenyl ring with another hetero- or carbocycle is explored.

Although the activity levels are modest (most active derivative giving an MIC<sub>50</sub> of ~0.5 mM), one can see a relationship in Table 1 for *S. aureus*, where all *trans*-cinnamaldehydes that are more electrophilic than TC are also more biologically active. For example, *p*-bromocinnamaldehyde (Fig. 1, structure 8) is nearly five times more active than TC, with the less electrophilic *p*-methoxycinnamaldehyde (Fig. 1, structure 2) almost 17 times less active than TC, and *p*-dimethylaminocinnamaldehyde (Fig. 1, structure 6) showing no antibacterial activity. However, the most electrophilic derivatives *p*-nitro (Fig. 1, structure 4) and *p*-cyanocinnamaldehyde (Fig. 1, structure 7), while more active than TC, were not the most active derivatives tested. This may be due to differences in their cellular uptake or other



**Scheme 1.** (i) Arylaldehyde (0.628 mmol, 1 eq.), (triphenylphosphoranyliden) acetaldehyde (0.690 mmol, 1.1 eq.), toluene (5 mL), 80 °C, 24 h.



**Scheme 2.** (i)  $\text{H}_2\text{SO}_4$ , MeOH, 4 h, reflux (ii) DIBAL-H, toluene, 0 °C, (iii)  $\text{MnO}_2$ , DCM, 35 °C, 7 h.

cellular processes. A similar overall effect can be seen in *E.coli*, where *p*-bromocinnamaldehyde again proved to be the most active derivative, although here both *p*-Cl (Fig. 1, structure 3) and *p*- $\text{CO}_2\text{CH}_3$  (Fig. 1, structure 9) are less active than TC even though they are more electrophilic. We suggest that differences in activity for the *p*-Cl versus the *p*-Br derivatives may be as a result of differences in cellular uptake. *E.coli* is Gram-negative, where the bacterial cell wall is more complex than that of *S. aureus*, and consists of a thin layer of peptidoglycan that is surrounded by an outer membrane [29]. *S. aureus* is a Gram-positive bacteria where the cell wall consists largely of peptidoglycan and more easily facilitates the access of hydrophobic molecules to both the cell wall and the cytoplasm within [29].

Table 2 examines the position of the substituent on the phenyl ring. While *p*-bromocinnamaldehyde and *o*-bromocinnamaldehyde (Fig. 1, structure 11) have very similar electronic properties, the bacterial inhibition observed varied greatly, with *o*-bromocinnamaldehyde proving to be 10 times less active than the *para* derivative. Although a limited number of *ortho* and *meta* substituted derivatives were studied, this result suggests that sterics may also play an important role. The associated steric bulk of the bromo substituent at the *ortho*-position could be expected to block the electrophilic  $\beta$ -carbon site, preventing nucleophilic attack and thus hindering the reactivity of the cinnamaldehyde. Thus, the observed antimicrobial activity against both *S. aureus* and *E. coli*, with respect to the bromo-substituted cinnamaldehydes, decreased from *para*- to *meta*- (Fig. 1, structure 12) to *ortho*-substituted, Table 2, as steric hindrance increased. This same steric effect is observed with respect to *p*-methoxycinnamaldehyde, where the *o*-methoxycinnamaldehyde (Fig. 1, structure 5) was less active against both *S. aureus* and *E. coli*, Table 2.

To further probe the ability of TC derivatives to act as electrophilic agents, we utilized a cysteamine based NMR assay system to identify thiol-trapping agents and drugs, as described by Appendino and co-workers [47]. The same group also reported using this assay system to

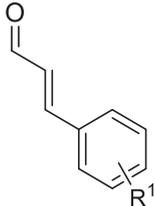
establish TC as a thiol-trapping agent, where TC reacted with cysteamine at the  $\beta$  carbon and at the carbonyl carbon [48]. In our hands, employment of the cysteamine assay system on TC produced the same results to that obtained by the Appendino group, i.e. complete consumption of TC in under 5 min at room temperature. Its application to *p*-bromocinnamaldehyde and *p*-nitrocinnamaldehyde also showed complete consumption of the cinnamaldehyde.

The effect of replacement of the phenyl ring with an alternative hetero- or carbocycle was also examined, where the new derivatives were shown to be much less active against *S. aureus* than those derivatives with a phenyl ring, Table 3. Here, only the 2-pyridine derivative (Fig. 1, structure 15) exhibited superior activity than TC, in that case against *E. coli*.

An in vivo toxicity study was carried out using the larvae of the greater wax moth, *Galleria mellonella* (*G. mellonella*), as described by Rowan et al. (Fig. S33 in ESI) [49]. The larvae of *G. mellonella*, have been used as an in vivo model in a number of studies to investigate the virulence of human pathogens, due to the similarities between the innate immune system of insects and mammals [50,51]. *G. mellonella* larvae have also been used in studies that evaluated the therapeutic effect of current and novel antimicrobial agents, as well as the in vivo tolerance of novel antimicrobial agents [52–53,54]. Three test concentrations (1 mM, 10 mM, 25 mM) were used for each of TC, *p*-bromocinnamaldehyde, *m*-bromocinnamaldehyde, and *o*-bromocinnamaldehyde. After injection, the larvae were incubated at 37 °C, for three days, and monitored for survival and melanisation at 24-hour intervals. High survival rates were observed in all cases (see Table S2a in the ESI), indicating the low toxicity of TC and the bromo derivatives, and that the enhanced activity of *p*-bromocinnamaldehyde relative to TC is not due to a toxic effect.

Multiple mechanisms of action have been reported for TC in the literature [60], including binding to FtsZ [55], lowering the uptake or use of glucose [23], and altering bacterial cell membrane integrity and

**Table 1**  
MIC<sub>50</sub> (mM) against *S. aureus* and *E.coli*, and calculated global electrophilicities.



Structure number	R <sup>1</sup>	<i>S. aureus</i> MIC <sub>50</sub> (mM)	<i>E. coli</i> MIC <sub>50</sub> (mM)	$\omega$ (eV) <sup>a</sup>	$\omega$ (eV) <sup>b</sup>
8	<i>p</i> -Br	0.51	0.511	2.32	1.27
7	<i>p</i> -CN	0.938	0.970	2.86	1.58
9	<i>p</i> - $\text{CO}_2\text{CH}_3$	1.75	2.05	2.54	1.43
3	<i>p</i> -Cl	1.83	3.16	2.32	1.26
4	<i>p</i> -NO <sub>2</sub>	1.84	1.18	3.31	1.81
1	H	2.32	1.93	2.10	1.15
10	<i>p</i> -CH <sub>3</sub>	3.14	3.31	2.01	1.10
2	<i>p</i> -OCH <sub>3</sub>	8.57	4.86	1.87	1.00
6	<i>p</i> -N(CH <sub>3</sub> ) <sub>2</sub>	No inhibition observed	No inhibition above 12%	1.59	0.82

Commercial antibiotics ampicillin trihydrate, tetracycline and streptomycin sulfate were used as controls (see ESI). MIC<sub>50</sub> in mg/mL are given in the ESI.

<sup>a</sup> Global Electrophilicity Index calculated via  $\epsilon_{\text{HOMO}}$  and  $\epsilon_{\text{LUMO}}$ .

<sup>b</sup> Global Electrophilicity Index calculated via IP and EA.

**Table 2**  
MIC<sub>50</sub> (mM) against *S. aureus* and *E. coli*, and calculated global electrophilicities.

Structure number	R <sup>1</sup>	<i>S. aureus</i> MIC <sub>50</sub> (mM)	<i>E. coli</i> MIC <sub>50</sub> (mM)	$\omega$ (eV) <sup>a</sup>	$\omega$ (eV) <sup>b</sup>
8	<i>p</i> -Br	0.51	0.511	2.32	1.27
12	<i>m</i> -Br	0.57	1.51	2.34	1.29
11	<i>o</i> -Br	6.59	5.64	2.24	1.25
2	<i>p</i> -OCH <sub>3</sub>	8.57	4.86	1.87	1.00
5	<i>o</i> -OCH <sub>3</sub>	9.63	7.26	1.85	1.01

MIC<sub>50</sub> in mg/mL are given in the ESI.

<sup>a</sup> Global Electrophilicity Index calculated via  $\epsilon_{\text{HOMO}}$  and  $\epsilon_{\text{LUMO}}$ .

<sup>b</sup> Global Electrophilicity Index calculated via IP and EA.

permeability [56]. On the basis that TC has an established ability to permeabilize bacterial cell membranes [56], this mechanism of action was probed further and expanded to include *p*-bromocinnamaldehyde (most active derivative) and *p*-methoxycinnamaldehyde (one of the least active derivatives). The effect of the derivatives on the cell wall/membrane after being added to a *S. aureus* or *E. coli* culture, was monitored after 2-, 4- and 6-hours incubation, with results expressed in terms of protein and amino acid leakage (see Figs. S31 & S32 in ESI). Much higher leakage was observed, in terms of both proteins and amino acids, for the Gram-negative *E. coli* compared to the Gram-positive *S. aureus* (Figs. S31 & S32 in ESI), something which has been noted previously in the literature [57]. This leaked material may be composed of constituents of the membrane itself or cell wall-related material. Proteomic analysis would be required to identify the leaked material and determine the extent to which the bacteria cell is disrupted. The amount of leakage observed was very different across the three cinnamaldehydes examined. For both bacteria, TC caused the highest leakage of amino acids and proteins. It is interesting that while *p*-bromocinnamaldehyde exhibited the greatest inhibition of all fifteen cinnamaldehydes evaluated it did not correlate to an ability to induce leakage, generating less leakage than TC. This may indicate that while this compound is capable of interacting with the cell wall of both bacteria it is not the primary mechanism of action. These observations coincide with previous reports regarding the multiple possible modes of action of TC [5,29,58].

### 3. Conclusion

In conclusion, a range of *trans*-cinnamaldehydes were synthesized and their antibacterial activity against *S. aureus* and *E. coli* assessed and compared to naturally occurring antibacterial *trans*-cinnamaldehyde. The cinnamaldehydes generated only low mM levels of activity, with the *p*-Br phenyl derivative showing a 4.5 fold increase in activity compared to that of the parent *trans*-cinnamaldehyde. However, based on the cinnamaldehyde structures evaluated in this study, a relationship could be observed between electrophilicity and

bacteriostatic activity, where *trans*-cinnamaldehydes that are more electrophilic than TC tend to be more biologically active. Calculated electrophilicity values for the cinnamaldehyde derivatives were reported and the use of a known NMR spectroscopic assay for the identification of thiol trapping drug molecules was also described. The positive results from this assay, along with the relationship between electrophilicity and antibacterial activity, gave some further insight into a possible antimicrobial mechanism of action for *trans*-cinnamaldehydes at a molecular level, where cinnamaldehydes could act as electrophilic species. Some *trans*-cinnamaldehyde derivatives were also assessed for their ability to permeabilize the bacterial cell wall in vitro, which suggested that while some compounds were capable of interacting with the cell wall of both bacteria, it is not the primary mechanism of action of the cinnamaldehyde derivatives. Future work would entail identifying some of the proteins released, as well as the origin of these proteins [59]. The in vivo toxicity of some derivatives were also evaluated using the larvae of the greater wax moth, *Galleria mellonella*, with all derivatives tested showing low toxicity levels.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data (procedures, NMR spectra, HPLC chromatograms) associated with this letter can be found online, at <https://doi.org/10.1016/j.rechem.2019.100013>.

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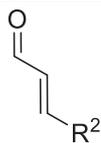
**Table 3**  
MIC<sub>50</sub> (mM) against *S. aureus* and *E. coli*, and calculated global electrophilicities.

Structure number	R <sup>2</sup>	<i>S. aureus</i> MIC <sub>50</sub> (mM)	<i>E. coli</i> MIC <sub>50</sub> (mM)	$\omega$ (eV) <sup>a</sup>	$\omega$ (eV) <sup>b</sup>
14	1-Napthalene	3.55	9.88	2.24	1.21
15	2-Pyridine	No inhibition above 49.5%	1.12	2.27	1.25
13	2-Thiophene	No inhibition above 25.1%	8.57	2.23	1.17

MIC<sub>50</sub> in mg/mL are given in the ESI.

<sup>a</sup> Global Electrophilicity Index calculated via  $\epsilon_{\text{HOMO}}$  and  $\epsilon_{\text{LUMO}}$ .

<sup>b</sup> Global Electrophilicity Index calculated via IP and EA.



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