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Synthesis and 5-lipoxygenase inhibitory activity of new cinnamoyl and caffeoylclusters

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ABSTRACT

Novel cinnamoyl and caffeoyl clusters were synthesized by multiple Cu(I)-catalyzed [1,3]-dipolar cycloadditions and their anti-5-lipoxygenase inhibitory activity was tested. Caffeoyl cluster showed an improved 5-lipoxygenase inhibitory activity compared to caffeic acid, with caffeoyl trimer **16** and tetramer **19** showing the best 5-lipoxygenase inhibitory activity.

5-Lipoxygenase (5-LO) is the key enzyme in the metabolism of arachidonic acid (AA) to leukotriene A4 (LTA4).[1] Further metabolism of LTA4 produces LTB4, a potent chemotactic agent for leukocytes that is thought to be a key component in a variety of diseases,[2] including inflammatory bowel disease and atherosclerosis. LTA_4 can also be converted to the peptidoleukotrienes LTC4, LTD4, and LTE4,[3] which are implicated in allergic hyper reactivity disorders such as asthma.[2b] Elevated levels of these LTs, associated with several inflammatory and allergic disorders, have been found in various pathologic tissues.[1]

In search of pharmacological strategies that intervene with LTs, a large number of different types of low molecular weight inhibitors that potently suppress LTs synthesis have been developed in the past 20 years.[4] Among the known inhibitors of the 5-LO are a variety of polyhydroxylated natural products such as caffeic acid,[5] and flavonoids.[6] The rational design of efficient inhibitors requires perfect knowledge about the target itself. In the case of 5-LO, much of our knowledge is based upon studies on soybean lipoxygenase.[7]

Caffeic and cinnamic acid derivatives are widely distributed in plants and exhibit a broad spectrum of biological activities including anti-oxidative,[8] anti-inflammatory,[9] antiviral,[10] and anti-cancer effects.[11]

Several modified caffeic acid amides were recently demonstrated for anti-lipoxidation and exhibited more stable characteristics.[12] Furthermore, some caffeic acid amide analogs, such as *N*-caffeoyl-*β*-phenethylamine were reported to have inhibitory effects on prostaglandin H synthase and have potential for the inhibition of 5-LO.[13] However, despite the large number of therapeutic indications and the strong need for efficient and safe drugs that target the 5-LO pathway, zileuton is the only 5-LO inhibitor marketed for the therapy of human subjects.[1]

Multivalent interactions have several advantages over monomeric ones and are often used by nature to control a wide variety of cellular processes.[14] Consequently, multivalent arrangements of ligands generally favor the formation of physiologically relevant associations.[14]

Thus, the development of novel multivalent macromolecules or dendrimers bearing caffeoyl or cinnamoyl moieties that inhibit 5-LO is an important challenge. To the best of our knowledge, clusters having peripheral covalently-bound caffeoyl or cinnamoyl units have not been described. Such inhibitors may provide therapeutic benefit for the pharmacological treatment of inflammatory and allergic disorders, cardiovascular diseases, and cancer.

Here we present the design and synthesis of novel caffeoyl clusters that proved to be potent inhibitors of 5-LO. Since the hydroxyl groups within the caffeic acid catechol moiety may also play an important role in the inhibitory activity, we also examined the effect of the presence of these groups by the synthesis of the cinnamoyl cluster analogs. A simple approach to the synthesis of multivalent caffeoyl- or cinnamoyl-bearing clusters is the attachment of acid moieties to structurally simple hyper-branched molecules. Taking advantage of the click ligation technique, we were able to use terminal alkyne- or azide-functionalized cores to introduce caffeoyl or cinnamoyl units into dendrimers in a "clicked" divergent fashion.

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Click chemistry has attracted much attention recently because of its high specificity, quantitative yield, and tolerance to various groups. Several reports have described the synthesis of clusters or dendrimers through the click reaction in either a convergent or a divergent manner.[15] The synthetic route to the mono and bivalent derivatives is outlined in Scheme 1. The novel propargyl **1**, **3** and azido **2**, **4** esters and were prepared from propargyl alcohol or azidoethanol[16] with cinnamic or acetylated caffeic acid in pyridine in quantitative yields. Using the combination of caffeic/cinnamic propargyl esters **1**, **3** and the azido analogs **2**, **4** as starting materials, we carried out the 1,3-dipolar cycloaddition[15] under standards conditions ($CuSO_4$, ascorbic acid, THF: H_2O, rt) to give, after hydrolysis, the corresponding triazole linked derivatives **5**, **7**, **9**, and **11** in 69%, 74%, 78%, and 82% yields, respectively (Scheme 1).

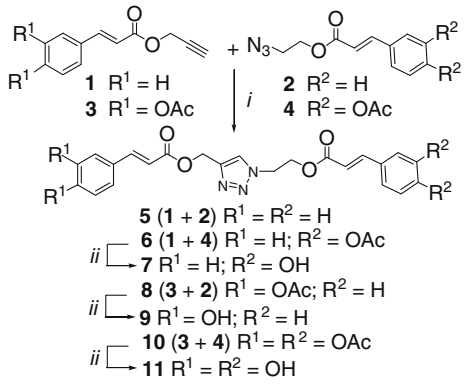
The pentaerythrityl core acts as a versatile building block that has been employed in dendrimer syntheses. To the best of our knowledge, no pentaerythritol-based cinnamoyl or caffeoyl clusters have been reported. Using pentaerythritol-based azide platforms functionalized with three **12** and four **13** azide groups,[17] click chemistry provided, as expected, tris and tetrameric clusters **14**, **15**, **17**, and **18**[18] according to Scheme 2. Finally, complete hydrolysis of the acetyl ester protecting groups in **15** and **18** to afford **16** and **19**[18] was accomplished under catalytic trans-esterification conditions using K_2CO_3 in CH_2Cl_2: MeOH (**16**: 69%, **19**: 72%).

A retrosynthetic analysis reveals two possibilities for the synthesis of new clusters bearing 1,2,3-triazole linkages, the azide or the alkyne functions can be located on either the pentaerythritol core or on the cinnamoyl or the caffeoyl moieties. According to Scheme 3, nitrogen linked caffeic and cinnamic moieties were obtained this time with the tetra alkyne **20**[19] and the corresponding azide **2** or **4** under the same click reaction conditions. Thus, acetyl hydrolysis (K_2CO_3, CH_2Cl_2: MeOH) provided the extended cluster analogs.

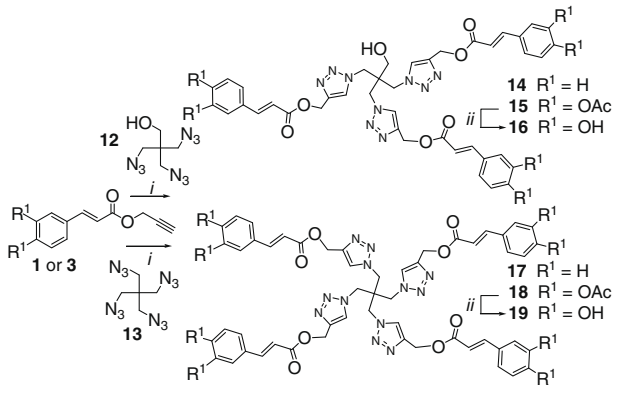
For the next higher member of the family, the same synthetic strategy was used. Under the same conditions described above, cinnamoyl and caffeoyl hexamers could be obtained starting from the hexa-azide **24**[15,20] and the corresponding alkynes **1** and **3**. (Scheme 4).

In all cases, analysis of the ^1H, ^{13}C NMR spectra of our clusters revealed calculated integrations for the triazole protons respective to the vinylic protons and complete disappearance of the acetylenic signals, thus confirming, together with MS and IR data, completion of the multivalent Cu(I)-catalyzed azide–alkyne cycloadditions.

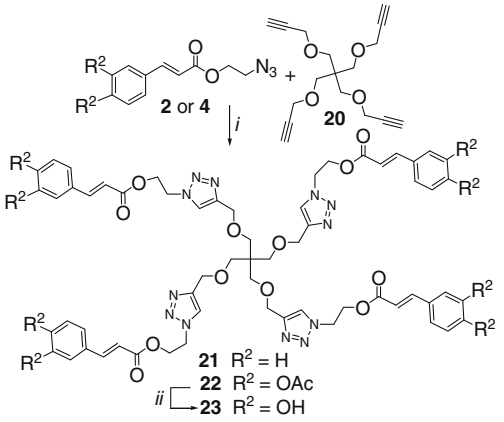
The unprecedented clusters prepared in this study displayed some structural differences that are governed by the pentaerythritol and bis-pentaerythritol scaffolds as well as by the number of cinnamoyl/caffeoyl units and their relative orientation. These disparities offer a unique opportunity to estimate their 5-LO inhibitory activities. The inhibitory activity of our compounds, with valencies ranging from 1 to 6 U, was measured in cell lysates of HEK 293 cells stably transfected with a pcDNA3.1 vector express-



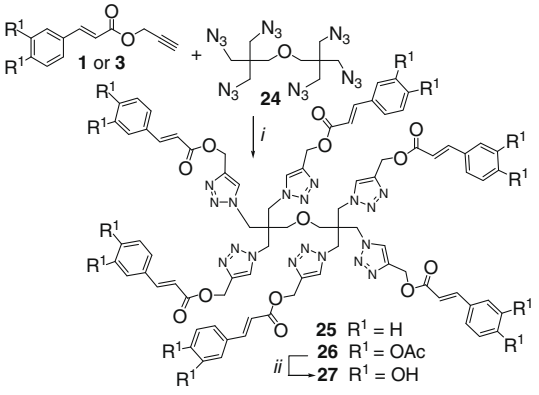
Scheme 1. Reagents and conditions: (i) $CuSO_4$, ascorbic acid, THF: H_2O, rt, 12 h, **5** (69%), **6** (80%), **8** (76%), **10** (78%); (ii) K_2CO_3 in CH_2Cl_2/MeOH (1:1, v/v), rt, 4 h, **7** (74%), **9** (78%), **11** (82%).



Scheme 2. Reagents and conditions: (i) $CuSO_4$, ascorbic acid, THF: H_2O, rt, 12 h, **14** (80%), **15** (78%), **17** (82%), **18** (79%); (ii) K_2CO_3 in CH_2Cl_2/MeOH (1:1, v/v), rt, 4 h, **16** (69%), **19** (72%).



Scheme 3. Reagents and conditions: (i) $CuSO_4$, ascorbic acid, THF: H_2O, rt, 12 h, **21** (82%), **22** (75%); (ii) K_2CO_3 in CH_2Cl_2/MeOH (1:1, v/v), rt, 4 h, **23** (69%).



Scheme 4. Reagents and conditions: (i) **1** or **3**, $CuSO_4$, ascorbic acid, THF: H_2O, rt, 12 h, **25** (82%), **26** (78%); (ii) K_2CO_3 in CH_2Cl_2/MeOH (1:1, v/v), rt, 4 h, **27** (69%).

ing human 5-LO. Measurement of 5-LO activity in cell lysates was performed as previously described with modifications.[21]

From the structure-activity point of view, all of the clusters bearing the cinnamic acid moiety **5**, **14**, **17**, **21**, and **25**, showed less inhibitory activity than the corresponding clusters bearing the caffeic acid moiety **7**, **9**, **11**, **16**, **19**, **23**, and **27** (Fig. 1), reinforcing the pharmacophoric contribution of the catechol entity to the mechanism of action against 5-LO activity.

As shown in Figure 1, compounds exhibiting one (**7**,**9**), two (**11**), three (**16**) or four (**19**) caffeic acid entities were equivalent and potent inhibitors of 5-LO activity compared to caffeic acid itself. The trimer **16** and tetramer **19** showed the most potent inhibition of the 5-LO activity (91% and 92%, respectively, Fig. 1). The inhibitory activity of tetramer **19** is more pronounced than that of tetramer **23** suggesting that the position of the triazole may be important with regard to modulating the inhibitory activity since these two compounds differ by the relative positioning of the triazole ring.

The noticeable activity enhancement observed for **16** and **19** must be ascribed to the special geometry arrangement and thus can accommodate the clustering of a few trimeric or tetrameric targets. The incorporation of additional caffeoyl units in hexamer **27** resulted in a less potent inhibition of 5-LO. This result would be consistent with a loss of activity due to enthalpy-entropy compensation for higher valency compounds.

Although the 5-LO activity shown in Figure 1 represents the sum of all 5-LO products detected (5-hydroxyeicosatetraenoic acid, LTB_4 and its *trans* isomers), similar results were observed for each of these 5-LO products separately (data not shown).

To better determine the potency of the compounds that demonstrated significant inhibitory activities against 5-LO at 1 μM, four promising inhibitors were selected (**7**, **11**, **16**, and **19**) for further investigation in concentration-response studies, and the results are summarized in Table 1.

The inhibitory activities of selected compounds were compared based on the number of caffeic acid moieties they contained (IC_{50} per Caf. Ac. in Table 1). All these compounds concentration-dependently inhibited 5-LO products synthesis and showed prominent inhibitory activities with IC_{50} values ranging from 0.66 to 0.79 μM, which were comparable to the inhibitory activity of zileuton (IC_{50} = 0.5–1 μM[22]). On the basis of corrected values on a per caffeoyl residue, dimer **11**, trimer **16**, and tetramer **19**, readily surpassed the activity of caffeic acid by more than 10-fold.

In conclusion, an alkyne-azide cycloaddition based route to various cinnamoyl and caffeoyl clusters featuring 1,2,3-triazole rings has been achieved. The results demonstrate that caffeoyl clusters

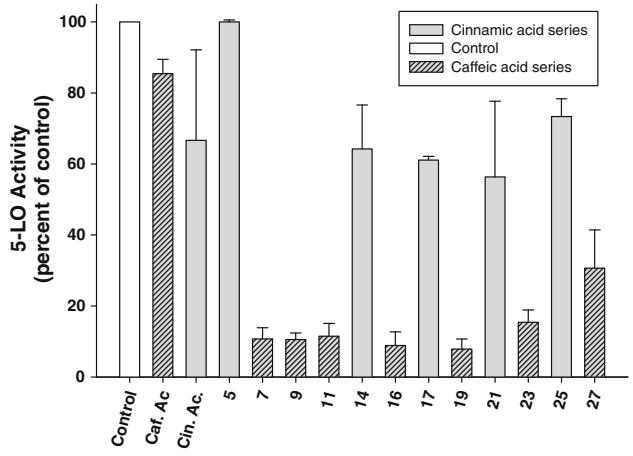


Figure 1. 5-LO Activity in cell lysates preincubated with the different test compounds (1 μM).

Table 1
Determination of IC_{50} values of selected inhibitors

Compound	IC_{50} (μM)	IC_{50} (μM) per Caf. Ac.[a]	R IC_{50}[b]
7	0.68	0.68	36.7
11	0.74	1.48	16.8
16	0.79	2.37	10.5
19	0.66	2.46	10.1
Caf. Ac.	25	25	1

[a] IC_{50} on a per caffeoyl residue basis.
[b] Relative IC_{50} values based on caffeic acid as standard.

are good lead compounds in the design and synthesis of more potent 5-LO inhibitors.

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18. Compound **18**. To a magnetically stirred solution of azide **13** (40 mg, 0.17 mmol) and propargyl derivative **1** (246 mg, 0.81 mmol), dissolved in a 1:1 mixture of water and tetrahydrofuran (3 mL), were sequentially added $CuSO_4$ (8.5 mg, 0.034 mmol, 5% per azide), and ascorbic acid (6 mg, 0.034 mmol, 5% per azide). The mixture was stirred for about 12 h until disappearance of the starting material (TLC, 5% $MeOH/CH_2Cl_2$). After addition of water (15 mL), the crude reaction was repeatedly extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were treated with brine

(20 mL), and then dried over anhydrous magnesium sulfate. After concentration under reduced pressure, the residue was purified by silica gel circular chromatography (chromatotron®, model 7924, Harrison Research) eluting with dichloromethane and gradually increasing the polarity to MeOH/CH_2Cl_2 (3:97) to afford 193 mg (79% yield) of the required tetra cluster **18**. Colorless oil; Rf = 0.33 (5% MeOH/CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.25 (s, 24H, 8× OAc), 4.44 (s, 8H, CH_2N), 5.38 (s, 8H, CH_2O), 6.41 (d, 4H, *J* = 17.6 Hz, =*CH*CO), 7.20 (d, 4H, *J* = 8.2 Hz, Har), 7.35 (br s, 8H, Har), 7.63 (d, 4H, *J* = 17.6 Hz, =*CH*Car), 8.30 (s, 4H, =*CH*N); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.6, 20.6, 46.6, 49.2, 57.5, 86.1, 118.4, 122.8, 123.9, 126.5, 127.8, 132.9, 132.4, 142.43, 143.6, 166.1, 167.9, 168.0; HRMS Calcd for $C_{69}H_{64}O_{24}N_{12}$ + (H^+): 1445.42347. Found: 1445.42314. Compound **19**. To a solution of **18** (62 mg, 0.043 mmol) in acetone (5 mL) was added 3N HCl (2 mL). The reaction mixture was refluxed for 3 h. The solution was then diluted with EtOAc (25 mL), washed with brine, dried ($MgSO_4$), concentrated, and purified silica gel circular chromatography (chromatotron®, model 7924, Harrison Research) eluting with (CH_3CN/H_2O; 95:5) to give 34 mg (72% yield) of the free hydroxyl tetra cluster **19**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.72 (s, 8H, CH_2N), 5.30 (s, 8H, CH_2O), 6.30 (d, 4H, *J* = 18.6 Hz, =*CH*CO), 6.85 (m, 8H, Har), 7.05 (m, 4H, Har), 7.12 (br s, 8H, OH), 7.60 (d, 4H, *J* = 17.6 Hz, =*CH*Car), 8.35 (s, 4H, =*CH*N); ^{13}C NMR (50 MHz, $CDCl_3$): δ 44.5, 50.2, 59.3, 127.5, 142.9, 115.3, 117.6, 118.2, 121.3, 129.4, 143.2, 145.6, 142.5, 166.3; HRMS Calcd for $C_{53}H_{48}O_{16}N_{12}$ + (H^+): 1109.33895. Found: 1109.33678.

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21. (a) Cells were lysed in phosphate-buffered saline containing 1 g/L glucose. Following the addition of 2 mM EDTA and 5 mM $CaCl_2$ lysates were then pre-incubated with each of our compounds at the indicated concentration for 5 min at 37 °C. The 5-LO reaction was initiated with the addition of 1 mM ATP and 40 μM arachidonic acid followed by incubation at 37 °C for 20 min.[21b] Reactions were stopped with addition of 0.5 vol methanol and samples were processed for separation and quantification of 5-LO products (5-HETE, LTB_4 and its *trans* isomers) by reverse-phase HPLC with UV detection as previously described;[21c].; (b) Werz, O.; Szellas, D.; Steinhilber, D. *Eur. J. Biochem.* **2000**, *267*, 1263; (c) Borgeat, P.; Picard, S.; Vallerand, P.; Bourgoin, S.; Odeimat, A.; Sirois, P.; Poubelle, P. E. *Methods Enzymol.* **1990**, *187*, 98.
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