



## New synthesized polyoxygenated diarylheptanoids suppress lipopolysaccharide-induced neuroinflammation

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### ABSTRACT

In neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease, Parkinson's disease and multiple sclerosis, neuroinflammation induced by the microglial activation plays a crucial role. In effort to develop effective anti-neuroinflammatory compounds, different new linear polyoxygenated diarylheptanoids were synthesized. In LPS-triggered BV-2 microglial cells their ability to reduce the concentration of IL-6 and TNF- $\alpha$  pro-inflammatory cytokines was evaluated. Moreover, their effect on NF- $\kappa$ B and ATP citrate lyase (ACLY), a recently emerged target of metabolic reprogramming in inflammation, was assessed. Finally, we turned our attention to inflammatory mediators derived from the cleavage of citrate catalyzed by ACLY: prostaglandin E<sub>2</sub>, nitric oxide and reactive oxygen species. All compounds showed null or minimal cytotoxicity; most of them had a great anti-neuroinflammatory activity. Diarylheptanoids **6b** and **6c**, bearing a halide atom and benzyl ether protective groups, exhibited the best effect since they blocked the secretion of all inflammatory mediators analyzed and reduced NF- $\kappa$ B and ACLY protein levels.

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### 1. Introduction

Chronic neuroinflammation has a pivotal role in the onset and progression of a broad range of neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease and multiple sclerosis [1]. Neuroinflammation is a consequence of the activation of microglia, the resident immune cells of the central nervous system. Microglial cells influence brain development and constantly survey the microenvironment of parenchyma, but they also act as the first line of defence against injury and invading pathogens. When the homeostasis is disrupted, microglia becomes activated and undergoes morphological and phenotypical changes into one of the two extremes of polarization: the classically activated M1 or alternatively activated M2 macrophages. M1 microglia secretes pro-inflammatory cytokines

(e.g. TNF- $\alpha$ , IL-1 $\beta$ , IL-6), chemokines, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), reactive oxygen species (ROS), and reactive nitrogen species [2], which elicit detrimental effects on adjacent neurons and further exacerbate tissue inflammation and damage. By contrast, in M2 microglial cells the expression of anti-inflammatory mediators is up-regulated and they contribute in neuroprotection and tissue recovery and remodelling [3]. Therefore, neuroinflammation is a double-edged sword because of its beneficial or harmful effects on central nervous system, which depend on the type of microglia activation and the duration of inflammatory response. In fact, acute events scavenge harmful substances playing a protective role for nervous tissue while chronic ones are always dangerous due to the production and release of pro-inflammatory and cytotoxic factors [4]. In neurodegenerative diseases the equilibrium between microglial surveillance and activation is disrupted and growing evidence demonstrates that the control of microglia activation could attenuate their severity [5]. Therefore, the discovery of anti-neuroinflammatory compounds able to inhibit the microglia activation could be helpful in neurodegenerative disorders.

Diarylheptanoids (C6–C7–C6), a class of oxygenated compounds

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with a 1,7-diphenylheptane skeleton, have been isolated from wood, bark or leaves of many hardwoods including Betulaceae, Leguminosae, Myricaceae and Proteaceae. They are increasingly recognized as potential therapeutic agents for their wide spectrum of biological and pharmacological activities such as anti-inflammatory, antioxidant, antitumor, estrogenic, leishmanicidal, melanogenesis, hepatoprotective, neuroprotective proprieties [6]. To this class of natural products belongs myricanol, a cyclic and not symmetric diarylheptanoid extracted from *Myrica* species, and curcumin, the main curcuminoid isolated from *Curcuma longa* (Fig. 1). It has been shown that myricanol reduces the levels of the microtubule-associated protein tau resulting in anti-Alzheimer's disease effect [7] and curcumin exerts neuroprotective and anti-inflammatory activities [8–10]. In particular, curcumin inhibits PGE<sub>2</sub>, nitric oxide (NO), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) release following lipopolysaccharide (LPS) stimulation in RAW 264.7 murine macrophages [11] and switches the polarization of LPS-activated BV2 microglial cells from M1 phenotype to a predominantly M2 phenotype [12].

Taking into consideration the biological importance of linear diarylheptanoids in general, and myricanol and curcumin in particular, we planned a straightforward access to a series of linear diarylheptanoid derivatives using the cross-metathesis reaction on readily available precursors **1a,b**, **2a,b** and **3** [13–15]. Terminal olefins **1–3** [14] were easily prepared and used to give access to new linear diarylheptanoids and to the cyclic one, myricanol. Herein some of these were differently protected, halogenated and reduced. Eleven of the new synthesized diarylheptanoids were tested on a cell line model of neuroinflammation: LPS-triggered BV-2 cells. Their effect on different mediators of inflammation was evaluated in comparison with curcumin and celecoxib, a COX-2 selective non-steroidal anti-inflammatory drug (Fig. 1). Celecoxib, in a rat model with LPS-triggered neuroinflammation, significantly decreased the rate of microglia activation and levels of IL-1 $\beta$  and TNF- $\alpha$  pro-inflammatory cytokines [16]. Finally, the ability of the new synthesized diarylheptanoids to reduce NF- $\kappa$ B and ACLY – a metabolic pro-inflammatory enzyme – was also investigated.

## 2. Material and methods

### 2.1. Cell culture, treatment and cell viability assay

The immortalized murine BV-2 microglial cell line was maintained in Roswell Park Memorial Institute 1640 Medium supplemented with 10% heat inactivated fetal bovine serum, 4 mM L-Glutamine, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin at 37 °C and 5% CO<sub>2</sub>. Where indicated BV-2 cells were activated with 200 ng/ml LPS from *Salmonella enterica* serotype typhimurium (LPS, Sigma–Aldrich, St. Louis, MO, United States). Cell viability was evaluated by using CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) according to manufacturer's instructions. The details are given in Supplementary data.

### 2.2. Enzyme-linked immunosorbent assay (ELISA)

The concentration of pro-inflammatory cytokines IL-6 (Cat. n° 32670069U1, ImmunoTools, Friesoythe, Germany) and TNF- $\alpha$  (Cat. n° 32673019U1, ImmunoTools) in cell culture media was measured with ELISA kits following the manufacturer's recommendations. The levels of PGE<sub>2</sub> were quantified by using DetectX® Prostaglandin E2 High Sensitivity Immunoassay Kit (Arbor Assays, Ann Arbor, MI, USA) as per manufacturer's protocol.

### 2.3. ROS and NO detection

Reactive oxygen species and nitric oxide levels were measured by using 6-Carboxy-2',7'-Dichlorodihydrofluorescein Diacetate and 4-Amino-5-Methylamino-2',7'-Difluorofluorescein Diacetate (Thermo Fisher Scientific, San Jose, CA, USA), respectively, as previously described [17].

### 2.4. Western blotting

Western blotting experiments were carried-out as reported previously [18] using anti-NF- $\kappa$ B/p65 (ab7970), anti-ATP citrate lyase (ab157098) or anti- $\beta$ -actin (ab8227) primary antibodies (Abcam, Cambridge, MA). More details are reported in

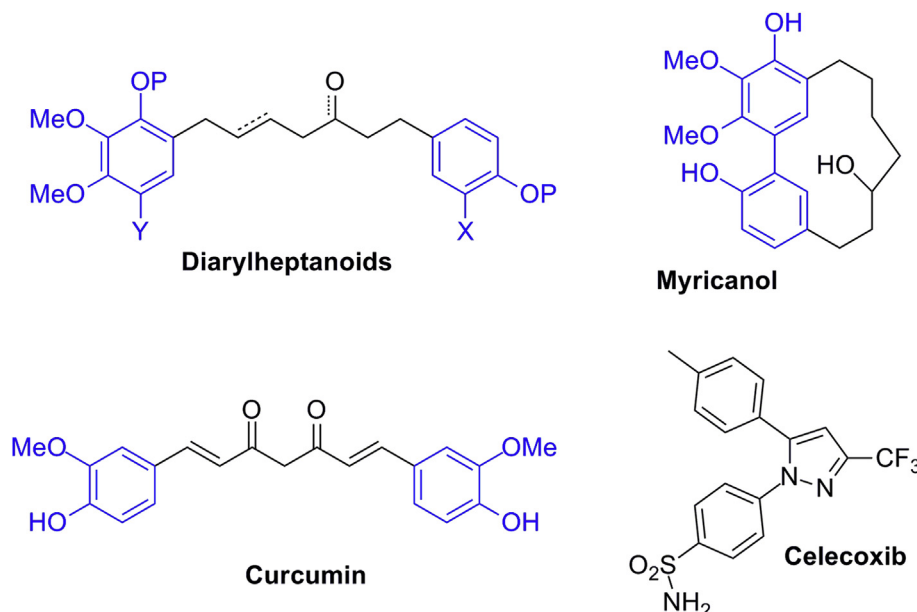


Fig. 1. Diarylheptanoids, myricanol, curcumin and celecoxib structures.

## Supplementary data.

## 2.5. Statistical analysis

Statistical significance of differences was determined by using Student's t-test. Results are shown as means  $\pm$  SD of, at least, four independent experiments. Differences were considered as significant (\* $p < 0.05$ ), very significant (\*\* $p < 0.01$ ) and highly significant (\*\*\*) ( $p < 0.001$ ).

## 3. Results

## 3.1. Preparation of diarylheptanoids 4–6

The requisite allylphenols **1a** and **1b** were accessed in two steps from commercially available 2,3-dimethoxyphenol [13]. Coupling partners **2a**, **b** and **3** were readily prepared from commercial methyl 3-(4-benzyloxyphenyl)propanoate [14,15]. With the key fragments **1a**, **1b**, **2a**, **2b** and **3** in hands, we attempted cross metathesis (CM) reactions as depicted in Scheme 1 [14,19,20]. Hence, treatment of **1a** (4 equiv.) with **2a** or **2b** (1 equiv.) in the presence of G-II catalyst (5mol%) in dichloromethane from  $-78^\circ\text{C}$  to room temperature provided diarylheptanoid **4a** and **4c** in a gratifying 81% and 83% yield, respectively, if compared to the examples of CM at low temperature reported in literature [21].

Addition of G-II catalyst at  $-78^\circ\text{C}$  was mandatory to obtain good yields. Excess of partner **1a** and its homocoupled were almost fully recovered after chromatography.

Cross metathesis between **1b** and **2a** afforded coupling product **4b** in only 35% yield without formation of homocoupling product resulting from dimerization of **1b**. While positive effect of free OH (phenol or alcohol) on CM reactions, already reported in literature [22–24], could explain the excellent yield obtained to produce **4a** (81% yield), an opposite behavior was observed in the preparation of compound **4b** (Scheme 1) despite the presence of two free OH on the starting catechol **1b**.

Probably the lower solubility of catechol **1b** in DCM at  $-78^\circ\text{C}$  prevented the metathesis reaction which started only increasing the temperature ( $20^\circ\text{C}$ ). No dimerization of **1b** was observed or after column chromatography. Moreover reaction between **1a** and

**2b** gave rise to compound **4c** in high yield (82%) and cross metathesis between **1a** and **3** afforded alkene **4e** in only 32% of yield.

Subsequent transformations were performed in order to have different protections and different functionalities (double bond, ketone or halide) on the same parent scaffold. Therefore, the acetylation of **4a** by  $\text{Ac}_2\text{O}$  in pyridine afforded **4d** in a quantitative yield, while the methylation of **4e** furnished the allylketone **4f** in 30% of yield.

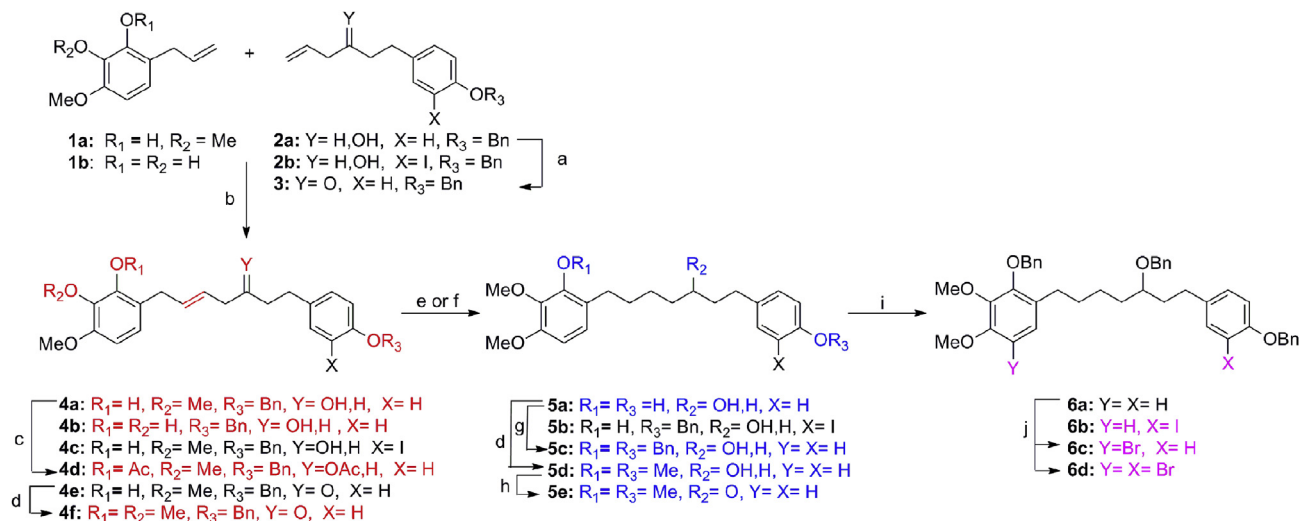
Hydrogenation of the double-bond and concurrent debenzyla-tion of **4a** resulted in the quantitative formation of **5a** which was selectively protected on phenols as methyl ethers **5d**. Chemo-selective hydrogenation of **4c** by  $o\text{-NO}_2\text{PhSO}_2\text{Cl}$  and  $\text{N}_2\text{H}_2$  [25] furnished **5b** in high yield, preserving the benzyl protecting group and the iodine atom of **4c**. Oxidation of compound **5d** using DMP in DCM afforded the ketone **5e** with 63% yield. Compounds **5a** and **5b** were completely protected on phenol and carbinol functions as tribenzylethers **6a** and **6b** using  $\text{BnBr}$ ,  $\text{NaH}$ ,  $\text{NaI}$  in DMF. Subsequently regioselective bromination of **6a** furnished the mono-brominated **6c** and the di-brominated **6d** in quantitative yield when 1.2 or 2.4 equiv of  $\text{NBS}$  was used, respectively.

## 3.2. Novel polyoxygenated diarylheptanoids do not affect microglial cell viability

BV-2 microglial cells were treated with curcumin, celecoxib or the novel polyoxygenated diarylheptanoids at 0.01, 0.1 and  $1\ \mu\text{M}$  and their cytotoxic effects were determined by MTT assay after 72 h (Fig. S1). Curcumin and celecoxib as well as the new-synthesized diarylheptanoids exhibited minimal or null toxic effect. We chose the lowest concentration ( $0.01\ \mu\text{M}$ ) as optimal concentration to evaluate the anti-inflammatory activity.

3.3. Impact of treatment with the linear polyoxygenated diarylheptanoids on IL-6, TNF- $\alpha$  and NF- $\kappa\text{B}$ 

When stimulated with lipopolysaccharide, the macrophages secrete different mediators among which IL-6 and TNF- $\alpha$  have a primary role for the initiation and propagation of inflammation [26]. Therefore, to probe the effect of the novel molecules on IL-6



**Scheme 1.** a) refs 13 and 14; b) Grubbs-II, DCM,  $-78^\circ\text{C}$  to rt, 81% yield **4a**, 35% yield **4b**, 82% yield **4c**, 32% yield **4e**; c)  $\text{Ac}_2\text{O}$ , Py, rt, 99%; d) methylation of **4e** and **5a** by  $\text{MeI}$ ,  $\text{K}_2\text{CO}_3$ , acetone, 30% yield **4f** and 40% yield **5d**; e) hydrogenation of **4a** by  $\text{H}_2$ ,  $\text{Pd/C}$ ,  $\text{MeOH}$ , rt, 1 h, 99% yield **5a**; f) hydrogenation of **4c** by  $o\text{-NO}_2\text{PhCl}$ ,  $\text{N}_2\text{H}_2 \cdot \text{H}_2\text{O}$ ,  $\text{ACN}$ ,  $0^\circ\text{C}$  91% yield **5c**; g) benzoylation of **5a** by  $\text{BnBr}$ ,  $\text{K}_2\text{CO}_3$ , TBAI acetone, reflux, 99% yield **5c**; h) DMP, DCM, 63%; i) benzoylation of **5a** and **5b** by  $\text{BnBr}$ ,  $\text{NaH}$ ,  $\text{NaI}$ , DMF 75% yield **6a** and 71% yield **6b**; j) bromination of **6a** by  $\text{NBS}$  (1.1 equiv or 2.2 equiv.), TFA,  $\text{ACN}$  99% yield **6c** and 99% yield **6d**.

and TNF- $\alpha$  secretion, BV-2 cells were stimulated with LPS in the presence or absence of each compound at the concentration of 0.01  $\mu$ M (Fig. 2). Twenty-four hours later, the levels of IL-6 and TNF- $\alpha$  were measured in cell culture media. LPS induced a significant release of IL-6 (Fig. 2A; LPS vs. CONTROL,  $p < 0.001$ ) and TNF- $\alpha$  (Fig. 2B; LPS vs. CONTROL,  $p < 0.001$ ). Celecoxib resulted more active than curcumin in lowering IL-6 levels compared to cells activated only with LPS (Fig. 2A, \*\*\* $p < 0.001$ ). The majority of the tested compounds was able to reduce the secretion of IL-6; exceptions were molecules **4a**, **5a**, **5c** and **5d**, which had no significant effect (Fig. 2A). Interestingly, compound **4b** was effective as celecoxib in lowering IL-6 levels, while compounds **5e**, **6c** and **6d** had an effect similar to curcumin. In the meantime, the diarylheptanoid **6d** did not affect TNF- $\alpha$  release following LPS stimulation as well as compounds **4b**, **4d**, **5a**, **5d** and **5e** (Fig. 2B). The remaining molecules **4a**, **4f**, **5c**, **6b** and **6c** acted like celecoxib and curcumin in letting TNF- $\alpha$  concentration down (Fig. 2B, compounds vs. LPS, \*\*\* $p < 0.001$ ).

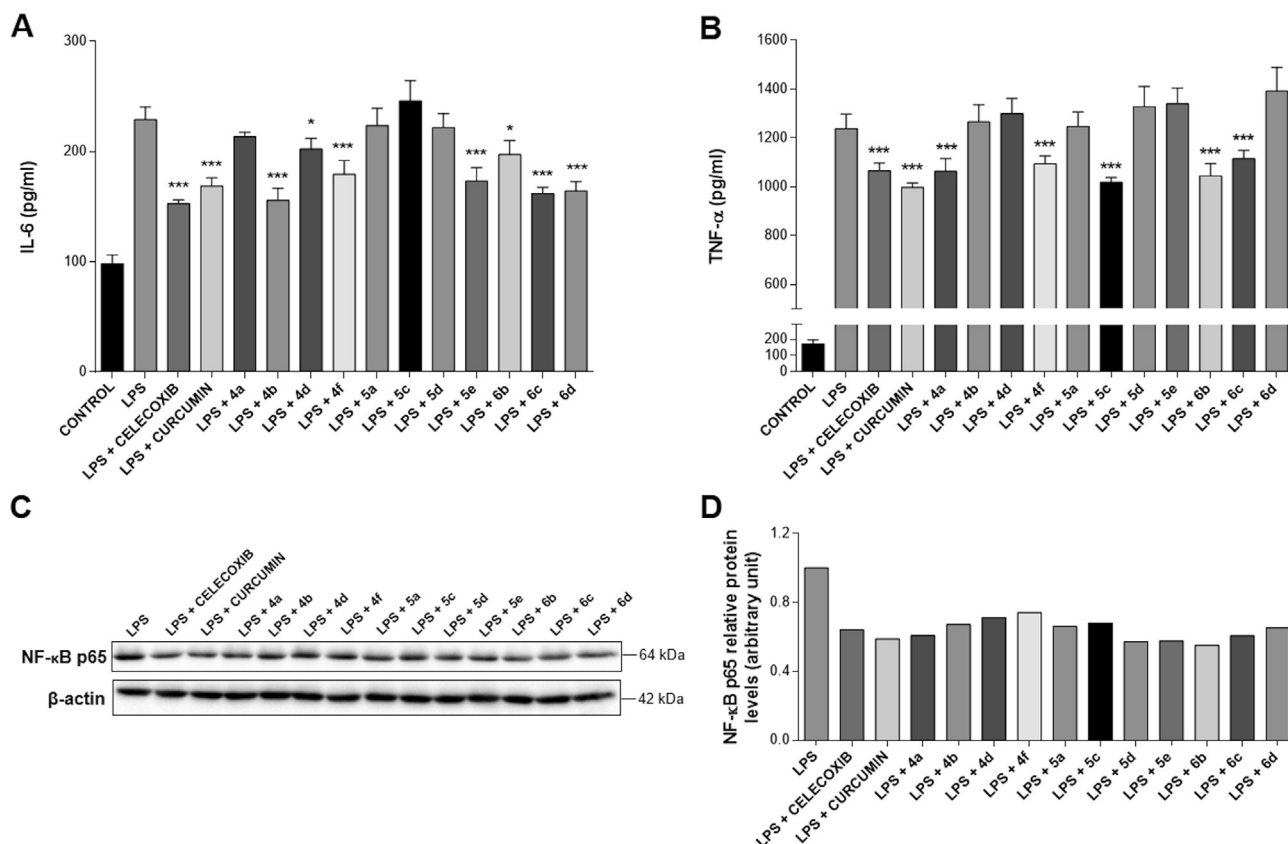
Then we investigated the effect of the new synthesized compounds on NF- $\kappa$ B, a crucial transcription factor whose activation leads to the induction of multiple genes regulating the immune and the inflammatory responses [27]. Therefore, to verify if NF- $\kappa$ B could be a target for the polyoxygenated diarylheptanoids, LPS-triggered BV-2 cells were treated with the novel compounds, curcumin, or celecoxib. The protein levels of NF- $\kappa$ B subunit p65 were quantified via Western blot. Surprisingly, all compounds were able to reduce the expression of NF- $\kappa$ B in the same manner as celecoxib and

curcumin (Fig. 2C). Molecules **4d** and **4f** were the less active; however, they led to the reduction of about 30% in NF- $\kappa$ B protein levels with respect to BV-2 cells stimulated only with LPS (Fig. 2D).

#### 3.4. Effect of the novel polyoxygenated diarylheptanoids on ACLY expression and PGE<sub>2</sub>, NO and ROS production

Since the new synthesized compounds reduced NF- $\kappa$ B expression and ACLY up-regulation in LPS-induced macrophages is under control of NF- $\kappa$ B transcription factor [28], we evaluated the protein levels of ACLY (Fig. 3A). Of note, ACLY is a metabolic enzyme - recently identified as a new player of the immunometabolism - which splits glucose-derived citrate into acetyl-CoA and oxaloacetate (OAA). Acetyl-CoA provides units for lipid elongation, including arachidonic acid, precursor of prostaglandins. OAA leads to NADPH production, necessary for NADPH oxidase and iNOS to generate ROS and NO, respectively, in LPS-activated macrophages [29]. In BV-2 cells primed with LPS, curcumin and celecoxib were able to reduce ACLY expression levels (Fig. 3A). Among the new synthesized diarylheptanoids, the molecule **6d** was the most active, even better than celecoxib, in lowering ACLY protein; on the contrary, molecules **4b**, **4d** and **5e** had no activity. The remaining compounds were able to reduce the expression of ACLY (Fig. 3A).

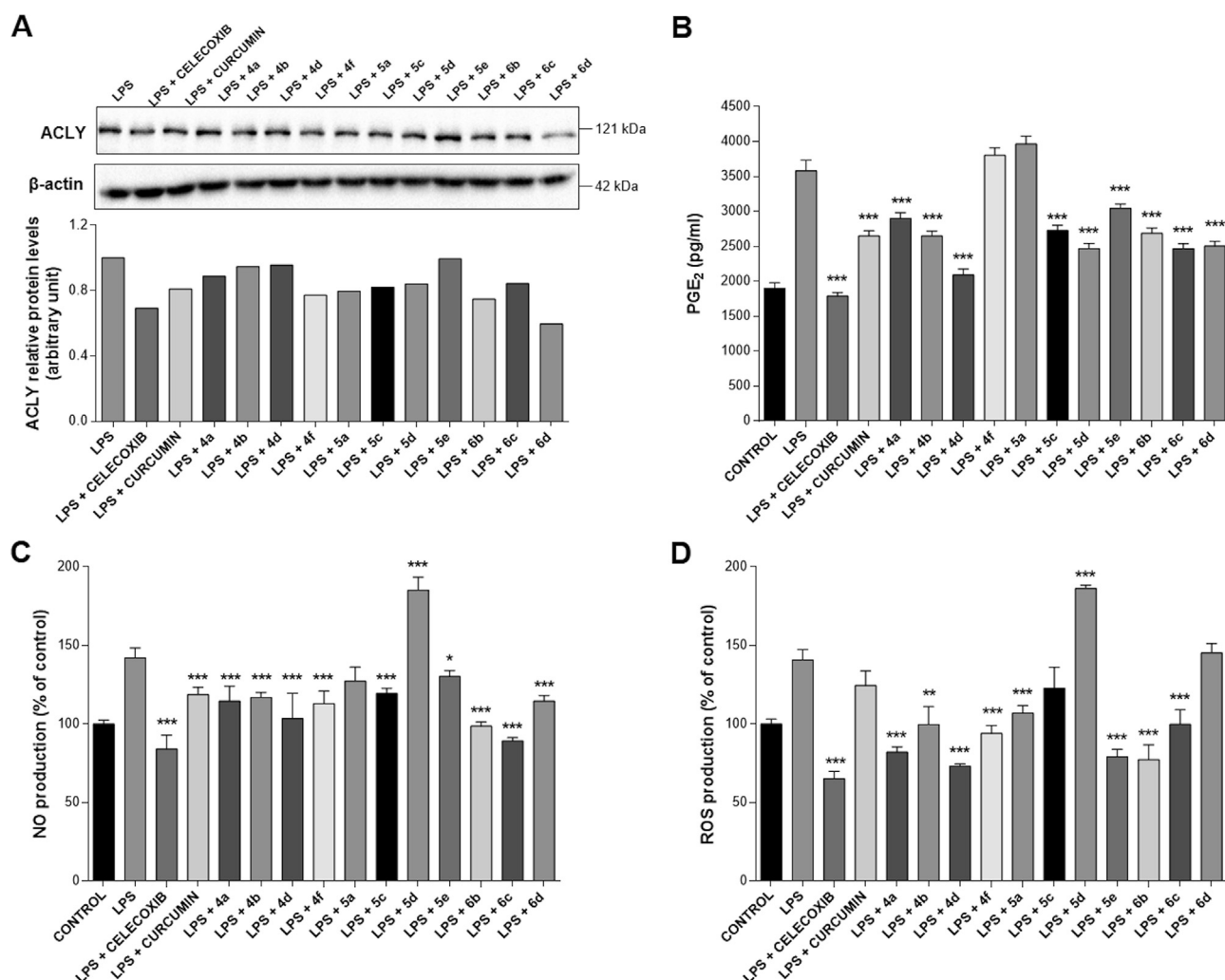
Taking into consideration the inhibitory effect of ACLY by some of novel diarylheptanoids, we decided to monitor the levels of inflammatory mediators linked to the citrate pathway: PGE<sub>2</sub>, NO and ROS. When BV-2 microglial cells were activated with LPS we found



**Fig. 2.** Effect of polyoxygenated diarylheptanoids on IL-6 and TNF- $\alpha$  release and NF- $\kappa$ B/p65 expression levels.

BV-2 cells were treated with DMSO (CONTROL) or 200 ng/ml LPS alone (LPS) or LPS plus 0.01  $\mu$ M celecoxib, curcumin, **4a**, **4b**, **4d**, **4f**, **5a**, **5c**, **5d**, **5e**, **6b**, **6c** and **6d** compounds. After 24 h IL-6 (A) and TNF- $\alpha$  (B) concentrations were quantified. Means  $\pm$  S.D. of four duplicate independent experiments are shown; differences between CONTROL and LPS were highly significant ( $p < 0.001$  Student's t-test). Where indicated differences between LPS and cells treated with LPS plus compounds were significant (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , Student's t-test). C) The expression levels of subunit p65 of NF- $\kappa$ B were evaluated via western blotting. The intensity of immunolabelled protein bands depicted in C was quantified by densitometric scanning and NF- $\kappa$ B p65 levels were normalized against  $\beta$ -actin to have NF- $\kappa$ B p65 relative protein levels (D).





**Fig. 3.** Effect of polyoxygenated diarylheptanoids on ACLY expression levels and PGE<sub>2</sub>, NO and ROS production.

BV-2 microglial cells were treated with or 200 ng/ml LPS alone (LPS) or LPS plus 0.01  $\mu$ M celecoxib, curcumin, **4a**, **4b**, **4d**, **4f**, **5a**, **5c**, **5d**, **5e**, **6b**, **6c** and **6d** compounds. **A**) By using specific antibodies, the expression levels of ACLY and  $\beta$ -actin were detected (upper panel). The intensity of immunolabelled protein bands was analyzed by densitometric scanning and ACLY levels were normalized against  $\beta$ -actin to have ACLY relative protein levels (lower panel). **B–C–D**) PGE<sub>2</sub> concentration was measured after 48 h of LPS treatment (**B**) while NO and ROS 24 h later (**C–D**). Means  $\pm$  S.D. of four triplicate independent experiments are shown. In **B–C–D** differences between CONTROL and LPS were highly significant ( $p < 0.001$ , Student's *t*-test). Where indicated, differences between LPS and cells treated with LPS plus compounds were significant (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , Student's *t*-test).

a strong increase in PGE<sub>2</sub> levels compared with untreated cells (Fig. 3B, CONTROL vs. LPS  $p < 0.001$ ). Celecoxib worked better than curcumin in modulating the production of PGE<sub>2</sub>, bringing the levels of PGE<sub>2</sub> back to those of the untreated cells (Fig. 3B, CELECOXIB vs. LPS \*\*\* $p < 0.001$ ). The majority of the tested diarylheptanoids acted as curcumin with the exception of compound **4d**, **4f**, **5a** and **5e**. Molecules **4f** and **5a** had no effect: the levels of PGE<sub>2</sub> in BV-2 cells were similar to cells treated only with LPS. On the contrary, compound **4d** was the only tested diarylheptanoid more active than curcumin but less effective than celecoxib in reducing PGE<sub>2</sub> levels (Fig. 3B).

Then, efforts were dedicated to NO and ROS evaluation. Intense production of both ROS and NO was observed in various diseases [30] and can be correlated to neurodegenerative disorders [31,32]. Celecoxib resulted more active than curcumin in lowering NO and ROS levels compared to cells activated only with LPS (Fig. 3C–D,  $p < 0.001$ ). The majority of the tested compounds had similar effect of curcumin with the exception of **6b** and **6c**, the most active in the reduction of NO with an effect similar to celecoxib (Figs. 3C, **6b**, **6c** vs. LPS, \*\*\* $p < 0.001$ ). By contrary, all the tested polyoxygenated

diarylheptanoids, except **6d**, showed a higher reduction of ROS levels with respect to the leading curcumin. Compounds **4a**, **4d**, **5e** and **6b** had the strongest inhibitor effect on LPS-induced ROS production in BV-2 cells (Fig. 3D, compounds vs. LPS, \*\*\* $p < 0.001$ ). Meanwhile, compound **5d** was completely inactive: it even led to a significant increase in NO (Fig. 3C, **6c** vs. LPS, \*\*\* $p < 0.001$ ) as well as in ROS (Fig. 3D, **6c** vs. LPS, \*\*\* $p < 0.001$ ) levels.

#### 4. Discussion

In our investigation, we reported the prospect of new eleven linear polyoxygenated diarylheptanoids as a source of alternative anti-neuroinflammatory compounds. To this end, they were synthesized according to known methodology [14] and screened in LPS-triggered BV-2 microglial cells. The tested diarylheptanoids could be classified into three class: a) with double bond (**4a**, **4b**, **4d** and **4f**); b) without double bond (**5a**, **5c**, **5d** and **5e**) and c) with one or two halides on the aryl rings and completely protected on hydroxyl groups (**6b**, **6c** and **6d**). Our findings showed that the acetylation of OHs had made more efficient compound **4d** in lowering

PGE<sub>2</sub>, NO and ROS levels with respect to **4a** and **4b** with one and two free phenolic group, respectively. Moreover, the presence of the carbonyl and the additional methyl ether in compound **4f** made it with similar activity of **4b** in reducing NO and ROS production and none inhibitory effect on PGE<sub>2</sub> release. An opposite behaviour was observed in IL-6 and TNF- $\alpha$  secretion, where compound **4d** was completely inactive while **4f** had a high ability. Furthermore, molecule **4b** reduced IL-6 concentration of about 30% similarly to celecoxib and **4a** had a strong inhibitory effect on TNF- $\alpha$  secretion. From the second class of compounds (**5a**, **5c**, **5d**, **5e**) it is apparent that the absence of the double bond leads to a reduction in activity. For example, compound **5d** did not have any inhibitory effect of IL-6 and TNF- $\alpha$  release and it even led an increase in NO and ROS levels with respect to BV-2 cells activated with LPS. Beneficial effects were found with benzyl ethers derivatives (third class) and in presence of just one halide (iodine or bromine) on one aryl ring, as in **6b** and **6c**, respectively. These last compounds reduced the concentration of all inflammatory mediators analyzed and NF- $\kappa$ B and ACLY protein levels, while the presence of two bromine in the compound **6d** induced loss of activity in lowering TNF- $\alpha$ , NO and ROS levels. However, the compound **6d** had the strongest inhibitory effect on ACLY.

The great anti-neuroinflammatory activity of the compounds **6b** and **6c** could be linked to their lipophilic nature and to a possible passive membrane permeability. Despite molecular weights equal to 757 and 710 Da and predicted partition coefficients, logP, of  $11.88 \pm 0.47$  and  $11.69 \pm 0.59$  for the molecules **6b** and **6c** respectively, anti-neuroinflammatory effects for compounds with similar values beyond Lipinski's Rule of 5 were recorded [33,34]. In addition, it has been recently demonstrated that linear diarylheptanoids with a halide atom and the benzyl ethers as protective groups on the rings have biological activity [35].

Noteworthy, all polyoxygenated diarylheptanoids exhibited an inhibitory effect on NF- $\kappa$ B, transcription factor that regulates the expression of many genes involved in the inflammatory response among which IL-6, TNF $\alpha$  and ACLY genes [27,28]. Therefore, the effects exerted by the novel diarylheptanoids on these latter targets could be whether direct inhibitory effects or consequence of NF- $\kappa$ B inhibition.

Surprisingly, the new synthesized molecules showed anti-neuroinflammatory features at 0.01  $\mu$ M, a very low concentration with respect to the natural linear diarylheptanoids from the rhizomes of *Dioscorea nipponica* able to inhibit NO production in LPS-triggered BV-2 cells at a concentration higher than 10  $\mu$ M (IC<sub>50</sub> of about 14  $\mu$ M) [36] and to the reported cyclic ether diarylheptanoids [37], which showed anti-inflammatory at 30  $\mu$ M.

All these results shed light on chemical groups and/or modifications that look promising to improve anti-neuroinflammatory activity. Future investigation could demonstrate whether these compounds have other molecular targets in inflammation.

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## 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.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.bbrc.2020.06.122>.

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