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Short Note

Ethyl (E)-(3-(4-((4-Bromobenzyl)Oxy)Phenyl)Acryloyl)Glycinate

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Abstract: In an attempt to develop new potent anti-inflammatory agents, a cinnamic -amino acid hybrid molecule was synthesized and in silico drug-likeness, in vitro COX-2 inhibition, and pharmacokinetic properties were studied. The results showed high cyclooxygenase inhibitory activity (IC_{50} = 6 μM) and favorable pharmacokinetic properties, being orally bioavailable according to Lipinski's rule of five, making this compound a possible lead to design and develop potent COX inhibitors. The new compound, in comparison with its cinnamic acid precursor (E)-(3-(4-((4-bromobenzyl)oxy)phenyl)acrylic acid, showed improved biological activities. Compound ethyl (E)-(3-(4-((4-bromobenzyl)oxy)phenyl)acryloyl)glycinate can be used as a lead for the synthesis of more effective hybrids.

Keywords: cinnamic acid; amino acid; hybrids; COX-2

Citation: Fotopoulos, I.; Papaioannou, G.-C.; Hadjipavlou-Litina, D. Ethyl (E)-(3-(4-((4-Bromobenzyl)Oxy) Phenyl)Acryloyl)Glycinate. *Molbank* **2022**, *2022*, M1378. https://doi.org/10.3390/M1378

Academic Editor: Stefano D'Errico

Received: 24 May 2022
Accepted: 2 June 2022
Published: 4 June 2022

1. Introduction

Natural products are considered as a useful tool for medicinal chemistry to design new compounds with significant biological properties. Cinnamic acids are an important group of natural compounds presenting a plethora of biological activities, among them anti-inflammatory [1,2] and neuroprotective properties [3].

Amino acid glycine is implicated as a precursor for a variety of significant molecules such as glutathione, porphyrins, purines, heme, and creatine acting as neurotransmitter in central nervous system presenting antioxidant, anti-inflammatory, cryoprotective, and immunomodulatory activities in peripheral and nervous tissues. The brain's function is mainly governed by the activity of several neurotransmitters, among them the most important being glycine, which exerts both inhibitory and sedative activities [4]. Several neuroinflammatory pathologies such as Alzheimer's disease have been linked to the disruption of the glycinergic neuro-transmittance. This disruption is highly correlated to the activation of several pro-inflammatory enzymes such as cyclooxygenase 2 (COX-2). Glycine presents a neuroprotective effect against neuroapoptosis, neuroinflammation, synaptic dysfunction, and memory impairment. Thus, COX-2 inhibitors including in their structure the glycine moiety could offer protection against the onset of Alzheimer's disease.

Hybridization is a contemporary approach used in the design of new and more potent biologically active agents combining in a single molecular entity, the pharmacophore groups of more than one molecule considering a new compound with better biological activity, better pharmacokinetic properties and lower toxicity in relation to the parent compounds. The design of hybrids has attracted the interest of medicinal chemistry the last decade, due to their better pharmacokinetic properties and improved biological activities. Increasing efforts to compete with inflammatory conditions, establishing new synthetic approaches have been presented. In many multifactorial diseases, the role of inflammation was found to be significant.

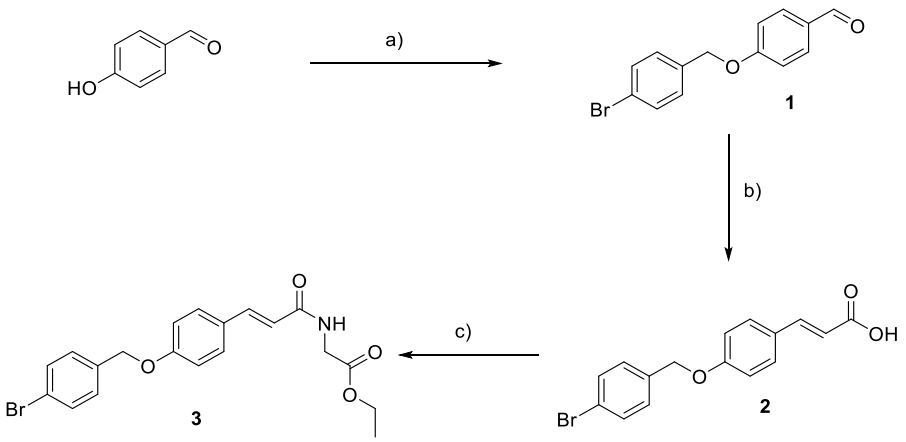
In light of the above, continuing our efforts in the design of cinnamic hybrids [5], we report the synthesis of a new cinnamic acid-glycine hybrid combining two pharmacophore units in a single molecule. Earlier studies[7] showed that (E)-(3-(4-((4-bromobenzyl)oxy)

phenyl)acrylic acid[7] inhibits soybean lipoxygenase (LOX) (IC_{50} = 80 μM) and exhibits antioxidant activities without presenting any COX-2 inhibitory activity. In silico studies showed that the combination (E)-(3-(4-((4-bromobenzyl)oxy)phenyl)acrylic acid[7] with glycinate group in a hybrid molecule leads to an anti-COX-2 entity. The newly synthesized compound **3** was studied for its biological activity in vitro and in silico for drug-likeness and for pharmacokinetic properties. The results showed that compound **3** does not violate Lipinski's rule of five, and that it could be a possible lead to design new and more potent COX inhibitors.

2. Results and Discussion

2.1. Chemistry

The synthesis of ethyl (E)-(3-(4-((4-bromobenzyl)oxy)phenyl)acryloyl)glycinate (3) was achieved via a three-step synthetic procedure as described in Scheme 1. The synthesis entailed the reaction of 4-hydroxybenzaldehyde with 4-bromo-benzyl bromide and potassium carbonate (K_2CO_3), refluxed in acetone [6], to yield the corresponding 4-(4-bromobenzyl)oxy)-benzaldehyde (**1**), which was recrystallized from ethyl acetate/petroleum ether, which is a known compound [4]. Compound (**1**) was refluxed with malonic acid in pyridine/piperidine to yield (E)-3-(4-((4-bromobenzyl)oxy)phenyl)acrylic acid (**2**) [7] (yield 76%). We developed a modified Steglich esterification [8] synthetic procedure using acetonitrile (MeCN) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI HCl) in an effort to eliminate the need for additional purification steps and to follow a green chemistry, making the solvent system less hazardous to the environment and human health. Thus, (E)-3-(4-((4-bromobenzyl)oxy)phenyl)acrylic acid reacted with glycine ethyl ester hydrochloride, EDCI HCl, 4-dimethylaminopyridine (DMAP) in MeCN under microwave irradiation (MWI), to yield the final product (**3**) (yield 95%).



Scheme 1. (**a**) 4-hydroxybenzaldehyde (4.1 mmol), 4-bromobenzylbromide (4.1 mmol), K_2CO_3 (8.2 mmol), acetone (10 mL), reflux, 1 h. (**b**) **1** (4.1 mmol), malonic acid (6.15 mmol), piperidine (1.025 mmol), pyridine (10 mL), reflux, 6 h, (**c**) **2** (0.15 mmol), glycine ethyl ester hydrochloride (0.225 mmol), EDCI HCl (0.225 mmol), DMAP (0.600 mmol), MeCN (2 mL), MWI, 50 °C, 100 W, 30 min.

The structure of the final compound **3** was confirmed via ^{1}H, ^{13}C NMR (nuclear magnetic resonance) and HRMS (high-resolution mass spectrum) analysis. In the ^{1}H NMR spectrum, cinnamic moiety's double bond protons appeared as doublets at 7.60 ppm and 6.33 ppm with a *J*-coupling value of 15.6 Hz, indicative of the *trans*-isomerism. The amide proton was assigned as a singlet at 6.09 ppm while the ethyl ester protons were recorded at 4.17 ppm and 1.31 ppm. In the ^{13}C NMR spectrum, the signals of the amide and ester carbons were observed at 170.3 and 166.3 ppm respectively. In the HRMS spectrum, the $[M+H]^+$ ion was recorded at m/z 418.0647.

2.2. Drug-Likeness Studies

In drug design, drug-likeness is a very useful concept with respect to ADMET factors (absorbance–distribution–elimination–metabolism–toxicity), which can be developed from the molecular structure of a molecule before its synthesis and biological evaluation. Lipinski's rule of five is usually performed to evaluate drug-likeness [9]. Thus, compound **3** was analyzed in silico, using the online platform Molinspiration, which theoretically calculates the molecular properties (http://www.molinspiration.com/ 14 June 2021). The BBB (blood brain barrier) penetrating potency of compound **3** was also calculated using the online platform Molsoft (https://molsoft.com/mprop/ 17 August 2021). The results are shown in Table 1.

Table 1. Drug likeness and blood–brain barrier penetration studies of the newly synthesized compound **3**.

milogP [a]	TPSA [b]	No. of atoms	No of O and N [c]	No of OH and NH [d]	No of Violations	No of Rotational Bonds [e]	Volume [f]	MW [g]	BBB score [h]
4.47	64.64	26	5	1	0	9	336.06	418.29	4.21

[a] Lipophilicity calculated in a logarithmic scale (milogP); [b] Topological polar surface area (TPSA); [c] Number of hydrogen bond acceptors; [d] Number of hydrogen bond donors; [e] Number of rotatable bonds; [f] Molecular volume; [g] Molecular weight; [h] Blood–brain barrier score.

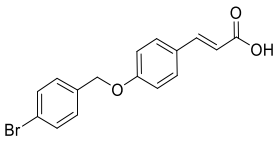
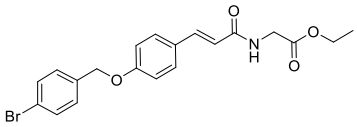
From the results, it was shown that compound **3** does not present any violations of Lipinski's rules, which makes it a competent pharmacochemical entity. Compound **3** has a molecular weight lower than 500 and a calculated lipophilicity value lower than 5, so it can be easily transported and absorbed by membranes. The high lipophilicity often contributes to low solubility and poor cell–membrane permeability and oral absorption. Furthermore, the number of rotatable bonds was less than 10, suggesting that the molecule's flexibility will not hinder its absorption and distribution. The topological polar surface area (TPSA) is highly correlated with the hydrogen bonding of a compound and applied for the prediction of intestinal absorption and blood–brain barrier penetration [10]. The TPSA of compound (**3**) was below the limits of 160 $Å^2$ and 90 $Å^2$, indicating good oral bioavailability and blood–brain barrier permeability, respectively. This is in accordance with its BBB score, which was close to the highest score calculated by the Molsoft platform. Thus, compound **3** is considered to be easily transferred and absorbed. Since the number of hydrogen bond donors and acceptors was lower than 10 and 5 respectively, compound **3** is considered active following *per os* administration.

2.3. Biological Evaluation

In the present study, the new compound **3** was evaluated as ovine cyclooxygenase inhibitor, compared with Indomethacin, a dual cyclooxygenase -1 (COX-)1 and COX-2 inhibitor, as a reference compound.

In our studies, compound **3** exhibited an interesting selective inhibitory activity of COX-2, with an IC_{50} value of 6 μM without any LOX inhibitory activity while the starting material, the corresponding cinnamic acid derivative **2**, did not exhibit any COX-2 inhibition (Table 2). This result supports the importance of this specific structural hybridization of the cinnamic group for the anti-COX-2 activity.

Table 2. Inhibition of ovine COX-2 (IC_{50} value) for compound **3**.

Compound	Structure	COX Inhibition (IC_{50}) μM
2		no
3		6 μM
	Indomethacin #	1.12 μM

no: No activity under the reported conditions; # dual cyclooxygenase -1 (COX-)1 and COX-2 inhibitor.

3. Materials and Methods

3.1. General Information

All chemicals, solvents, and biochemical reagents were of analytical grade and purchased from commercial sources (Merck, Merck KGaA, Darmstadt, Germany, Fluka, Sigma-Aldrich Laborchemikalien GmbH, Hannover, Germany, Alfa Aesar, Karlsruhe, Germany, Fluorochem Ltd., Derbyshire, United Kingdom and Sigma, St. Louis, MO, USA) and used without further purification. Ovine cyclooxygenase-2 was obtained from Cayman Chemicals.

Melting points (uncorrected) were determined on a MEL-Temp II (Lab. Devices, Holliston, MA, USA). For the in vitro tests, a 590 nm, double beam spectrophotometer Shimadzu UV-Vis Spectrophotometer PharmaSpec UV-1700 was used. The ^{1}H Nucleic Magnetic Resonance (NMR) spectra were recorded at 250 MHz on a Bruker Avance III spectrometer, in $CDCl_3$, or at 500 MHz on an Agilent 500/54 (DD2) spectrometer, in DMSO. ^{13}C-NMR spectra were obtained at 63 MHz (Bruker Avance III spectrometer) in $CDCl_3$ or at 125 MHz on an Agilent 500/54 (DD2) spectrometer, in DMSO with tetramethylsilane as internal reference unless otherwise stated. Chemical shifts are expressed in (ppm) and coupling constants *J* in Hz. High Resolution Mass Spectra were determined on an Agilent Q-TOF G6540B with dual AJS ESI-MS spectrometer, using MeOH as the solvent. Spectra data are given in the Supplementary Materials. Reactions were monitored by thin-layer chromatography on F_{254} silica plates (Merck and Fluka Chemie GmbH Buchs, Steinheim, Switzerland).

3.2. Chemistry General Procedure

3.2.1. Synthesis of 4-(Bromobenzyl)Oxy-Benzaldehyde (**1**)

In a round-bottomed flask containing acetone (10 mL), 4-hydroxybenzaldehyde (500 mg, 4.1 mmol, 1.0 eq.) and 4-bromobenzylbromide (1.02 g, 4.1 mmol, 1.0 eq.) were consequently added. Then, K_2CO_3 (1.13 g, 8.2 mmol, 2.0 eq.) was added, and the mixture was refluxed for 1 h. The reaction was monitored by TLC. The solvent was evaporated, and the residue was diluted with ethyl acetate and washed with water (2 × 15 mL) and brine (1 × 15 mL). Finally, the organic layer was dried (Na_2SO_4) and the solvent was evaporated to dryness to yield a white solid quantitatively [6]. The final product was recrystallized from ethyl acetate/petroleum ether and was subsequently used for the next step.

3.2.2. Synthesis of (E)-3-(4-((4-Bromobenzyl)Oxy)Phenyl)Acrylic Acid (**2**)

In a round-bottomed flask, pyridine (10 mL), the appropriate aldehyde (1.19 g, 4.10 mmol, 1 equiv.), and malonic acid (640 mg, 6.15 mmol, 1.5 equiv.) were consecutively added. Then, piperidine was added (0.1 mL, 1.025 mmol, 0.25 equiv.) and the mixture was refluxed. Upon completion of the staring material (TLC monitoring), the mixture was

cooled to 0 °C and then acidified with 2M HCl (aq). The precipitated solid was filtered off, washed with water, dried, and recrystallized from water. Yield: 76%, m.p.: 228–230 °C (H_2O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.62 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.51 (d, *J* = 16.0 Hz, 1H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 8.8 Hz, 2H), 6.36 (d, *J* = 16.0 Hz, 1H), 5.13 (s, 2H). ^{13}C—NMR (125 MHz, DMSO-d_6): δ 167.8, 159.8, 143.6, 136.2, 131.4, 129.9, 129.8, 127.2, 121.0, 116.7, 115.2, 68.49. LC—MS (ESI) *m*/*z*: $[M + H]^+$ = 334.

3.2.3. Synthesis of Ethyl (E)-(3-(4-((4-Bromobenzyl)Oxy)Phenyl)Acryloyl)Glycinate (**3**)

Two mL of MeCN, compound **2** (50 mg, 0.15 mmol, 1.0 equiv.), glycine ethyl ester hydrochloride (31 mg, 0.225 mmol, 1.5 equiv.), EDCI HCl (28 mg, 0.225 mmol, 1.5 equiv.), and DMAP (115 mg, 0.6 mmol, 4 equiv.) were inserted in a CEM-Discovery microwave oven vial, capped with a Teflon cap. The mixture was irradiated in Discover SPS microwave mode, at 50 °C, 100W for 30 min. Upon completion (monitoring by TLC), the mixture was poured onto a separating funnel and 15 mL of ethyl acetate were added. The organic solvent was washed with 2 × 15 mL of 2M HCl solution and then 2 × 15 mL of saturated $NaHCO_3$ solution. The organic layer was collected, dried upon $MgSO_4$, and evaporated to yield an off-white solid. Yield: 95%; Off-white solid; M.p. 157–60 °C. ^{1}H—NMR (250 MHz, $CDCl_3$): δ 7.60 (d, *J* = 15.6 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 6.33 (d, *J* = 15.6 Hz, 1H), 6.09 (brs, 1H), 5.04 (s, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 4.17 (d, *J* = 5.0 Hz, 2H), 1.31 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$): δ 170.3, 166.3, 160.1, 141.5, 135.7, 131.9, 129.7, 129.2, 128.0, 122.2, 117.8, 115.3, 69.5, 61.8, 41.8, 14.3. HRMS (ESI) *m*/*z*: $[M + H]^+$ Calculated for $C_{20}H_{21}BrNO_4$ 418.0648, Found 418.0647.

3.3. Biological In Vitro Assays

Each experiment was performed in triplicate and the standard deviation was found as less than 10% of the mean. For the in vitro assays, a stock DMSO solution of the respective compound was prepared from which several dilutions were created.

Inhibition of Ovine Cyclooxygenase-2

Arachidonic acid (AA) was used as a substrate and as co-substrate *N*, *N*, *N*, *N*- tetramethylphenylenediamine (TMPD) was inserted. The reaction mixture (1 mL) consisted of 0.75 mM heme, 128 mM TMPD, 80 mM AA, and 1.5 mg enzyme (ovine COX-2 in 0.1 M Tris/HCl (pH 8.5). The oxidation of the substrate was measured at room temperature by monitoring the increase of absorbance at 611 nm [11]. Indomethacin was used as a reference COX-2 inhibitor. The results are depicted in Table 2.

4. Conclusions

In the present study, a new cinnamic-glycine hybrid was synthesized as a potential COX-2 inhibitor. The synthetic procedure provides an alternative methodology for the preparation of (*E*)-cinnamyl hybrid amide underlining the utility of acetonitrile as a less hazardous solvent system for carbodiimide coupling reactions. Its chemical structure was confirmed by ^{1}H-NMR, ^{13}C-NMR, and HRMS analyses. The hybrid compound was studied in silico for its drug-likeness and pharmacokinetic properties and no violation of Lipinski's rule of five was noticed. The in vitro biological experiments showed high anti-COX-2 activity with an IC_{50} value of 6 μM, making the combination of a (E)-(3-(4-((4-bromobenzyl)oxy)phenyl)acrylic acid moiety with glycine ester a promising scientific project and this compound a possible lead in order to develop new and more potent COX inhibitors.

Further investigations are in progress to determine the anti-inflammatory and anti-Alzheimer's disease activity of this hybrid.

Supplementary Materials: The following are available online. Figure S1: ^{1}H-NMR spectrum of compound **3**, Figure S2: ^{13}C-NMR spectrum of compound **3**, Figure S3: HRMS spectrum of compound **3**.

Author Contributions: Author Contributions D.H.-L. contributed to the supervision, design, conceptualization, project administration, validation, and writing. I.F. and G.-C.P. contributed to the writing, synthesis, biological evaluation, and analysis of the data. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in this article.

Acknowledgments: The authors are grateful to Catherine Gabriel for running the HRMS calculations and to Dimitris Matiadis (National Centre for Scientific Research "DEMOKRITOS" for running the NMR experiments on the 250 MHz Bruker Avance III spectrometer.

Conflicts of Interest: The authors declare no conflict of interest.

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