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Research paper

Design, synthesis and evaluation of semi-synthetic triazole-containing caffeic acid analogues as 5-lipoxygenase inhibitors

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ABSTRACT

In this work the synthesis, structure–activity relationship (SAR) and biological evaluation of a novel series of triazole-containing 5-lipoxygenase (5-LO) inhibitors are described. The use of structure-guided drug design techniques provided compounds that demonstrated excellent 5-LO inhibition with IC_{50} of 0.2 and 3.2 μM in cell-based and cell-free assays, respectively. Optimization of binding and functional potencies resulted in the identification of compound **13d**, which showed an enhanced activity compared to the parent bioactive compound caffeic acid **5** and the clinically approved zileuton **3**. Compounds **15** and **16** were identified as lead compounds in inhibiting 5-LO products formation in neutrophils. Their interference with other targets on the arachidonic acid pathway was also assessed. Cytotoxicity tests were performed to exclude a relationship between cytotoxicity and the increased activity observed after structure optimization.

1. Introduction

5-Lipoxygenase (5-LO) is a crucial enzyme of the arachidonic acid (AA) cascade, involved in the first two catalytic reactions in the biosynthesis of leukotrienes (LTs) [1]. The role of LTs as inflammatory mediators is well established, with pathophysiological implications in different diseases like asthma, allergic rhinitis as well as cardiovascular diseases and certain types of cancer [2].

Some new 5-LO inhibitors of plant origin are currently under clinical development for the treatment of osteoarthritis, such as a combination of baicalein **1** and catechin **2**, named Flavocoxid (Limbrel) [3]. Nevertheless, the only 5-LO inhibitor to have reached the market so far is zileuton **3**, which has some major drawbacks such as liver toxicity, low potency, and a short half-life due to rapid metabolic breakdown (Fig. 1) [4].

With the rising number of indications for anti-LT therapy, 5-LO inhibitor drug development becomes increasingly important. A variety of polyphenols have been reported to be capable of preventing the formation of 5-LO products, among which prominent dietary compounds such as curcumin **4**, and caffeic acid **5** are worth mentioning (Fig. 2) [5].

The current interest in polyphenols has been driven primarily by their abundance in human diet and in plants [6]. Moreover, several polyphenols can be isolated as products from the waste streams of the food industry, thus providing a potentially sustainable source of these compounds. However, the health effects of polyphenols are

Abbreviations: 5-LO, 5-Lipoxygenase; LT, Leukotriene; AA, Arachidonic Acid; SAR, Structure-Activity Relationship; CuAAC, Copper(I)-catalysed Azide Alkyne Cycloaddition; DCC, Dicyclohexylcarbodiimide; DCM, Dichloromethane; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; FLAP, Five Lipoxygenase Activating Protein; FCC, Flash Column Chromatography; TLC, Thin Layer Chromatography.

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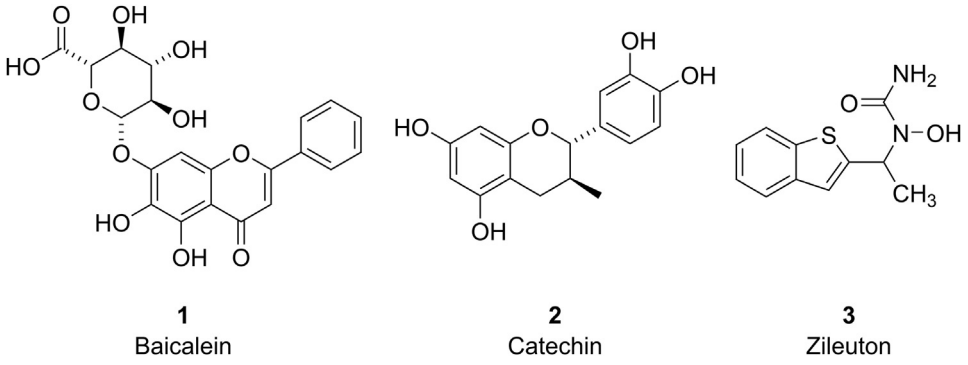


Fig. 1. Zileuton and 5-LO inhibitors of plant origin.

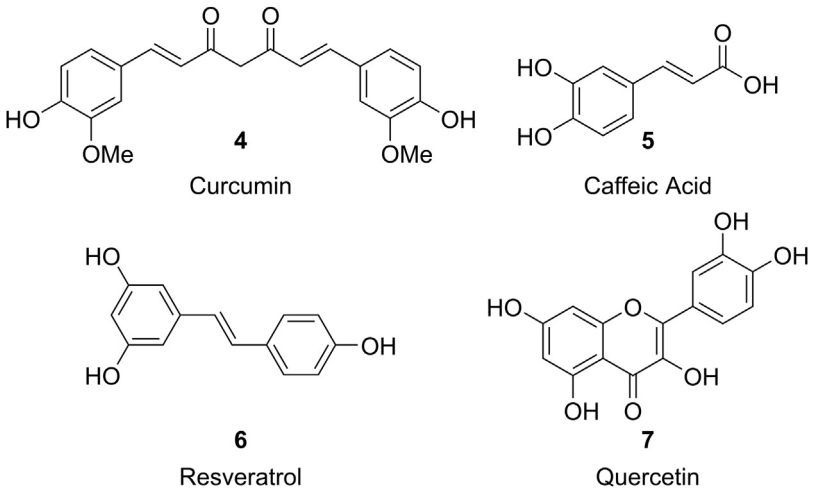


Fig. 2. Naturally occurring bioactive polyphenols.

negatively influenced by the bioavailability and solubility issues often associated with these phytochemicals, which largely reduce their use in pharmaceuticals [7]. Recent attempts to overcome these issues include the pegylation of curcumin **4** [8] and the glycosylation of resveratrol **6** and curcumin **4** [9].

A new approach is explored in the present work with the incorporation of a triazole-containing linker into polyphenol molecules. Triazoles are, in fact, not only passive linkers capable of giving metabolic stability and increasing water solubility through hydrogen-bond formation, but are also important pharmacophores able to interact with different targets. They have been proven to be determinant for the activities in antifungal, antibacterial, and anticancer agents, HIV protease inhibitors [10] and anti-inflammatory compounds [11].

2. Results and discussion

2.1. Synthesis of analogues

In this study a new class of semi-synthetic polyphenols related to caffeic acid has been designed. Structural modifications have been made in order to evaluate the structure–activity relationship (SAR) on 5-LO. Particularly, the linker length (A), the linker nature (B), the substitution on the aromatic portion (C) and the functionalization of the hydroxyl group (D) have been considered (Fig. 3).

The retrosynthetic analysis of these compounds suggested an initial esterification or amidation of the caffeic acid followed by the

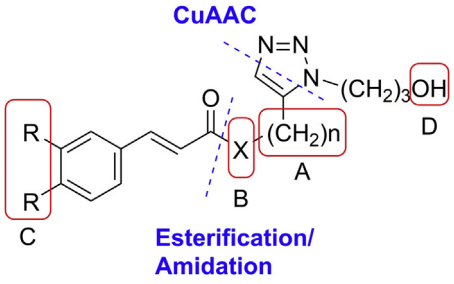
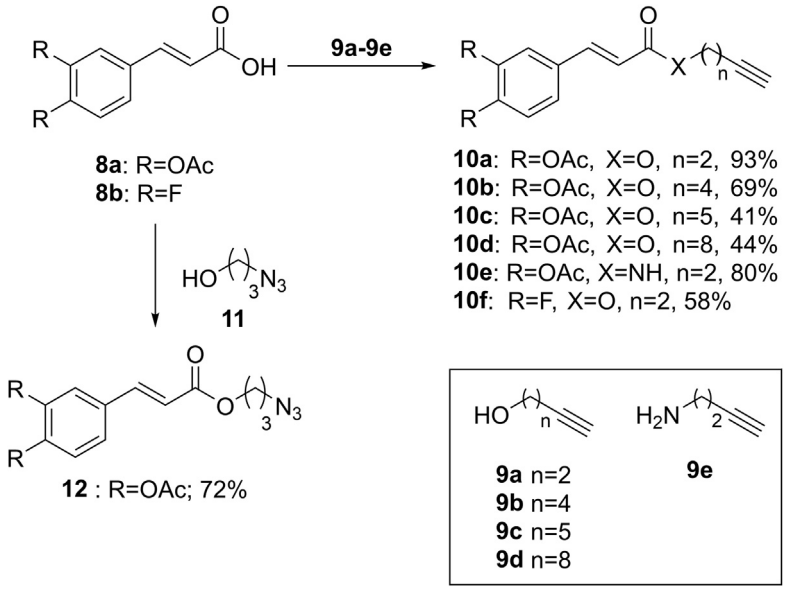


Fig. 3. Structural modifications on the triazolyl caffeic acid.

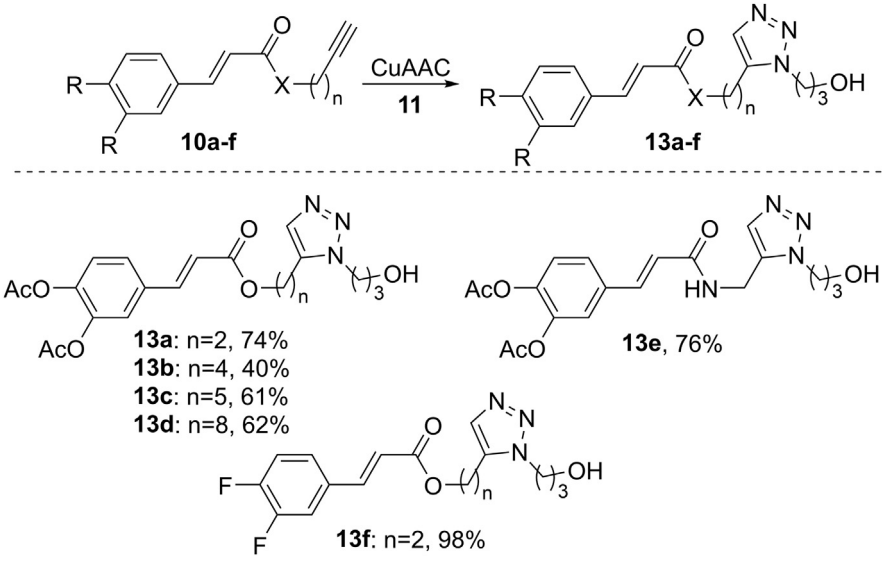
formation of the triazole cycle, by means of a copper(I) catalysed azide-alkyne cycloaddition (CuAAC, Fig. 3) [12]. Carboxylic acids **8a** and **8b** were easily converted into **10a**–**10f** and **12** after refluxing in benzene in presence of DMF and an excess of thionyl chloride. Propargyl alcohols **9a**–**9d**, propargylamine **9e** or the 3-azidopropan-1-ol **11** were then added and reacted at room temperature to furnish **10a**–**10f** and **12** in good yields (Scheme 1).

Propargyl derivatives **10a**–**10f** were subjected to the classical CuAAC conditions, by reacting with the azidoalcohol **11** in presence of catalytic amounts of Cu(I)iodide (Scheme 2).

The molecular diversity achieved in this library of compounds could be further increased by preparing analogue **14**, which represents a regioisomer of **13a**, in order to evaluate a possible distinct activity against 5-LO. Compound **14** was smoothly obtained by



Scheme 1. Synthetic procedures for esters (**10a**–**10f**, **12**). Reagents and conditions: thionyl chloride (25 mmol), **8a**–**8b** (1 mmol), DMF (0.1% mol), dry benzene (1 mL), reflux, then concentrate to dryness, addition of dry benzene (4 mL), pyridine (0.1% mol) dropwise and **9a**–**9e** (1.2 mmol) at r.t.



Scheme 2. Synthetic procedures for the preparation of triazoles (**13a-13f**). Reagents and conditions: *t*-BuOH–H_2O (5 mL, ratio 1:1), **10a-10f** (1.2 mmol), **11** (1 mmol), Cu(I)iodide (0.1 mol), 125 °C, 1 h.

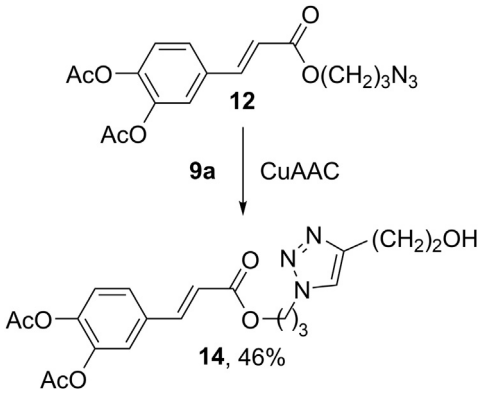
reaction of **12** with propargyl alcohol **9a** in the CuAAC conditions applied above (Scheme 3).

The orthogonal derivatives **15** and **16** were prepared to further enhance the molecular diversity within this family of compounds, and to widen the SAR study. Compound **15** was prepared from **10a** and **12**, whereas compound **16** was prepared from **10a** and 3-azidopropyl-6-acetoxy-2,5,7,8-tetramethylchromane-2-carboxylate, **17** (Fig. 4).

2.2. Biological evaluation

In order to study the ability of the new compounds to directly inhibit 5-LO, a cell-free assay using purified human recombinant 5-LO enzyme and arachidonic acid (20 μM) as the substrate was applied[13]. To study the inhibitory potency on 5-LO product formation in intact cells, human neutrophils stimulated with the Ca^{2+}-ionophore A23187 together with exogenous AA (20 μM) were used [14]. This dual strategy allows the identification of direct 5-LO inhibitors and provides insights into their potency in a physiologically relevant intact cell system, which generally constitutes a cut out for actives [15]. 5-LO products formed (LTB_4, its two trans-isomers, and 5-H(P)ETE) were analysed by RP-HPLC and the 5-LO inhibitor zileuton (IC_{50} = 0.5–1 μM) served as reference compound [16].

With reference to the poor 5-LO inhibitor caffeic acid **5**, acetylation of the two hydroxyl moieties yielding compound **8a** failed to improve potency (Table 1, entry 3). However, esterification of **8a** with **9a**–**d** yielding **10a**–**d** led to highly potent derivatives in intact neutrophils with IC_{50} values 0.18–1.8 μM (Table 1, entries 4–7). Note that the compounds showed low inhibition of 5-LO in the cell-free assay, implying that suppression of 5-LO product synthesis requires the cellular environment and could be related to interference with



Scheme 3. CuAAC towards the synthesis of compound **14**.

other enzymes within LT biosynthesis. The corresponding amide **10e** of bioactive **10a** was less potent, favouring the ester over amide function (Table 1, entry 8). Replacement of the acetoxy residues at the benzene of **10a** by fluorine yielding **10f** abolished 5-LO inhibition (Table 1, entry 9). Exchange of the alkyne of **10a** for the azide in **12** was tolerated (Table 1, entry 10).

The triazole caffeic acid esters **13a**–**d** suppressed the 5-LO activity in the cell-free and cell-based assays and progressive elongation of the chain length from **13a** to **13d** increased 5-LO inhibitory potency. Compound **13d** turned out as most potent lead compound with IC_{50} values of 0.2 and 3.2 μM, in cell-based and cell-free assays, respectively (Table 1, entry 14). The corresponding amide **13e** of the ester **13a** was inactive (cell-based and cell-free assays, Table 1, entry 15) and exchange of the acetoxy residues in **13a** by fluorine in **13f** led to an inactive derivative (Table 1, entry 16). Moreover, the isomeric triazole derivative **14** did not affect the 5-LO inhibitory potency in cell-free assays but resulted in loss of activity in neutrophils (Table 1, entry 17).

For the orthogonal derivatives **15** and **16**, which actually failed to significantly suppress the activity of isolated 5-LO, potent inhibition of 5-LO in intact cells was evident with IC_{50} values of 0.6 and 0.16 μM, respectively (Table 1, entries 18 and 19). Together, compounds **10d, 15** and **16** are potent inhibitors of cellular 5-LO product formation but **15** and **16** are less efficient against isolated 5-LO. Compound **13d** showed the highest potency for inhibiting 5-LO within this series (IC_{50} values 0.2 μM and 3.2 μM in cell based and cell-free assays respectively). The chain length of the bridge between the polyphenol and the triazole core was apparently critical for the activity profile. In fact, the gain in potency by linker elongation from **13a** to **13d** suggests the relevance of the hydrophobic interactions in the 5-LO binding site. Based on the fact that some compounds were much more potent against 5-LO in intact cells than in the isolated 5-LO, it appeared possible that these compounds may suppress cellular 5-LO product synthesis by interference with 5-lipoxygenase activating protein (FLAP). Thus, in addition to the 5-LO activity assay in neutrophils and on the isolated enzyme, compound **10a, 15** and **16** were tested in a HEK cell system that stably expresses 5-LO with or without FLAP (Fig. 5) [18].

All three tested compounds inhibited 5-LO product formation at 1 μM in cells expressing only 5-LO. Of significance, co-expression of FLAP even reduced the potencies against inhibition of 5-LO. In particular, compounds **15** and **16**, that failed to reduce 5-LO activity of the isolated enzyme, were highly potent and blocked 5-LO activity at a concentration of 1 μM completely. These results exclude FLAP as target of the compounds prepared herein, but clearly supports the inhibitory effect of the compounds on the 5-LO product formation in intact cells.

The logP of compounds herein presented was calculated through Marvin Sketch software 5.4.1.1. [19]. Elongating the linker length from compound **10a** (logP 1.99) to **10d** (logP 4.80) and from compound **13a** (logP 1.01) to **13d** (logP 3.65) leads to an increase in the value of logP due to the increase in lipophilicity. Turning the linker from an ester to an amide leads to a decrease in the logP value confirming an enhanced hydrophilic character of compounds **10e** (logP 1.08) and **13e** (logP 0.10) compared to their ester analogues **10a** (logP 1.99) and **13a** (logP 1.01). Substituting the groups on the aromatic ring with fluorine atoms instead, leads to an increase in logP values **10f** (logP 3.07), **13f** (logP 2.09) compared to their homologues **10a** and **13a** respectively. The orthogonal derivatives **15** and **16** which possess similar inhibitory activity against neutrophils (0.6 and 0.16 μM respectively) have shown logP values of 3.04 and 5.70 respectively, making theoretically compound **15** a better choice for a drug candidate than compound **16**. Compound **16** possess a logP value slightly higher than the acceptable range defined by Lipinski (5.70 *vs* 5.00). All the compounds herein presented have acceptable logP values (see supporting information for details).

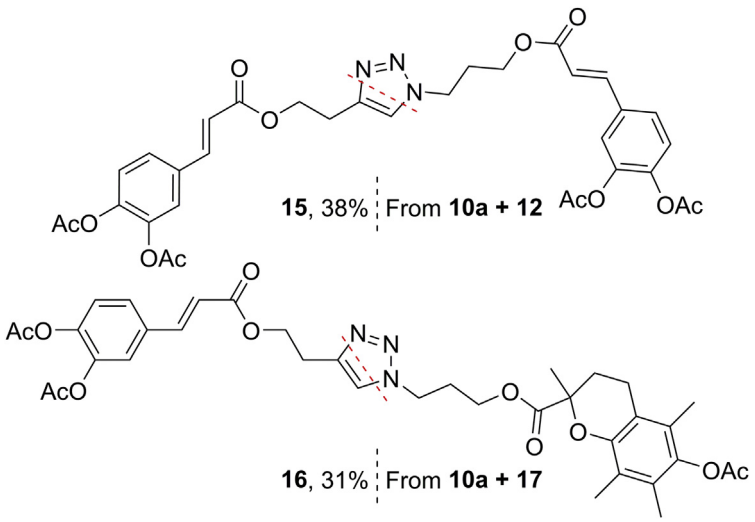
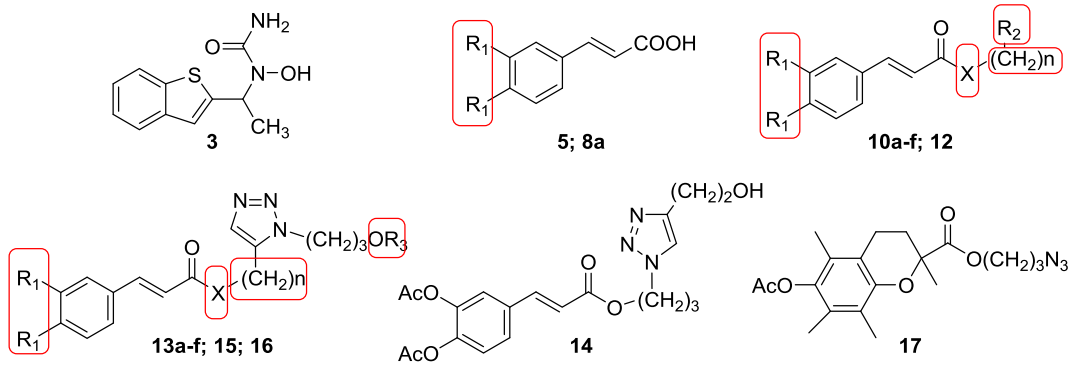


Fig. 4. Orthogonal compounds derived from caffeic acid and Trolox.

Table 1
Effects of new semi-synthetic compounds on the activity of 5-LO in cell-based (neutrophils) and cell-free (isolated 5-LO) assays.



Entry	Cmpd	Substituents	5-LO in neutrophils IC_{50} (μM)[a]	Isolated 5-LO IC_{50} (μM)[a]
1	**3**		1.3 ± 0.3	0.54 ± 0.11 [17]
2	**5**	R_1 = OH	>10 (104 ± 4.0)	8.5 ± 0.6
3	**8a**	R_1 = OAc	>10 (84.7 ± 3.5)	>10 (90.3 ± 1.6)
4	**10a**	R_1 = OAc; R_2 = CCH; X = O; n = 2	1.8 ± 0.2	>10 (50.2 ± 3.7)
5	**10b**	R_1 = OAc; R_2 = CCH; X = O; n = 4	0.92 ± 0.34	>10 (84.3 ± 1.8)
6	**10c**	R_1 = OAc; R_2 = CCH; X = O; n = 5	0.47 ± 0.15	>10 (70.7 ± 4.7)
7	**10d**	R_1 = OAc; R_2 = CCH, X = O; n = 8	0.18 ± 0.01	>10 (71.8 ± 4.6)
8	**10e**	R_1 = OAc; R_2 = CCH; X = NH; n = 2	8.23 ± 0.15	>10 (53.5 ± 3.1)
9	**10f**	R_1 = F; R_2 = CCH; X = O; n = 2	>10 (88.2 ± 3.9)	>10 (52.6 ± 3.0)
10	**12**	R_1 = OAc; R_2 = N_3; n = 2	1.3 ± 0.4	>10 (73.9 ± 8.2)
11	**13a**	R_1 = OAc; R_3 = H; X = O; n = 2	9.9 ± 1	7.4 ± 0.5
12	**13b**	R_1 = OAc; R_3 = H; X = O; n = 4	6.1 ± 0.5	7.6 ± 0.3
13	**13c**	R_1 = OAc; R_3 = H; X = O; n = 5	3.1 ± 0.6	6.7 ± 0.3
14	**13d**	R_1 = OAc; R_3 = H; X = O; n = 8	0.2 ± 0.03	3.2 ± 0.2
15	**13e**	R_1 = OAc; R_3 = H; X = NH; n = 2	>10 (103.7 ± 4.3)	>10 (53.6 ± 14.3)
16	**13f**	R_1 = F; R_3 = H; X = O; n = 2	>10 (101.4 ± 6.6)	>10 (90.3 ± 9.7)
17	**14**		>10 (75.1 ± 4.3)	7.6 ± 3.0
18	**15**	R_1 = OAc; R_3 = **5**; X = NH; n = 2	0.6 ± 0.09	>10 (81.3 ± 10.4)
19	**16**	R_1 = OAc; R_3 = Trolox[b]; X = NH; n = 2	0.16 ± 0.01	>10 (60.5 ± 8.2)
20	**17**		2.6 ± 0.1	>10 (86.3 ± 4.8)

[a] Remaining activity in % at 10 μm.
[b] 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

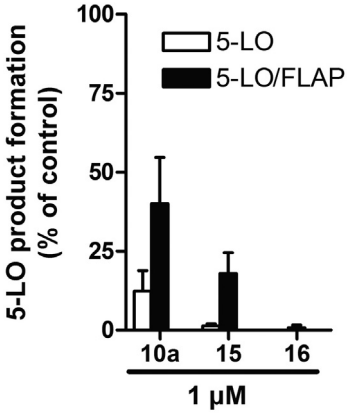


Fig. 5. Effect of compounds **10a, 15** and **16** on 5-LO product formation in HEK 293 cells stably transfected with 5-LO with and without FLAP.

2.3. Cytotoxicity assays

A decrease in cellular 5-LO product formation due to cytotoxic effects of the compounds in the library has been ruled out using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. After incubation of U937 cells for 24 h in the presence of representative compounds, cell viability was evaluated and expressed as EC_{50}, which is the concentration required to inhibit MTT-reducing mitochrondrial dehydrogenase activity by 50% (Table 2).

All the compounds tested caused only weak cytotoxic effects at similar concentrations as caffeic acid **5**. Compound **13f**, which was one of the less active 5-LO inhibitors among the compounds tested, most potently suppressed cell viability with an EC_{50} value of 5 μM (Table 2, entry 5). These data suggest that inhibition of cellular 5-LO product synthesis is unrelated to potentially cytotoxic effects of the compounds in the present library.

Table 2
Effects on cell viability of representative compounds on U937.

Entry	Cmpd	EC_{50} (μM)
1	**5**	9.9 ± 0.5
2	**10a**	9.8 ± 1.0
3	**13d**	10.2 ± 0.4
4	**13e**	10.3 ± 1.0
5	**13f**	5.1 ± 0.3
6	**15**	9.6 ± 0.5
7	**16**	10.1 ± 0.6

2.4. Molecular docking studies

A docking protocol was used to predict, at a molecular level, the possible interactions between 5-LO and the ligands reported in this work. All the compounds were docked into 5-LO (PDB ID: 3O8Y) and those with the lowest predicted binding energies were considered the most active compounds (Fig. 6). In general, compounds **13a**–**e** presented the lowest predicted binding energy. As reference, the calculated binding energy of caffeic acid is −6.5 kcal/mol. It is worth noting that in general compounds with an IC_{50} < 10 μM presented a predicted binding energy ≤−9.2 (with the exception of caffeic acid **5**).

Caffeic acid **5** and zileuton **3** were docked as references. Caffeic acid **5** is stabilized in the 5-LO binding pocket by the formation of hydrogen bonds with Ala424, blocking the binding site of arachidonic acid in 5-LO (Figs. 7-A). This binding site has been reported before for ketoconazole and ketaminazole in 5-LO. [20]. In a similar way, the binding site of zileuton **3** is located in the inner part of the binding pocket and is stabilized by the formation of a network of hydrogen bonds with the main chains of Leu420 and Ala424 and the side chain of Asn425 (Figs. 7-B).

Interestingly, the phenyl ring of compound **13d**, the most active inhibitor in both isolated 5-LO and intact neutrophils, is in close proximity to Leu420, Ala424 and Asn425, similarly as for caffeic acid **5**, whereas the rest of the molecule extends until fulfilling the binding site of arachidonic acid (Figs. 8-A). Compound **13f** does not form any hydrogen bond with the 5-LO pocket compared to **13d**, which is interacting with Asn425. Although the fluorines in compound **13f** could participate in hydrogen bonding, they are not oriented properly for this interaction. Moreover, the hydrogen bond between the triazole group and the catalytic Fe^{2+} likely could prevent the compound **13f** to get deeper in the binding site (Figs. 8-B). It has been observed before that the interaction with the catalytic Fe^{2+} contributes favourably to model the ligand binding, as the reduction of the ferric atom by the ligand is a very effective mode of action for 5-LO inhibition [20].

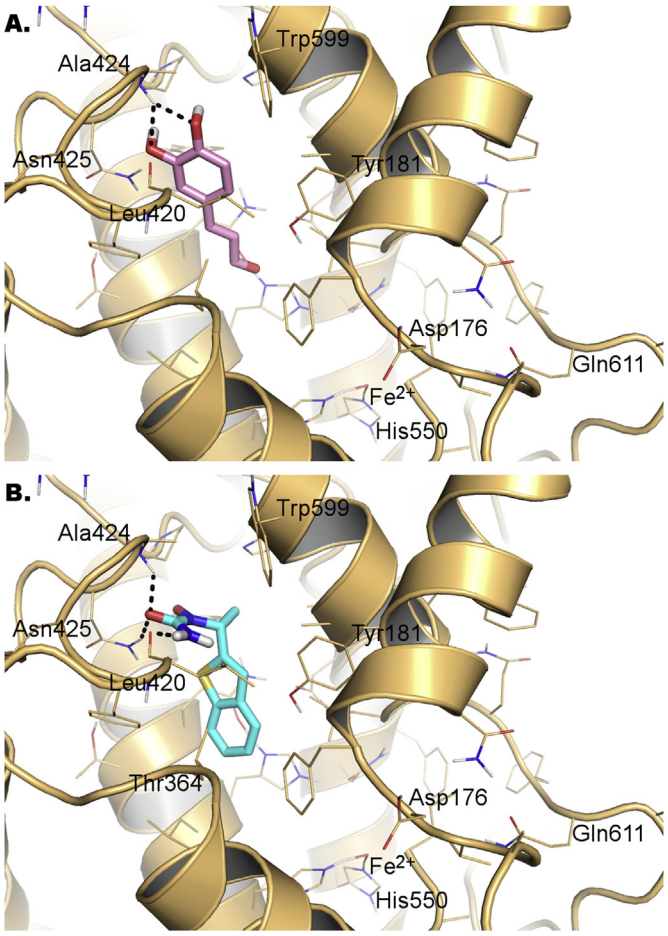


Fig. 7. Suggested binding conformations of caffeic acid **5** (A), zileuton **3** (B).

3. Conclusions

A new series of caffeic acid analogues bearing a triazole motif has been developed as potential 5-LO inhibitors. A clear profile in the structure–activity relationship emerged in dependence on the triazole containing linker. Lead compound, **13d**, was identified as a direct 5-LO inhibitor, with a demonstrated excellent and higher potency than the clinically approved zileuton **3** (IC_{50} = 0.5–1 μM) in neutrophils and slightly higher against isolated 5-LO. The linker length proved to be a crucial factor in determining potency against 5-LO. Compounds lacking of the triazole group (**10a**–**d**) showed noteworthy activity in the cell based assays but not against the isolated enzyme, suggesting the triazole core as crucial element to specifically direct the selectivity of these compounds on 5-LO. The orthogonal derivatives **15** and **16,** although failing to inhibit 5-LO directly, proved to be powerful inhibitors of cellular 5-LO product formation. Experiments conducted to evidence FLAP as possible alternative target for compounds **10a**, **15** and **16** indicated that their behaviour is not connected with inhibitory activity towards FLAP. Possibly, the compounds act as non-redox type 5-LO inhibitors that are up to 100-fold more potent in intact cells as compared to cell-free assays [21]. Further studies are currently undergoing to get more insights on the molecular mode of action of this class of compounds.

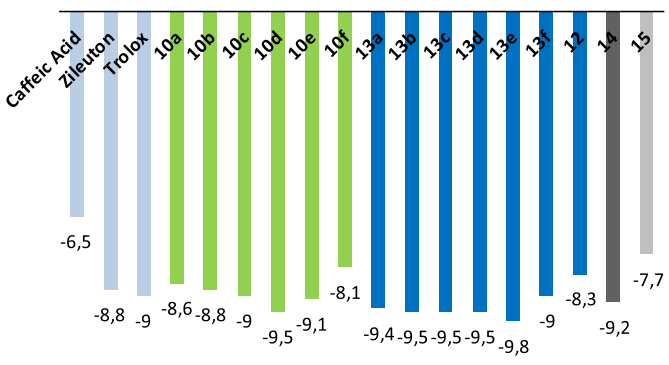


Fig. 6. Predicted binding energy of the compounds in the library on 5-LO (kcal/mol).

4. Experimental section

4.1. Instrumentation and reagents

Flash column chromatography (FCC) was performed using Breckland Scientific silica gel 60, particle size 40–63 nm under air pressure. Analytical thin layer chromatography (TLC) was performed using silica gel 60 F254 pre-coated glass backed plates and visualized by ultraviolet radiation (254 nm) and/or potassium permanganate or ammonium molybdate as appropriate. Isolated yields are reported to 0 decimal places and "quant." signifies a yield of 99.5% or higher. ^{1}H NMR spectra were recorded on Bruker DRX-400 (400 MHz) or DRX-600 (600 MHz) spectrometer. Chemical

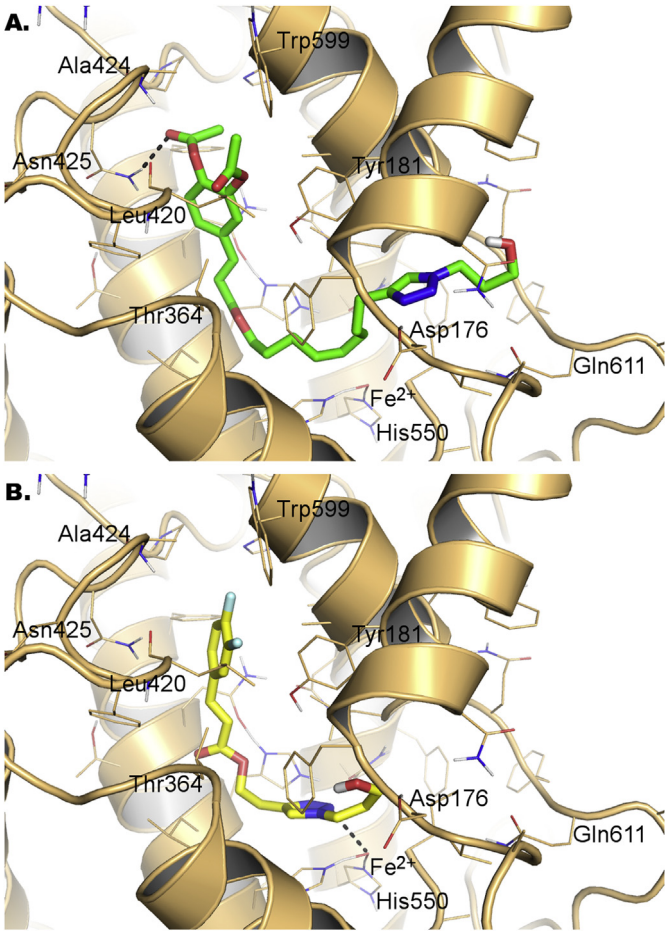


Fig. 8. Suggested binding conformations of **13d** (A) and **13f** (B).

shifts are reported in ppm with the resonance resulting from incomplete deuteration of the solvent as the internal standard ($CDCl_3$: 7.26 ppm, or DMSO-*d6*: 2.54 ppm, q). ^{13}C NMR spectra were recorded on Bruker DRX-400 (100 MHz) or DRX-600 (150 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$: 77.0 ppm, t or DMSO-*d6*: 30.73, ep.). Data are reported as follows: chemical shift δ/ppm, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet or combinations thereof. ^{13}C signals are singlets unless otherwise stated), coupling constants are reported in Hz, integration (^{1}H only). ^{1}H NMR signals are reported to 2 decimal places and ^{13}C signals to 1 decimal place unless rounding would produce a value identical to another signal. In this case, an additional decimal place is reported for both signals concerned. ^{19}F NMR signals are reported to 2 decimal places and trifluorotoluene was used as internal standard. High resolution mass spectrometry (HRMS) was performed on a Waters Micromass LCT spectrometer using electrospray ionization, time-of-flight analysis and Micromass MS software HRMS signals are reported to 4 decimal places and are within ±5 ppm of theoretical values. Infrared spectra were recorded neat as thin films on a Perkin–Elmer Spectrum One FTIR spectrometer and only selected peaks are reported (br = broad). Melting points were collected using a Stanford Research Systems Optimelt automated melting point system using a gradient of 1 °C per min. Unless stated otherwise, reagents were obtained from commercial sources and used without purification. The removal of solvent under reduced pressure was carried out on a standard rotary evaporator.

*4.1.1. General procedure for the preparation of compound **8a***

To a mixture of caffeic acid (1.0 eq., 1.0 mmol) and pyridine (1.0 eq., 1.0 mmol) was added acetic anhydride (5.0 eq., 5.0 mmol) and stirred at room temperature until the reaction was completed. The reaction mixture was then diluted with DCM (5 mL) and washed with 3 M HCl (3 × 5 mL) and with brine (3 × 5 mL). The organic layer was then dried over $MgSO_4$ and concentrated to dryness to afford the pure product.

*4.1.1.1. (E)-3-(3,4-Diacetyl)caffeic acid **8a**.* White solid; yield 95%; mp 125 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.66 (d, J = 2.0 Hz, 1H), 7.63 (dd, J = 8.4 Hz, 1H), 7.57 (d, J = 16.0 Hz, 1H), 7.31 (d, J = 8.35 Hz, 1H), 6.53 (d, J = 16.0 Hz, 1H), 3.32 (s, br, 1H), 2.29 (s, br, 3H) 2.28 (s, br, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.07, 168.98, 167.27, 143.2, 142.22, 142.05, 130.0, 126.6, 124.0, 122.9, 120.2, 20.3, 20.2. FT-IR (Neat, ν_{max} cm^{-1}) 1755, 1676, 1630. HRMS *m/z* calculated for $C_{13}H_{13}O_6$ $[M+H]^+$ 265.0712, found 265.0707.

*4.1.2. General procedure for the preparation of compounds **10a–10f** and **12***

A mixture of diacetylcaffeic acid **8a** or 3,4-difluoro cinnamic acid **8b** (1.0 eq., 1.0 mmol), thionyl chloride (25.0 eq., 25.0 mmol), DMF (0.001 eq., 0.001 mmol) and dry benzene (1.0 mL) was refluxed for 4 h. The excess of thionyl chloride was removed under vacuum. The residue was dissolved in dry benzene (4 mL) and pyridine (0.001 eq., 0.001 mmol) was added dropwise. The appropriate reagent **9a–e** or **11** was added (1.2 eq., 1.2 mmol) and the mixture was stirred overnight at room temperature under argon atmosphere. The resulting mixture was concentrated and the residue dissolved in DCM (10 mL), washed with water (3 × 10 mL), washed with brine (3 × 10 mL), dried over $MgSO_4$ and solvents were removed under vacuum to afford the crude material. The purification was performed as stated for each compound.

*4.1.2.1. (E)-4-(3-(But-3-yn-1-yloxy)-3-oxoprop-1-en-1-yl)-1,2-phenylene diacetate **10a**.* White solid; yield 93%; mp 82 °C. Purified by FCC (EtOAc/petroleum ether 30:70). Recrystallized from EtOAc/hexane (10:90). R_f 0.23 (EtOAc/petroleum ether 30:70). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (d, J = 16.0 Hz, 1H), 7.41 (dd, J = 8.4 Hz, 1.9 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.32 (t, J = 6.8 Hz, 2H), 2.61 (m, J = 6.8 and 2.7 Hz, 2H), 2.31 (s, 3H), 2,30 (s, 3H), 2.03 (t, J = 2.7 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0, 167.9, 166.2, 143.6, 143.3, 142.4, 133.2, 126.4, 123.9, 122.8, 118.8, 80.0, 70.0, 62.3, 20.7, 20.6, 19.1. FT-IR (Neat, ν_{max} cm^{-1}) 3272, 1765, 1714, 1638. HRMS *m/z* calculated for $C_{17}H_{17}O_6$ $[M+H]^+$ 317.1020, found 317.1016. Elemental analysis calculated for $C_{17}H_{16}O_6$ requires C 64.55%, 5.10%, found C 64.26%, H 5.12%.

*4.1.2.2. (E)-4-[3-(Hex-5-yn-1-yloxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate **10b**.* White solid; yield 69%; mp 48 °C. Purified by FCC (EtOAc/petroleum ether 30:70). Recrystallized from hexane. R_f 0.35 (EtOAc/petroleum ether 30:70). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 16.0 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.33 (overlapping, br, s, 1H), 7.18 (d, J = 8.3 Hz, 1H), 6.35 (d, J = 16.0 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 2.26 (s, 3H), 2.25 (s, 3H), 2.22 (overlapping m, 2H), 1.96 (s, 1H), 1.86–1.73 (m, 2H), 1.65–1.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 167.6, 166.2, 143.2, 142.5, 142.2, 133.0, 126.1, 123.7, 122.5, 119.0, 83.7, 68.7, 64.0, 27.6, 24.8, 20.50, 20.46, 18.0. FT-IR (Neat, ν_{max} cm^{-1}) 3290, 2933, 2864, 1762, 1707, 1638, 1507. HRMS *m/z* calculated for $C_{19}H_{21}O_6$ $[M+H]^+$ 345.1338, found 345.1347. Elemental analysis calculated for $C_{19}H_{20}O_6$ requires C 66.27%, 5.85%, found C 66.23%, H 5.87%.

4.1.2.3. (E)-4-[3-(Hept-6-yn-1-yloxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***10c***. White solid; yield 41%; mp 68 °C. Purified by FCC (EtOAc/petroleum ether 30:70). Recrystallized from hexane. R_f 0.23 (EtOAc/petroleum ether 30:70). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 16.0 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.34 (br, s, 1H), 7.19 (d, J = 8.3 Hz, 1H), 6.36 (d, J = 16.0 Hz, 1H), 4.18 (t, J = 6.6 Hz, 2H), 2.27 (s, 3H), 2.26 (s, 3H), 2.19 (td, J = 6.6 and 2.3 Hz, 2H), 1.94 (t, J = 2.4 Hz, 1H), 1.76–1.64 (m, 2H), 1.62–1.43 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 167.8, 166.4, 143.3, 142.6, 142.3, 133.2, 126.2, 123.8, 122.6, 119.2, 84.1, 68.4, 64.4, 28.1, 27.9, 25.0, 20.48, 20.45, 18.2. FT-IR (Neat, ν_{max} cm^{-1}) 3294, 2940, 2861, 1764, 1705, 1638. HRMS *m/z* calculated for $C_{20}H_{23}O_6$ $[M+H]^+$ 359.1489, found 359.1479. Elemental analysis calculated for $C_{20}H_{22}O_6$ requires C 67.03%, H 6.19%, found C 66.99%, H 6.24%.

4.1.2.4. (E)-4-[3-(Dec-9-yn-1-yloxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***10d***. White solid; yield 44%; mp 49 °C. Purified by FCC (EtOAc/petroleum ether 30:70). Recrystallized from hexane. R_f 0.43 (EtOAc/petroleum ether 30:70). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 16.0 Hz, 1H), 7.40 (dd, J = 8.4 and 1.9 Hz, 1H), 7.36 (d, J = 1.8 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 6.38 (d, J = 16.0 Hz, 1H), 4.19 (t, J = 6.7 Hz, 2H), 2.31 (s, 3H), 2.30 (s, 3H), 2.21–2.15 (m, 3H), 1.94 (t, J = 2.6 Hz, 1H), 1.74–1.64 (m, 2H), 1.59–1.46 (m, 4H), 1.38 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.34, 168.26, 167.0, 143.7, 142.9, 142.7, 133.7, 126.6, 124.2, 123.0, 119.8, 85.0, 68.4, 65.1, 29.4, 29.3, 29.0, 28.9, 28.7, 26.2, 21.0, 20.9, 18.7. FT-IR (Neat, ν_{max} cm^{-1}) 3303, 2932, 2862, 1764, 1709, 1638. HRMS *m/z* calculated for $C_{23}H_{29}O_6$ $[M+H]^+$ 401.1959, found 401.1956. Elemental analysis calculated for $C_{23}H_{28}O_6$ requires C 68.98%, H 7.05%, found C 68.87%, H 7.03%.

4.1.2.5. (E)-4-(3-(But-3-yn-1-ylamino)-3-oxoprop-1-en-1-yl)-1,2-phenylene diacetate ***10e***. White solid, yield 80%; mp 143 °C. Purified by FCC (EtOAc/petroleum ether 60:40). Recrystallized from EtOAc/hexane (90:10). R_f 0.32 (EtOAc/petroleum ether 60:40). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (d, J = 15.6 Hz, 1H), 7.37 (dd, J = 8.4 and 1.7 Hz, 1H), 7.34 (d, J = 1.6 Hz, 1H), 7.19 (d, J = 8.3 Hz, 1H), 6.33 (d, J = 15.5 Hz, 1H), 5.97 (s, 1H), 3.54 (q, J = 6.3 Hz, 2H), 2.47 (td, J = 6.3 and 2.5 Hz, 2H), 2.30 (s, 3H), 2.30 (s, 3H), 2.04 (t, J = 2.5 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.09, 168.07, 165.4, 142.9, 142.3, 139.2, 133.7, 126.2, 123.7, 122.2, 121.7, 81.5, 70.1, 38.2, 20.58, 20.55, 19.4. HRMS *m/z* found 316.1191 requires 316.1185. FT-IR (Neat, ν_{max} cm^{-1}) 3274, 3241, 3073, 1755, 1658, 1614, 1569. HRMS *m/z* calculated for $C_{17}H_{18}NO_5$ $[M+H]^+$ 316.1191, found 316.1185. Elemental analysis calculated for $C_{17}H_{17}NO_5$ requires C 64.75%, H 5.43%, N 4.44%, found C 64.37%, H 5.38%; N 4.30%.

4.1.2.6. (E)-but-3-yn-1-yl-3-(3,4-difluorophenyl)acrylate ***10f***. White solid; yield 58%; mp 48 °C. Purified by FCC (EtOAc/petroleum ether 10:90). Recrystallized from petroleum ether. R_f 0.4 (EtOAc/petroleum ether 10:90). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 16.0 Hz, 1H), 7.40–7.30 (m, 1H), 7.28–7.23 (m, 1H), 7.18 (dt, J = 16.6 and 8.2 Hz, 1H), 6.37 (d, J = 16.0 Hz, 1H), 4.32 (t, J = 6.8 Hz, 2H), 2.61 (td, J = 6.8 and 2.6 Hz, 2H), 2.03 (t, J = 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 152.9, 151.9 (d, J = 13.2 Hz), 150.3 (d, J = 12.9 Hz), 149.4 (d, J = 13.2 Hz), 142.9, 124.9 (dd, J = 6.6 and 3.6 Hz), 118.9, 117.9 (dd, J = 17.8 and 0.7 Hz), 116.5 (dd, J = 17.7 and 0.9 Hz), 80.2, 77.5, 77.2, 76.8, 70.1, 62.6, 19.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ −63.61 (d, J = 3.5 Hz), -134.83 (d, J = 20.9 Hz), −137.41 (d, J = 20.8 Hz). FT-IR (Neat, ν_{max} cm^{-1}) 3296, 1710, 1640, 1612, 1602, 1513. HRMS *m/z* calculated for $C_{13}H_{11}F_2O_2$ $[M+H]^+$ 237.0722, found 237.0710. Elemental analysis calculated for $C_{13}H_{10}F_2O_2$ requires C 66.10%, H 4.27%, found C 66.07%, H 4.30%.

4.1.2.7. (E)-4-(3-(3-Azidopropoxy)-3-oxoprop-1-en-1-yl)-1,2-phenylene diacetate ***12***. Yellow oil; yield 72%. Purified by FCC (EtOAc/petroleum ether 40:60). R_f 0.28 (EtOAc/petroleum ether 30:70). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 16.0 Hz, 1H), 7.36 (dd, J = 8.4, 1.9 Hz, 1H), 7.33 (d, J = 1.8 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 6.34 (d, J = 16.0 Hz, 1H), 4.23 (t, J = 6.2 Hz, 2H), 3.38 (t, J = 6.7 Hz, 2H), 2.25 (s, 3H), 2.24 (s, 3H), 1.96–1.88 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.35, 168.25, 166.7, 143.9, 143.5, 142.8, 133.4, 126.7, 124.3, 123.1, 119.2, 61.9, 48.5, 28.5, 20.93, 20.89. FT-IR (Neat, ν_{max} cm-1) 2096, 2933, 2864, 1770, 1710, 1639. HRMS *m/z* calculated for $C_{16}H_{18}N_3$ O6 $[M+H]^+$ 348.1190, found 348.1189.

4.1.3. General procedure for the preparation of compounds ***13a–f***

A catalytic amount of Cu(I)iodide (0.1 eq., 0.1 mmol) was added to a stirred solution of **11** (1.0 eq., 1.0 mmol) and **10a–f** (1.2 eq., 1.2 mmol) in a H_2O/*t*-butanol (5 mL, ratio 1:1). The mixture was stirred at 125 °C for 1 h until the consumption of **4** as checked by TLC (EtOAc/petroleum ether 80:20). The reaction was allowed to warm to room temperature and then diluted with water, extracted with DCM (3 × 10 mL), dried over $MgSO_4$, concentrated and purified by silica column chromatography with the appropriate eluent system to afford the pure triazole derivatives.

4.1.3.1. (E)-4-(3-(2-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl) ethoxy)-3-oxoprop-1-en-1-yl)-1,2-phenylene diacetate ***13a***. Yellow oil; yield 74%. Purified by column chromatography (EtOAc/petroleum ether 70:30). *Rf* 0.20 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, J = 16.0 Hz, 1H), 7.45 (s, 1H), 7.40 (dd, J = 8.4 and 1.8 Hz, 1H), 7.37 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 6.38 (d, J = 16.0 Hz, 1H), 4.52–4.48 (m, 4H), 3.64 (q, J = 5.6 Hz, 2H), 3.20–3.12 (m, 2H), 2.35 (s, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.15–2.10 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.3, 168.1, 166.5, 143.7, 143.3, 142.5, 133.3, 126.6, 124.1, 122.9, 122.3, 119.1, 63.5, 58.8, 47.0, 32.7, 31.1, 25.7, 20.77, 20.76. FT-IR (Neat, ν_{max} cm^{-1}) 2954 (br), 1771, 1710, 1638, 1505. HRMS *m/z* calculated for $C_{20}H_{24}O_7N_3$ $[M+H]^+$ 418.1609, found 418.1605.

4.1.3.2. (E)-4-(3-(4-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl) butoxy)-3-oxoprop-1-en-1-yl)-1,2-phenylene diacetate ***13b***. Yellow oil; yield 40%. Purified by FCC (EtOAc/petroleum ether 70:30). *Rf* 0.22 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 16.0 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.35 (br, s, 2H), 7.20 (d, J = 8.3 Hz, 1H), 6.35 (d, J = 16.0 Hz, 1H), 4.46 (t, J = 6.7 Hz, 2H), 4.20 (t, J = 5.4 Hz, 2H), 3.61 (t, J = 5.6 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.29 (s, 3H), 2.28 (s, 3H), 2.10–2.07 (m, 2H), 1.82–1.71 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 168.1, 166.8, 147.7, 143.6, 142.9, 142.5, 133.4, 126.5, 124.0, 122.8, 121.4, 119.4, 64.5, 58.8, 46.9, 32.8, 28.3, 25.9, 25.3, 20.7, 20.7. FT-IR (Neat, ν_{max} cm-1) 2948 (br), 1770, 1706, 1638, 1505 HRMS *m/z* calculated for $C_{22}H_{28}O_7N_3$ $[M+H]^+$ 446.1927, found 446.1927.

4.1.3.3. (E)-4-[3-(5-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***13c***. Yellow oil; yield 61%. Purified by FCC (EtOAc/petroleum ether 70:30). R_f 0.22 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (d, J = 16.0 Hz, 1H), 7.36 (dd, J = 8.4 and 1.9 Hz, 1H), 7.33 (d, J = 1.9 Hz, 1H), 7.31 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.34 (d, J = 16.0 Hz, 1H), 4.41 (t, J = 6.9 Hz, 2H), 4.15 (t, J = 6.6 Hz, 2H), 3.57 (t, J = 5.8 Hz, 2H), 3.12 (s, 1H), 2.69 (t, J = 7.6 Hz, 2H), 2.26 (s, 3H), 2.25 (s, 3H), 2.07–2.01 (m, 2H), 1.73–1.63 (m, 4H), 1.47–1.37 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0, 167.9, 166.5, 147.7, 143.3, 142.6, 142.3, 133.1, 126.3, 123.8, 122.6, 121.1, 119.2, 64.4, 58.4, 46.7, 32.6, 28.8, 28.2, 25.4, 25.3, 20.5, 20.5. FT-IR (Neat, ν_{max} cm^{-1}) 2939, 2860, 1770, 1707, 1639, 1505. HRMS *m/z* calculated for $C_{23}H_{30}O_7N_3$ $[M+H]^+$ 460.2071, found 460.2084.

4.1.3.4. (E)-4-[3-((8-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl) octyl)oxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***13d***. White solid; yield 62%; mp 73 °C. Purified by FCC (EtOAc/petroleum ether 70:30). Recrystallized from hexane. R_f 0.25 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 16.0 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.36 (s, 1H), 7.29 (s, 1H), 7.21 (d, J = 8.4 Hz, 1H), 6.38 (d, J = 16.0 Hz, 1H), 4.47 (t, J = 6.7 Hz, 2H), 4.18 (t, J = 6.6 Hz, 2H), 3.63 (t, J = 5.8 Hz, 2H), 2.70 (t, J = 7.6 Hz, 2H), 2.30 (s, 3H), 2.29 (s, 3H), 2.13–2.07 (m, 2H), 1.70–1.65 (m, 4H), 1.40–1.30 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 167.9, 166.6, 143.3, 142.5, 142.3, 133.3, 126.3, 123.8, 122.6, 120.9, 119.4, 77.1, 64.7, 58.8, 46.6, 32.6, 29.3, 29.1, 29.0, 29.0, 28.6, 25.8, 25.5, 20.6, 20.5. FT-IR (Neat, ν_{max} cm^{-1}) 3460, 2920, 2853, 1760, 1731, 1702, 1635, 1504. HRMS (ESI$^+$) calculated for $C_{26}H_{36}O_7N_3$ *m/z* 502.2567, found 502.2553. Elemental analysis calculated for $C_{26}H_{35}O_7N_3$ requires C 62.11%, H 7.00%, N 8.38%, found C 62.12%, H 6.97%, N 8.30%.

4.1.3.5. (E)-4-[3-((2-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl) ethyl)amino)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***13e***. White solid; yield 76%; mp 84 °C. Purified by FCC (MeOH/DCM 5:95). R_f 0.25 (MeOH/DCM 10:90). Recrystallized from EtOAc/hexane (20:80). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 (d, J = 15.6 Hz, 1H), 7.45 (s, 1H), 7.37 (dd, J = 8.4 and 2.0 Hz, 1H), 7.33 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 6.48 (m, 1H), 6.33 (d, J = 15.6 Hz, 1H), 4.51 (t, J = 6.7 Hz, 2H), 3.78–3.75 (m, 2H), 3.64–3.61 (m, 2H), 2.99 (t, J = 6.3 Hz, 2H), 2.32 (s, 3H), 2.31 (s, 3H), 2.19 (s, 1H), 2.16–2.10 (m, 2H), 1.97 (t, J = 4.9 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 168.2, 165.7, 142.9, 142.3, 142.3, 139.0, 133.8, 126.2, 123.8, 122.3, 122.0, 58.6, 46.9, 38.8, 32.4, 29.7, 25.5, 20.6, 20.6. FT-IR (Neat, ν_{max} cm^{-1}) 3348, 3243, 3060, 2933, 2256, 1768, 1663.21, 1620, 1574, 1505. HRMS *m/z* calculated for $C_{20}H_{25}O_6N_4$ [M+H]$^+$ 417.1778, found 417.1774. Elemental analysis calculated for $C_{20}H_{24}O_6N_4$ requires C 57.69%, 5.81%, N 13.45%, found C 58.19%, H 6.04%, N 10.25%.

4.1.3.6. (E)-2-[1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl]ethyl-3-(3,4-difluorophenyl)acrylate ***13f***. White solid; yield 98%; mp 82 °C. Purified by FCC (EtOAc/petroleum ether 70:30). Recrystallized from petroleum ether. R_f 0.11 (EtOAc/petroleum ether 80:20). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 16.0 Hz, 1H), 7.47 (s, 1H), 7.34–7.27 (m, 1H), 7.20 (s, 1H), 7.16–7.06 (m, 1H), 6.30 (d, J = 16.0 Hz, 1H), 4.46 (dt, J = 13.2 and 6.6 Hz, 4H), 3.62 (t, J = 5.5 Hz, 2H), 3.10 (t, J = 6.2 Hz, 2H), 2.14–2.01 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9 (s), δ 152.0 (dd, J = 96.8, 13.0 Hz), 149.5 (d, J = 79.7 Hz) 143.7 (s), 142.6–142.3 (m), 131.2 (dd, J = 6.0 and 4.1 Hz), 124.5 (dd, J = 6.6 and 3.5 Hz), 121.7 (s), 118.6 (d, J = 2.3 Hz), 117.5 (d, J = 17.8 Hz), 116.0 (d, J = 17.7 Hz), 63.2 (s), 58.6 (s), 46.7 (s), 32.4 (s), 25.4 (s). ^{19}F NMR (376 MHz, CDCl3) δ −62.75 (s), −133.92 (s), −133.98 (s), −136.52 (d, J = 20.9 Hz). FT-IR (Neat, ν_{max} cm-1) 3328, 3124, 3055, 2962, 2885, 1712, 1638, 1600, 1516. HRMS *m/z* calculated for $C_{16}H_{18}O_3N_3F_2$ [M+H]$^+$ 338.1316, found 338.1316. Elemental analysis calculated for $C_{16}H_{17}N_3O_3F_2$ requires C 56.97%, H 5.08%, N 12.46%, found C 56.97%, H 5.19%, N 12.16%.

4.1.3.7. (E)-4-[3-(3-(4-(2-Hydroxyethyl)-1H-1,2,3-triazol-1-yl)propoxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***14***. White solid; yield 46%; mp 78 °C. Purified by FCC (EtOAc). R_f 0.14 (EtOAc). Recrystallized from EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 16.0 Hz, 1H), 7.44 (s, 1H), 7.40 (dd, J = 8.5 and 1.9 Hz, 1H), 7.37 (d, J = 1.7 Hz, 1H), 7.22 (d, J = 8.3 Hz, 1H), 6.35 (d, J = 16.0 Hz, 1H), 4.46 (t, J = 6.9 Hz, 2H), 4.23 (t, J = 5.9 Hz, 2H), 3.92 (t, J = 5.8 Hz, 2H), 2.93 (t, J = 5.9 Hz, 2H), 2.34–2.31 (m, overlapping s, 2H), 2.30 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 168.1, 166.5, 143.8, 143.6, 142.6, 133.1, 126.6, 126.6, 124.1, 122.9, 122.1, 118.7, 61.7, 61.3, 47.3, 29.6, 28.8, 20.8, 20.7. FT-IR (Neat, ν_{max} cm^{-1}) 2933, 2099, 1770, 1710, 1639, 1505. HRMS *m/z* calculated for $C_{20}H_{23}O_6N_3$ [M+H]$^+$ 359.1501, found 4359.1495.

4.1.3.8. 4-[(E)-3-(3-(4-(2-(((E)-3-(3,4-Diacetoxyphenyl)acryloyl)oxy) ethyl)-1H-1,2,3-triazol-1-yl)propoxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***15***. White solid; yield 40%; mp 125 °C. Purified by FCC (EtOAc). Recrystallized from EtOAc/hexane (20:80). R_f 0.27 (EtOAc/petroleum ether 80:20). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, J = 16.0 and 2.3 Hz, 2H), 7.43 (s, 1H), 7.40 (dd, J = 8.4 and 2.2 Hz, 2H), 7.37 (s, 2H), 7.23 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 8.5, 1H), 6.40–6.34 (m, 2H), 4.50–4.47 (m, 4H), 4.25 (t, J = 5.9 Hz, 2H), 3.20–3.10 (m, 2H), 2.37–2.33 (m, 2H), 2.31 (s, 3H), 2.30 (s, 3H), 2.29 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 168.1, 168.1, 168.1, 166.5, 166.4, 144.4, 143.8, 143.7, 143.6, 143.3, 142.6, 142.6, 133.3, 133.2, 126.6, 124.1, 124.07, 122.9, 122.9, 121.9, 119.1, 118.7, 63.5, 61.3, 47.3, 29.7, 25.7, 25.7, 20.8, 20.8, 20.7, 20.7. FT-IR (Neat, ν_{max} cm^{-1}) 2962, 1768, 1708, 1638, 1504. HRMS *m/z* calculated for $C_{33}H_{34}O_{12}N_3$ [M+H]$^+$ 664.2137, found 664.2130 Elemental analysis calculated for $C_{33}H_{33}O_{12}N_3$ requires C 59.73%, H 5.01%, N 6.33%, found C 59.06%, H 4.97%, N 6.02%.

4.1.3.9. (E)-4-[3-(2-(1-(3-((6-acetoxy-2,5,7,8-tetramethylchromane-2-carbonyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)ethoxy)-3-oxoprop-1-en-1-yl]-1,2-phenylene diacetate ***16***. White solid; yield 30%; mp 58 °C. Purified by FCC (EtOAc/petroleum ether 20:80). R_f 0.44 (EtOAc/petroleum ether 30:70). Recrystallized from hexane. ^{1}H NMR (600 MHz, DMSO) δ 7.75 (s, 1H), 7.69–7.65 (m, 1H), 7.64 (d, J = 15.5 Hz, 2H), 7.31 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 16.0 Hz, 1H), 4.38 (t, J = 6.2 Hz, 2H), 4.22–4.07 (m, 2H), 4.04–3.88 (m, 2H), 3.02 (t, J = 6.1 Hz, 2H), 2.67–2.33 (m, 4H), 2.28 (d, J = 3.6 Hz, 8H), 2.09 (s, 3H), 2.04 (s, 2H) 1.93 (s, 3H), 1.86 (s, 3H), 1.85–1.76 (m, 2H), 1.55 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.0, 169.5, 168.6, 168.5, 166.4, 149.3, 144.0, 143.6, 143.4, 142.8, 141.4, 133.3, 127.4, 127.1, 125.5, 124.6, 123.6, 123.0, 122.2, 119.4, 117.7, 77.5, 63.6, 62.2, 46.3, 30.3, 29.2, 25.5, 25.4, 20.8, 20.7, 20.7, 20.6, 13.2, 12.3, 12.1. FT-IR (Neat, ν_{max} cm^{-1}) 2935, 1749, 1712, 1638, 1505. HRMS *m/z* calculated for $C_{36}H_{42}O_{11}N_3$ [M+H]$^+$ 692.2814, found 692.2823. Elemental analysis calculated for $C_{36}H_{41}N_3O_{11}$ requires C 62.51%, H 5.97%, N 6.07% found C 61.76%, H 5.90%, N 5.92.

4.1.4. General procedure for the preparation of compound ***17***

To 6-acetyl-2,5,7,8-tetramethylchromane-2-carboxylic acid (1.0 eq.) in dichloromethane was added DCC (1.0 eq.) and DMAP (1.0 eq.). The mixture was allowed to stir for 10 min at room temperature before the azide or alcohol (1.0 eq.) was added. The mixture was stirred at room temperature overnight under an atmosphere of argon. The resulting mixture was filtered, washed with a 10% solution of $KHSO_4$ three times and dried over $MgSO_4$ before the solvent removed *in vacuo* to afford the crude material.

4.1.4.1. 3-Azidopropyl 6-acetoxy-2,5,7,8-tetramethylchromane-2-carboxylate ***17***. Colourless oil; yield 78%. Purified by FCC (EtOAc/petroleum ether 40:60). *Rf* 0.24 (EtOAc/petroleum ether 60:30) ^{1}H NMR (400 MHz, DMSO-d6) δ 4.13 (m, 1H), 3.97–4.03 (m, 1H), 3.10–3.03 (m, 2H), 2.68–2.56 (m, 1H), 2.50–2.32 (m, 2H), 2.29 (s, 3H), 2.09 (s, 3H), 1.96 (s, 3H), 1.87 (s, 3H), 1.86–1.77 (m, 1H), 1.75–1.65 (m, 2H), 1.57 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d6) δ 172.4, 168.8, 148.7, 140.9, 126.5, 124.9, 121.7, 117.0, 77.0, 61.8, 47.2, 29.9, 27.5, 25.1, 20.2, 20.2, 12.7, 11.8, 11.6. FT-IR (Neat, νmax cm^{-1}) 2934, 2097, 1731, 1752. HRMS *m/z* calculated for $C_{19}H_{25}N_3O_5Na$ [M+Na]$^+$ 398.1705, found 398.1692.

4.2. Biological assays

4.2.1. Expression and purification of human recombinant 5-LO

E. coli Bl21 (DE3) cells were transformed with pT3–5LO plasmid,

lysed in 50 mM triethanolamine/HCl pH 8.0 plus EDTA (5 mM), soybean trypsin inhibitor (60 μg/mL), phenylmethanesulphonyl fluoride (1 mM), dithiothreitol (1 mM) and lysozyme (1 mg/mL) and then sonicated (3 × 15 s). The homogenate was centrifuged at 10,000 × g for 15 min and the remaining supernatant at 40,000 × g for 70 min at 4 °C. 5-LO in the supernatant was partially purified by affinity chromatography on an ATP-agarose column as described by Fisher et al., in 2003 [22]. Semi-purified 5-LO was diluted in PBS containing EDTA (1 mM) and ATP (1 mM) and immediately used for activity assays.

4.2.2. Activity assay for human recombinant 5-LO

Human recombinant 5-LO was pre-incubated with the test compounds for 10 min at 4 °C and pre-warmed for 30 s at 37 °C. 5-LO product formation was initiated by addition of 2 mM $CaCl_2$ and 20 μM arachidonic acid. After 10 min at 37 °C, the reaction was terminated by addition of 1 mL ice-cold methanol. Formed 5-LO metabolites (all-trans isomers of LTB4 and 5-H(P)ETE) were analysed by RP-HPLC as described by Koeberle et al., in 2009 [23].

4.2.3. Determination of 5-LO product formation in neutrophils

Freshly isolated neutrophils (1×10^7/mL) were pre-incubated with the test compounds for 15 min at 37 °C. Then, 5-LO product formation was started by addition of 2.5 μM Ca^{2+}-ionophore A23187 plus 20 μM AA. The reaction was stopped after 10 min at 37 °C with 1 mL of methanol. Major 5-LO metabolites (LTB4 and its all-trans isomers and 5-H(P)ETE) were extracted and analysed by HPLC as described by Werz et al. [24]. Cysteinyl-LTs C4, D4 and E4 and oxidation products of LTB4 were not determined.

4.2.4. Determination of 5-LO product formation in stably transfected HEK 293 cells

Two different HEK cell lines were tested (HEK_5-LO; HEK-5LO/FLAP) as described by Pergola et al. [18]. 1×10^6 cells were suspended in 1 mL PGC buffer (PBS; 0.1% glucose, 1 mM $CaCl_2$, pre-incubated with the test compound for 10 min at 37 °C and subsequently stimulated by 2.5 μM Ca^{2+}-ionophore A23187) plus 2 μM arachidonic acid for 10 min at 37 °C. The reaction was stopped by 1 mL methanol, and the metabolites (all-trans isomers of LTB4 and 5-H(P)ETE) were extracted and analysed by HPLC as described above.

4.2.5. Cytotoxicity assays

U937 (lymphoblast from lung) a cell line derived from malignant cells of a pleural effusion of 37 year old caucasian male with diffuse histiocytic lymphoma were grown in RPMI 1640 (Gibco) added with 10% FBS (Foetal Bovine Serum), 10% Hepes buffer, 5% penicillin\streptomycin at 37 °C in a humidified 5% CO_2 atmosphere for 72 h 100 μL of cells at the density of 1×10^6/mL were seeded into 96-well plates (Nunc) and treated with the different compounds at different concentrations (Caffeic acid at 1, 10, 20 μM; **10a**,**13d**, **13e**,**13f**, **15**, **16** at 3, 10, 20 μM) and analysed by MTT assay after 24 h incubation. MTT stock solution (5 mg/mL) was added to each culture being assayed to equal one-tenth the original culture volume and incubated for 4 h. At the end of the incubation period, we added acidic isopropanol (100 μL of 0.04N HCl in absolute isopropanol). Absorbance of converted dye was measured at a wavelength of 570 nm with background subtraction at 630–690 nm using ELISA-reader (Victor, Perkin–Elmer). We expressed the effect on cell viability as EC_{50}, which is the concentration required to inhibit MTT-reducing mitochrondrial dehydrogenase activity by 50% [25]. The absorbance of untreated cells (RPMI + DMSO) was taken as 100% viability to calculate cytotoxicity.

4.2.6. Statistics

Data obtained from biological experiments are expressed as mean ± S.E.M. of single determinations performed in three or four independent experiments at different days. IC50 values obtained from at least four different compound concentrations were calculated by nonlinear regression using SigmaPlot 9.0 (Systat Software Inc., San Jose, USA) one site binding competition. Statistical evaluation of the data was performed by one-way ANOVA followed by a Bonferroni or Tukey–Kramer post-hoc test for multiple comparisons respectively. A p value <0.05 (*) was considered significant.

4.3. Computational analysis

4.3.1. Ligand and protein preparation for docking

The structure of all compounds used in this study was optimized using the MMFF94x force field. The torsional root and branches of the ligands were chosen using AutoDockTools (version May_03_13), allowing flexibility for all rotatable bonds of the ligand [26]. Subsequently, AutoDockTools was used to assign Gasteiger–Marsilli atomic charges to all ligands. On the other hand, all water molecules were removed from the 5-LO crystal structure (PDB ID: 3O8Y). It is worth mentioning that this structure of 5-LO was crystallized without any ligand. In order to obtain an active conformation of this enzyme, the coordinates of arachidonic acid were extracted from crystal structure 3V99 and docked in 3OY8 using a rigid docking approach. Afterwards, the complex was submitted to a structure optimization using the MMFF94x force field and the resulting protein structure was used for the calculations in this study. Also, AutoDockTools was used to merge all non-polar hydrogen atoms and to assign Gasteiger charges for each atom of the macromolecule.

4.3.2. Molecular docking

Docking calculations were performed with AutoDock Vina 1.1.2 software [27]. The searching area was defined by a box of 30 × 20 × 30 Å centred at the coordinates of the catalytic Fe^{2+}. In this research, the conformational search of the ligand was carried using and exhaustiveness value of 8 to generate maximum 9 binding modes with a maximum difference in energy of 4 kcal/mol between the best and the worst conformation. The conformation with the lowest predicted binding energy was used for further analysis. In order to maintain the reproducibility of this study, all the calculations were performed using a seed equal to 1.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.07.011.

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