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Research paper

Synthesis and biological evaluation of C(5)-substituted derivatives of leukotriene biosynthesis inhibitor BRP-7

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ABSTRACT

Pharmacological intervention with 5-lipoxygenase (5-LO) pathway leading to suppression of leukotriene (LT) biosynthesis is a clinically validated strategy for treatment of respiratory and cardiovascular diseases such as asthma and atherosclerosis. Here we describe the synthesis of a series of C(5)-substituted analogues of the previously described 5-LO-activating protein (FLAP) inhibitor BRP-7 (IC_{50} = 0.31 μM) to explore the effects of substitution at the C(5)-benzimidazole (BI) ring as a strategy to increase the potency against FLAP-mediated 5-LO product formation. Incorporation of polar substituents on the C(5) position of the BI core, exemplified by compound **11** with a C(5)-nitrile substituent, significantly enhances the potency for suppression of 5-LO product synthesis in human neutrophils (IC_{50} = 0.07 μM) and monocytes (IC_{50} = 0.026 μM).

1. Introduction

Leukotrienes (LTs) are important pro-inflammatory mediators, which play key roles in the progression of inflammation in various ailments including asthma, chronic obstructive pulmonary disease (COPD), arthritis, allergy, cancer, and atherosclerosis [1,2]. LTs are produced by the 5-lipoxygenase (5-LO) pathway, which is activated by the release of arachidonic acid (AA) via cytosolic phospholipase ($cPLA_2$) in response to inflammatory stimuli. Liberated AA is then converted to the unstable epoxide intermediate LTA_4 by 5-LO, which is a junction point for production of either LTB_4 by LTA_4 hydrolase or cysteinyl-LTs (LTC_4, D_4 and E_4) by LTC_4 synthase [3]. LTB_4 acts as ligand for the G protein-coupled receptors (GPCR) BLT_1 and BLT_2, which promotes the inflammatory response as well as neutrophil and eosinophil chemotaxis. The cysteinyl-LTs activate the GPCRs $CysLT_1$ and $CysLT_2$ causing bronchoconstriction, airway edema and mucus secretion.

In intact cells, the 5-LO-mediated formation of LTA_4 from AA also requires a concomitant involvement of the 5-LO-activating protein (FLAP) that transfers AA to 5-LO for efficient metabolism. FLAP may function as a regulatory protein for 5-LO at the nuclear membrane where the LT synthetic complex is assembled [4]. Pharmacological concepts to interfere with LT biosynthesis essentially include direct 5-LO inhibitors or compounds that block the function of FLAP [2,5]. Although intensive efforts have been made to develop LT biosynthesis inhibitors, only a single 5-LO inhibitor, namely zileuton (**1**), is in clinical use for the treatment of asthma and/or allergic rhinitis [6]. Meanwhile, 5-LO inhibitor setileuton (**2**, MK-0633) and several FLAP inhibitors such as fiboflapon (GSK2190915, **3**), AZD6642 (**4**) and BI665915 (**5**) have been reported to be in various phases of preclinical and clinical studies for treatment of respiratory diseases such as asthma and COPD [7–10] (Fig. 1). Moreover, beneficial pharmacology of LT biosynthesis inhibitors in atherosclerosis was demonstrated, highlighting the potential value of this therapeutic target for cardiovascular diseases besides respiratory pathologies [11–15].

Based on the renowned interest in LT biosynthesis inhibitors and as a result of our continuing efforts to develop novel

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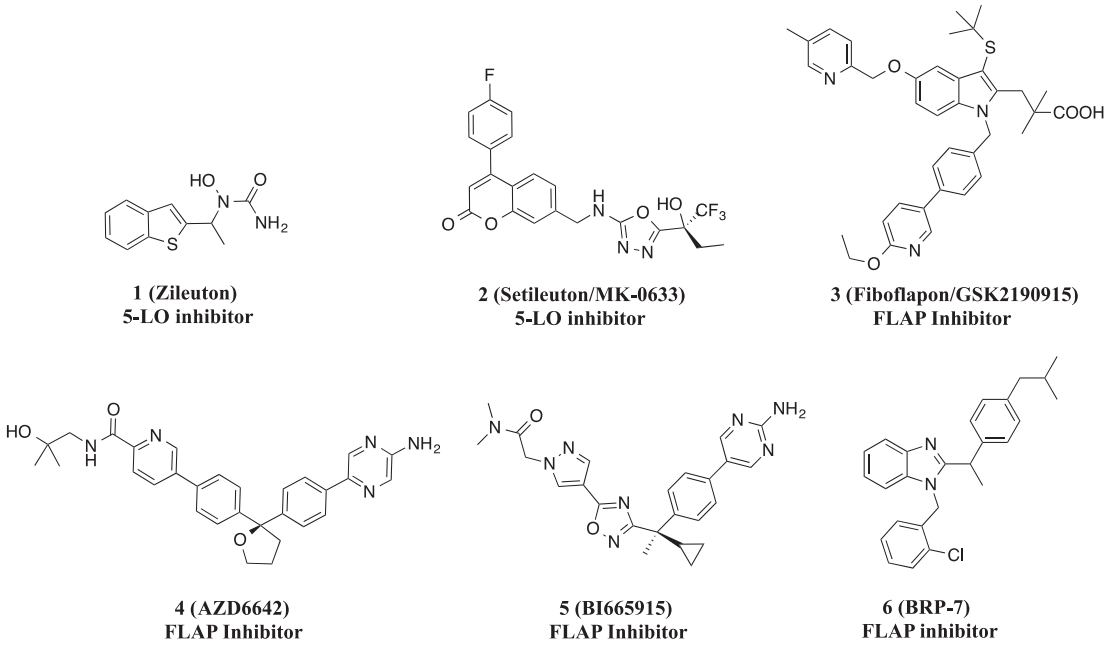


Fig. 1. Chemical structures of LT biosynthesis inhibitors.

compounds along these lines [16–23], we recently identified a non-acidic benzimidazole derivative (**6**, BRP-7) bearing an ibuprofen fingerprint with reasonable potency to suppress 5-LO product synthesis in intact neutrophils (IC_{50} = 0.31 μM) [16]. Our detailed pharmacological investigation revealed BRP-7 as a potent inhibitor of LT biosynthesis targeting FLAP without affecting 5-LO [22,23].

Our preliminary structure-activity relationships (SAR) studies indicated that (*i*) the benzyl group in 1-position of the benzimidazole (BI) is absolutely essential and *ortho* substitution increases the potency, (*ii*) the isobutyl substitution clearly governs the potency and larger or smaller hydrophobic groups in this position are not tolerated, (*iii*) C(5)-BI substitution may favor inhibition of LT biosynthesis and (*iv*) the chirality at asymmetric carbon linked at C(2)-BI was not an important determinant of the biological activity since a negligible difference in the inhibitory activity of individual isomers over racemic BRP-7 was previously revealed [16,23]. Since the number of substitutions was limited to conclusively explain the contribution of C(5)-BI substitution on pharmacological activity, here we explored different substituents at C(5)-BI to obtain broad and non-correlated variation in their lipophilic, electronic and steric properties. This substitution-focused SAR-study resulted in the discovery of highly active compounds that might be of importance to understand the role of C(5)-substitution of the BI in order to develop efficient inhibitors of LT biosynthesis.

2. Results and discussion

2.1. Chemistry

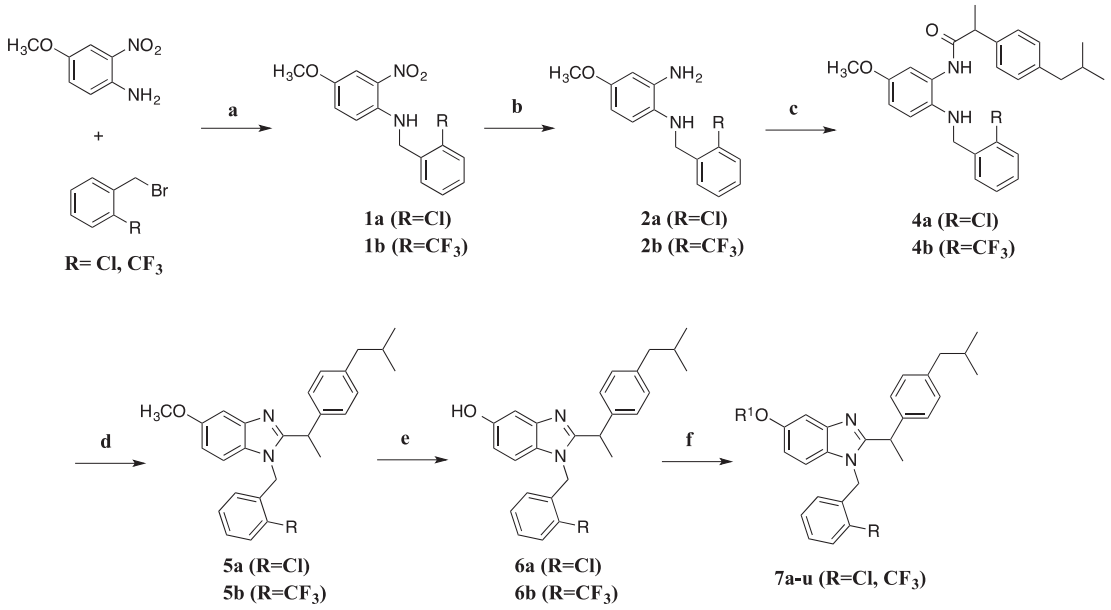
For the synthesis of compounds **7a-u**, we utilized the synthetic procedure outlined in Scheme 1 by following our published procedures [16]. All compounds were synthesized as racemates since we previously showed a negligible difference in the inhibitory potency of individual enantiomers of BRP-7 [23]. Starting from 2-nitro-4-methoxyaniline, benzylation and further reduction of the nitro group generated **2a-b**. Following the described conditions shown in Scheme 1, the 5-methoxybenzimidazole derivatives **5a-b** were obtained. Hydrolysis of the methyl ether was accomplished by HBr to generate the 5-hydroxy derivatives (**6a-b**). The 5-hydroxybenzimidazole derivatives were then alkylated with appropriate alkyl halides to obtain the target compounds **7a-u**. For the synthesis of 5-cyanobenzimidazole derivative **11** (Scheme 2), *N*-2-chlorobenzyl-4-cyano-2-nitroaniline **8** was first prepared from 2-chloro-5-cyanonitrobenzene by nucleophilic substitution of the chlorine atom by 2-chlorobenzylamine. Reduction of the nitro group followed by the standard procedures described in Scheme 2 resulted in the 5-cyanobenzimidazole derivative **11**.

All compounds were purified by automated flash chromatography and checked for purity with UPLC before being tested in biological assays (purity was >97%). The structures of the compounds were confirmed by high-resolution mass spectrometry (HRMS), ^{1}H and ^{13}C NMR spectral data.

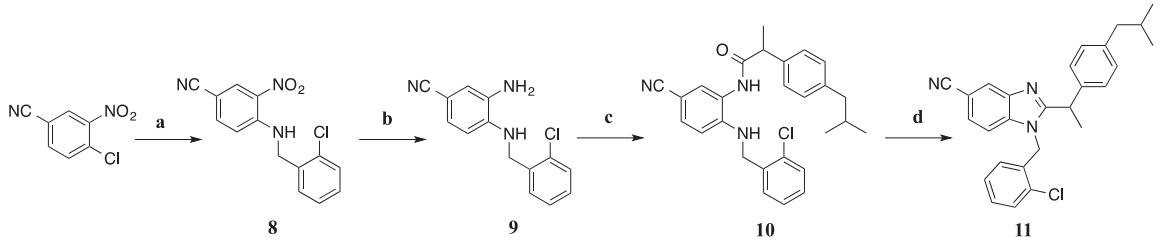
2.2. Biological evaluation and SAR

We previously established that *ortho*-substituted *N*-benzyl group of BRP-7 derivatives governed the potent inhibition of 5-LO product formation, and lipophilic and electron-withdrawing *o*-CF_3 substitution on *N*-benzyl slightly improved the activity (IC_{50} = 0.25 μM) as compared to *N*-*o*-chlorobenzyl derivative BRP-7 (IC_{50} = 0.31 μM) [16]. In addition, we observed slightly improved potency by substitutions at C(5) of BRP-7, applying a limited number of substituents such as hydroxyl, methoxy, chlorine and 2-pyridinylmethyloxy (IC_{50} = 0.12–0.19 μM) [16]. Based on this initial screen, we introduced further modifications by varying the substituent at C(5)-BI basically to obtain compounds with different physicochemical characteristics (compounds **7a**–**u** in Table 1).

LT biosynthesis is initiated by $cPLA_2$-mediated release of AA, which is subsequently converted to LTA_4 by activated 5-LO with the aid of FLAP that is essential for cellular LT biosynthesis [4]. Note that FLAP has no concrete (enzymatic) activity that can be monitored in a cell-free assay with a select read-out. Therefore, we analyzed the test compounds in a 'FLAP-dependent' cell-based assay for suppression of 5-LO (i.e. LTB_4 and its *trans* isomers and 5-HPETE) product formation as described before [22]. In this assay, neutrophils are challenged with Ca^{2+} ionophore A23187 that causes



Scheme 1. Synthesis of C(5)-substituted analogues of BRP-7. Reagents and conditions: (a) DIEA, DMF; (b) Pd/C, H_2 or $SnCl_2$, EtOH; (c) ibuprofen (**3**), EDC, DMAP, DCM; (d) AcOH, Δ; (e) HBr 48%; (f) alkyl halides, K_2CO_3, AcCN.



Scheme 2. Synthesis of C(5)-nitrile-substituted analogue of BRP-7. Reagents and conditions: (a) 2-chlorobenzylamine, MeOH; (b) $SnCl_2$, EtOH; (c) ibuprofen acyl chloride, DIEA, DCM; (d) Dioxan, HCl, Δ.

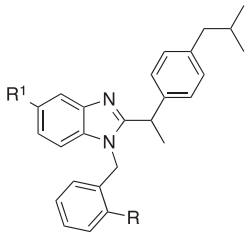
substantial formation of 5-LO products as a result of elevated intracellular Ca^{2+} concentration where FLAP plays an indispensable role in facilitating substrate (AA) transfer to 5-LO [3]. The initial screening of **7a-u** for their ability to inhibit 5-LO product formation in this assay was performed at 1 and 10 μM concentrations of test compounds. Analyzed 5-LO products include 5-H(P)ETE and all *trans*-isomers of LTB_4, as well as LTB_4 in intact cells.

Among the tested compounds, only compounds carrying 2-pyridinylmethyloxy (**7a**), 4- or 2-cyanobenzyloxy (**7h** and **7l**), 2-allyloxy (**7o**), 3,3-dimethylallyloxy (**7p**), 2-pentynoxy (**7r**), *n*-butyloxy (**7s**) and methoxycarbonylmethyloxy (**7t**) substituents showed significant inhibition of 5-LO product synthesis at 1 μM (Table 1). Interestingly, other *p*-substituted analogues equipped with nonpolar or bulky substituents (**7b**–**7g**) failed to inhibit cellular 5-LO product synthesis. This suggests that introducing lipophilicity or increasing the steric bulk at *p*-phenyl at C(5)-BI (**7b**–**7g**) impaired the potency except for **7h** (with polar *p*-CN), indicating that voluminous and nonpolar substituents at this position are not well accommodated. Accordingly, variations of the nonpolar substituents in *o*-position of the phenyl ring (i.e., compounds **7i**–**7k**) abolished the inhibitory activity whereas significant suppression of 5-LO product formation at 1 μM could be retained with *o*-CN substitution (**7l**) providing an H-bond acceptor. *m*-Substituted phenyl analogues (**7m**–**7n**) were not pursued further as they showed marked loss of inhibitory activity (Table 1). In addition, multiple alkoxy substitutions (**7o**–**7s**) and ester group (**7t**) were quite beneficial and well tolerated at C(5) position of the BI core for inhibition of 5-LO product synthesis (Table 1). Since, **7a**, **7o** and **7t** were the only derivatives that elicited almost complete inhibition of 5-LO product formation at 1 μM, more detailed concentration-response studies were performed in order to calculate the IC_{50} values, which were determined at 0.15, 0.17 and 0.21 μM, respectively (Table 2). Suppression of 5-LO product synthesis by **7l** (35%) was more pronounced when *N*-2-CF_3-benzyl was replaced with *N*-2-chlorobenzyl (**7u,** 61%), in which the IC_{50} value for **7u** was determined at 0.12 μM (Table 2).

On the basis of the contribution of the polar nitrile moiety to the inhibitory activity, and also considering this moiety as a prevalent pharmacophoric element contributing to drug-like properties of many known drug molecules [24], we chose to synthesize the 5-nitrile derivative of BRP-7 (**11,** Scheme 2). Interestingly, 5-nitrile substitution resulted in the highly active derivative (**11**), which outperformed BRP-7, as the potency of **11** in the cell-based assay was improved by 4.4-fold (Table 2). In fact, compound **11** concentration-dependently inhibited the formation of 5-LO-derived products in A23187-stimulated human neutrophils with an IC_{50} value of 0.07 μM.

Besides neutrophils, monocytes are also a major source of LTs [25,26]. We thus determined the effects of **11** on 5-LO product formation in human monocytes stimulated with A23187. Compound **11** potently inhibited the formation of 5-LO products (LTB_4 and its *trans* isomers and 5-H(p)ETE) in A23187-stimulated

Table 1
Effects of test compounds on 5-LO product synthesis in a cell-based (intact neutrophils) assay.



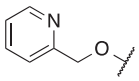
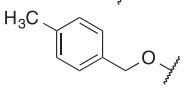
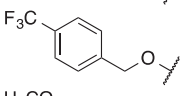
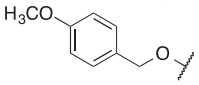
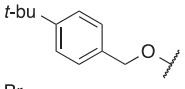
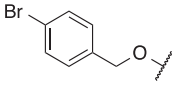
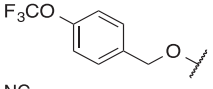
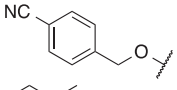
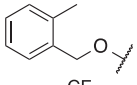
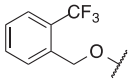
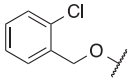
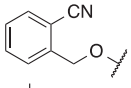
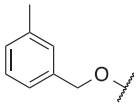
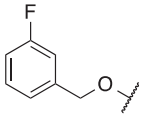
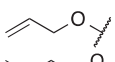
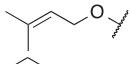
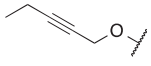
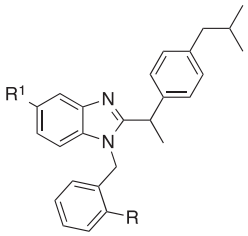
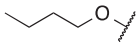
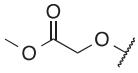
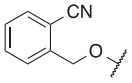
Cmpd.	R^1	R	5-LO product formation in neutrophils, remaining activity (% of control) at	
			1 μM	10 μM
7a		CF_3	0.0 ± 0.0	n.d.
7b		CF_3	92.6 ± 6.3	90.5 ± 14.9
7c		CF_3	91.7 ± 9.6	94.3 ± 14.1
7d		CF_3	81.6 ± 2.9	80.1 ± 7.5
7e		CF_3	94.9 ± 16.9	94.0 ± 9.5
7f		CF_3	108.1 ± 10.9	94.4 ± 9.6
7g		CF_3	99.9 ± 6.5	n.d.
7h		CF_3	71.1 ± 17.6	n.d.
7i		CF_3	97.1 ± 13.7	89.7 ± 4.5
7j		CF_3	90.9 ± 8.3	n.d.
7k		CF_3	92.2 ± 15.7	92.2 ± 15.5
7l		CF_3	65.2 ± 19.4	n.d.
7m		CF_3	98.2 ± 2.9	n.d.
7n		CF_3	92.0 ± 9.8	85.9 ± 8.3
7o		CF_3	7.5 ± 2.4	0.6 ± 0.5
7p		CF_3	60.2 ± 9.3	n.d.
7r		CF_3	55.6 ± 19.7	n.d.

Table 1 (*continued*)



Cmpd.	R^1	R	5-LO product formation in neutrophils, remaining activity (% of control) at	
			1 μM	10 μM
7s		CF_3	58.1 ± 24.3	n.d.
7t		CF_3	1.9 ± 1.7	n.d.
7u		Cl	39.9 ± 14.5	15.6 ± 6.9
11		Cl	0.0 ± 0.0	n.d.
12	**MK-886**		2.7 ± 1.2	n.d.

Compounds were tested in neutrophils stimulated with 2.5 μM A23187. Data are given as percentage of control at 1 and 10 μM inhibitor concentration (means ± SE, n = 4–6). The 100% value of the control (0.3% DMSO, vehicle) corresponds to an average of 47 ng 5-LO products per 10^6 neutrophils; n.d., not determined. The FLAP inhibitor 3-[3-(tert-butylthio)-1-(4-chlorobenzyl)-5-isopropyl-1H-indol-2-yl]-2,2-dimethylpropanoic acid (**12**, MK-886, see Fig. S1 for chemical structure) was used (1 μM) as reference compound.

monocytes with an IC_{50} = 0.026 μM, which was more potent than that observed for neutrophils (Fig. 2A). In the presence of 10 μM exogenous AA, **11** still inhibited 5-LO activity with a slightly reduced potency (IC_{50} = 0.035 μM; Fig. 2B), which was seen with BRP-7 and other FLAP inhibitors whose potencies are also reduced by addition of exogenous AA [22,27,28]. In contrast to neutrophils, monocytes may convert LTA_4 to LTC_4 by LTC_4 synthase, and compound **11** also efficiently suppressed the synthesis of cysteinyl (cys)-LTs in monocytes stimulated with lipopolysaccharide (LPS) plus *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), which was more physiologically relevant assay condition, with an IC_{50} of 0.012 μM,.

Because there is no enzymatic function for FLAP (an integral membrane protein) has yet been discovered and since FLAP is operative only in a complex cellular environment, proof of direct functional interference of a test compound with this protein is laborious. To confirm that compound **11** acts on FLAP and not on 5-LO to suppress cellular 5-LO product formation, we assayed **11** in a cell-free 5-LO activity assay using isolated human recombinant 5-LO as the enzyme source. As shown in Fig. 3A, **11** failed to substantially inhibit 5-LO activity up to 10 μM, while the direct 5-LO inhibitor zileuton (3 μM) blocked enzyme activity by > 80% (not shown) as expected and as reported in previous studies by us [17,29]. Moreover, we analyzed **11** in a cell-based model suitable for studying FLAP functionality in 5-LO product biosynthesis [30]. Thus, HEK293 cells stably expressing solely 5-LO (HEK_5-LO) and HEK cells expressing both 5-LO and FLAP (HEK_5-LO/FLAP) were investigated; the FLAP inhibitor MK-886 was used as reference control [31]. Compound **11** failed to suppress A23187-induced 5-LO product synthesis in HEK_5-LO (devoid of FLAP) but efficiently reduced 5-LO product biosynthesis in HEK cells expressing both 5-LO and FLAP; the same pattern was also found for MK-886 (Fig. 3B). Together, our data suggest that the efficient inhibition of cellular 5-

Table 2
Inhibition of 5-LO product formation by selected compounds in cell-based assay.[a]

Cmpd.	IC_{50} μM
7a	0.15
7o	0.17
7t	0.21
7u	0.12
11	0.07

[a] IC_{50} values in the cell-based assay were obtained from intact neutrophils challenged with 2.5 μM ionophore.

LO product formation by **11** is due to interference with FLAP.

2.3. Docking studies and molecular dynamic simulations of *11* with FLAP

Although biological assays were carried out for racemates, both stereoisomers (*S* and *R*) were considered for rationalizing the inhibitory activity of **11** by means of molecular modeling. Thus, we combined docking studies of (*S*)- and (*R*)-**11** with molecular dynamic simulations (MDs) using the FLAP crystal structure (PDB code: 2Q7M) [32]. This computational strategy allows to investigate the binding mode of inhibitors taking into account ligand-induced conformational changes of FLAP. Docking studies focused on the binding site located between the chains B and C to predict the top scored pose (Glide SP scores are −6.74 for (*S*)-**11** and −6.96 for (*R*)-**11**) showing C(5)-nitrile analogue of BRP-7 (**11**) interacting with residues C-Val20, C-Val21, C-Asn23, C-Gly24, C-Phe25, C-Ala27, C-His28, C-Glu31, B-Val61, B-Ala63, B-Tyr112, B-Ile113, B-Lys116, B-Phe114, B-Lys116, B-Ile119, B-Leu120, B-Phe123, in agreement with our previous reports [16,17]. These starting complexes have then been submitted to 10 ns MD study (Fig. S3). As a result, the binding orientations of the stereoisomers of **11** to FLAP are characterized by several interactions (Fig. 4A–B). For the purpose of clarity, we report the most conserved interactions derived from the MD simulations (occupancy ≥ 30%). The BI is engaged in a strong cation–π interaction with Lys116 (occupancy, 56% for (*S*)-stereoisomer and 31% for (*R*)-stereoisomer) orienting the *o*-Cl-benzyl moiety to form π–π contacts with Phe123 (occupancy, 30% for (*S*)-stereoisomer and 70% for (*R*)-stereoisomer), in agreement with our previously reported data on the arachidonate-binding site of FLAP (Fig. 4A–B) [17]. After analyzing the water molecules with *VMD 1.9.2* [33] during MD simulations, we also realized that there exist several bridging water molecules maintaining similar contacts between C(5)-nitrile of both stereoisomers and Lys116, polar head of POPC membrane and His28.

3. Conclusion

FLAP inhibition is a promising strategy for the pharmacological therapy of respiratory and cardiovascular disorders, and several FLAP inhibitors have proceeded to human clinical trials, however, no drug molecule has yet reached the market, despite the promising potential of these molecules [34]. We recently presented BRP-7 as a novel FLAP inhibitor chemotype, which lacks typical pharmacophoric moieties of other published FLAP inhibitors [16,22,34].

In the present study, modifications of the C(5)-position of the BI ring in BRP-7 with differently substituted benzyloxy or alkyloxy

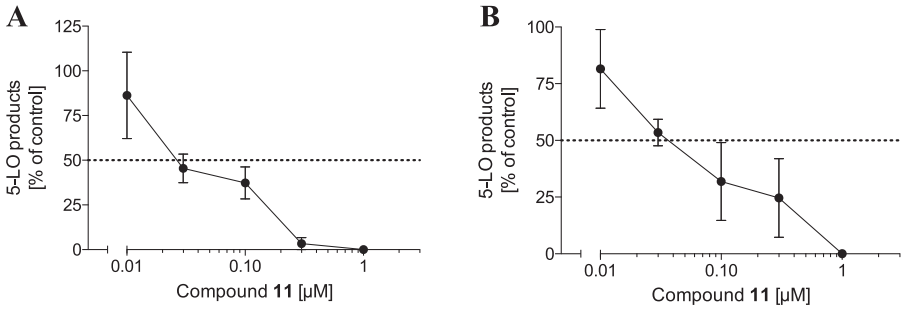


Fig. 2. Inhibition of 5-LO product formation by 5-CN-BRP-7 (**11**) in human monocytes. Effect of compound **11** on LTB_4 and 5-H(P)ETE synthesis in monocytes stimulated with A23187 (2.5 μM) in the absence (**A**) or presence (**B**) of exogenous AA (10 μM).

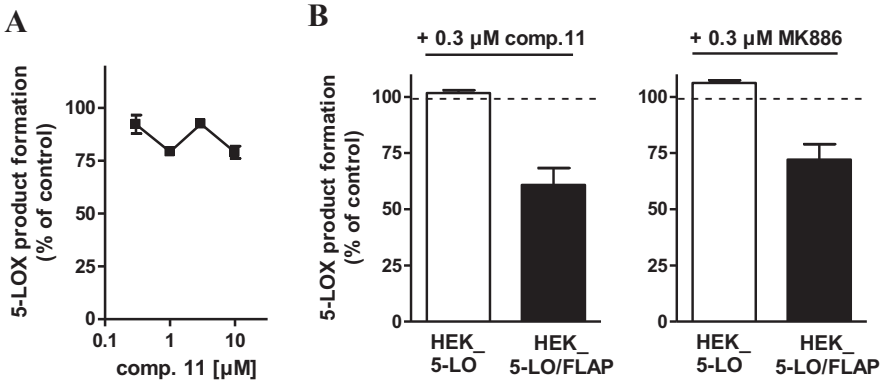


Fig. 3. Effects of compound **11** on 5-LO activity in a cell-free assay and in HEK293 cells. (A) Isolated human recombinant 5-LO was preincubated with **11** and 5-LO activity was induced by addition of 20 μM AA. (B) HEK293 cells, transfected with 5-LO alone (HEK_5-LO) or with 5-LO and FLAP (HEK_5-LO/FLAP), were pre-incubated with **11** or MK-886 (or vehicle control), and activated with 2.5 μM A23187 plus 3 μM AA. 5-LO products (*trans*-isomers of LTB_4 and 5-HETE) were determined by HPLC. Data, given as % residual activity of uninhibited vehicle control (= 100%), are means ± S.E.M., n = 3.

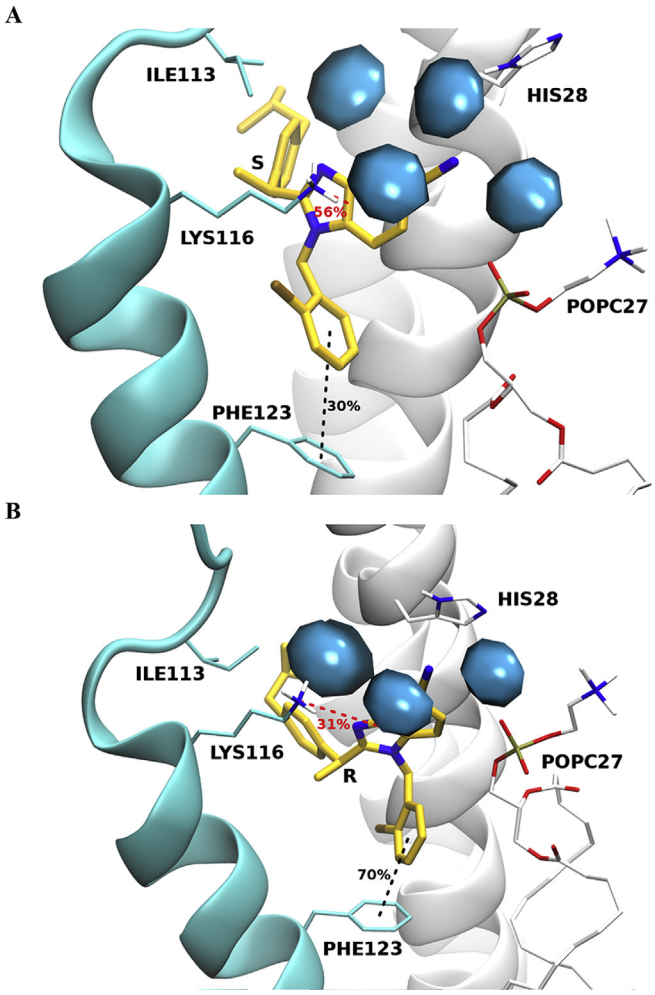


Fig. 4. Binding mode analysis of A) (*S*)- and B) (*R*)-stereoisomers of **11** during interaction with FLAP (PDB code: 2Q7M). Ligand protein interactions during 10 ns long MD simulations are represented by their occupancy values. Water molecules are mapped volumetrically around C(5)-nitrile by calculating occupancies and combining with average values with VolMap tool *VMD 1.9.2*.

groups are described. The various nonpolar or bulky substituents on the benzyl group were not tolerated and resulted in mostly inactive (**7b-g, i-k, m, n**) or weak inhibitors, such as **7h, l** and **u**, which are equipped with polar nitrile at *p*- and *o*-positions. Modifications of C(5)-position with smaller alkoxy analogues were also beneficial (**7o-s**) leading to improved inhibitors. Finally, the direct addition of a nitrile group at C(5)-BI of BRP-7 (yielding **11**) improved the potency to inhibit cellular 5-LO product synthesis with IC_{50} values in the range of 0.012–0.07 μM. These results can be explained by molecular modeling studies, which indicate the formation of additional hydrogen bonds when **11** is bound in the arachidonate-binding site of FLAP. The nitrile group of **11** is able to interact with polar amino acids (i.e. Lys116 and His28) and polar membrane heads through water-mediated hydrogen bond interactions. This may explain why a C(5)-nitrile substitution (Compound **11**) led to a more effective molecule (IC_{50} = 0.07 μM) as compared to the parent BRP-7 (IC_{50} = 0.31 μM). The modeling studies may also help to understand why the *o*-nitrile substituted (**7u**), allyloxy (**7o**) and ester (**7t**) derivatives are highly effective as well (IC_{50} = 0.12–0.21 μM), since they all form similar H-bond interactions with Lys116 at the membrane opening due to effective positioning of C(5)-substituents (Fig. S2). The inactive analogues do not seem to form this H-bond interaction because they cannot be positioned in an effective way as active derivatives in the vicinity of Lys116 (unpublished observations). In conclusion, substitution of the C(5)-position of the BI ring in BRP-7 might be a valuable means to develop new BRP-7 derivatives with greater potency. Further exploration of this position is under investigation in our laboratory.

4. Experimental section

4.1. Chemistry

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard. All chemical shifts were recorded as δ (ppm). High resolution mass spectra data (HRMS) were collected using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in ESI (+) method, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geraetetechnik, Germany). Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using hexane–EtOAc or DCM–MeOH solvent gradients. The purity of the final compounds was determined to be >97% by UPLC with UV detector.

4.1.1. General procedure for the alkylation of 5-hydroxy benzimidazole derivatives **6a-b**

To a solution of compound **6a-b** (1 equiv) in acetonitrile was added K_2CO_3 (1.7 equiv) followed by corresponding benzyl halides (1.5 equiv). The mixture was refluxed for 5 h then filtered and evaporated in vacuo. Purification of the crude product by automated flash chromatography provided the title compound.

*4.1.1.1. 2-(1-(4-Isobutylphenyl)ethyl)-5-(pyridin-2-ylmethoxy)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7a**).* Obtained by the reaction of picolylchloride with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 57.5%; mp 135.0–136.0 °C. ^1H NMR ($CDCl_3$): δ 0.81 (3H, d, *J* = 6.4 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 1.71–1.75 (1H, m), 2.12 (3H, d, *J* = 7.2 Hz), 2.35 (2H, d, *J* = 6.8 Hz), 4.49 (1H, q, *J* = 7.2 Hz), 5.60 (2H, s), 5.72 (2H, s), 6.11 (1H, d, *J* = 7.6 Hz), 7.00 (2H, d, *J* = 8.0 Hz), 7.11–7.23 (4H, m), 7.39 (1H, t, *J* = 7.8 Hz), 7.54 (1H, d, *J* = 8.0 Hz), 7.75 (1H, d, *J* = 7.6 Hz), 7.86–7.89 (1H, m), 8.03 (1H, s), 8.18 (1H, d, *J* = 7.2 Hz), 8.47 (1H, t, *J* = 7.2), 8.88 (1H, d, *J* = 5.2 Hz). ^{13}C NMR ($CDCl_3$): δ 20.48, 22.20, 22.24, 29.99, 38.16, 44.76, 45.15, 66.63, 100.78, 112.49, 117.75, 123.99 (q, $^1J_{C\text{-}F}$ = 275 Hz), 125.37, 125.79, 125.91, 126.67 (q, $^3J_{C\text{-}F}$ = 6.0 Hz), 127.05, 127.09 (q, $^2J_{C\text{-}F}$ = 30.5 Hz), 127.15, 128.63, 130.28, 130.39, 131.82, 132.75, 134.86, 142.07, 142.28, 145.28, 151.67, 155.33, 156.54. HRMS (*m*/*z*) $[M+H]^+$ calcd for $C_{33}H_{33}F_3N_3O$: 544.2576; found, 544.2570.

*4.1.1.2. 2-(1-(4-Isobutylphenyl)ethyl)-5-((4-methylbenzyl)oxy)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7b**).* Obtained by the reaction of 4-methylbenzyl bromide with **6b** and purified by flash column chromatography (0% → 20% EtOAc in hexane). Yield 60.0%; mp 137.6–138.3 °C. ^1H NMR ($CDCl_3$): δ 0.82 (3H, d, *J* = 6.4 Hz), 0.84 (3H, d, *J* = 6.4 Hz), 1.70–1.76 (1H, m), 1.80 (3H, d, *J* = 6.8 Hz), 2.33 (2H, d, *J* = 6.8 Hz), 2.36 (3H, s), 4.15 (1H, q, *J* = 6.8 Hz), 5.09 (2H, s), 5.32 (1H, d, *J* = 18.4 Hz), 5.39 (1H, d, *J* = 18.4 Hz), 6.19 (1H, d, *J* = 7.6 Hz), 6.88–6.93 (4H, m), 7.05 (2H, d, *J* = 8.0 Hz), 7.13 (1H, t, *J* = 7.6 Hz), 7.19 (2H, d, *J* = 7.2 Hz), 7.24–7.28

(1H, m), 7.36 (2H, d, $J = 7.6$ Hz), 7.48 (1H, s), 7.65 (1H, d, $J = 7.6$ Hz). HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{36}F_3N_2O$: 557.2780; found, 557.2798.

*4.1.1.3. 2-(1-(4-Isobutylphenyl)ethyl)-1-(2-(trifluoromethyl)benzyl)-5-((4-(trifluoromethyl)benzyl)oxy)-1H-benzo[d]imidazole (**7c**).* Obtained by the reaction of 4-trifluoromethylbenzyl bromide with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 70.8%; mp 98.0–99.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.83 (3H, d, $J = 6.8$ Hz), 1.72–1.76 (1H, m), 2.12 (3H, s), 2.36 (2H, d, $J = 7.2$ Hz), 4.45 (1H, s), 5.24 (2H, s), 5.57 (2H, s), 6.10 (1H, d, $J = 6.8$ Hz), 7.01 (2H, d, $J = 8.0$ Hz), 7.06–7.21 (5H, m), 7.39 (1H, t, $J = 7.4$ Hz), 7.60 (2H, d, $J = 8.0$ Hz), 7.67 (2H, d, $J = 8.0$ Hz), 7.75 (1H, d, $J = 8.0$ Hz), 7.92 (1H, s). HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{33}F_6N_2O$: 611.2497; found, 611.2517.

*4.1.1.4. 2-(1-(4-Isobutylphenyl)ethyl)-5-((4-methoxybenzyl)oxy)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7d**).* Obtained by the reaction of 4-meyhoxybenzyl bromide with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 67.0%; mp 231.3–232.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.83 (3H, d, $J = 6.8$ Hz), 1.70–1.76 (1H, m), 2.13 (3H, d, $J = 4.8$ Hz), 2.35 (2H, d, $J = 7.2$ Hz), 3.84 (3H, s), 4.48 (1H, s), 5.15 (2H, s), 5.57 (2H, s), 6.10 (1H, d, $J = 6.8$ Hz), 6.87–6.90 (1H, m), 6.99–7.12 (7H, m), 7.20 (2H, d, $J = 7.6$ Hz), 7.32 (1H, t, $J = 8.0$ Hz), 7.38 (1H, t, $J = 7.6$ Hz), 7.74 (1H, d, $J = 8.0$ Hz), 7.91 (1H, s); ^{13}C NMR ($CDCl_3$): δ 20.47, 22.21, 22.25, 30.01, 38.08, 44.79, 55.31, 70.79, 99.21, 111.71, 112.99, 113.95, 118.24, 119.88, 124.00 (q, $^1J_{C\text{-}F} = 272$ Hz), 125.65, 126.11, 126.70 (q, $^3J_{C\text{-}F} = 6.1$ Hz), 127.07, 127.11, (q, $^2J_{C\text{-}F} = 31.7$ Hz), 128.62, 129.75, 130.27, 130.66, 132.75, 134.96, 137.40, 142.04, 154.45, 158.15, 159.84. HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{36}F_3N_2O_2$: 573.2729; found, 573.2725.

*4.1.1.5. 5-((4-(tert-Butyl)benzyl)oxy)-2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7e**).* Obtained by the reaction of 4-*t*-butylbenzyl bromide with **6b** and purified by flash column chromatography (0% → 4% MeOH in DCM). Yield 49.8%; mp 72.4–73.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.79 (3H, d, $J = 6.4$ Hz), 0.81 (3H, d, $J = 6.4$ Hz), 1.31 (9H, s), 1.68–1.75 (1H, m), 1.80 (3H, d, $J = 6.4$ Hz), 2.31 (2H, d, $J = 7.2$ Hz), 4.11 (1H, m), 5.08 (2H, s), 5.32 (1H, d, $J = 18.0$ Hz), 5.39 (1H, d, $J = 18.0$ Hz), 6.17 (1H, d, $J = 8.4$ Hz), 6.90 (4H, s), 7.04 (2H, d, $J = 7.6$ Hz), 7.11 (1H, t, $J = 7.4$ Hz), 7.24–7.39 (5H, m), 7.49 (1H, s), 7.63 (1H, d, $J = 7.6$ Hz). HRMS (m/z): $[M+H]^+$ calcd for $C_{38}H_{42}F_3N_2O$: 599.3249; found: 599.3248.

*4.1.1.6. 5-((4-Bromobenzyl)oxy)-2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7f**).* Obtained by the reaction of 4-bromobenzyl bromide with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). Yield 72.8%; mp 143.8–144.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.83 (3H, d, $J = 6.8$ Hz), 1.70–1.76 (1H, m), 1.80 (3H, d, $J = 6.8$ Hz), 2.34 (2H, d, $J = 6.8$ Hz), 4.11 (1H, q, $J = 6.8$ Hz), 5.08 (2H, s), 5.33 (1H, d, $J = 18.0$ Hz), 5.39 (1H, d, $J = 18.0$ Hz), 6.19 (1H, d, $J = 7.6$ Hz), 6.86–6.93 (4H, m), 7.04 (2H, d, $J = 8.4$ Hz), 7.13 (1H, t, $J = 7.6$ Hz), 7.24–7.38 (1H, m), 7.34 (2H, d, $J = 8.4$ Hz), 7.44 (1H, d, $J = 2.0$ Hz), 7.51 (2H, d, $J = 8.4$ Hz), 7.65 (1H, d, $J = 7.6$ Hz). HRMS (m/z): $[M+H]^+$ calcd for $C_{34}H_{33}BrF_3N_2O$: 621.1728; found: 621.1727.

*4.1.1.7. 2-(1-(4-Isobutylphenyl)ethyl)-5-((4-(trifluoromethoxy) benzyl)oxy)-1-(2-(trifluoromethyl)benzyl)-1H-benzo[d]imidazole (**7g**).* Obtained by the reaction of 4-trifluoromethoxybenzyl bromide with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). Yield 79.6%; mp 171.0–172.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.84 (3H, d, $J = 6.8$ Hz), 1.69–1.76 (1H, m), 1.80 (3H, d, $J = 6.8$ Hz), 2.33 (2H, d, $J = 7.2$ Hz), 4.11 (1H, d, $J = 6.8$ Hz), 5.13 (2H, s), 5.33 (1H, d, $J = 18.0$ Hz), 5.39 (1H, d, $J = 18.0$ Hz), 6.19 (1H, d, $J = 7.6$ Hz), 6.88–6.95 (4H, m), 7.05 (2H, d, $J = 8.0$ Hz), 7.11–7.15 (1H, m), 7.23–7.29 (3H, m), 7.45 (1H, d, $J = 2.0$ Hz), 7.50 (2H, d, $J = 8.4$ Hz), 7.65 (1H, d, $J = 7.6$ Hz). HRMS (m/z) $[M+H]^+$ calcd for $C_{35}H_{33}F_6N_2O_2$: 627.2446; found: 627.2468.

*4.1.1.8. 4-(((2-(1-(4-Isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazol-5-yl)oxy)methyl)benzonitrile (**7h**).* Obtained by the reaction of α-bromo-*p*-tolunitrile with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 78.5%; mp 107.2–108.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.83 (3H, d, $J = 6.8$ Hz), 1.71–1.76 (1H, m), 2.13 (3H, s), 2.36 (2H, d, $J = 7.6$ Hz), 4.54 (1H, s), 5.26 (2H, s), 5.58 (2H, s), 6.09 (1H, m), 7.01 (2H, d, $J = 7.2$ Hz), 7.08–7.20 (5H, m), 7.40 (1H, t, $J = 7.6$ Hz), 7.61 (2H, d, $J = 7.6$ Hz), 7.71 (2H, d, $J = 8.0$ Hz), 7.75 (1H, d, $J = 8.0$ Hz), 7.97 (1H, s). ^{13}C NMR ($CDCl_3$): δ 20.69, 22.21, 22.25, 30.02, 38.19, 44.79, 69.96, 99.39, 111.92, 112.05, 117.94, 118.60, 123.99 (q, $^1J_{C\text{-}F} = 272$ Hz), 125.71, 126.55, 126.76 (q, $^3J_{C\text{-}F} = 5.3$ Hz), 127.13, 127.16 (q, $^2J_{C\text{-}F} = 30.3$ Hz), 127.89, 128.65, 130.29, 130.68, 132.51, 132.75, 135.05, 141.33, 142.09, 154.82, 157.47. HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{33}F_3N_3O$: 568.2576; found: 568.2579.

*4.1.1.9. 2-(1-(4-Isobutylphenyl)ethyl)-5-((2-methylbenzyl)oxy)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7i**).* Obtained by the reaction of 2-methylbenzyl bromide with **6b** and purified by flash column chromatography (0% → 20% EtOAc in hexane). Yield 71.6%; mp 143.3–144.4 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.83 (3H, d, $J = 6.8$ Hz), 1.70–1.77 (1H, m), 1.81 (3H, d, $J = 6.8$ Hz), 2.34 (2H, d, $J = 6.8$ Hz), 2.39 (3H, s), 4.12 (1H, q, $J = 6.8$ Hz), 5.11 (2H, s), 5.33 (1H, d, $J = 18.0$ Hz), 5.40 (1H, d, $J = 18.0$ Hz), 6.21 (1H, d, $J = 8.0$ Hz), 6.92–6.94 (4H, m), 7.06 (2H, d, $J = 8.0$ Hz), 7.14 (1H, t, $J = 7.2$ Hz), 7.21–7.29 (4H, m), 7.45–7.47 (1H, m), 7.52 (1H, s), 7.66 (1H, d, $J = 7.2$ Hz). HRMS (m/z) $[M+H]^+$ calcd for $C_{35}H_{36}F_3N_2O$: 557.2780; found: 557.2779.

*4.1.1.10. 2-(1-(4-Isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-5-((2-(trifluoromethyl)benzyl)oxy)-1H-benzo[d]imidazole (**7j**).* Obtained by the reaction of 2-trifluoromethylbenzyl bromide with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 73.5%. mp 127.0–128.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, $J = 6.8$ Hz), 0.83 (3H, d, $J = 6.8$ Hz), 1.72–1.76 (1H, m), 2.13 (3H, d, $J = 4.4$ Hz), 2.36 (2H, d, $J = 7.2$ Hz), 4.50 (1H, m), 5.31 (2H, s), 5.57 (2H, s), 6.11 (1H, d, $J = 7.2$ Hz), 7.00–7.32 (4H, m), 7.21–7.23 (3H, m), 7.37–7.50 (2H, m), 7.61 (1H, t, $J = 7.2$ Hz), 7.69–7.76 (3H, m), 7.93 (1H, s). HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{33}F_6N_2O$: 611.2497; found: 611.2498.

*4.1.1.11. 5-((2-Chlorobenzyl)oxy)-2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7k**).* Obtained by the reaction of 2-chlorobenzyl bromide with **6b** and purified by flash column chromatography (0% → 20% EtOAc in hexane). Yield 85.0%; mp 99.2–100.2 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, d, $J = 6.4$ Hz), 0.82 (3H, d, $J = 6.4$ Hz), 1.69–1.76 (1H, m), 1.80 (3H, d, $J = 6.4$ Hz), 2.32 (2H, d, $J = 6.8$ Hz), 4.11 (1H, m), 5.23 (2H, s), 5.34 (1H, d, $J = 18.8$ Hz), 5.40 (1H, d, $J = 18.8$ Hz), 6.19 (1H, d, $J = 7.6$ Hz), 6.91–6.93 (4H, m), 7.05 (2H, d, $J = 7.6$ Hz), 7.13 (1H, t, $J = 7.6$ Hz), 7.25–7.30 (3H, m), 7.39–7.60 (3H, m), 7.65 (1H, d, $J = 7.6$ Hz). ^{13}C NMR ($CDCl_3$): δ 21.78, 22.27, 22.33, 30.07, 38.49, 43.33, 44.88, 68.04, 103.77, 109.76, 113.28, 124.01 (q, $^1J_{C\text{-}F} = 272$ Hz), 125.99 (q, $^3J_{C\text{-}F} = 5.8$ Hz), 126.36, 126.83 (q, $^2J_{C\text{-}F} = 30.2$ Hz), 126.91, 126.95, 127.35, 128.70, 128.83, 129.35, 129.49, 130.31, 132.28, 132.60, 134.23, 134.99,

137.45, 139.44, 140.33, 155.18, 157.76. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{33}ClF_3N_2O$: 577.2234; found: 577.2236.

*4.1.1.12. 2-(((2-(1-(4-Isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazol-5-yl)oxy)methyl)benzonitrile (**7l**).* Obtained by the reaction of α-bromo-*o*-tolunitrile with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 84.3%, mp 150.3–150.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.8 Hz), 1.72–1.76 (1H, m), 2.14 (3H, s), 2.36 (2H, d, *J* = 7.6 Hz), 4.49 (1H, s), 5.33 (2H, s), 5.58 (2H, s), 6.12 (1H, s), 7.01 (2H, d, *J* = 6.8 Hz), 7.08–7.22 (5H, m), 7.40–7.50 (2H, m), 7.67–7.76 (4H, m), 7.80 (1H, s). ^{13}C NMR ($CDCl_3$): δ 20.65, 22.22, 22.25, 30.02, 38.20, 44.79, 44.92, 69.37, 100.12, 111.98, 112.45, 117.10, 117.84, 124.01 (q, $^1J_{C\text{-}F}$ = 272 Hz), 125.80, 126.70 (q, $^3J_{C\text{-}F}$ = 5.3 Hz), 127.11 (q, $^2J_{C\text{-}F}$ = 31.2 Hz), 127.12, 128.65, 129.08, 129.86, 130.30, 130.61, 132.85, 133.15, 133.36, 134.94, 139.14, 142.07, 154.83, 157.75. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{33}F_3N_3O$: 568.2576; found: 568.2573.

*4.1.1.13. 2-(1-(4-Isobutylphenyl)ethyl)-5-((3-methylbenzyl)oxy)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7m**).* Obtained by the reaction of 3-methylbenzyl bromide with **6b** and purified by flash column chromatography (0% → 20% EtOAc in hexane). Yield 54.0%, mp 129.6–130.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, d, *J* = 6.4 Hz), 0.82 (3H, d, *J* = 6.4 Hz), 1.68–1.75 (1H, m), 1.80 (3H, d, *J* = 6.8 Hz), 2.33 (2H, d, *J* = 6.8 Hz), 2.37 (3H, s), 4.11 (1H, q, *J* = 6.8 Hz), 5.09 (2H, s), 5.32 (1H, d, *J* = 18.0 Hz), 5.39 (1H, d, *J* = 18.0 Hz), 6.20 (1H, d, *J* = 7.6 Hz), 6.91–6.93 (4H, m), 7.05 (2H, d, *J* = 8.0 Hz), 7.11–7.14 (2H, m), 7.24–7.29 (4H, m), 7.48 (1H, s), 7.65 (1H, d, *J* = 7.6 Hz). ^{13}C NMR ($CDCl_3$): δ 20.41, 21.40, 22.22, 22.26, 30.02, 38.05, 44.74, 44.80, 71.04, 99.10, 111.70, 118.30, 124.01 (q, $^1J_{C\text{-}F}$ = 272 Hz), 124.91, 125.63, 126.03, 126.72 (q, $^3J_{C\text{-}F}$ = 5.8 Hz), 127.06, 127.10 (q, $^2J_{C\text{-}F}$ = 31.2 Hz), 128.55, 128.58, 128.62, 129.08, 130.28, 130.66, 132.40, 132.75, 134.94, 135.73, 138.41, 142.05, 154.41, 158.31. HRMS (*m/z*) $[M+H]^+$ calcd for $C_{35}H_{36}F_3N_2O$: 557.2780; found: 557.2777.

*4.1.1.14. 5-((3-Fluorobenzyl)oxy)-2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7n**).* Obtained by the reaction of 3-fluorobenzyl bromide with **6b** and purified by flash column chromatography (0% → 4% DCM in MeOH). The oily product was triturated by cold isopropanol. Yield 92.5%, mp 84.3–84.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, d, *J* = 6.4 Hz), 0.82 (3H, d, *J* = 6.4 Hz), 1.66–1.76 (1H, m), 1.81 (3H, d, *J* = 7.2 Hz), 2.32 (2H, d, *J* = 7.2 Hz), 4.12 (1H, q, *J* = 7.2 Hz), 5.12 (2H, s), 5.33 (1H, d, *J* = 18.0 Hz), 5.39 (1H, d, *J* = 18.0 Hz), 6.18 (1H, d, *J* = 8.0 Hz) 6.91–6.94 (4H, m), 6.97–7.05 (3H, m), 7.10–7.20 (4H, m), 7.31–7.36 (1H, m), 7.45 (1H, s), 7.64 (1H, d, *J* = 7.6 Hz). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{33}F_4N_2O$: 561.2529; found: 561.2526.

*4.1.1.15. 5-(Allyloxy)-2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl)benzyl)-1H-benzo[d]imidazole (**7o**).* Obtained by the reaction of allyl bromide with **6b** and purified by flash column chromatography (0% → 30% EtOAc in Hexane). The oily product was triturated by acetone-petroleum ether mixture. Yield 51.1%, mp 93.5–94.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, d, *J* = 6.4 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 1.70–1.77 (1H, m), 1.81 (3H, d, *J* = 6.8 Hz), 2.34 (2H, d, *J* = 6.8 Hz), 4.11 (1H, q, *J* = 6.8 Hz), 4.60 (2H, d, *J* = 5.2 Hz), 5.28–5.31 (2H, m), 5.38 (1H, d, *J* = 18 Hz), 5.45 (1H, dd, *J* = 17.2, 1.6 Hz), 6.06–6.16 (1H, m), 6.20 (1H, d, *J* = 8.0 Hz), 6.84–6.93 (4H, m), 7.05 (2H, d, *J* = 8.4 Hz), 7.13 (1H, t, *J* = 7.8 Hz), 7.25–7.28 (1H, m), 7.42 (1H, d, *J* = 2.4 Hz), 7.65 (1H, d, *J* = 7.6 Hz). ^{13}C NMR ($CDCl_3$): δ 21.80, 22.27, 22.33, 30.07, 38.49, 43.30, 44.88, 69.53, 103.47, 109.66, 113.28, 117.64, 124.01 (q, $^1J_{C\text{-}F}$ = 271 Hz), 125.96 (q, $^3J_{C\text{-}F}$ = 5.7 Hz), 126.37, 126.83 (q, $^2J_{C\text{-}F}$ = 31.0 Hz), 126.95, 127.31, 129.46, 130.13, 132.26, 133.56, 134.29, 139.52, 140.29, 143.09, 155.19, 157.66. HRMS (*m/z*) $[M+H]^+$ calcd for $C_{30}H_{32}F_3N_2O$: 493.2467; found: 493.2474.

*4.1.1.16. 2-(1-(4-Isobutylphenyl)ethyl)-5-((3-methylbut-2-en-1-yl) oxy)-1-(2-(trifluoromethyl)benzyl)-1H-benzo[d]imidazole (**7p**).* Obtained by the reaction of 3,3-dimethylallyl bromide with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 68.6%, mp 159.0–160.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, d, *J* = 6.0 Hz), 0.78 (3H, d, *J* = 6.0 Hz), 1.65–1.74 (1H, m), 1.76 (6H, s), 1.80 (3H, d, *J* = 6.8 Hz), 2.31 (2H, d, *J* = 6.8 Hz), 4.64 (2H, d, *J* = 6.4 Hz), 4.78 (1H, m), 5.45 (t, 1H, *J* = 6.4 Hz), 5.70 (1H, d, *J* = 18.0 Hz), 5.84 (1H, d, *J* = 18.0 Hz), 6.20 (1H, d, *J* = 7.6 Hz), 6.96 (2H, d, *J* = 8.0 Hz), 7.06–7.24 (4H, m), 7.34–7.42 (3H, m), 7.76 (1H, d, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 18.33, 20.45, 22.21, 22.25, 25.86, 30.01, 38.05, 44.79, 65.89, 98.62, 111.60, 118.38, 118.49, 124.02 (q, $^1J_{C\text{-}F}$ = 272 Hz), 125.66, 125.79, 126.69 (q, $^3J_{C\text{-}F}$ = 5.3 Hz), 127.08, 127.10, (q, $^2J_{C\text{-}F}$ = 31.3 Hz), 128.59, 130.25, 130.71, 132.74, 134.99, 139.55, 141.99, 154.26, 158.43. HRMS (*m/z*) $[M+H]^+$ calcd for $C_{32}H_{36}F_3N_2O$: 521.2780; found: 521.2775.

*4.1.1.17. 2-(1-(4-Isobutylphenyl)ethyl)-5-(pent-2-yn-1-yloxy)-1-(2-(trifluoromethyl) benzyl)-1H-benzo[d]imidazole (**7r**).* Obtained by the reaction of 1-bromopent-3-yne with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 76.7%, mp 118.0–119.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, *J* = 6.4 Hz), 1.18 (3H, t, *J* = 7.0 Hz) 1.62–1.77 (1H, m), 2.15 (3H, d, *J* = 6.8 Hz), 2.28 (2H, q, *J* = 7.0 Hz), 2.37 (2H, d, *J* = 7.0 Hz), 4.55 (1H, d, *J* = 6.8 Hz), 4.80 (2H, s), 5.58 (2H, s), 6.13 (1H, s), 7.04–7.41 (8H, m), 7.75 (1H, d, *J* = 7.6 Hz), 7.85 (1H, s). ^{13}C NMR ($CDCl_3$): δ 12.55, 13.53, 20.60, 22.22, 22.25, 30.02, 38.18, 44.80, 44.96, 57.71, 73.11, 90.54, 99.56, 111.73, 118.20, 124.00 (q, $^1J_{C\text{-}F}$ = 271.2 Hz), 125.82, 126.34, 126.73 (q, $^3J_{C\text{-}F}$ = 5.3 Hz), 126.98 (q, $^2J_{C\text{-}F}$ = 30.3 Hz), 127.15, 127.58, 130.27, 130.67, 132.35, 132.83, 134.92, 142.03, 154.70, 157.34. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{32}H_{34}F_3N_2O$: 519.2623; found: 519.2632.

*4.1.1.18. 5-Butoxy-2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl)benzyl)-1H-benzo[d]imidazole (**7s**).* Obtained by the reaction of 1-bromobutane with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 69.4%, mp 109.0–110.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.8 Hz), 0.98 (3H, t, *J* = 7.2 Hz) 1.47–1.53 (2H, m), 1.73–1.82 (3H, m), 2.12 (3H, bs), 2.35 (2H, d, *J* = 7.6 Hz), 4.07 (2H, t, *J* = 6.8 Hz), 4.46 (1H, bs), 5.56 (2H, s), 6.10 (1H, m), 7.00–7.03 (4H, m), 7.20–7.40 (4H, m), 7.74–7.77 (2H, m). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{36}F_3N_2O$: 507.2780; found: 507.2778.

*4.1.1.19. Methyl 2-((2-(1-(4-isobutylphenyl)ethyl)-1-(2-(trifluoromethyl)benzyl)-1H-benzo[d]imidazol-5-yl)oxy)acetate (**7t**).* Obtained by the reaction of methyl bromoacetate with **6b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 83.1%, mp 158.0–159.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.8 Hz), 1.72–1.76 (1H, m), 2.12 (3H, d, *J* = 7.2 Hz), 2.36 (2H, d, *J* = 7.2 Hz), 3.85 (3H, s), 4.49 (1H, q, *J* = 7.2 Hz), 4.78 (2H, s), 5.57 (2H, s), 6.10 (1H, d, *J* = 8.0 Hz), 7.01 (2H, q, *J* = 8.2 Hz), 7.07–7.23 (5H, m), 7.40 (1H, t, *J* = 8.0 Hz), 7.73–7.76 (2H, m). HRMS (*m/z*) $[M+H]^+$ calcd for $C_{30}H_{32}F_3N_2O_3$: 525.2365; found: 525.2363.

*4.1.1.20. 2-(((1-(2-Chlorobenzyl)-2-(1-(4-isobutylphenyl)ethyl)-1H-benzo[d]imidazol-5-yl)oxy)methyl)benzonitrile (**7u**).* Obtained by the reaction of α-bromo-*o*-tolunitrile with **5b** and purified by flash column chromatography (0% → 40% EtOAc in hexane). The oily product in ether was solidified by addition of 1–2 eq of conc. HCl. Yield 61.5%, mp 179.0–180.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6.4 Hz), 1.71–1.80 (1H, m), 2.12 (3H, bs), 2.37 (2H, d, J = 7.2 Hz), 4.50 (1H, bs), 5.31 (2H, s), 5.42 (2H, s), 6.20 (1H, d, J = 7.6 Hz), 7.00–7.05 (3H, m), 7.14 (2H, s), 7.22–7.26 (3H, m), 7.43–7.50 (2H, m), 7.66–7.75 (3H, m), 7.97 (1H, s). ^{13}C NMR ($CDCl_3$): δ 20.56, 22.38, 22.31, 30.06, 38.19, 44.83, 46.02, 69.22, 99.95, 112.11, 112.39, 117.15, 117.61, 126.54, 127.10, 127.67, 129.07, 129.81, 129.92, 130.02, 130.11, 130.32, 132.19, 133.15, 133.37, 135.16, 139.18, 142.03, 154.60, 157.58. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{33}ClN_3O$: 534.2312; found: 534.2311.

*4.1.1.21. 1-[(2-Chlorophenyl)methyl]-2-{1-[4-(2-methylpropyl) phenyl]ethyl}-1H-benzimidazol-5-carbonitrile (**11**).* Compound **10** (1 equiv) was refluxed in 5 ml dioxane and 1.5 ml conc. HCl for 1.5 h. The reaction mixture was evaporated in vacuo, and dissolved in 20 ml EtOAc. The organic phase was extracted with 5% $NaHCO_3$ (30 ml × 2). Combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was purified by automated flash chromatography. Yield 71%, mp 114.0–114.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, d, J = 6.8 Hz), 0.86 (3H, d, J = 6.8 Hz), 1.73–1.78 (1H, m), 1.82 (3H, d, J = 6.8 Hz), 2.36 (2H, d, J = 7.6 Hz), 4.18 (1H, q, J = 6.8 Hz), 5.27 (2H, s), 6.11 (1H, d, J = 7.6 Hz), 6.92–6.98 (3H, m), 7.06 (2H, d, J = 8.0 Hz), 7.13–7.19 (2H, m), 7.39 (1H, dd, J = 7.6, 0.8 Hz) 7.44 (1H, dd, J = 8.4, 1.6 Hz), 8.20 (1H, d, J = 0.8 Hz). ^{13}C NMR ($CDCl_3$): δ 21.69, 22.31, 22.35, 30.07, 38.69, 44.81, 44.90, 105.49, 110.46, 119.91, 124.75, 126.27, 126.45, 126.94, 127.24, 129.06, 129.67, 129.72, 132.04, 132.28, 138.47, 138.79, 140.73, 142.17, 160.12; HRMS *(m/z)*: $[M+H]^+$ *calcd* for $C_{27}H_{27}ClN_3$ 428.1894; found 428.1893.

4.2. Biological studies

4.2.1. Cells

Human neutrophils were freshly isolated from leukocyte concentrates obtained at the Institute of Transfusion Medicine University Hospital Jena (Germany). In brief, venous blood was taken from healthy adult donors that did not take any medication for at least 7 days and leukocyte concentrates were prepared by centrifugation at 4000 g for 20 min at 20 °C. Neutrophils were immediately isolated by dextran sedimentation, centrifugation on Nycoprep cushions (PAA Laboratories, Linz, Austria), and hypotonic lysis of erythrocytes as described previously [22]. Cells were finally resuspended in phosphate-buffered saline pH 7.4 (PBS) containing 1 mg/ml glucose and 1 mM $CaCl_2$ (PGC buffer).

4.2.2. Determination of 5-LO product formation in intact cells

For assays in intact cells, neutrophils or monocytes [5×10^6 and 2×10^6 mL^{-1}, respectively; in PBS (pH 7.4) containing 1 mg mL^{-1} glucose and 1 mM $CaCl_2$ (PGC buffer); incubation volume, 1 mL] were pre-incubated with the compounds or vehicle (0.1% DMSO) for 15 min at 37 °C. Then, 2.5 μM A23187 without or with exogenous AA was added, and the reaction was stopped after 10 min with 1 mL methanol, and 30 μL 1 N HCl plus 200 ng PGB_1 and 500 μL of PBS were added. The samples were then subjected to solid phase extraction on C18-columns (100 mg, UCT, Bristol, PA). 5-LO products (LTB_4 and its *trans*-isomers, and 5-H(P)ETE) were analyzed by RP-HPLC and UV detection and quantities calculated on the basis of the internal standard PGB_1 as reported elsewhere [35]. For the determination of cysLTs in supernatants of monocytes, a cysLT ELISA kit from Enzo Life Sciences International Inc. (Lörrach, Germany) was used.

4.2.3. Determination of the activity of isolated 5-LO in cell-free assays

5-LO activity in cell-free assays was assessed using recombinant 5-LO expressed in *E. coli* BL21 cells (transformed with pT3-5-LO plasmid) and purified on an ATP-agarose column as described before [36]. Semi-purified 5-LO (0.5 μg/ml) were diluted with ice-cold PBS containing 1 mM EDTA, and 1 mM ATP was added. Samples were pre-incubated with the test compounds or vehicle (0.1% DMSO) as indicated. After 15 min at 4 °C, samples were pre-warmed for 30 s at 37 °C, and 2 mM $CaCl_2$ plus 20 μM arachidonic acid was added to initiate 5-LO product formation. After 10 min at 37 °C, the reaction was stopped by addition of 1 ml ice-cold methanol, and the formed metabolites were analyzed by RP-HPLC as described [35]. 5-LO products include the all-trans isomers of LTB_4 and 5-H(P)ETE.

4.2.4. Determination of 5-LO product synthesis in HEK293 cells

Transfection of HEK293 cells with pcDNA3.1/neom (+)_5-LO and pcDNA3.1/Hygro (−)_FLAP and verification of protein expression was performed as described before [30]. HEK293 cells were harvested by trypsinization and centrifugation (1000 × g; 10 min; 4 °C). 5-LO product synthesis in intact cells was determined as previously described [30]. Briefly, 1×10^6 cells in 1 ml PGC buffer were stimulated with 2.5 μM A23187 plus 3 μM exogenous AA for 10 min at 37 °C. The reaction was stopped by addition of 1 ml ice-cold methanol and 5-LO products (all-trans isomers of LTB_4 and 5-H(P)ETE) were extracted and analyzed as described above.

4.3. Computational methods

Docking and MD simulation protocols are reported in Supporting Information.

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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.2016.07.004.

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