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Identification of novel benzimidazole derivatives as inhibitors of leukotriene biosynthesis by virtual screening targeting 5-lipoxygenase-activating protein (FLAP)

Erden Banoglu [a,*], Burcu Çalışkan [a], Susann Luderer [b], Gökçen Eren [a], Yagmur Özkan [a], Wolfram Altenhofen [c,†], Christina Weinigel [d], Dagmar Barz [d], Jana Gerstmeier [e], Carlo Pergola [e], Oliver Werz [e,*]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Etiler, 06330 Yenimahalle, Ankara, Turkey
[b] Department of Pharmaceutical Analytics, Pharmaceutical Institute, Eberhard-Karls-University Tuebingen, Auf der Morgenstelle 8, D-72076 Tuebingen, Germany
[c] Chemical Computing Group, AG, Kaiser-Wilhelm-Ring 11, 50676 Koeln, Germany
[d] Institute of Transfusion Medicine, University Hospital Jena, 07743 Jena, Germany
[e] Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, D-07743 Jena, Germany

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ABSTRACT

Pharmacological suppression of leukotriene biosynthesis by 5-lipoxygenase (5-LO)-activating protein (FLAP) inhibitors is a promising strategy to intervene with inflammatory, allergic and cardiovascular diseases. Virtual screening targeting FLAP based on a combined ligand- and structure-based pharmacophore model led to the identification of 1-(2-chlorobenzyl)-2-(1-(4-isobutylphenyl)ethyl)-1*H*-benzimidazole (**7**) as developable candidate. Compound **7** potently suppressed leukotriene formation in intact neutrophils (IC_{50} = 0.31 μM) but essentially failed to directly inhibit 5-LO suggesting that interaction with FLAP causes inhibition of leukotriene synthesis. For structural optimization, a series of 46 benzimidazole-based derivatives of **7** were synthesized leading to more potent analogues (**70**–**72**, **82**) with IC_{50} = 0.12–0.19 μM in intact neutrophils. Together, our results disclose the benzimidazole scaffold bearing an ibuprofen fingerprint as a new chemotype for further development of anti-leukotriene agents.

1. Introduction

Leukotrienes (LTs) are potent lipid mediators with central roles in the initiation and amplification of the inflammatory response by regulating the recruitment and activation of leukocytes in inflamed tissues.[1,2] The first two steps in LT biosynthesis involve the key enzyme 5-lipoxygenase (5-LO) that converts arachidonic acid (AA) into 5-hydroperoxyeicosatetraenoic acid (5-HpETE) followed by dehydration to the unstable epoxide LTA_4. These initial oxygenation/dehydration steps are aided in the cellular environment by the 5-LO-activating protein (FLAP), which acts as substrate transfer/supply protein for 5-LO at the nuclear membrane.[3] LTA_4 is then rapidly converted to either LTB_4 by LTA_4 hydrolase or to LTC_4 by LTC_4 synthases. LTC_4 is further metabolized to LTD_4 and then to LTE_4 which are collectively named cysteinyl(cys)-LTs. LT signals are specifically transduced via distinct receptors (BLT1 and BLT2 for LTB_4; CysLTR1 for LTD_4, CysLTR2 for LTC_4; and $CysLT_ER$/P2Y12 for LTE_4) resulting in a number of biological activities including neutrophil and eosinophil chemotaxis and activation of inflammatory responses by LTB_4, as well as bronchoconstriction, airway edema, and hypersecretion of mucus by cysLTs.[2]

There are two major pharmacological strategies pursued in order to intervene with LTs: (I) antagonism of cys-LTs at CysLTR1 and (II) inhibition of 5-LO product (i.e., cys-LTs, LTB_4 and 5-H(p)ETE) biosynthesis, where inhibitors of 5-LO product synthesis may be advantageous over CysLTR1 antagonists because of their interference with all 5-LO-derived products. However, while CysLTR1 antagonists (e.g., montelukast) are successfully applied in asthma therapy,[4] the development of LT biosynthesis inhibitors is less advanced.[5,6] So far, the direct 5-LO inhibitor zileuton is the only marketed LT synthesis inhibitor, but it is not widely prescribed due to significant side-effects, poor pharmacokinetics, and multiple

Abbreviations: AA, arachidonic acid; $cPLA_2$, cytosolic phospholipase A_2; FLAP, 5-lipoxygenase-activating protein; 5-HpETE, 5-hydroperoxyeicosatetraenoic acid; 5-LO, 5-lipoxygenase; LT, leukotriene; PGC buffer, phosphate-buffered saline pH 7.4 containing 1 mg/mL glucose and 1 mM $CaCl_2$; SAR, structure–activity relationship.

* Corresponding authors. Tel.: +90 312 2023240; fax: +90 312 2235018 (E.B.); tel.: +49 3641 949801; fax: + 49 3641 949802 (O.W.).
E-mail addresses: banoglu@gazi.edu.tr (E. Banoglu), Oliver.Werz@uni-jena.de (O. Werz), Oliver.Werz@uni-jena.de (O. Werz).
† Present address: Leukocare AG, Am Klopferspitz 19, D-82152 Martinsried, Germany.

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dosing.[7] Recently, a new 5-LO inhibitor (MK-0633, setileuton) was reported to be under investigation for the treatment of asthma and atherosclerosis.[8] Besides targeting 5-LO, inhibition of LT biosynthesis may also be achieved by targeting FLAP. Early candidates like MK-886, BAY X1005 and MK-591 that all target FLAP underwent phase I and II studies and demonstrated clinical benefits in allergic asthma trials but were not further developed for unpublished reasons.[9] However, the recently developed FLAP inhibitors such as AM803, AM643, and AM103 (which are deduced from MK886) were shown to be efficacious in preclinical studies of inflammatory diseases as well as in trials with patients suffering from asthma and are currently under clinical investigations.[9–13] Though no FLAP inhibitor has yet reached the market, FLAP is currently considered a promising and clinically relevant target for pharmacological intervention with LT-related disorders.[14]

The aim of this study was the identification of new FLAP inhibitors by a novel combined structure- and ligand-based virtual screening approach. Thus, we first compiled diverse sets of published FLAP inhibitors to be used as queries for a combined ligand- and structure-based pharmacophore model. We then used this model to screen libraries of commercially available compounds for novel chemical scaffolds with potential inhibitory activity on cellular 5-LO product formation. From a list of 37 candidates, 8 molecules were 'cherry-picked' and tested for inhibition of 5-LO product synthesis in intact cells and the most active derivative (**7**) was selected as lead for structure–activity relationship (SAR) studies. Eventually, this approach led to the identification of a new chemotype bearing a benzimidazole core structure as a potent inhibitor of LT biosynthesis in intact cells with bioactivities in the submicromolar range.

2. Results and discussion

2.1. Building of the combined ligand- and structure-based pharmacophore model for virtual screening

The analysis of the available crystal structure of FLAP[15] was found to be insufficient for a reliable structure-based drug design due to the low resolution. Therefore, our current work combined structure- and ligand-based evidences in order to identify novel chemotypes as potential FLAP inhibitors. The workflow of the applied strategy was: (i) collection of chemically diverse sets of known FLAP inhibitors; (ii) flexible alignment of their structures onto the X-ray ligand; (iii) generation of a ligand-based pharmacophore model from the aligned ligands, including an exterior volume that indicates the shape of the alignment; (iv) use of this model for the virtual screening of a commercially available compound database; (v) docking of the initial hits from step 4 into the active site using a pharmacophore-biased placement algorithm, and (vi) selection of the hits on the basis of diversities in side chain interaction fingerprints among the survived molecules obtained after post processing of the docking results.

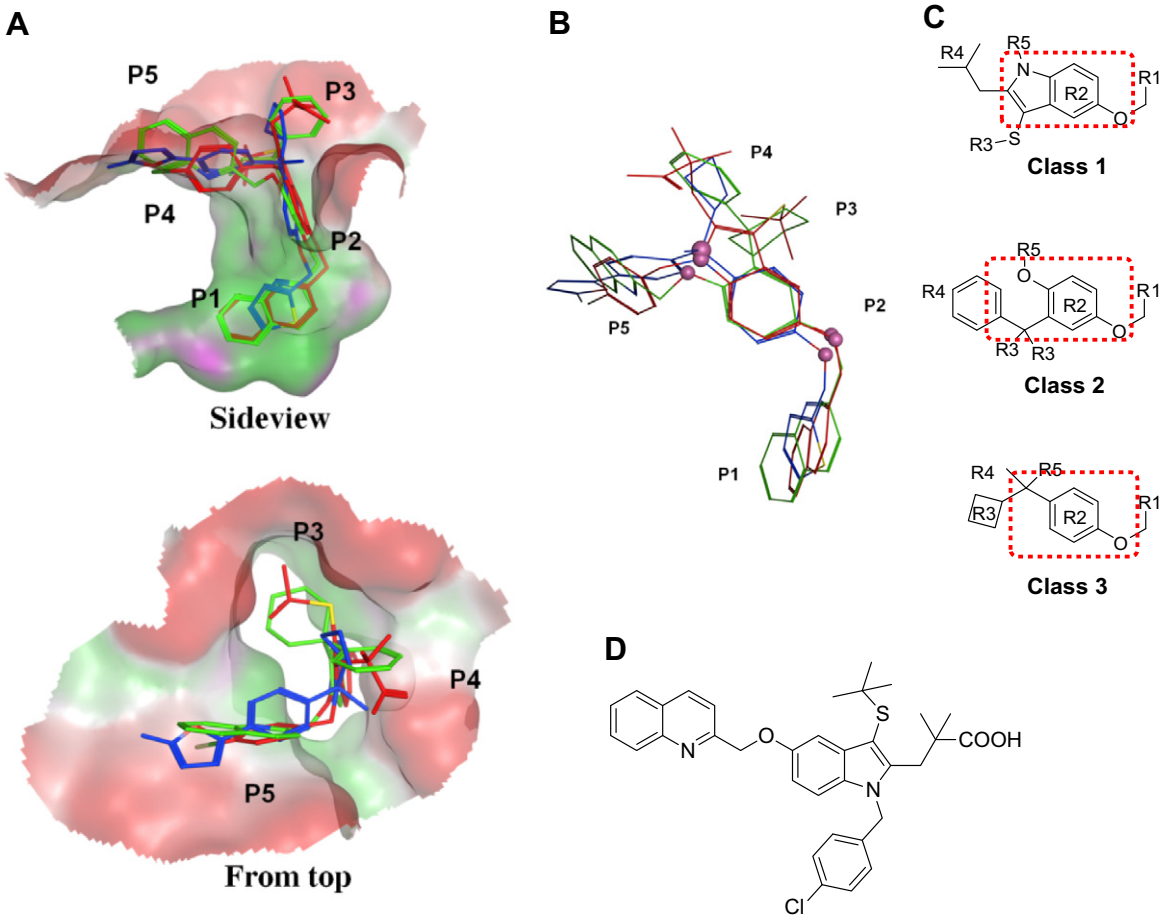


Figure 1. (A) The FLAP active site identified by five subpockets (P1–P5), where P1 and P2 are rather buried and P3–P5 are on the water accessible exterior. (B) Alignment of literature known active inhibitors from each chemical class (Class 1: 1,2-disubstituted indoles, Class 2: 2,2-bisaryl-bicycloheptanes, Class 3: diaryl-substituted alkanes) showing P1–P5 exit vectors. (C) The presentation and aligned scaffolds of the chemical classes of literature known active FLAP inhibitors, where R1–5 correspond to the respective subpockets P1–5. (D) Chemical structure of the well-known FLAP inhibitor **85** (MK-591).

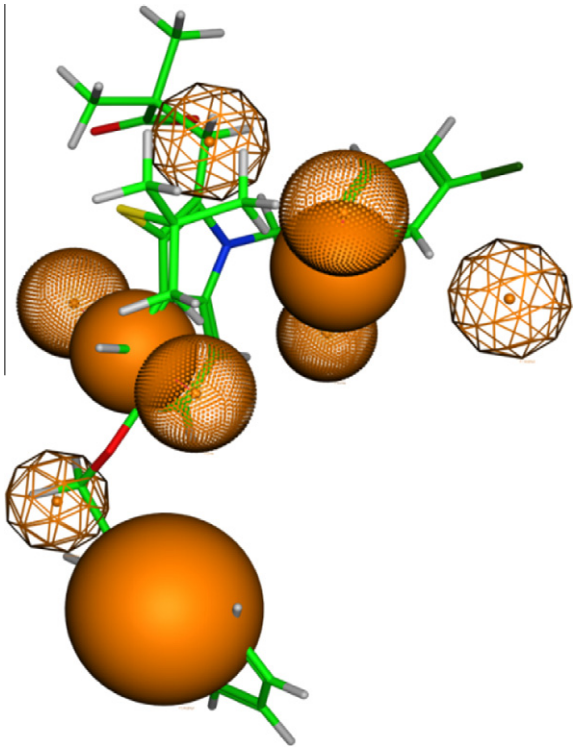


Figure 2. Constructed ligand-based pharmacophore model for FLAP inhibitors. The solid spheres represent the core pattern of aromatic rings. Projected Pi-Ring features (dotted) constrain the directionality of the interaction. Features shown in line mode are optional aromatic or hydrophobic features.

The development of the pharmacophore model was initiated by aligning a set of 202 known active FLAP inhibitors (collected from published reports[16–21]) to the crystallographic data of the complex of compound **85** (3-[3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-(quinolin-2-ylmethoxy)-2,3-dihydro-1*H*-indol-2-yl]-2,2-dimethylpropanoic acid, MK-591, Fig. 1) and FLAP (PDB code: 2Q7M) available in the Brookhaven Protein Data Bank[15] by using the software MOE. The known FLAP inhibitors involved three distinct structural classes, that is, 1,2-disubstituted indoles (class 1), 2,2-bisaryl-bicycloheptanes (class 2), and diaryl-substituted alkanes (class 3) where the available X-ray ligand **85** was a member of the class 1 structural type (Fig. 1). Alignment scores and pocket side-chain atoms were used to identify the best matching among the multiple poses per molecule produced by the algorithm.

Relevant structural features from the aligned training set were transformed into a consensus pharmacophore model using Ph4 consensus of MOE. Optimizing query simplicity resulted in a six-featured pharmacophore query which consists of three aromatic rings and three hydrophobic groups in the shown spatial arrangements as common features of the aligned ligands (Fig. 2). The 3D pharmacophore query was used to screen a precompiled collection of 2.8 mio vendor compounds (database available from Chemical Computing Group, 153 mio conformations) where 1792 compounds matching the desired pharmacophoric features were identified. To confirm the hits, they were docked using MOE and prioritized for biological testing.

2.2. Molecular docking and scoring

FLAP is a homotrimer with three binding sites located between adjacent monomers.[15] We used the FLAP crystal structure in complex with compound **85** (PDB code: 2Q7M) and chains B and C were selected for docking studies. All hit compounds retrieved from the pharmacophore-based virtual screening were subjected to molecular docking to the FLAP binding site located between the chains B and C with the key residues B-Phe123, B-Leu120, B-Ile119, B-Lys116, B-Phe114, B-Ile113, B-Tyr112, B-Thr66, B-Asp63, C-Val61, C-His28, C-Ala27, C-Phe25 and C-Asn23. As a result, 1494 of 1792 hits were successfully docked. Then, the docking poses that survived were filtered according to forcefield refinement and rescoring function. Thus, 192 virtual hits were identified and selected as promising candidates, and the protein–ligand interaction fingerprints (PLIFs) application of MOE was used to identify most diverse interaction pattern. As a result, a subset of 37 hits was selected based on diversity observed in PLIFs. Finally, 8 candidates (Fig. 3) were 'cherry-picked' from this subset based on chemical intuition (e.g. compound **7**) and literature knowledge (e.g. compound **8**) considering the geometrical and pharmacophoric features as well as overall lipophilicity of known FLAP inhibitors for evaluation of

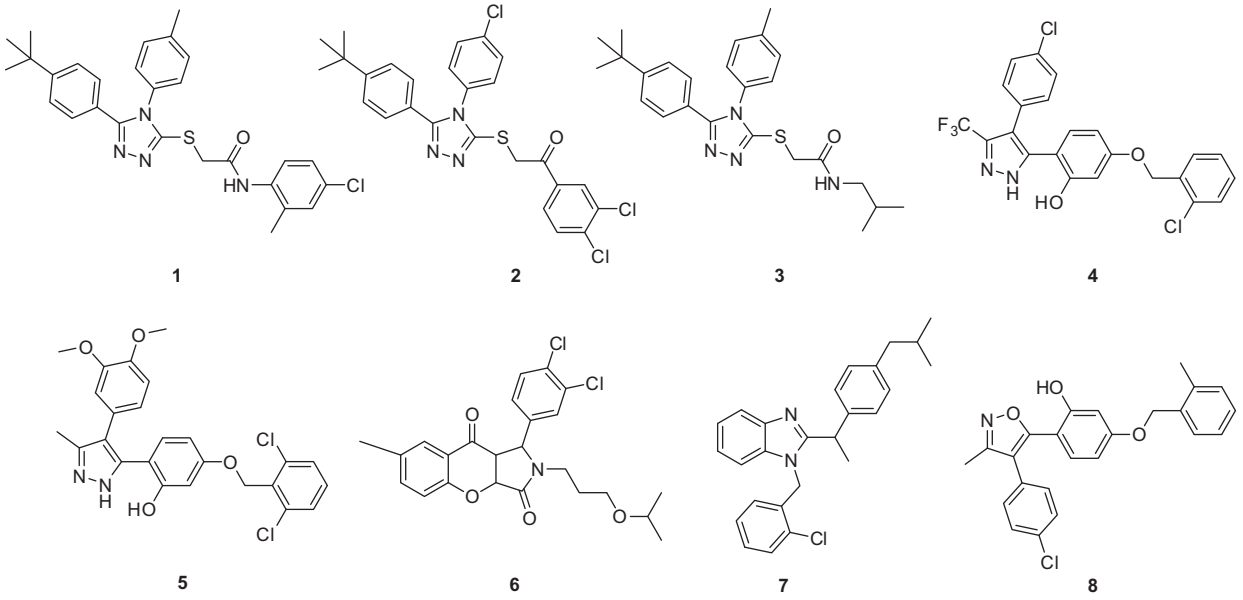


Figure 3. Structures of potentially active FLAP inhibitors identified by the virtual screening study.

inhibition of 5-LO product synthesis in cell-based and cell-free assays.

2.3. Analysis of inhibition of 5-LO product synthesis by the virtual screening hits

For 5-LO product biosynthesis in intact cells, the substrate AA is released from membrane phospholipids by cytosolic phospholipase A_2 ($cPLA_2$), hooked up by FLAP, and then transferred by FLAP to 5-LO for metabolism.[3] However, when there is ample supply of substrate, for example under cell-free assay conditions (i.e., using isolated 5-LO or cell homogenates), FLAP is dispensable for 5-LO product synthesis.[2] Thus, FLAP inhibitors fail to inhibit 5-LO product formation in cell-free test systems.[5] In order to differentiate between putative FLAP inhibitors and direct 5-LO inhibitors, we have studied the compounds in a 'FLAP-dependent', cell-based assay of 5-LO product synthesis using human neutrophils challenged by Ca^{2+}-ionophore A23817, as well as in a 'FLAP-independent', cell-free 5-LO activity assay using human recombinant 5-LO expressed in *Escherichia coli*. To evaluate the compounds in neutrophils, the direct 5-LO products 5-H(p)ETE and all-trans isomers of LTB_4, as well as LTB_4, were analyzed.

Compounds **1**, **2** and **3** barely inhibited 5-LO product formation with IC_{50} values >10 μM both in the cell-based and the cell-free system (Table 1). Compounds **4**, **5**, and **8** were more potent inhibitors as they reduced 5-LO product synthesis in intact neutrophils (IC_{50} = 4.4–9.1 μM), but had similar effects also in the cell-free assay. Also, compound **6** significantly inhibited 5-LO activity in the cell-free assay. From these data we conclude that compounds **4**, **5**, **6** and **8** primarily inhibit 5-LO leading to reduced cellular 5-LO product formation. In contrast, compound **7** (a benzimidazole derivative bearing the isobutylphenylethyl fingerprint of ibuprofen) potently suppressed cellular 5-LO product formation with IC_{50} = 0.31 μM but was much less active on cell-free 5-LO (only 23% inhibition at 10 μM, Table 1), suggesting that **7** is not a direct 5-LO inhibitor but rather affects FLAP, thereby suppressing 5-LO product synthesis in neutrophils. The FLAP reference inhibitor **83** (3-[3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-isopropyl-1*H*-indol-2-yl]-2,2-dimethylpropanoic acid, MK-886) and the direct 5-LO reference inhibitor **84** ((*E*)-*N*-hydroxy-*N*-(3-(3-phenoxyphenyl)-allyl)acetamide, BWA4C) inhibited 5-LO product formation in these assays (Table 1) as expected.

Table 1
Inhibition of 5-LO product formation by screening hits in cell-free and cell-based assays

Compd	5-LO product formation at 10 μM, cell-free assay (% of control)	5-LO product formation at 10 μM, cell-based assay (% of control)	IC_{50}, cell-based assay (μM)
1	85.3 ± 16.7	91.3 ± 29.5	>10
2	116.0 ± 32.3	57.9 ± 13.2	>10
3	62.0 ± 13.0	80.8 ± 7.3	>10
4	52.8 ± 28.9	40.9 ± 9.9	8.8
5	58.0 ± 22.7	43.6 ± 6.1	9.1
6	55.1 ± 14.1	78.8 ± 17.4	>10
7	77.1 ± 15.4	4.0 ± 1.6	0.31
8	16.0 ± 2.0	10.0 ± 7.5	4.4
83	87.8 ± 7.3	2.7 ± 1.2	0.017
84	4.4 ± 0.9	3.1 ± 2.2	0.11

Values (mean ± SE, n = 3–4) for 5-LO product formation under cell-free conditions (40,000×*g* supernatants of lysates of *E. coli* expressing human recombinant 5-LO incubated with 20 μM AA) and in intact human neutrophils challenged with 2.5 μM ionophore are given as percentage of control at 10 μM inhibitor concentration. The 100% values of the controls (0.3% DMSO, vehicle) correspond to an average of 44 ng 5-LO products per 10^6 neutrophils and of 310 ng 5-LO products per ml in the cell-free assay. The FLAP inhibitor 3-[3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-isopropyl-1*H*-indol-2-yl]-2,2-dimethylpropanoic acid (MK-886, compound **83**) and the 5-LO inhibitor (*E*)-*N*-hydroxy-*N*-(3-(3-phenoxyphenyl)-allyl)acetamide (BWA4C, compound **84**) were used (10 μM, each) as reference compounds, respectively. IC_{50} values in the cell-based assay were obtained from intact neutrophils challenged with 2.5 μM ionophore.

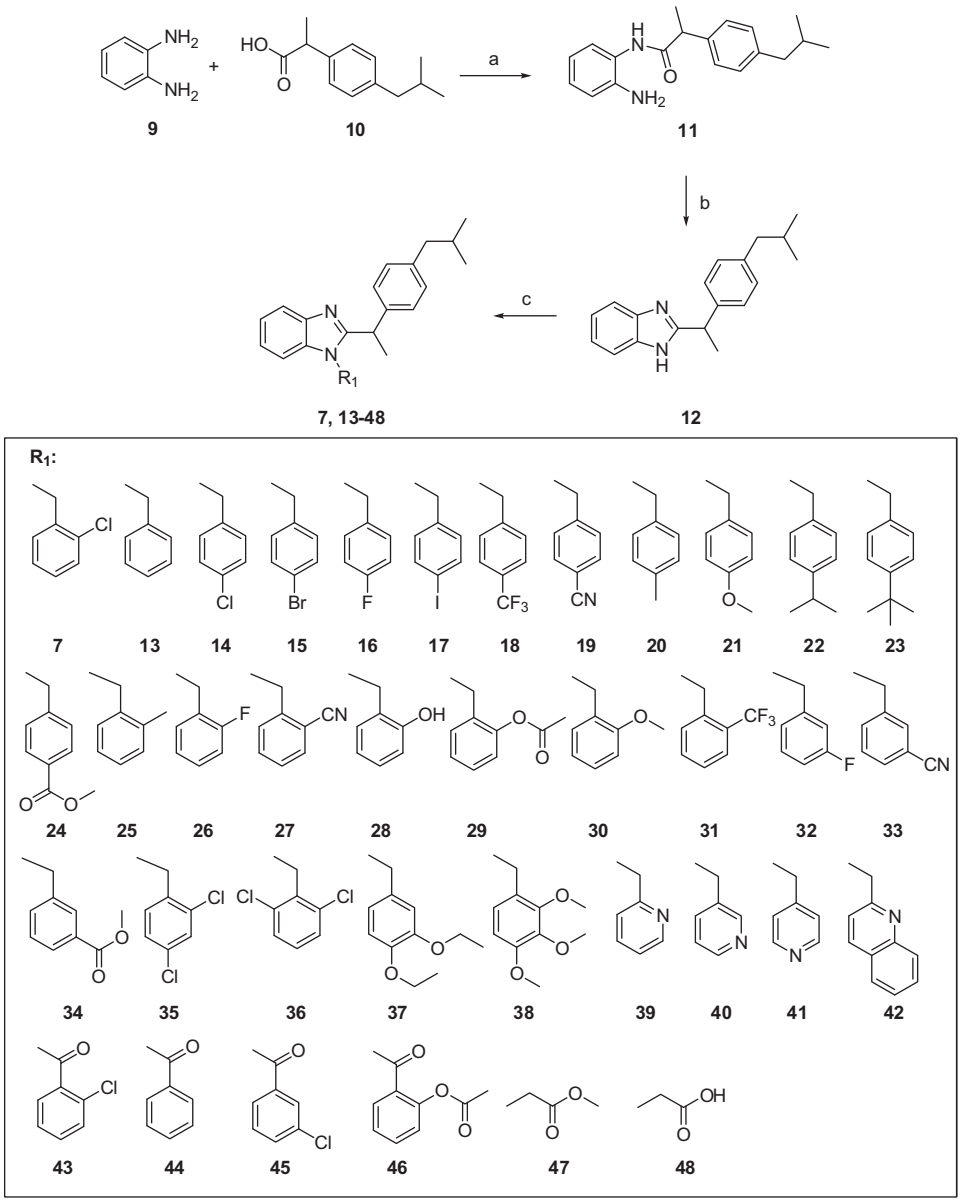
2.4. Chemistry

Based on the favorable profile of compound **7** as inhibitor of LT biosynthesis, we prepared further derivatives in order to perform SAR studies. For the synthesis of compounds **7** and **13–48**, we utilized the synthetic procedure outlined in Scheme 1, following published standard procedures. For the synthesis of benzimidazole derivative **12**, we acquired a two-step procedure whereby 1,2-phenylenediamine **9** was first treated with the activated carboxylic acid **10** to yield the monoacylated derivative **11** which subsequently converted to the target benzimidazole through cyclodehydration by refluxing in pure acetic acid.[22] For the N-alkylation of compound **12**, the required benzyl/benzoyl halides were either obtained from commercial sources or prepared first through sodium borohydride reduction of appropriate benzaldehydes using PEG400 as a phase transfer catalyst,[23] and second, halogenation of the resulting benzyl alcohol by standard agents such as PBr_3, $SOCl_2$ and CBr_4. The target compounds **7** and **13–48**, except for **28** and **29**, were synthesized by N-benzylation of the NH-benzimidazole **12** with the obtained benzyl halides using different basic media such as NaH in DMF, KOH in DMSO, and TEA in THF to obtain the optimal reaction yields. The generation of **28** was accomplished conveniently by directly reacting **12** with 2-hydroxybenzyl alcohol under neat conditions.[24] By this method, 2-hydroxybenzyl derivative **28** was successfully synthesized in moderate yield by taking advantage of the in situ generated *o*-methylenequinone as an electrophile. Subsequent acetylation of **28** by acetic acid anhydride in pyridine resulted in 2-acetoxybenzyl derivative **29**.

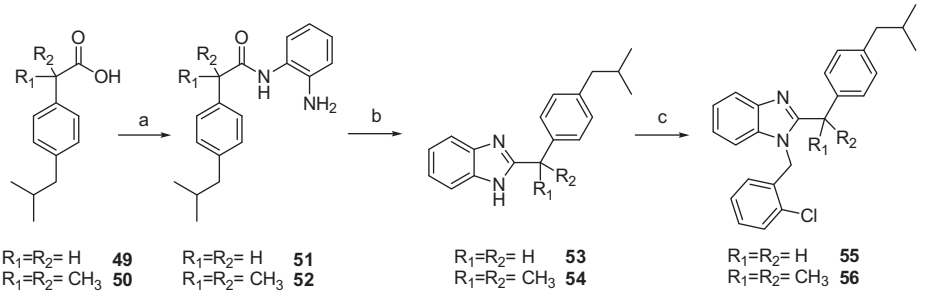
Synthesis of the desmethyl (**55**) and the geminal dimethyl (**56**) analogues of **7** was carried out as outlined in Scheme 2 by using ibufenac (**49**) and the geminal dimethyl analogue of ibuprofen (**50**) as starting intermediates. Synthesis of **49** was achieved by a conventional Willgerodt–Kindler synthesis of thiomorpholide from 4-isobutylacetophenone, which was subsequently converted to **49** by hydrolysis.[25] For the preparation of **50**, esterification of ibuprofen followed by methylation under basic conditions yielded the geminal dimethyl ester which was readily hydrolyzed to the acid by using Triton B.[26] For **63**, carrying a methyl group instead of an isobutyl substituent in compound **7**, the starting intermediate **57** was prepared according to general published reports (Scheme 3). For the synthesis of **57**, ethyl *p*-tolylacetate was methylated with methyl iodide under standard conditions and the ester was quantitatively hydrolyzed to the acid. The commercially available ketoprofen (**58**) was used instead of **49** for the synthesis of **64** with benzoyl substituent. The monoacylation, cyclization to benzimidazole, and N-benzylation reactions for compounds **49**, **50**, **57** and **58** were carried out as described for compound **7** (Schemes 2 and 3).

The analogues of compound **7** carrying a substituent at 5-position were obtained by the series of reactions given in Scheme 4. Starting from 4-methoxyaniline (**65**), N-acetylation followed by nitration and hydrolysis afforded 2-nitroaniline derivative **66** which was benzylated and reduced to generate **68**. After following the standard conditions shown in Scheme 1, the 5-methoxybenzimidazole derivative **70** was obtained. Hydrolysis of the methyl ether was done by HBr to yield the 5-hydroxy derivative **71**. The 5-hydroxybenzimidazole derivative was then alkylated either with 2-picolylchloride or α-chloroquinaldine to obtain the target compounds **72** and **73**, respectively.

For the synthesis of 5-chlorobenzimidazole derivatives (Scheme 5), *N*-benzyl- or *N*-2-chlorobenzyl-4-chloro-2-nitroanilines **75** and

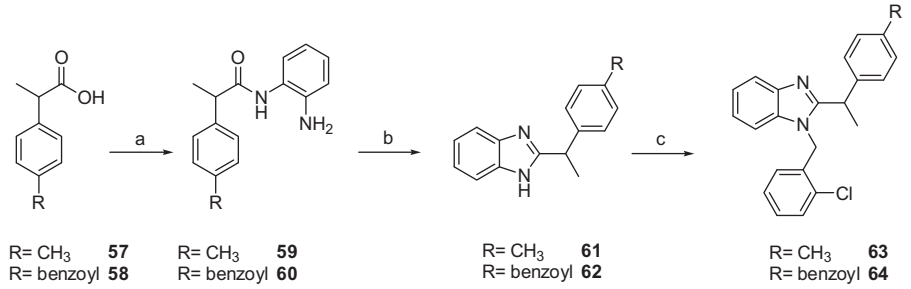


Scheme 1. Synthetic strategy for the synthesis of *N*-benzyl analogues of compound **7** Reagents and conditions: (a) EDC, DMAP, CH_2Cl_2, rt. (b) AcOH, reflux; (c) alkylhalide, KOH/DMSO or NaH/DMF or TEA/THF at rt.

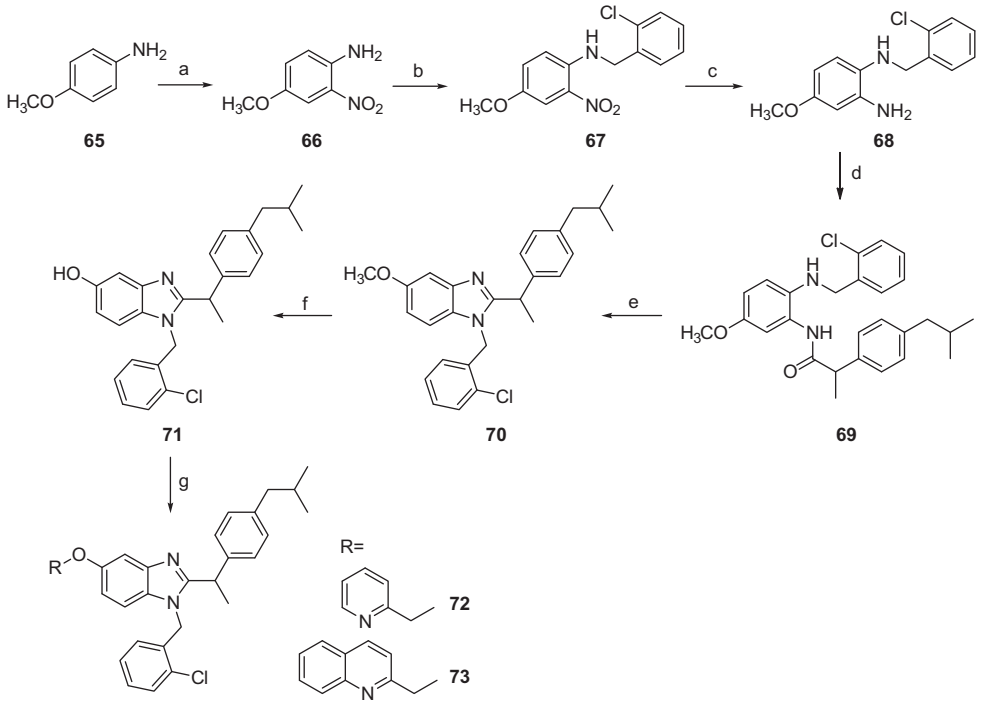


Scheme 2. Synthesis of achiral analogues of compound **7**. Reagents and conditions: (a) o-phenylenediamine, EDC, DMAP, CH_2Cl_2, rt. (b) AcOH, reflux. (c) 2-chlorobenzylbromide, KOH/DMSO, rt.

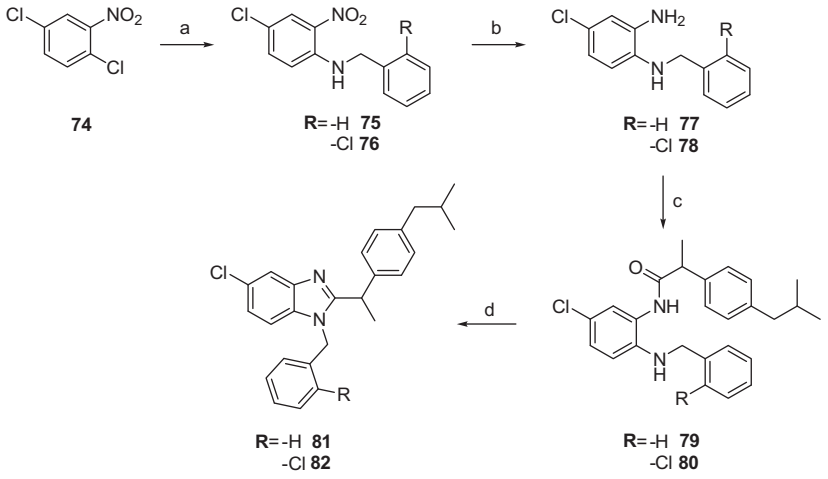
76 were prepared from 2,5-dichloronitrobenzene by nucleophilic substitution of the chlorine atom by benzylamine derivatives. Reduction of the nitro group followed by the standard procedures described in Scheme 1 resulted in the 5-chlorobenzimidazole derivatives **81** and **82**. All compounds were purified by automated flash chromatography and checked for purity with UPLC before



Scheme 3. Synthesis of **63** and **64**. Reagents and conditions: (a) *o*-phenylenediamine, EDC, DMAP, CH_2Cl_2, rt. (b) AcOH, reflux. (c) 2-chlorobenzylbromide, KOH/DMSO, rt.



Scheme 4. Synthesis of C5-substituted analogues of compound **7**. Reagents and conditions: (a) (i) Ac_2O, AcOH (ii) HNO_3, AcOH (iii) Claisen's alkali. (b) 2-Chlorobenzylbromide, DIPEA, DMF. (c) $SnCl_2{\cdot}2\ H_2O$, EtOH (d) ibuprofen, oxalyl chloride, TEA, DMF (e) AcOH, reflux. (f) HBr 48% (g) 2-picolylchloride or α-chloroquinaldine, Cs_2CO_3, DMF.



Scheme 5. Synthesis of C5-chloro-substituted analogues of compound **7**. Reagents and conditions: (a) benzylamine or 2-chlorobenzylamine, EtOH (b) $SnCl_2{\cdot}2\ H_2O$, EtOH (c) ibuprofen, oxalyl chloride, TEA, DMF (d) AcOH, reflux.

being tested in biological assays (purity was >97%). The structures of the compounds were confirmed by high resolution mass spectrometry (HRMS), elemental analysis, IR and ^{1}H NMR spectral data.

2.5. Structure–activity relationships (SAR) studies

In order to deduce SARs, we concentrated on four distinct areas of the lead compound **7**. Thus, we approached (i) modification of the *N*-benzyl group, (ii) removal of the chirality by preparing desmethyl and geminal dimethyl analogs of **7**, (iii) replacement of the isobutyl moiety of **7** with smaller or larger groups, and (iv) incorporation of different substituents at the C5-benzimidazole ring. Together, by installation of distinct chemical functionalities with the aforementioned modifications, forty-six benzimidazole derivatives were synthesized to investigate their inhibitory effect on 5-LO product formation in the cell-based (intact neutrophils) assay (Table 2). First, the substitution pattern at the aromatic ring of the benzyl group was studied as shown by the comparison of the 4-substituted (**14**–**24**), 2-substituted (**25**–**31**) and 3-substituted analogues (**32**–**34**). Removal of 2-chlorine, resulting in an unsubstituted benzyl (**13**), decreased the potency (IC_{50} = 0.98 μM) versus compound **7** (IC_{50} = 0.31 μM). Movement of the chlorine substituent to 4-position (**14**) also caused a significant loss of activity (IC_{50} = 4.8 μM). Substitutions at 4-position other than chlorine, i.e. electron withdrawing (**15**–**19**) and electron donating groups (**20**–**21**), were detrimental resulting in compounds with IC_{50} values between 2.3 and 6.0 μM. Increasing the steric bulk at 4-position (**22**–**24**) also impaired the potency versus **7**, indicating that voluminous substituents at this position are not well accommodated. In contrast, variations of the substituents in 2-position (i.e., compounds **25–31**) slightly affected the inhibitory activity (except for **28**) and potent, concentration-dependent suppression of 5-LO product formation (IC_{50} = 0.25–0.7 μM) could be retained. Interestingly, in this series, a free 2-hydroxyl group caused a significant loss of activity (**28**, IC_{50} = 4.5 μM), but acetylation (**29**) or methylation (**30**) of this residue recovered the activity (IC_{50} = 0.25 and 0.44 μM, respectively). Although the reason for this is not clear, hydrogen bond forming properties or general increased polarity of the hydroxy-derivative **28** is conceivable. Of interest, voluminous groups are seemingly tolerated at 2-position, deduced from the almost 2-fold increased potency of the ester **29**. 3-Substituted benzyl analogues (**32**–**34**) were not pursued further as they showed marked loss of inhibitory activity. When the dichloro substitution pattern was investigated, the 2,4-substitution (**35**) was superior over 2,6-substitution (**36**) with IC_{50} values of 0.5 and 4.4 μM, respectively. Multiple alkoxy substitutions on the phenyl ring (**37** and **38**) were detrimental, consistent with the result that voluminous substituents are not well tolerated on the benzyl moiety other than in 2-position. We also investigated the effects of introducing a heteroaromatic group into the molecule. When the phenyl was replaced by 2-pyridinyl (**39**), 3-pyridinyl (**40**) or 4-pyridinyl (**41**), the compounds were still active, albeit the potency decreased (IC_{50} = 1.95–2.5 μM). Introducing a larger quinoline (**42**) diminished the inhibitory activity which again was in agreement with the hypothesis that voluminous substituents in place of the *N*-benzyl group are detrimental.

We next examined the necessity of the *N*-benzyl group by replacing it with a benzoyl substituent. When **43**, the derivative of **7** carrying a 2-chlorobenzoyl moiety instead of *N*-benzyl, was analyzed, a loss of activity was clearly apparent (IC_{50} >10 μM). Similarly, unsubstituted benzoyl (**44**), and 3-chlorobenzoyl (**45**) derivatives appeared to be ineffective. However, incorporation of a 2-acetoxy-substituent to the benzoyl group partially restored the inhibitory activity in this series (IC_{50} = 6 μM), supporting the previous finding with compound **29** that a 2-acetoxy substituent favors inhibitory activity. Finally, replacement of benzyl by an alkyl carboxylic acid (**48**) or by its ester (**47**) was found to be detrimental suggesting the significance of the *N*-benzyl group for inhibition of LT biosynthesis. Taken together, 2-substituted *N*-benzyl groups clearly govern potent inhibition of LT biosynthesis.

Since all compounds were evaluated as racemates, our second approach was to investigate the effect of chirality and the importance of the methyl group on the chiral carbon for the inhibitory potency of lead compound **7**. As seen in Table 2, either deletion of the methyl group (**55**) or insertion of a second methyl at the chiral carbon (**56**) only slightly influenced the inhibitory potency versus **7**, with IC_{50} values of 0.5 and 0.7 μM, respectively.

Our third goal was to understand the role of the isobutyl fingerprint of **7** for the potency of compounds and we briefly examined this by replacing the isobutyl with smaller methyl (**63**) and also with bulky benzoyl (**64**) groups. However, these derivatives also

Table 2
Effects of test compounds on 5-LO product synthesis in a cell-based (intact neutrophils) assay

Compd	5-LO product formation in intact neutrophils	
	Remaining activity at 1 μM (% of control)	IC_{50} (μM)
7	19.4 ± 4.9	0.31
13	47.7 ± 6.2	0.98
14	66.5 ± 11.7	4.8
15	79.7 ± 6.4	6
16	65.8 ± 10.7	3.4
17	78.2 ± 3.6	5
18	83.9 ± 5.6	4.9
19	100.4 ± 35.2	2.3
20	67.1 ± 8.9	2.9
21	63.0 ± 7.8	3.8
22	83.8 ± 6.6	7.2
23	98.3 ± 7.0	>10 (77.8 ± 10.6)[a]
24	99.8 ± 13.1	>10
25	18.1 ± 10.0	0.5
26	43.9 ± 7.3	0.7
27	43.5 ± 9.8	0.45
28	84.8 ± 8.3	4.5
29	8.4 ± 3.1	0.25
30	9.2 ± 4.9	0.44
31	4.89 ± 2.25	0.25
32	71.1 ± 11.9	4.0
33	91.1 ± 18.5	n.d.
34	109.2 ± 10.8	n.d.
35	24.6 ± 12.5	0.5
36	89.4 ± 2.2	4.4
37	96.1 ± 16.2	>10 (91.5 ± 13)[a]
38	120.3 ± 4.7	9.4
39	85.3 ± 11.4	2.5
40	93.6 ± 20.5	2.8
41	74.1 ± 14.1	1.95
42	112.6 ± 13.6	4.4
43	103.5 ± 11.3	>10 (58.8 ± 6.5)[a]
44	88.7 ± 6.0	10
45	92.2 ± 18.1	>10 (58.7 ± 9.5)[a]
46	94.4 ± 2.9	6
47	86.0 ± 17.5	>10 (56.7 ± 16.9)[a]
48	135.0 ± 23.6	>10 (85.5 ± 6.9)[a]
55	25.6 ± 11.2	0.5
56	33.3 ± 10.3	0.7
63	80.9 ± 13.9	2.8
64	90.2 ± 2.3	3
70	7.8 ± 7.0	0.12
71	4.5 ± 0.3	0.19
72	7.0 ± 5.2	0.18
73	72.0 ± 11.6	>10 (53.4 ± 5.44)[a]
81	44.9 ± 11.6	0.83
82	18.08 ± 4.27	0.16

Compounds were tested in neutrophils stimulated with 2.5 μM A23187. Data are given as percentage of control (100%) at 10 μM inhibitor concentration (means ± SE, n = 4–6) or as IC_{50} values (means). The 100% value of the control (0.3% DMSO, vehicle) corresponds to an average of 44 ng 5-LO products per 10^6 neutrophils.
[a] Remaining activity at 10 μM; n.d., not determined.

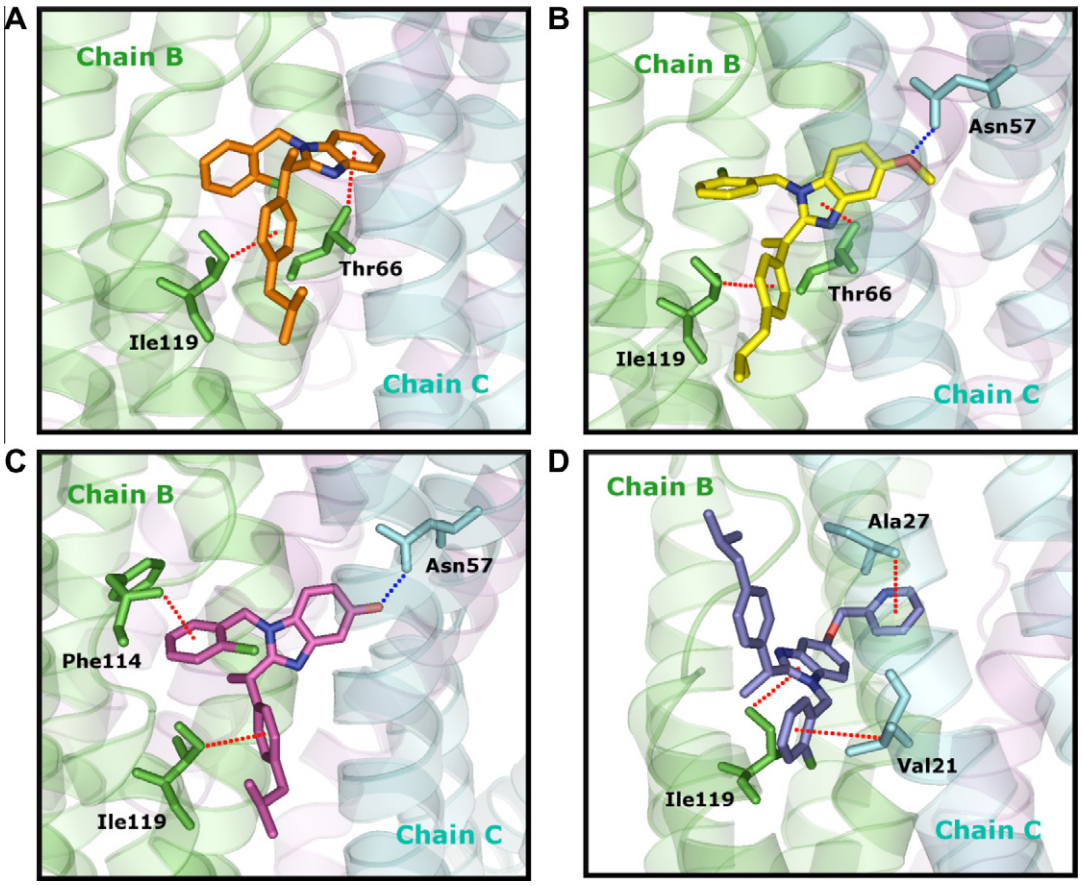


Figure 4. Docking of the compounds **7** (A), **70** (B), **71** (C), and **72** (D) at the active site of FLAP. Hydrophobic interactions are presented in red, while hydrogen bonds are in blue.

showed diminished inhibitory potency indicating that the isobutyl substituent was favorable for bioactivity.

Finally, we evaluated the incorporation of various substituents at the C(5) of the benzimidazole. At this stage, we aimed to anticipate two improvements on **7**: (i) the inhibitory potency and (ii) the probable metabolic stability by blocking the C(5) position at the benzimidazole, since benzimidazole derivatives generally tend to be converted to 5-hydroxy derivatives.[27,28] Of interest, we found that electron donating substituents at the C(5) position enhance the inhibitory potency. Thus, 5-methoxy (**70**) and 5-hydroxy (**71**) analogues are more potent inhibitors than **7** with IC_{50} of 0.12 and 0.19 μM, respectively. Pyridine and quinoline moieties are found in a variety of LT biosynthesis inhibitors and also are established pharmacophores for FLAP inhibitors.[9,11,29] Therefore, we sequentially incorporated a 2-pyridinylmethyl (**72**) and 2-quinolynylmethyl (**73**) into the C(5) position of **7**. While the pyridine moiety was well tolerated (IC_{50} = 0.18 μM), the quinoline group caused a complete loss of inhibitory potency. This may indicate that larger substituents at this position are not tolerated and may hamper the binding to FLAP. Finally, we synthesized the mono- and dichloro derivatives **81** and **82** of **7** as examples of electron withdrawing substituent which essentially retained the inhibitory potency (IC_{50} = 0.83 and 0.16 μM, respectively) of **7**.

Together, compounds **7**, **25**–**27**, **28**–**31**, **35**, **55**, **56** and the highly active analogues **70**–**72** and **82** potently suppress LT biosynthesis in intact neutrophils. In a final step, we performed molecular modeling studies of the most active compounds by using the 3D-structure of FLAP in complex with the inhibitor **85**.[15] In our proposed poses (Fig. 4), compounds **7, 70** and **71** form hydrophobic interactions with the fundamental amino acid B-Ile119 through the phenyl moiety positioned at C(2) of the benzimidazole. In addition, the benzimidazole ring of compounds **7** and **70** is stabilized by forming CH-π interactions with B-Thr66, while **71** interacts with the B-Phe114 through its *N*-chlorobenzyl group. However, the most potent inhibitors **70** and **71** form an additional H-bond with the C-Asn57 through their hydroxy or methoxy groups located at C(5) of the benzimidazole, which was not present in the case of compound **7**. Compound **72** adopts a different binding conformation versus **70** and **71** in which the 2-pyridinylmethyl group now occupies the position of the benzimidazole ring in these compounds, however maintaining similar hydrophobic interaction network such as CH-π interactions with B-Ile119, C-Val21 and C-Ala27. The binding energies and the stability of the formed ligand–protein complexes were in good agreement with the observed biological data (Table S1, Supplementary data).

3. Conclusions

There is currently a strong interest in the development of FLAP inhibitors because of their promising potential as therapeutics in inflammatory and allergic disorders as well as in cardiovascular disease.[9–13] Here, we have implemented for the first time a rapid virtual screening on a large number of compounds from vendor libraries based on a combined ligand- and structure-based FLAP pharmacophore. Though recently discussed as a future opportunity, drug design solely based on the available structure of FLAP was found to be insufficient due to low resolution. Out of eight candidates that were tested, compound **7**, a benzimidazole derivative bearing the isobutylphenylethyl fingerprint of ibuprofen, was identified as potent suppressor of cellular LT biosynthesis without

inhibiting 5-LO activity directly. SAR investigations revealed that the 2-substituted *N*-benzyl group and the isobutyl substituent of **7** clearly govern potent inhibition of LT biosynthesis, and that large substituents on the C5-benzimidazole are not tolerated. This suggests the benzimidazole scaffold bearing an ibuprofen fingerprint as a new chemotype for further development of anti-LT agents. A possible description of the putative FLAP binding mode for the novel benzimidazole-based compounds was also proposed. In conclusion, our results proved these fast synthetically accessible benzimidazole-based compounds as a new and innovative scaffold useful in the rational design of novel developmental candidates as anti-LT drugs and provide valuable insights into the chemical decorations functional for the design of further analogues belonging to this class of inhibitors.

4. Experimental

4.1. Compounds and chemistry

^{1}H 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 at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). High resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in either 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 benzimidazole derivatives

4.1.1.1. Method A (for compounds 7, 13–27, 30–42, 47, 48, 55, 56, 63, 64). To an ice-cooled solution of NaH (60% in oil, 1.5 equiv) in 10 mL DMF or to a solution of KOH (1.5 equiv) in 10 mL DMSO at rt, the appropriate benzimidazole derivative (**12**, **53**, **54**, **61** or **62**) (1 equiv) was added and stirred for 0.5 h. After addition of the corresponding alkyl halide (1 equiv), stirring was continued for 3 h. The reaction mixture was diluted with water, extracted with EtOAc (30 mL × 3), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The final product was purified by automated flash chromatography.

4.1.1.2. Method B (for compounds 43–46). To a solution of benzimidazole derivative **12** (1 equiv) in anhydrous THF, the appropriate acyl chloride (1.1 equiv) and TEA (1.5 equiv) was added. After stirring at rt overnight, the eluent was evaporated in vacuo and the residue was dissolved in 20 mL DCM. The organic phase was extracted with 5% $NaHCO_3$ (3 × 30 mL), brine (3 × 30 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated in vacuo to give the product which was subsequently purified by automated flash chromatography.

4.1.1.3. 1-(2-Chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (7). Yield 82%; mp 105.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, dd, J = 6.8 and 2.0 Hz), 1.75 (1H, m), 1.82 (3H, d, J = 7.2 Hz), 2.35 (2H, d, J = 7.2 Hz), 4.15 (1H, q, J = 7.2 Hz), 5.24 (2H, dd, J = 17.6 and 17.6 Hz), 6.18 (1H, d, J = 7.2 Hz), 6.90 (1H, m), 6.94–7.08 (3H, m), 7.13–7.37 (6H, m), 7.90 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}ClN_2$ 403.1941; found 403.1925; Anal. Calcd for $C_{26}H_{27}ClN_2$: C, 77.50; H, 6.75; N, 6.95. Found: C, 77.64; H, 6.75; N, 6.92.

4.1.1.4. 1-Benzyl-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (13). Yield 67%; mp 107.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (6H, d, J = 6.8 Hz), 1.78 (1H, m) 1.81 (3H, d, J = 6.8 Hz), 2.40 (2H, d, J = 7.2), 4.18 (1H, q, J = 7.0 Hz), 5.04–5.19 (2H, dd, J = 16.8 and J = 16.8), 6.87 (2H, m), 7.02 (2H, d, J = 8.0 Hz), 7.09 (2H, d, J = 8.0 Hz), 7.13–7.28 (6H, m), 7.89 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{28}N_2$ 369.2331; found 369.2324; Anal. Calcd for $C_{26}H_{28}N_2$: C, 84.74; H, 7.66; N, 7.60; found: C, 84.67; H, 7.96; N, 7.63.

4.1.1.5. 1-(4-Chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (14). Yield 69%; mp 180.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.4 Hz), 1.76 (1H, m), 1.81 (3H, d, J = 7.2 Hz), 2.39 (2H, d, J = 7.2 Hz), 4.16 (1H, q, J = 7.0 Hz), 5.01–5.17 (2H, dd, J = 16.8 Hz and J = 16.8 Hz), 6.76 (2H, d, J = 8.8 Hz), 7.01 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8.0 Hz), 7.10–7.27 (5H, m), 7.89 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}ClN_2$ 403.1941. Found 403.1931; Anal. Calcd for $C_{26}H_{27}ClN_2$: C, 77.50; H, 6.75; N, 6.95. Found: C, 77.57; H, 6.37; N, 6.98.

4.1.1.6. 1-(4-Bromobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (15). Yield 77%; mp 168.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.4 Hz), 1.76 (1H, m), 1.81 (3H, d, J = 7.2 Hz), 2.39 (2H, d, J = 7.2 Hz), 4.16 (1H, q, J = 7.0 Hz), 4.99–5.15 (2H, dd, J = 17.2 Hz and J = 16.8 Hz), 6.68 (2H, d, J = 8.8 Hz), 6.99 (2H, d, J = 8.0 Hz), 7.06 (2H, d, J = 8.0 Hz), 7.07 (1H, m), 7.18–7.30 (4H, m), 7.88 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}BrN_2$ 447.1436; found 447.1416; Anal. Calcd for $C_{26}H_{27}ClN_2$: C, 69.80; H, 6.08; N, 6.26. Found: C, 69.83; H, 5.82; N, 6.20.

4.1.1.7. 1-(4-Fluorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (16). Yield 94%; mp 169.4 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.4 Hz), 1.76–1.82 (4H, m), 2.39 (2H, d, J = 7.2 Hz), 4.19 (1H, q, J = 7.1 Hz), 5.02 (2H, dd, J = 16.4 Hz and J = 16.8 Hz), 6.78–6.91 (4H, m), 7.01 (2H, d, J = 8.0 Hz), 7.08 (2H, d, J = 8.4 Hz), 7.11–7.28 (3H, m), 7.88 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}FN_2$ 387.2237; found 387.2235; Anal. Calcd for $C_{26}H_{27}FN_2$: C, 80.80; H, 7.04; N, 7.25. Found: C, 80.71; H, 7.05; N, 7.17.

4.1.1.8. 1-(4-Iodobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (17). Yield 64%; mp 140.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.8 Hz), 1.76 (1H, m), 1.82 (3H, d, J = 7.2 Hz), 2.39 (2H, d, J = 7.2 Hz), 4.16 (1H, q, J = 7.1 Hz), 4.98–5.14 (2H, dd, J = 16.8 Hz and J = 17.2 Hz), 6.56 (2H, d, J = 8.4 Hz), 7.00–7.16 (5H, m), 7.16–7.29 (2H, m), 7.50 (2H, d, J = 8.8 Hz), 7.89 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}IN_2$ 495.1297; found 495.1313; Anal. Calcd for $C_{26}H_{27}IN_2$: C, 63.16; H, 5.50; N, 5.67. Found: C, 63.36; H, 5.54; N, 5.49.

4.1.1.9. 2-[1-(4-Isobutylphenyl)ethyl]-1-[4-(trifluoromethyl)benzyl]-1*H*-benzimidazole (18). Yield 40%; mp 132.3 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6.8 Hz), 1.75 (1H, m), 1.83 (3H, d, J = 7.2 Hz), 2.37 (2H, d, J = 7.2 Hz), 4.16 (1H, q, J = 7.2 Hz), 5.12–5.26 (2H, dd, J = 17.2 Hz and J = 17.2 Hz), 6.89 (2H, d, J = 8.0 Hz), 6.97 (2H, d, J = 8.0 Hz), 7.05–7.07 (3H, m), 7.19 (1H, m), 7.28 (1H, m), 7.44 (2H, d, J = 8.4 Hz), 7.91 (1H, d, J = 7.6 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{27}F_3N_2$ 437.2205;

found 437.2194; Anal. Calcd for $C_{27}H_{27}F_3N_2$: C, 74.29; H, 6.23; N, 6.42. Found: C, 74.25; H, 6.22; N, 6.38.

4.1.1.10. 4-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)benzonitrile (19). Yield 32%; mp 158.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, dd, J = 6.4 Hz and J = 6.4 Hz), 1.74 (1H, m), 1.82 (3H, d, J = 7.2 Hz), 2.37 (2H, d, J = 7.2 Hz), 4.14 (1H, m), 5.10–5.28 (2H, dd, J = 17.6 Hz and J = 17,6 Hz), 6.86 (2H, d, J = 8.4 Hz), 6.96 (2H, d, J = 8.0 Hz), 7.02–7.31 (5H, m), 7.45 (2H, d, J = 6.8 Hz), 7.90 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{27}N_3$ 394.2283; found 394.2272; Anal. Calcd for $C_{27}H_{27}N_3$: C, 82.41; H, 6.92; N, 10.68. Found: C, 81.81; H, 6.90; N, 10.38.

4.1.1.11. 2-[1-(4-Isobutylphenyl)ethyl]-1-(4-methylbenzyl)-1*H*-benzimidazole (20). Yield 89%; mp 138.8 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (6H, d, J = 6.8 Hz), 1.79 (4H, m), 2.28 (3H, s), 2.41 (2H, d, J = 7.6 Hz), 4.19 (1H, q, J = 6.8 Hz), 4.99–5.14 (2H, dd, J = 16.8 Hz and J = 16.8 Hz), 6.77 (2H, d, J = 8.0 Hz), 7.01–7.27 (9H, m), 7.88 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{30}N_2$ 383.2487; found 383.2486; Anal. Calcd for $C_{27}H_{30}N_2$: C, 84.77; H, 7.90; N, 7.32. Found: C, 84.48; H, 7.54; N, 7.26.

4.1.1.12. 2-[1-(4-Isobutylphenyl)ethyl]-1-(4-methoxybenzyl)-1*H*-benzimidazole (21). Yield 76%; mp 118.4 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (6H, d, J = 6.8 Hz), 1.79–1.81 (4H, m), 2.42 (2H, d, J = 7.2 Hz), 3.74 (3H, s), 4.21 (1H, q, J = 7.0 Hz), 4.96–5.14 (2H, dd, J = 16.8 Hz and J = 16.4 Hz), 6.76 (2H, d, J = 8.4 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.04 (2H, d, J = 7.6 Hz), 7.11 (2H, d, J = 7.6 Hz), 7.13–7.27 (3H, m), 7.88 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{30}N_2O$ 399.2436; found 399.2429; Anal. Calcd for $C_{27}H_{30}N_2O$: C, 81.37; H, 7.59; N, 7.03. Found: C, 80.97; H, 7.98; N, 7.02.

4.1.1.13. 2-[1-(4-Isobutylphenyl)ethyl]-1-(4-isopropylbenzyl)-1*H*-benzimidazole (22). Yield 30%; mp 76.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (6H, d, J = 6.8 Hz), 1.21 (6H, d, J = 7.2 Hz), 1.66–1.84 (4H, m), 2.42 (2H, d, J = 7.2 Hz), 2.84 (1H, m), 4.21 (1H, q, J = 6.8 Hz), 5.14 (2H, dd, J = 16.8 Hz and J = 16.8 Hz), 6.82 (2H, d, J = 7.6 Hz), 7.03–7.27 (9H, m), 7.88 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{29}H_{34}N_2$ 411.2800; found 411.2780; Anal. Calcd for $C_{29}H_{34}N_2$: C, 84.83; H, 8.35; N, 6.82. Found: C, 84.45; H, 8.42; N, 6.81.

4.1.1.14. 1-(4-*tert*-Butylbenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (23). Yield 72%; mp 72 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (6H, d, J = 6.8 Hz), 1.26 (9H, s), 1.82 (4H, m), 2.42 (2H, d, J = 7.2 Hz), 4.22 (1H, q, J = 8.0 Hz), 5.18 (2H, dd, J = 16.8 Hz and J = 16.4 Hz), 6.82 (2H, d, J = 8.0 Hz), 7.03 (2H, d, J = 7.6 Hz), 7.10 (2H, d, J = 8.0 Hz), 7.16–7.27 (5H, m), 7.87 (1H, d, J = 6.8 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{30}H_{36}N_2$ 425.2957; found 425.2957; Anal. Calcd for $C_{30}H_{36}N_2$: C, 84.86; H, 8.55; N, 6.60. Found: C, 84.77; H, 8.53; N, 6.60.

4.1.1.15. Methyl 4-({2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)benzoate (24). Yield 74.5%; mp 127.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6.4 Hz), 1.76 (1H, m), 1.81 (3H, d, J = 7.2 Hz), 2.37 (2H, d, J = 6.8 Hz), 3.88 (3H, s), 4.15 (1H, q, J = 7.0 Hz), 5.05–5.26 (2H, dd, J = 17.2 Hz and J = 17.2 Hz), 6.86 (2H, d, J = 8.4 Hz), 6.98 (2H, d, J = 7.6 Hz), 6.98–7.09 (5H, m), 7.18–7.29 (2H, m), 7.87 (1H, m); HRMS (m/z): $[M+H]^+$ calcd for $C_{28}H_{30}N_2O_2$ 427.2386; found 427.2382; Anal. Calcd for $C_{28}H_{30}N_2O_2$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.90; H, 7.18; N, 6.52.

4.1.1.16. 2-[1-(4-Isobutylphenyl)ethyl]-1-(2-methylbenzyl)-1*H*-benzimidazole (25). Yield 43%; mp 93.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (6H, d, J = 6.6 Hz), 1.74–1.80 (4H, m), 2.26 (3H, s), 2.38 (2H, d, J = 7.2 Hz), 4.09 (1H, q, J = 8.6, Hz), 4.98–5.10 (2H, dd, J = 17.6 Hz and J = 17.2 Hz), 6.25 (1H, d, J = 8.0 Hz), 6.92–7.29 (10H, m), 7.90 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{30}N_2$ 383.2487; found 383.2482; Anal. Calcd for $C_{27}H_{30}N_2$: C, 84.77; H, 7.90; N, 7.32. Found: C, 84.95; H, 8.15; N, 7.38.

4.1.1.17. 1-(2-Fluorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (26). Yield 69.4%; mp 96.3 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (6H, d, J = 6.4 Hz), 1.77 (1H, m), 1.81 (3H, d, J = 7.2 Hz), 2.38 (2H, d, J = 7.6 Hz), 4.24 (1H, q, J = 6.8 Hz), 5.20 (2H, s), 6.39 (1H, t, J = 7.2 Hz), 6.83 (1H, t, J = 7.6 Hz), 6.98–7.28 (9H, m), 7.88 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}FN_2$ 387.2237; found 387.2224; Anal. Calcd for $C_{26}H_{27}FN_2$: C, 80.80; H, 7.04; N, 7.25. Found: C, 80.44; H, 7.12; N, 7.17.

4.1.1.18. 2-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)benzonitrile (27). Yield 52%; mp 134.2 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, m), 1.75 (1H, m), 1.84 (3H, d, J = 6.8 Hz), 2.31 (2H, d, J = 7.6 Hz), 4.21 (1H, q, J = 7.0 Hz), 5.34–5.48 (2H, dd, J = 18.0 Hz and J = 18.0 Hz), 6.29 (1H, d, J = 8.0 Hz), 6.90 (2H, d, J = 8.0 Hz), 7.04–7.31 (7H, m), 7.62 (1H, d, J = 8.0 Hz), 7.91 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{27}N_3$ 394.2283; found 394.2283; Anal. Calcd for $C_{27}H_{27}N_3$: C, 82.41; H, 6.92; N, 10.68. Found: C, 82.09; H, 6.94; N, 10.50.

4.1.1.19. 2-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)phenol (28). An equimolar mixture of **12** and 2-hydroxybenzyl alcohol was heated under neat conditions at 160 °C with intense stirring. The reaction product was solidified with petroleum ether and purified by automated flash chromatography. Yield 35%; mp 224.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, d, J = 6,8 Hz), 1.75 (1H, m), 1.79 (3H, d, J = 7.2 Hz), 2.36 (2H, d, J = 7.2 Hz), 4.45 (1H, q, J = 7.2 Hz), 5.21–5.36 (2H, dd, J = 16.8 Hz and J = 17.2 Hz), 6.49 (1H, d, J = 6.8 Hz), 6.65 (1H, t, J = 7.4 Hz), 6.90 (1H, d, J = 7.6 Hz), 6.96 (2H, d, J = 8.4 Hz), 7.05 (1H, t, J = 7.7 Hz), 7.14 (2H, d, J = 7.6 Hz), 7.18–7.29 (3H, m), 7.80 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{28}N_2O$ 385.2280; found 385.2274; Anal. Calcd for $C_{26}H_{28}N_2O$: C, 81.21; H, 7.34; N, 7.29. Found: C, 81.29; H, 7.30; N, 7.26.

4.1.1.20. 2-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)phenyl acetate (29). Compound **28** (1 equiv), acetic anhydride (2.5 equiv) and DMAP (0.02 equiv) in pyridine was stirred for 3 h at room temperature. The reaction mixture was diluted by ice-cold water, and extracted with EtOAc. The organic phase dried, filtered and purified by automated flash chromatography. Yield 97%; mp 113.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (6H, d, J = 5.6 Hz), 1.75 (1H, m), 1.79 (3H, d, J = 7.6 Hz), 2.21 (3H, s), 2.40 (2H, d, J = 7.2 Hz), 4.01 (1H, q, J = 7.2 Hz), 5.06 (2H, dd, J = 17.6 Hz and J = 17.6 Hz), 6.39 (1H, d, J = 7.6 Hz), 6.97 (1H, t, J = 8.0 Hz), 7.01 (2H, d, J = 8.0 Hz), 7.09 (2H, d, J = 8.0 Hz), 7.13–7.29 (5H, m), 7.89 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{28}H_{30}N_2O_2$ 427.2386; found 427.2379; Anal. Calcd for $C_{28}H_{30}N_2O_2$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.96; H, 7.20; N, 6.10.

4.1.1.21. 2-[1-(4-Isobutylphenyl)ethyl]-1-(2-methoxybenzyl)-1*H*-benzimidazole (30). Yield 78.2%; mp 129.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.4 Hz), 1.78 (1H, m), 1.81 (3H, d, J = 6.8 Hz), 2.38 (2H, d, J = 6.8 Hz), 3.86 (3H, s, OCH_3), 4.24 (1H, q, J = 7.0 Hz), 5.09–5.19 (2H, dd, J = 17.6 Hz and J = 17.2 Hz), 6.35 (1H, d, J = 6.8 Hz), 6.68 (1H, t), 6.86 (1H, d, J = 7.6 Hz), 6.98 (2H, d, J = 7.6 Hz), 7.09–7.25 (6H, m), 7.87 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{30}N_2O$ 399.2436; found 399.2433; Anal. Calcd for $C_{27}H_{30}N_2O$: C, 80.28; H, 7.66; N, 6.88. Found: C, 80.29; H, 7.49; N, 6.85.

4.1.1.22. 2-[1-(4-Isobutylphenyl)ethyl]-1-[2-(trifluoromethyl)benzyl]-1*H*-benzimidazole (31). Yield 90%; mp 112.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, dd, J = 3.6 Hz and J = 3.2 Hz), 1.72 (1H, m), 1.82 (3H, d, J = 7.2 Hz), 2.32 (2H, d, J = 6.8 Hz), 4.14 (1H, q, J = 7.2 Hz), 5.33–5.42 (2H, dd, J = 17.6 Hz and J = 17.2 Hz), 6.18 (1H, d, J = 7.6 Hz), 6.91 (2H, d, J = 8.0 Hz), 7.03–7.30 (7H, m), 7.66 (1H, d, J = 7.6 Hz), 7.92 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{27}F_3N_2$ 437.2205; found 437.2208; Anal. Calcd for $C_{27}H_{27}F_3N_2$: C, 74.29; H, 6.23; N, 6.42. Found: C, 74.41; H, 6.03; N, 6.61.

4.1.1.23. 1-(3-Fluorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (32). Yield 40%; mp 112.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.4 Hz), 1.75–1.83 (4H, m), 2.40 (2H, d, J = 7.2 Hz), 4.18 (1H, q, J = 7.2 Hz), 5.18 (2H, dd, J = 17.2 Hz and J = 16.8 Hz), 6.51 (1H, d, J = 9.6 Hz), 6.63 (1H, d, J = 8.0 Hz), 6.89 (1H, m), 7.01 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8.4 Hz), 7.12–7.29 (4H, m), 7.88 (1H, d, J = 7.6 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}FN_2$ 387.2237; found 387.2244; Anal. Calcd for $C_{26}H_{27}FN_2$: C, 80.80; H, 7.04; N, 7.25. Found: C, 80.91; H, 7.25; N, 7.17.

4.1.1.24. 3-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)benzonitrile (33). Yield 41.5%; mp 142.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, dd, J = 6.4 Hz and J = 6.4 Hz), 1.77 (1H, m), 1.84 (3H, d, J = 6.8 Hz), 2.37 (2H, d, J = 6.8 Hz), 4.17 (1H, q, J = 7.2 Hz), 5.08–5.25 (2H, dd, J = 17.2 Hz and J = 17.2 Hz), 6.97 (2H, d, J = 8.4 Hz), 7.03–7.31 (8H, m), 7.46 (1H, d, J = 8.0 Hz), 7.90 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{27}N_3$ 394.2283; found 394.2293; Anal. Calcd for $C_{27}H_{27}N_3$: C, 82.41; H, 6.92; N, 10.68. Found: C, 82.55; H, 7.23; N, 10.66.

4.1.1.25. Methyl 3-({2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)benzoate (34). Yield 81.1%; mp 87.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (6H, dd, J = 6.4 Hz and J = 6.4 Hz), 1.76 (1H, m,), 1.82 (3H, d, J = 7.2 Hz), 2.37 (2H, d, J = 6.8 Hz), 3.89 (3H, s), 4.19 (1H, q, J = 6.8 Hz), 5.08–5.25 (2H, dd, J = 16.8 Hz and J = 17.2 Hz), 6.85 (1H, d, J = 8.0 Hz), 6.99 (2H, d, J = 7.6 Hz), 7.06–7.29 (7H, m), 7.72 (1H, s), 7.89 (1H, m); HRMS (m/z): $[M+H]^+$ calcd for $C_{28}H_{30}N_2O_2$ 427.2386; found 427.2401; Anal. Calcd for $C_{28}H_{30}N_2O_2$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.92; H, 7.18; N, 6.49.

4.1.1.26. 1-(2,4-Dichlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (35). Yield 70%; mp 115.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, d, J = 6.8 Hz), 1.72 (1H, m), 1.84 (3H, d, J = 7.2 Hz), 2.34 (2H, d, J = 7.6 Hz), 4.16 (1H, q, J = 7.2 Hz), 5.17–5.22 (2H, dd, J = 18.0 Hz and J = 18.0 Hz), 6.00 (1H, d, J = 8.4 Hz), 6.82 (1H, dd, J = 8.4 Hz and J = 0.2 Hz), 6.91 (2H, d, J = 8.0 Hz), 7.04 (3H, m), 7.20 (1H, t, J = 8.0 Hz and J = 7.2 Hz), 7.29 (1H, t, J = 8.0 Hz and J = 7.6 Hz), 7.35 (1H, d, J = 0.2 Hz), 7.90 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{26}Cl_2N_2$ 437.1551; found 437.1533; Anal. Calcd for $C_{26}H_{26}Cl_2N_2$: C, 71.39; H, 5.99; N, 6.40. Found: C, 71.14; H, 5.85; N, 6.54

4.1.1.27. 1-(2,6-Dichlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (36). Yield 47%; mp 143.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (6H, d, J = 6.8 Hz), 1.81 (1H, m), 1.89 (3H, d, J = 6.8 Hz), 2.42 (2H, d, J = 6.8 Hz), 4.58 (1H, q, J = 7.2 Hz), 5.28 (2H, dd, J = 15.6 Hz and J = 15.2 Hz), 6.81 (1H, d, J = 8.0 Hz), 7.01–7.09 (5H, m), 7.15–7.21 (2H, m), 7.28–7.31 (2H, m), 7.82 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{26}Cl_2N_2$ 437.1551; found 437.1548; Anal. Calcd for $C_{26}H_{26}Cl_2N_2 \cdot 0.15H_2O$: C, 70.95; H, 6.02; N, 6.37. Found: C, 70.86; H, 5.90; N, 6.39.

4.1.1.28. 1-(3,4-Diethoxybenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (37). Yield 48.2%; mp 115.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.8 Hz), 1.35 (3H, t, J = 6.8 Hz), 1.41 (3H, t, J = 6.8 Hz), 1.81 (4H, m), 2.40 (2H, d, J = 7.6 Hz), 3.85 (2H, q, J = 6.8 Hz), 4.02 (2H, q, J = 6.8 Hz), 4.21 (1H, q, J = 6.8 Hz), 4.92–5.15 (2H, dd, J = 16.4 Hz and J = 16.4 Hz), 6.39 (2H, m), 6.71 (1H, d, J = 8.4 Hz), 7.03 (2H, d, J = 7.6 Hz), 7.11 (2H, d, J = 7.6 Hz), 7.15–7.27 (3H, m), 7.86 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{30}H_{36}N_2O_2$ 457.2855; found 457.2840; Anal. Calcd for $C_{30}H_{36}N_2O_2$: C, 78.91; H, 7.95; N, 6.13. Found: C, 78.67; H, 8.06; N, 6.14.

4.1.1.29. 2-[1-(4-Isobutylphenyl)ethyl]-1-(2,3,4-trimethoxybenzyl)-1*H*-benzimidazole (38). Yield 51%; mp 152.4 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.8 Hz), 1.78 (1H, m), 1.82 (3H, d, J = 7.2 Hz), 2.39 (2H, d, J = 7.6 Hz), 3.64 (6H, s), 3.79 (3H, s) 4.24 (1H, q, J = 7.2 Hz), 4.98–5.16 (2H, dd, J = 16.8 Hz and J = 16.8 Hz), 6.06 (2H, s), 7.09 (2H, d, J = 8.0 Hz), 7.13–7.28 (5H, m), 7.89 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{29}H_{34}N_2O_3$ 459.2648; found 459.2644; Anal. Calcd for $C_{29}H_{34}N_2O_3$: C, 75.95; H, 7.47; N, 6.11. Found: C, 75.81; H, 7.53; N, 6.20.

4.1.1.30. 2-[1-(4-Isobutylphenyl)ethyl]-1-(pyridin-2-ylmethyl)-1*H*-benzimidazole (39). Yield 54%; mp 100.2 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6.8 Hz), 1.73 (1H, m), 1.83 (3H, d, J = 6.8 Hz), 2.54 (2H, d, J = 7.2 Hz), 4.29 (1H, q, J = 7.2 Hz), 5.31 (2H, dd, J = 17.2 Hz and J = 17.6 Hz), 6.31 (1H, d, J = 7.6 Hz), 6.95 (2H, d, J = 8.4 Hz), 7.08–7.34 (7H, m), 7.88 (1H, d, J = 8.0 Hz), 8.53 (1H, d, J = 4.8 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{27}N_3$ 370.2283. found 370.2297; Anal. Calcd for $C_{25}H_{27}N_3$: C, 81.26; H, 7.37; N, 11.37. Found: C, 81.25; H, 7.25; N, 11.27.

4.1.1.31. 2-[1-(4-Isobutylphenyl)ethyl]-1-(pyridin-3-ylmethyl)-1*H*-benzimidazole (40). Yield 45%; mp 89.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (6H, d, J = 6.4 Hz), 1.78 (1H, m), 1.82 (3H, d, J = 7.2 Hz), 2.38 (2H, d, J = 6.8 Hz), 4.14 (1H, q, J = 7.2 Hz), 5.04–5.26 (2H, dd, J = 16.8 Hz and J = 16.8 Hz), 6.87–7.30 (9H, m), 7.89 (1H, d, J = 8.0 Hz), 8.36–8.45 (2H, m); HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{27}N_3$ 370.2283. found 370.2265; Anal. Calcd for $C_{25}H_{27}N_3$: C, 81.26; H, 7.37; N, 11.37. Found: C, 81.43; H, 7.32; N, 11.21.

4.1.1.32. 2-[1-(4-Isobutylphenyl)ethyl]-1-(pyridin-4-ylmethyl)-1*H*-benzimidazole (41). Yield 77.6%; mp 157.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6,4 Hz), 1.75 (1H, m), 1.83 (3H, d, J = 7.2 Hz), 2.37 (2H, d, J = 6.8 Hz), 4.14 (1H, q, J = 7.2 Hz), 5.01–5.24 (2H, dd, J = 17.6 Hz and J = 17,6 Hz), 6.71 (2H, d, J = 5.2 Hz), 6.98 (2H, d, J = 7.6 Hz), 7.05–7.31 (5H, m), 7.91 (1H, d, J = 8.0 Hz), 8.41 (2H, d, J = 6.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{27}N_3$ 370.2283. found 370.2274; Anal. Calcd for $C_{25}H_{27}N_3$: C, 81.26; H, 7.37; N, 11.37. Found: C, 81.56; H, 7.55; N, 11.08.

4.1.1.33. 2-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}methyl)quinoline (42). Yield 44.3%; mp 108.4 °C. ^{1}H NMR ($CDCl_3$): δ 0.78 (6H, dd, J = 6.8 Hz and J = 6.8 Hz), 1.68 (1H, m), 1.85 (3H, d, J = 7.2 Hz), 2.98 (2H, d, J = 7.6 Hz), 4.36 (1H, q, J = 8.8 Hz), 5.49 (2H, dd, J = 17.2 Hz and J = 17.2 Hz), 6.40 (1H, d, J = 8.4 Hz), 6.89 (2H, d, J = 8.0 Hz), 7.12 (2H, d, J = 8.0 Hz), 7.15–7.29 (3H, m), 7.53 (1H, t, J = 7.2 Hz), 7.70–7.79 (3H, m), 7.90 (1H, d, J = 8.0 Hz), 8.05 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{29}H_{29}N_3$ 420.2440. found 420.2458; Anal. Calcd for $C_{29}H_{29}N_3$: C, 83.02; H, 6.97; N, 10.02. Found: C, 83.26; H, 7.16; N, 9.99.

4.1.1.34. 1-(2-Chlorobenzoyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-1*H*-benzimidazole (43). Yield 52%; mp 93.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, d, J = 6.8 Hz), 1.77 (1H, m), 1.84 (3H, d, J = 7.2 Hz), 2.38 (2H, d, J = 7.2 Hz), 4.95 (1H, m), 6.37 (1H, m), 6.99–7.09 (5H, m), 7.25–7.29 (3H, m), 7.45–7.51 (2H, m), 7.81

(1H, d, J = 7.6 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{25}ClN_2O$ 417.1734. found 417.1723; Anal. Calcd for $C_{26}H_{25}ClN_2O$: C, 74.90; H, 6.04; N, 6.72. Found: C, 74.64; H, 6.17; N, 6.74.

4.1.1.35. 1-Benzoyl-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (44). Yield 75%; oil. ^{1}H NMR ($CDCl_3$): δ 0.72 (6H, dd, J = 6.4 Hz and J = 6.4 Hz), 1.66 (1H, m), 1.86 (3H, d, J = 7.2 Hz), 2.54 (2H, d, J = 7.2 Hz), 4.85 (1H, q, J = 7.2 Hz), 6.52 (1H, d, J = 8.0 Hz), 6.91 (2H, d, J = 8.4 Hz), 7.01–7.05 (3H, m), 7.25–7.41 (5H, m), 7.57 (1H, m), 7.84 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{26}N_2O$ 383.2123. found 383.2142; Anal. Calcd for $C_{26}H_{26}N_2O$: C, 81.64; H, 6.85; N, 7.32. Found: C, 81.11; H, 6.92; N, 7.42.

4.1.1.36. 1-(3-Chlorobenzoyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (45). Yield 85%; oil. ^{1}H NMR ($CDCl_3$): δ 0.73 (6H, dd, J = 6.4 Hz and J = 6.4 Hz), 1.68 (1H, m), 1.86 (3H, d, J = 6.8 Hz), 2.33 (2H, d, J = 7.6 Hz), 4.82 (1H, q, J = 7.0 Hz), 6.55 (1H, d, J = 8.4 Hz), 6.93 (2H, d, J = 8.0 Hz), 7.01 (2H, d, J = 8.4 Hz), 7.07 (1H, m), 7.22–7.30 (4H, m), 7.53 (1H, m), 7.84 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{25}ClN_2O$ 417.1734. found 417.1739; Anal. Calcd for $C_{26}H_{25}ClN_2O\bullet1.0H_2O$: C, 71.79; H, 6.25; N, 6.44. Found: C, 71.82; H, 6.20; N, 6.52.

4.1.1.37. 2-({2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazol-1-yl}carbonyl)phenyl acetate (46). Yield 91%; oil. ^{1}H NMR ($CDCl_3$): δ 0.78 (6H, d, J = 6.8 Hz), 1.72 (1H, m), 1.83 (3H, d, J = 7.2 Hz), 2.35 (2H, d, J = 7.6 Hz), 4.85 (1H, q, J = 7.0 Hz), 6.66 (1H, d, J = 8.0 Hz), 6.96 (2H, d, J = 8.0 Hz), 7.03–7.27 (6H, m), 7.57 (1H, t, J = 7.6 Hz), 7.80 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{28}H_{28}N_2O_3$ 441.2178. found 441.2173; Anal. Calcd for $C_{28}H_{28}N_2O_3$: C, 76.34; H, 6.41; N, 6.36. Found: C, 76.19; H, 6.65; N, 6.32.

4.1.1.38. Methyl 2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole-1-carboxylate (47). Yield 33%; mp 103.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (6H, d, J = 6.6 Hz), 1.81 (1H, m), 1.85 (3H, d, J = 6.8 Hz), 2.41 (2H, d, J = 7.2 Hz), 3.56 (3H, s), 4.24 (1H, q, J = 7.2 Hz), 4.65 (2H, dd, J = 18.0 Hz and J = 18.0 Hz), 7.04 (2H, d, J = 8.4 Hz), 7.09 (2H, d, J = 8.4 Hz), 7.14–7.30 (3H, m), 7.86 (1H, m); HRMS (m/z): $[M+H]^+$ calcd for $C_{22}H_{26}N_2O_2$ 351.2073. found 351.2063; Anal. Calcd for $C_{22}H_{26}N_2O_2$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.19; H, 7.62; N, 7.74.

4.1.1.39. 2-[1-(4-Isobutylphenyl)ethyl]-1*H*-benzimidazole-1-carboxylic acid (48). Yield 58.2%; mp 173.8 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, d, J = 6.8 Hz), 1.61 (3H, d, J = 6.8 Hz), 1.76 (1H, m), 2.34 (2H, d, J = 7.2 Hz), 4.39 (1H, q, J = 6.8 Hz), 4.63 (2H, dd, J = 17.6 Hz and J = 17.2 Hz), 6.98 (2H, d, J = 8.0 Hz), 7.05 (2H, d, J = *8.0* Hz), 7.21–7.29 (3H, m), 7.73 (1H, m); HRMS (m/z): $[M+H]^+$ calcd for $C_{21}H_{24}N_2O_2$ 337.1916. found 337.1916; Anal. Calcd for $C_{21}H_{24}N_2O_2$: C, 74.97; H, 7.19; N, 8.33. Found: C, 74.90; H, 7.04; N, 8.13.

4.1.1.40. 1-(2-Chlorobenzyl)-2-(4-isobutylbenzyl)-1*H*-benzimidazole (55). Yield 34%; mp 100.1 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6.8 Hz), 1.76 (1H, m), 2.36 (2H, d, J = 7.6 Hz), 4.21 (2H, s), 5.31 (2H, s), 6.24 (1H, d, J = 7.2 Hz), 6.92–7.26 (9H, m), 7.37 (1H, d, J = 8.0 Hz), 7.88 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{25}N_2Cl$ 389.1785. found 389.1797; Anal. Calcd for $C_{25}H_{25}N_2Cl$: C, 77.20; H, 6.48; N, 7.20. Found: C, 77.57; H, 6.59; N, 7.15.

4.1.1.41. 1-(2-Chlorobenzyl)-2-[1-(4-isobutylphenyl)-1-methylethyl]-1*H*-benzimidazole (56). Yield 70%; mp 164.2 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, d, J = 6.4 Hz), 1.76 (1H, m), 1.78 (6H, s), 2.37 (2H, d, J = 6.8 Hz), 4.90 (2H, s), 6.33 (1H, d, J = 8.0 Hz), 6.91 (1H, d, J = 8.4 Hz), 6.98 (3H, m), 7.08 (2H, d, J = 8.4 Hz), 7.13–7.30 (4H, m), 7.90 (1H, d, J = 7.6 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{29}N_2Cl$ 417.2098. found 417.2080; Anal. Calcd for $C_{27}H_{29}N_2Cl$: C, 77.77; H, 7.01; N, 6.72. Found: C, 77.90; H, 7.34; N, 6.64.

4.1.1.42. 1-(2-Chlorobenzyl)-2-[1-(4-methylphenyl)ethyl]-1*H*-benzimidazole (63). Yield 64%; mp 132.2 °C. ^{1}H NMR ($CDCl_3$): δ 1.81 (3H, d, J = 7.2 Hz), 2.23 (3H, s), 4.13 (1H, q, J = 7.0 Hz), 5.22 (2H, m), 6.18 (1H, d, J = 7.4 Hz), 6.92 (1H, t, J = 8.0 Hz), 6.97 (2H, d, J = 7.6 Hz), 7.06 (2H, d, J = 8.0 Hz), 7.09–7.37 (5H, m), 7.90 (1H, d, J = 7.8 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{23}H_{21}N_2Cl$ 361.1472. found 361.1472; Anal. Calcd for $C_{23}H_{21}N_2Cl$: C, 76.55; H, 5.87; N, 7.76. Found: C, 76.73; H, 5.79; N, 7.68.

4.1.1.43. (4-{1-[1-(2-Chlorobenzyl)-1*H*-benzimidazol-2-yl]ethyl}phenyl)(phenyl)methanone (64). Yield 56%; mp 147.3 °C. ^{1}H NMR ($CDCl_3$): δ 1.86 (3H, d, J = 7.2 Hz), 4.27 (1H, q, J = 7.2 Hz), 5.20–5.36 (2H, dd, J = 18.0 Hz and J = 18.0 Hz), 6.14 (1H, d, J = 8.0 Hz), 6.88 (1H, t, J = 7.6 Hz), 7.11–7.66 (14H, m), 7.89 (1H, d, J = 8.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{29}H_{23}N_2OCl$ 451.1577. found 451.1565; Anal. Calcd for $C_{29}H_{23}N_2OCl$: C, 77.24; H, 5.14; N, 6.21. Found: C, 77.04; H, 5.04; N, 6.08.

4.1.1.44. 1-(2-Chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-5-methoxy-1*H*-benzimidazole (70). Compound **69** (1 equiv) was refluxed in 25 mL acetic acid for 4 h. The reaction mixture was evaporated in vacuo, and dissolved in 50 mL DCM. The organic phase was extracted with 5% $NaHCO_3$ (50 mL × 2) and brine (50 mL x 2). Combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The oily residue was purified by automated flash chromatography. Yield 77%; oil. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, d, J = 6.8 Hz), 1.74 (1H, m), 1.83 (3H, d, J = 6.8 Hz), 2.35 (2H, d, J = 6.8 Hz), 3.87 (3H, s), 4.13 (1H, q, J = 8.8 Hz), 5.15–5.25 (2H, dd, J = 18.0 Hz and J = 18.0 Hz), 6.17 (1H, d, J = 8.0 Hz), 6.82–7.15 (8H, m), 7.35 (1H, m), 7.38 (1H, d, J = 2.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{29}N_2OCl$ 433.2047; found 433.2049; Anal. Calcd for $C_{27}H_{29}N_2OCl\bullet0.4H_2O$: C, 73.67; H, 6.82; N, 6.36. Found: C, 73.57; H, 7.12; N, 6.40.

4.1.1.45. 1-(2-Chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazol-5-ol (71). Compound **70** (1 equiv), 48% HBr (66 equiv) in 2 mL acetic acid was refluxed for 2 h. The reaction mixture was diluted by distilled water, extracted with EtOAc (60 mL × 2). The organic phase was dried over Na_2SO_4, filtered, evaporated in vacuo, and the final product was purified by automated flash chromatography. Yield 60.3%; mp 213.2 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, dd, J = 6.8 Hz and J = 6.4 Hz), 1.74 (1H, m), 1.80 (3H, d, J = 6.8 Hz), 2.23 (2H, d, J = 6.8 Hz), 4.13 (1H, m), 5.19 (2H, m), 6.20 (1H, d, J = 7.6 Hz), 6.89 (1H, m), 6.90 (2H, d, J = 8.4 Hz), 6.94 (2H, d, J = 8.0 Hz), 7.08 (2H, d, J = 8.4 Hz), 7.14 (1H, m), 7.34–7.39 (2H, m); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}ClN_2O$ 419.1890. Found 419.1878; Anal. Calcd for $C_{26}H_{27}ClN_2O$: C, 74.54; H, 6.50; N, 6.69. Found: C, 74.01; H, 6.58; N, 6.66.

4.1.1.46. 1-(2-Chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-5-(pyridin-2-ylmethoxy)-1*H*-benzimidazole (72). To a solution of compound **71** (1 equiv) in DMF was added Cs_2CO_3 (4 equiv) followed by 2-picolylchloride (1 equiv) and TBAI (0.06 equiv). The mixture was heated overnight under N_2 then diluted with water and extracted with EtOAc (3 × 50 ml). The organic phase was dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography provided the title compound. Yield 60%; mp 128.6 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, d, J = 6.6 Hz), 1.74 (1H, m), 1.79 (3H, d, J = 7.2 Hz), 2.34 (2H, d, J = 6.8 Hz), 4.12 (1H, q, J = 6.8 Hz), 5.20 (2H, m), 5.27 (2H, s), 6.19 (1H, d, J = 7.4 Hz),

6.89–6.99 (5H, m), 7.07 (2H, d, J = 8.0 Hz), 7.13–7.22 (2H, m), 7.35 (1H, d, J = 7.6 Hz), 7.45 (1H, d, J = 8.0 Hz), 7.57 (1H, d, J = 7.6 Hz), 7.71 (1H, m), 8.62 (1H, d, J = 5.6 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{32}H_{32}ClN_3O$ 510.2312; found 510.2307; Anal. Calcd for $C_{26}H_{27}ClN_2O$: C, 75.35; H, 6.32; N, 8.24. Found: C, 75.35; H, 6.65; N, 8.09.

4.1.1.47. 2-[({1-(2-Chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazol-5-yl}oxy)methyl]quinoline (73). The title compound was synthesized by the same procedure for **72** starting from 2-(chloromethyl)quinoline. Yield 57%; mp 86.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (6H, dd, J = 6.8 Hz and J = 6.8 Hz), 1.74–1.79 (4H, m), 2.34 (2H, d, J = 7.2 Hz), 4.11 (1H, q, J = 7.0 Hz), 5.20 (2H, m), 5.45 (2H, s), 6.19 (1H, d, J = 7.2 Hz), 6.89–6.7.15 (8H, m), 7.35 (1H, d, J = 7.6 Hz), 7.48–7.55 (2H, m), 7.74 (2H, m), 7.82 (1H, d, J = 8.4 Hz), 8.11 (1H, d, J = 8.4 Hz), 8.20 (1H, d, J = 8.4 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{36}H_{34}ClN_3O$ 560.2469. Found 560.2476; Anal. Calcd for $C_{26}H_{27}ClN_2O\bullet0.7H_2O$: C, 75.49; H, 6.22; N, 7.33. Found: C, 75.32; H, 6.07; N, 7.15.

4.1.1.48. 1-Benzyl-5-chloro-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (81). The title compound was synthesized by the same procedure for **70** starting from compound **79**. Yield 52%; oil. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, d, J = 6.8 Hz), 1.78–1.81 (4H, m), 2.40 (2H, d, J = 7.6 Hz), 4.17 (1H, q, J = 7.6 Hz), 4.99–5.20 (2H, dd, J = 16.8 Hz and J = 16.8 Hz), 6.82–7.26 (11H, m), 7.84 (1H, d, J = 2.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}N_2Cl$ 403.1941. found 403.1937; Anal. Calcd for $C_{26}H_{27}N_2Cl$: C, 77.50; H, 6.75; N, 6.95. Found: C, 77.54; H, 6.76; N, 6.86.

4.1.1.49. 5-Chloro-1-(2-chlorobenzyl)-2-[1-(4-isobutylphenyl)ethyl]-1*H*-benzimidazole (82). The title compound was synthesized by the same procedure for **70** starting from compound **80**. Yield 35%; mp 107.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, dd, J = 2.0 Hz and J = 2.4 Hz), 1.74 (1H, m), 1.79 (3H, d, J = 6.8 Hz), 2.34 (2H, d, J = 7.2 Hz), 4.15 (1H, q, J = 6.8 Hz), 5.21 (2H, m), 6.12 (1H, d, J = 8.0 Hz), 6.89–7.15 (8H, m), 7.35 (1H, d, J = 8.0 Hz), 7.85 (1H, d, J = 2.0 Hz); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{26}N_2Cl_2$ 437.1551; found 437.1562; Anal. Calcd for $C_{26}H_{26}N_2Cl_2$: C, 71.39; H, 5.99; N, 6.40. Found: C, 77.71; H, 6.03; N, 6.59.

4.2. Molecular modelling studies

4.2.1. Computational approach

The energy calculation, pharmacophore search and docking studies for virtual screening were performed using the MOE 2010.10 suite (Chemical Computing Group, CCG). The 3D structures were generated in the protonated state assuming physiological conditions and energy minimized using MOE to a RMSD gradient of 0.05 kcal/mol Å with MMFF94× forcefield. Partial charges were automatically assigned. The molecular docking program GOLD 5.0.1 (Cambridge Crystallographic Data Centre, CCDC) was also used to confirm the binding modes of the synthesized compounds. Ligand receptor interactions were analyzed using the Ligand Interaction module available in MOE and visualizations were performed using Pymol (DeLano Scientific).

4.2.2. Pharmacophore model

The alignment was produced using a slightly relaxed representation of the X-ray ligand with MOE Align. For those known FLAP inhibitors containing chiral centers with undetermined stereochemistry, all possible isomers were enumerated and the collection of the lowest energy conformers aligned onto X-ray ligand pose. The minimization protocol applied decreasing flat bottom constraints to retain as much of the original geometry as possible while removing artificial strain. To generate the pharmacophore queries, the Ph4 consensus module of MOE, which calculates a consensus query consisting of most conserved features of a given alignment, was used. The final query was adjusted manually to optimize the hit rate of true positives. A volume constraint was added to further constrain the search.

4.2.3. Virtual screening

A presampled collection of 2.8 mio vendor compounds (153 mio conformations) was obtained from CCG libraries. The 3D search with MOE was done using the pharmacophore model described above. Only compounds that matched all the features of the query were retained as hits.

4.2.4. Postprocessing filtering

The hits retrieved from the chemical databases were filtered using molecular docking and protein-ligand interaction fingerprints (PLIFs) using MOE. The FLAP crystal structure in complex with compound **85** (PDB code: 2Q7M) was used and the chains B and C were selected for docking studies. For the first docking step, pharmacophore placement (six-point matching while the excluded volume is ignored) and Alpha HB were used as scoring functions. Then, the raw poses obtained from the first step were submitted to the second step in which FF refinement (receptor flexible, tether weight = 6) and rescoring function Affinity dG were used.

GOLD 5.0.1 was also used to confirm binding modes of the synthesized compounds. The active site was defined with 10 Å radius around the bound inhibitor **85**. Top 10 scored conformation of every ligand was allowed to be saved at the end of the calculation. The early termination option was used to skip the genetic optimization calculation when any five conformations of a particular compound predicted within the RMSD value of 1.5 Å. Gold score was tested as fitness function. The docking poses for each compound were stored in a MOE database and further processed.

The interaction fingerprint of a protein-ligand complex was calculated by the presence or absence of six types of intermolecular interactions: hydrogen bond with side chain donor, hydrogen bond with side chain acceptor, hydrogen bond with backbone donor, hydrogen bond with backbone acceptor, ionic attraction, and surface contact. The resulting fingerprints were extracted from MOE for the analysis.

4.3. Assay systems

4.3.1. Materials

Arachidonic acid, Ca^{2+} ionophore A23187, and all other fine chemicals were from Sigma (Deisenhofen, Germany), unless stated otherwise. HPLC solvents were from Merck (Darmstadt, Germany).

4.3.2. Cells

Human neutrophils were freshly isolated from leukocyte concentrates obtained at the Blood Center of the University Hospital Tuebingen (Germany) or 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 4,000×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.[30] Cells were finally resuspended in phosphate-buffered saline pH 7.4 (PBS) containing 1 mg/mL glucose and 1 mM $CaCl_2$ (PGC buffer).

4.3.3. Determination of 5-LO product formation in cell-based assays

For assays of intact cells, 5×10^6 freshly isolated neutrophils were resuspended in 1 mL PGC buffer. After preincubation with

the compounds for 15 min at 37 °C, 5-LO product formation was started by addition of 2.5 μM A23187. Treatment with 2.5 μM A23187 for 10 min appeared not toxic for neutrophils as determined by trypan blue staining and light microscopy. After 10 min at 37 °C, the reaction was stopped with 1 mL of methanol and 30 μL of 1 N HCl, 200 ng prostaglandin B_1 and 500 μL of PBS were added. Formed 5-LO metabolites were extracted and analyzed by HPLC as described.[30] 5-LO product formation is expressed as ng of 5-LO products per 10^6 cells, which includes LTB_4 and its all-trans isomers, and 5(*S*)-hydro(pero)xy-6-*trans*-8,11,14-cis-eicosatetraenoic acid (5-H(p)ETE). Cysteinyl-$LTsC_4$, D_4 and E_4 were not detected (amounts were below detection limit), and oxidation products of LTB_4 were not determined.

4.3.4. Expression and preparation of human recombinant 5-LO from *E. coli* and determination of 5-LO activity in cell-free systems

E. coli BL21 was transformed with pT3–5LO plasmid, recombinant 5-LO protein was expressed at 37 °C, and 40,000×*g* supernatants were prepared as described.[31] For determination of 5-LO activity in neutrophil homogenates, 5×10^6 freshly isolated neutrophils were resuspended in 1 mL of PBS containing 1 mM EDTA and sonicated (3 × 10 s). Aliquots of the *E. coli* lysate supernatants (corresponding to 2 mL *E. coli* cell culture) were diluted with PBS/EDTA plus 1 mM ATP to 1 mL and pre-incubated with the test compounds. After 5–10 min at 4 °C, samples were pre-warmed for 30 s at 37 °C, and 2 mM $CaCl_2$ plus 20 μM AA were added to start 5-LO product formation. The reaction was stopped after 10 min at 37 °C by addition of 1 mL ice-cold methanol and the formed metabolites (the two all-trans isomers of LTB_4 and 5-H(p)ETE) were analyzed by HPLC as described for intact cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.04.048.

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