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Indole derivatives as potent inhibitors of 5-lipoxygenase: Design, synthesis, biological evaluation, and molecular modeling

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Abstract—A series of novel indole derivatives was designed, synthesized and evaluated by cell-based assays for their inhibitory activities against 5-LOX in rat peritoneal leukocytes. Most of them (30 out of 35) showed an inhibitory potency higher than the initial screening hit **1a** (IC_{50} = 74 μM). Selected compounds for concentration–response studies showed prominent inhibitory activities with IC_{50} values ranging from 0.74 μM to 3.17 μM. Four compounds (**1m**, **1s**, **4a**, and **6a**) exhibited the most potent inhibitory activity compared to that of the reference drug (Zileuton), with IC_{50} values less than 1 μM. Molecular modeling studies for compounds **1a**, **3a**, **4a**, and **6a** were also presented. The excellent in vitro activities of this class of compounds may possess potential for the treatment of LT-related diseases.

5-Lipoxygenase (5-LOX) is the key enzyme in the biosynthesis of leukotrienes (LTs) through catalyzing the initial two steps in conversion of arachidonic acid to LTs.[1] LTB_4 and the cysteinyl-leukotrienes are potent constrictors of human airways, and have powerful pro-inflammatory properties.[2] Inhibition of 5-LOX may lead to the development of new therapeutic treatments for pathologies such as asthma, allergies, and other inflammatory disorders.[1,3,4] Recent studies have implicated a role of 5-LOX products involved in a number of other diseases, including cancer,[5] atherosclerosis,[6] stroke,[7] and osteoporosis.[8] Therapeutic potential of 5-LOX inhibition has been widely highlighted in recent years.[9–13]

Currently, a huge number of different types of small molecular inhibitors of 5-LOX have been described in the literature, classified depending on their mechanism of action. Three different types of inhibitors have been considered: redox inhibitors, iron chelators, and non-redox inhibitors of 5-LOX.[3] Zileuton (Fig. 1) was the first marketed 5-LOX inhibitor, with an iron chelating mechanism, used in the chronic treatment of asthma.[14] Although numerous efforts have been done toward 5-LOX in the past two decades, and there is a strong need for efficient drugs targeting 5-LOX pathway, no other 5-LOX inhibitor is available on the market currently.[8] Therefore, it is a great challenge to discover and develop novel efficient compounds as 5-LOX inhibitors, without disadvantages of former inhibitors.

Compounds with the indole core have been widely studied due to their capability of binding to many receptors with high affinity,[15] and a few indole derivatives have been reported for their inhibitory activities against 5-LOX.[3] Recently, Landwehr et al. reported a series of 2-amino-5-hydroxyindole derivatives as inhibitors of human 5-LOX.[16] By screening abundant substituted indole compounds of our in-house collection, 4-(1-benzyl-1*H*-indol-3-yl)-1-morpholinobutan-1-one (**1a**, Fig. 1) was identified, with an IC_{50} value of 74 μM, for inhibiting 5-LOX in the cell-based assay. Using the screening hit

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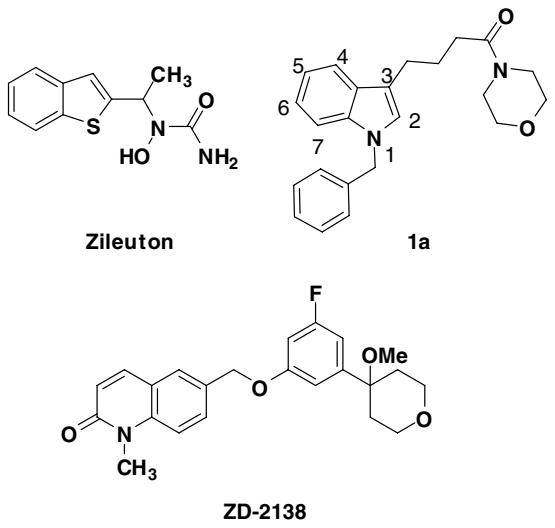


Figure 1. Representative structures of 5-LOX inhibitors and the screening hit **1a**.

1a as the benchmark compound, a series of novel indole derivatives (**1b**–**t**, **3a**–**e**, and **4**–**11**, Table 1) was designed, synthesized, and evaluated by the cell-based assay for their inhibitory activities against 5-LOX in rat peritoneal leukocytes. Molecular modeling studies were also carried out to investigate their binding interactions with 5-LOX.

Using different aryl groups substituted at the N1 position of **1a**, we synthesized compounds **1b**–**e**. Derivatives **1f**–**o** were designed by changing the length of the alkyl linkers between the indole core and the carbonyl moiety, using methylene or ethylene displacing the propylene linker of **1a**–**e**. Substituting the 2,5-positions of the indole core of **1a**–**e** with methyl, methoxy or chloro substituents, respectively, analogues **1p**–**t** were obtained. Compounds **3a**–**e** are the precursors of compounds **1** with no substitution at the N1 position. Using the Boc-piperazine, piperazine or 1-methanesulfonylpiperazine instead of the morpholino units of **1a**, **1p**, **3a**, and **3d**, we prepared analogues **4**–**7** (Table 1). Compound **8** is the reduction product of **1a**. The methoxytetrahydropyran moiety of ZD-2138 (Fig. 1) was a frequently reported pharmacophore of 5-LOX devoid of redox and iron-chelating properties.[17] Compounds **9**–**11** (Scheme 2) were designed and synthesized by introducing the non-redox 5-LOX pharmacophore to the 3-position of the indole core.

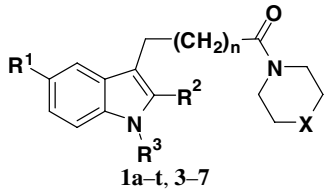
The synthesis of this class of indole derivatives is outlined in Schemes 1 and 2. Condensation of the appropriately substituted indole-3-carboxyl acid **2a**–**e** [18] with morpholine or Boc-piperazine afforded derivatives **3a**–**e**, and **4a**–**b**, which were subsequently reacted with various benzyl or benzoyl chlorides, respectively, in the presence of NaH in DMF, to produce derivatives **1b**–**t** and **5a**–**b**. Deprotection of **5a**–**b** using 50% TFA in dichloromethane afforded **6a**–**b**. Treatment of **6a** with mesyl chloride in pyridine gave the derivative **7**. Reduction the carbonyl of **1a** to a methylene by $LiAlH_4$ in THF afforded the derivative **8**.

Treatment of the commercially available tryptophol with mesyl chloride (MsCl) in CH_2Cl_2 formed 2-(1*H*-indol-3-yl)ethyl methanesulfonate, which was subsequently reacted with 3-fluoro-5-(4-methoxytetrahydro-2*H*-pyran-4-yl)phenol [19] in the presence of Cs_2CO_3 in DMF to produce the methoxytetrahydropyran derivative **9**. Compound **9** was converted to the *N*-benzyl product **10** by reacting with BnBr in the presence of NaH in DMF. Compound **11** was synthesized using indole-3-propyl carboxyl acid (**2d**) as the starting material. Acid **2d** was esterified in methanol in the presence of H_2SO_4 to afford the methyl ester **12**. *N*-benzylation of the free indole nitrogen of **12** with BnBr in the presence of NaH in DMF produced compound **13**, which was then reduced by $LiAlH_4$ in THF to give the indole-3-propyl alcohol intermediate **14**. The hydroxyl of **14** was then activated by MsCl and reacted with 3-fluoro-5-(4-methoxytetrahydro-2*H*-pyran-4-yl)phenol in the presence of Cs_2CO_3 in DMF to form the target product **11**.

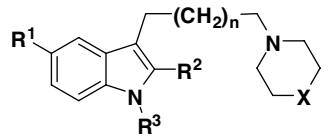
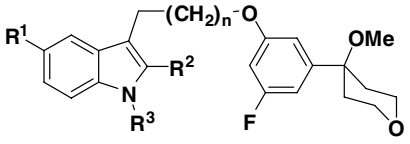
All derivatives (**1b**–**t**, **3a**–**e**, and **4**–**11**, Table 1) were evaluated by the cell-based assay for their inhibitory activities against 5-LOX in rat peritoneal leukocytes,[20] using Zileuton as the reference drug. As shown in Table 1, all the compounds showed modest to potent inhibitory activities against 5-LOX at 5 μM, with the inhibition range from 15.9% (**3c**) to 95.4% (**6a**). Most of them (30 out of 35) had inhibitory activities higher than that of the initial lead **1a** (25.8% inhibition at 5 μM; IC_{50} = 74 μM). Little change in activity was observed by introducing methyl (**1b**) or chloro (**1c**) to the benzyl of **1a**. While, replacing the benzyl substituent with benzoyl (**1d**), the activity was improved to 58.2% at 5 μM. When the alkyl linker between the indole core and the carbonyl moiety was changed from propylene to ethylene or methylene, the substituents' effects at the N1 position of **1a** were not in accordance with the derivatives with propylene linker derivatives (**1a**–**e**). The *N*1-benzyl derivative (**1f**) showed the most potent inhibitory activity among the compounds with ethylene linker (**1f**–**j**), with 63.8% inhibition at 5 μM. However, the *N*1-3,4-dichlorobenzyl derivative (**1m**) exhibited the most potent activity (81.9% inhibition at 5 μM) among the compounds with a methylene linker (**1k**–**o**) in the cell-based assay. Introducing methoxy or chloro substituents to the C5 position of the indole core (**1p**–**t**) also showed positive results. *p*-Chloro benzoyl substituent at the N1 position (**1r**) was more preferred than the corresponding benzyl substituted ones (**1p**–**q**) by the 5-methoxy indole core, with **1r** exhibiting 70.2% inhibition at 5 μM. On the contrary, when the 5-methoxy indole core was changed to the 5-chloro indole core, the *N*1-benzyl substituted derivative (**1s**, 80.2% inhibition at 5 μM) was more potent than the *p*-chloro benzoyl substituted one (**1t**, 37.9% inhibition at 5 μM). Results of N1 unsubstituted derivatives **3a**–**e** suggested that the inhibitory activity was affected greatly by the length of the alkyl linker between the indole core and the carbonyl moiety, and the propylene linker was preferred, regardless of the substituents in C5 position (CH_3O, Cl) of the indole.

An important advance in activity was achieved (95% inhibition at 5 μM) when the morpholino unit at the

Table 1. Structures and inhibitory activities against 5-LOX in rat peritoneal leukocytes of indole derivatives

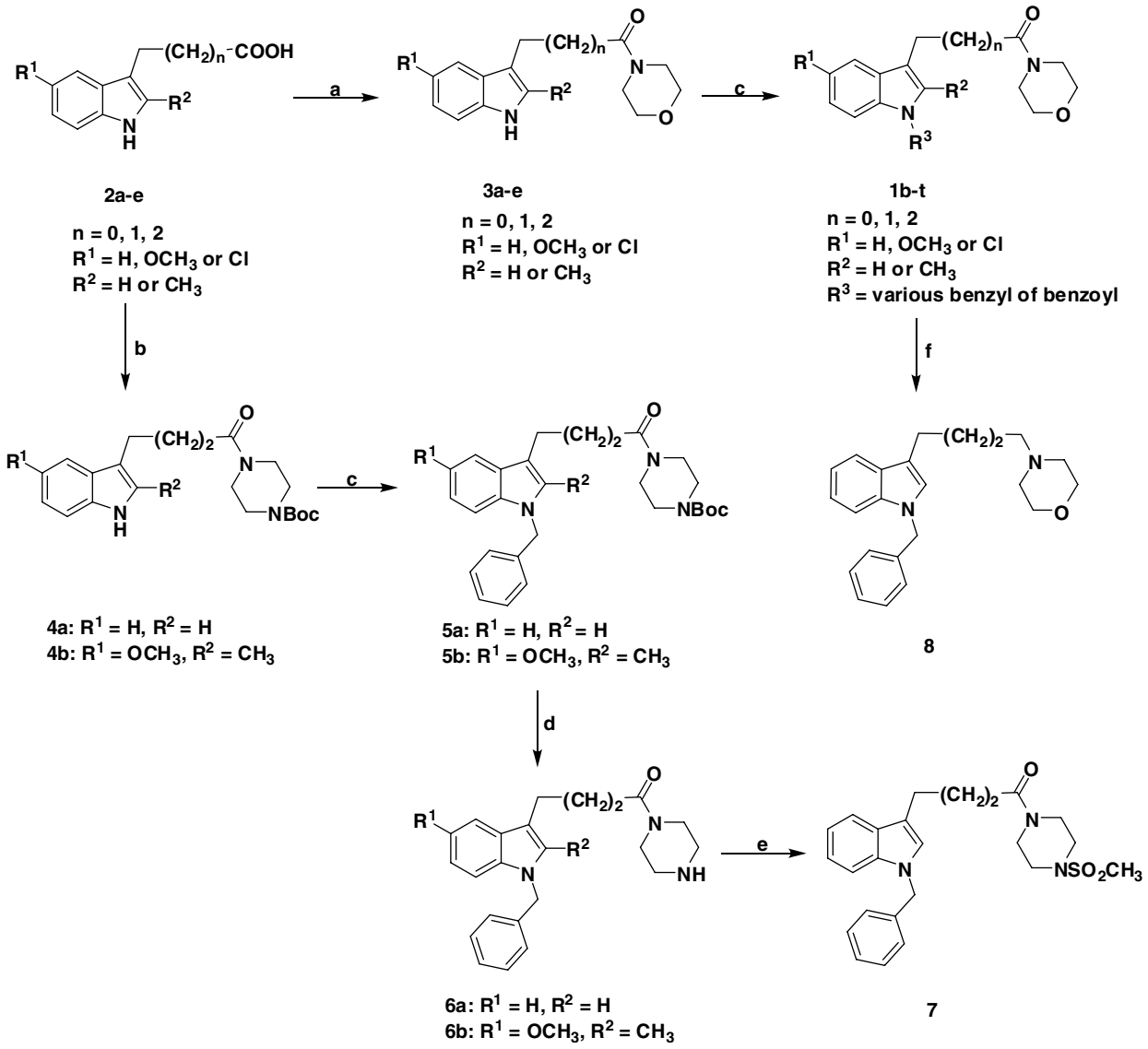


1a–t, 3–7

Compound	R^1	R^2	R^3	X	*n*	%Inhibition at 5 μM[a]
1a	H	H	Benzyl	O	2	25.8
1b	H	H	*p*-Methylbenzyl	O	2	33.9
1c	H	H	3,4-Dichlorobenzyl	O	2	26.7
1d	H	H	Benzoyl	O	2	58.2
1e	H	H	*p*-Chlorobenzoyl	O	2	32.4
1f	H	H	Benzyl	O	1	63.8
1g	H	H	Benzoyl	O	1	23.4
1h	H	H	*p*-Methylbenzyl	O	1	55.2
1i	H	H	3,4-Dichlorobenzyl	O	1	38.5
1j	H	H	*p*-Chlorobenzoyl	O	1	26.0
1k	H	H	Benzyl	O	0	41.4
1l	H	H	Benzoyl	O	0	21.0
1m	H	H	3,4-Dichlorobenzyl	O	0	81.9
1n	H	H	*p*-Methylbenzyl	O	0	34.6
1o	H	H	*p*-Chlorobenzoyl	O	0	22.4
1p	OCH_3	CH_3	Benzyl	O	2	54.2
1q	OCH_3	CH_3	*p*-Methylbenzyl	O	2	26.4
1r	OCH_3	CH_3	*p*-Chlorobenzoyl	O	2	70.2
1s	Cl	CH_3	Benzyl	O	2	80.2
1t	Cl	CH_3	*p*-Chlorobenzoyl	O	2	37.9
3a	H	H	H	O	2	62.3
3b	H	H	H	O	1	33.0
3c	H	H	H	O	0	15.9
3d	OCH_3	CH_3	H	O	2	72.9
3e	Cl	CH_3	H	O	2	71.3
4a	H	H	H	BocN	2	84.1
4b	OCH_3	CH_3	H	BocN	2	71.2
5a	H	H	Benzyl	BocN	2	38.2
5b	OCH_3	CH_3	Benzyl	BocN	2	51.5
6a	H	H	Benzyl	NH	2	95.4
6b	OCH_3	CH_3	Benzyl	NH	2	64.6
7	H	H	Benzyl	MsN	2	23.9
						
8	H	H	Benzyl	O	2	41.6
						
9	H	H	H		1	64.2
10	H	H	Benzyl		1	31.8
11	OCH_3	CH_3	Benzyl		2	46.5
Zileuton						86.9

[a] Values are means of three determinations and deviation from the mean is <10% of the mean value (Catalog No. 520111, Cayman Chemicals Inc.).

C3 position of **1a** was replaced by a piperazine (**6a**). Its N1 unsubstituted precursor **4a** also exhibited outstanding activity, with 84.1% inhibition at 5 μM. Mesylation of **6a** led to a tremendous decrease in ability against 5-LOX product synthesis, as shown by **7** (23.9% inhibition at 5 μM). Little improvement in activity was observed with the reduction of carbonyl in **1a** to a methylene (**8**). When the methoxytetrahydropyran

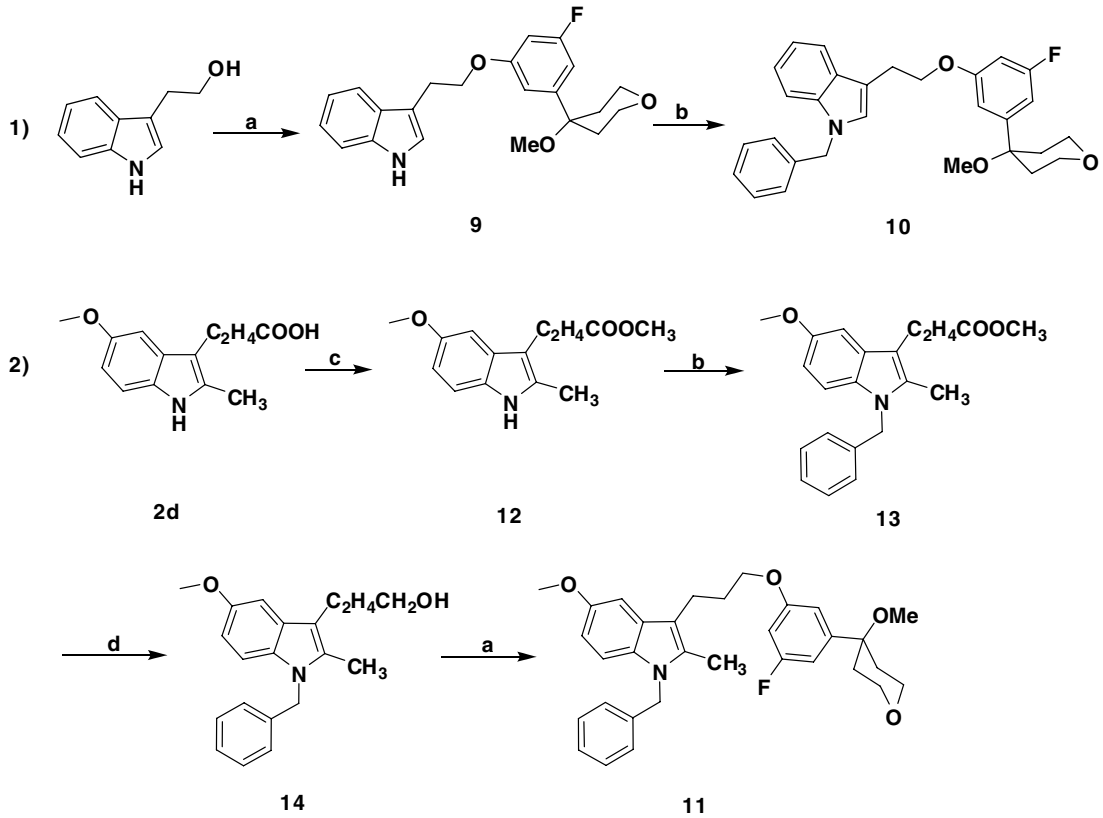


Scheme 1. Synthetic routes of indole derivatives **1b**–**t** and **3**–**8**. Reagents and conditions: (a) EDCI, DMAP, morpholine, DMF; (b) Boc-piperazine, EDCI, DMAP, DMF; (c) NaH, R^3Cl, DMF; (d) TFA, CH_2Cl_2; (e) MsCl, Py; (f) $LiAlH_4$, THF.

pharmacophore of 5-LOX was introduced to the C3 position (**9-11**), their activities were improved in comparison with **1a**. Among them, compound **9**, which had no substitution at the N1 position, was the most potent one, with 64.2% inhibition at 5 μM. So far, it seems that the N1 unsubstituted case was presumed to exhibit good inhibitory activities.

To determine the exact potency of the compounds that demonstrated significant inhibitory activities against 5-LOX at 5 μM, 10 compounds were selected (**1f**, **1m**, **1r**–**s**, **3a**, **3d**–**e**, **4a**, **6a**, and **9**) for further investigation in concentration–response studies, and the results are summarized in Table 2. All these compounds concentration-dependently inhibited 5-LOX product synthesis and showed prominent inhibitory activities with IC_{50} values ranging from 0.74 μM to 3.17 μM, which were 20 to 100 times more active than the initial screening hit **1a** (IC_{50} = 74 μM). Derivatives **1m**, **1s**, **4a**, and **6a** showed excellent inhibitory activities (IC_{50}s less than 1 μM) comparable to that of Zileuton (0.83 μM), which was in accordance with the previous report.[21]

Molecular modeling experiments were carried out to investigate the binding interactions between this series of compounds and the active site of 5-LOX. As described in our previous report,[22] 3D-model of 5-LOX was generated by homology modeling, based on rabit reticulocyte 15-LOX (PDB entry 1LOX at 2.40 Å resolution), which were found with sequence similarities of 40.68% to the target protein 5-LOX by using the BLAST software.[23] The missing atoms of residues 210, 211, 601, 602, and 177–187 of 15-LOX in its crystal structure were added with the Loop/Search module of InsightII,[24] followed by a loop relaxing. Then 10 of 3D models of 5-LOX were generated based on the coordinates of 15-LOX using MODELLER,[25] and all these 3D structures were optimized with conjugate gradient minimization scheme followed by a restrained simulated



Scheme 2. Synthetic routes of methoxytetrahydropyran attached indole derivatives **9**–**11**. Reagents and conditions: (a) (1) MsCl, CH_2Cl_2; (2) 3-fluoro-5-(4-methoxytetrahydro-2*H*-pyran-4-yl)phenol, Cs_2CO_3, DMF; (b) NaH, BnBr, DMF; (c) MeOH, H_2SO_4, reflux; (d) $LiAlH_4$, THF.

Table 2. Determination of IC_{50} values of selected compounds[a]

Compound	IC_{50} (μM)
1a	74.0
1f	3.17
1m	0.87
1r	2.25
1s	0.95
3a	2.06
3d	3.16
3e	1.33
4a	0.74
6a	0.85
9	2.38
Zileuton	0.83

[a] Values are means of three determinations and deviation from the mean is <10% of the mean value (Catalog No. 520111, Cayman Chemicals Inc.).

annealing molecular dynamics simulation. The model with the lowest value of the objective function was selected as a representative model for further study. More details of model equilibration by molecular dynamics have been described in our previous report.[22]

In accordance with a recent report,[26] the 5-LOX active site of our 3D-model consists of a deep bent-shaped cleft containing the non-heme iron cofactor. The bottom of the substrate binding pocket is defined by the side chains of Phe359, L420, Ala424, Asn425, and Ala603, and the wall of the binding channel is mainly lined with hydrophobic residues, except for Asn554 and four iron ligand residues around the iron center. Molecular modeling studies between the 5-LOX and compounds **1a**, **3a**, **4a**, and **6a** were carried out by using AutoDock 3.0.3 program,[27,28] and the docking parameters in our previous study [29] were adopted. For each compound, twenty possible binding conformations were generated, and the conformation with the lowest predicted binding free energy of the most occurring binding modes in 5-LOX active pocket was selected for further analysis. Figure 2 demonstrates the results of their binding modes.

Two different conformations have been found for the four compounds in the 5-LOX active pocket. As shown in Figure 2(A), compounds **1a** and **6a**, which have benzyl substitution at the N1 position, adopt an extended conformation, with the long subunit at C3 position extending deep into the cleft, in close proximity to Asn425. Whereas, a folded conformation shown in Figure 2(B) is taken by the *N*1-unsubstituted derivatives **3a** and **4a**, which fit well in the central hydrophobic part of the 5-LOX active site with their long subunits at C3 position bend back to the indole rings. The indole ring of compounds **1a** and **6a** is positioned in the middle of the cavity, interacting strongly with Leu368 and Leu607, and the phenyl group points to the entrance of the active pocket, having strong interactions with Ala410. The protonized NH of **6a** undergoes a strong hydrogen bond with $O^{\delta 1}$ of Asn425 (distance = 2.14 Å). This may contribute to the better IC_{50} value of **6a** (0.85 μM) than the corresponding morpholino derivative **1a**

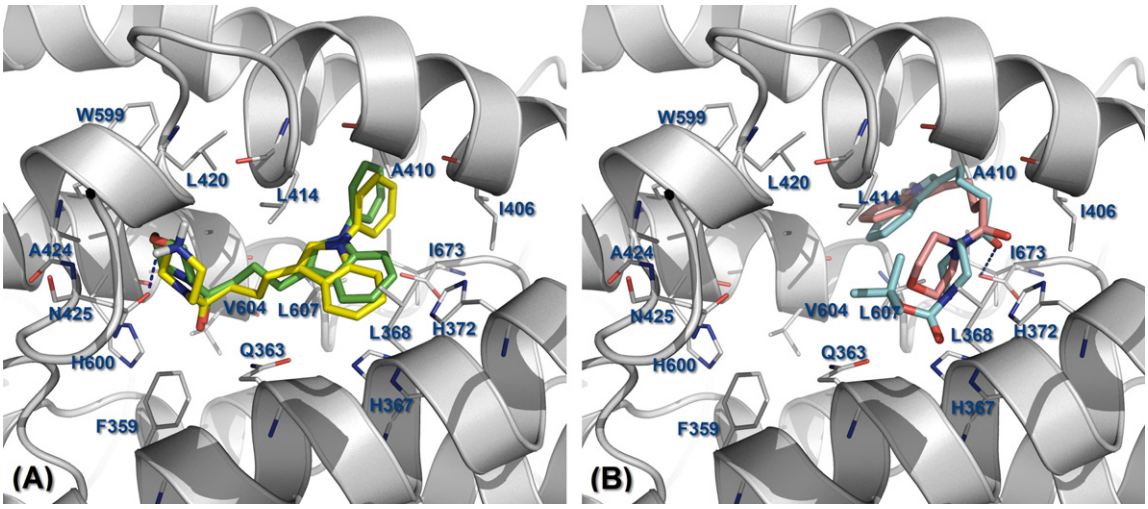


Figure 2. Binding models of compounds **1a**, **3a**, **4a** and **6a** in the binding cleft of 5-lipoxygenase via homology modeling. The left part (A) describes the binding models of compounds **1a** (green) and **6a** (yellow). The right part (B) shows the binding models of compounds **3a** (pink) and **4a** (blue).

(IC_{50} = 74 μM). However, in the folded conformation of compounds **3a** and **4a**, the indole rings are closely adjacent to Leu414, forming strong hydrophobic interactions with the isobutyl side chain of Leu414, and the alkyl linkers interact with the Ala410. The better inhibitory activity of **4a** (IC_{50} = 0.74 μM), in comparison with **3a** (IC_{50} = 2.06 μM), might be ascribed to the favorable hydrogen bond formed between the oxygen atom of the carbonyl of **4a** with the hydroxyl of the carboxyl of Ile 673 (distance = 2.61 Å). Additionally, the Boc part of **4a** also increases hydrophobic interactions between **4a** and the binding site of 5-LOX. It was noteworthy that the results of our docking studies were in agreement with the experimental data quite well, which validated the efficiency of our homology model of human 5-LOX.

In summary, a series of novel indole derivatives was designed and synthesized based on the initial hit **1a**. All the compounds were evaluated by the cell-based assay for their inhibitory ability against 5-LOX in rat peritoneal leukocytes. Most of the compounds (30 out of 35) exhibited inhibitory potency higher than that of compound **1a**. Selected compounds for concentration–response studies showed prominent inhibitory activities with IC_{50} values ranging from 0.74 μM to 3.17 μM, which were 20 to 100 times more active than **1a** (IC_{50} = 74 μM). Compounds **1m**, **1s**, **4a**, and **6a** showed potency comparable to that of Zileuton, with IC_{50} values less than 1 μM. Molecular modeling studies for compounds **1a**, **3a**, **4a**, and **6a** were in agreement with the experimental data quite well. The excellent in vitro activities of this class of compounds may possess potential for the treatment of LT-related diseases. Further studies are in progress.

Acknowledgments

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