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Recent development of lipoxygenase inhibitors as anti-inflammatory agents

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Inflammation is favorable in most cases, because it is a kind of body defensive response to external stimuli; sometimes, inflammation is also harmful, such as attacks on the body's own tissues. It could be that inflammation is a unified process of injury and resistance to injury. Inflammation brings extreme pain to patients, showing symptoms of rubor, swelling, fever, pain and dysfunction. As the specific mechanism is not clear yet, the current anti-inflammatory agents are given priority for relieving suffering of patients. Thus it is emergent to find new anti-inflammatory agents with rapid effect. Lipoxygenase (LOX) is a kind of rate-limiting enzyme in the process of arachidonic acid metabolism into leukotriene (LT) which mediates the occurrence of inflammation. The inhibition of LOX can reduce LT, thereby producing an anti-inflammatory effect. In this review, the LOX inhibitors reported in recent years are summarized, and, in particular, their activities, structure–activity relationships and molecular docking studies are emphasized, which will provide new ideas to design novel LOX inhibitors.

Introduction

Inflammation remains a major public health problem throughout the world in hospital and community settings. It plays a central role in cardiovascular disease and inflammatory conditions caused by a variety of factors, including autoimmunity.[1] Also, chronic and persistent inflammation can contribute to the development of cancer, and genetic damage can ignite the flame of cancer. The relationships among inflammation, innate immunity, and cancer are widely accepted in academia.[2]

Lipoxygenase (LOX) can catalyze fatty acid to produce a number of active metabolic products that are involved in a lot of vital diseases. For instance, type 1 and type 2 diabetes (or both), cardiovascular diseases, hypertension, renal diseases, and neurological conditions such as Alzheimer's disease and Parkinson's disease.[3] In particular, LOX is a kind of rate-limiting enzyme in the process of arachidonic acid (AA) metabolism into leukotriene (LT) which mediates the occurrence of inflammation. For example, blockade of LT production may result in reducing the pro-inflammatory cell populations induced and recruited, as well as ameliorating the negative effects of inflammation.[4,5] LOXs are mainly divided into four types of 5-LOX, 8-LOX, 12-LOX and 15-LOX, according to their ability to insert oxygen atoms into the relevant position of AA. Moreover, 5-LOX, 12-LOX and 15-LOX are related to specific disease conditions, such as asthma, atherosclerosis, and even cancer.[4,6] Therefore, it is critical to develop potential selective LOX inhibitors for treatment of such diseases. Additionally, in the synthesis of LT, the 5-LOX activating protein (FLAP) selectively transfers AA to 5-LOX and accelerates the synthesis of leukotriene epoxide LTA_4, which further produces a series of pro-inflammatory products.[7] FLAP selectively affects the activity of 5-LOX without effects on other LOXs. Thus there are two main strategies of blocking LT production and inhibiting 5-LOX and FLAP.

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Moreover, LOXs also occur in plants,[8] animals,[9] and specific bacteria.[10]

In this review, we summarize the LOX and FLAP inhibitors that have recently been discovered over the last 5 years, and in particular, their inhibitory potency, structure–activity relationships (SARs), and molecular docking studies are emphasized.

2. Structure and function of LOX

2.1 Structure of LOX

All subtypes of LOXs have a single polypeptide chain, which is folded into two major domains: C-terminal catalytic domain and N-terminal β-barrel domain.[11] Human LOXs are metalloenzymes of 60–100 kDa, ranging from 662 to 675 amino acid residues and sharing 35–80% sequential identity.[12,13] Their active site has a catalytic metal (high-spin ferrous and ferric ions) and an acyl chain, surrounded by the protein shell. In the C-terminal catalytic domain, there is a deep hydrophobic pocket, into which the substrate can be docked.[12] Especially, the C-terminal catalytic domain of human 5-LOX has a special lysine-rich region that may lead to instability. Practically, a particular –KKK– sequence of 5-LOX inclines it to non-turnover-based deactivation, which could be the reason why it is unstable inherently. Most LOXs possess a highly conserved Leu655 residue, while the unique feature of 5-LOX is Lys substituting for Leu at the position as part of a di- or tri-Lys peptide.[14] In terms of the amino acid sequence of LOXs, we compared human 5-LOX and rabbit 15-LOX to find several similar sequences such as –W^{354}VRSSDFHVH^{363}– in human 5-LOX and –W^{347}VRSSDFQVH^{356}– in rabbit 15-LOX, –H^{368}LLRTHL^{374}– in human 5-LOX and –H^{361}LLRGHL^{367}– in rabbit 15-LOX, –T^{428}GGGGHVQ^{435}– in human 5-LOX and –T^{421}GGGGHVQ^{428}– in rabbit 15-LOX, –P^{566}NAPPTMRAPPPTAK^{580}– in human 5-LOX and –P^{547}NAPCTMRLPPPTTK^{561}–, *etc.*[15] In particular, the mutation or deletion of His361, His366, His541 and highly conserved C-terminal isoleucine can lead to the abolishment of activity.[16,17] Moreover, the Phe353, Met419, Ile418 and Ile593 residues make up the binding pocket of LOX.[18]

2.2 Function of LOX

LOXs are a type of non-heme iron enzyme which dominate the metabolism of unsaturated fatty acids by different regiospecificities and stereospecificities.[6,19] The three main functions of LOXs are oxidization of substrates, transformation of hydroperoxy lipids and synthesis of LTs.[20] For instance, they catalyze oxygen atoms to insert into (1*Z*,4*Z*)-pentadiene sections of polyunsaturated fatty acids, giving the homologous (1*S*,2*E*,4*Z*)-hydroperoxides. LTs generated by 5-LOX are triggers of inflammation, which produce activated, sticky and migratory leukocytes, thereby leading to an increase of vascular permeability, and bronchi and vessel constriction.[21,22] On the other hand, when a mouse lacks 12*R*-LOX, its embryonic development will be normal. However, it will die from impairment of the epidermal lipid barrier once born.[23] For human beings, this may lead to ichthyosis, an epidermis skin disease.[24] In addition, the release of proteins from the organelle lumen and access of proteases to both lumenal and integral membrane proteins result in integration of 15-LOX and cell membrane.[25] More importantly, LOXs play a critical role in metabolism of AA into LTs which mediate inflammatory reactions, such as the disorder of respiratory, gastrointestinal and dermatological systems.[26] Besides, it is reported that pancreatic, breast and prostate cancers are linked to 12-LOX in platelets,[27–29] while colorectal and prostate cancers are related to 15-LOX in reticulocyte.[30,31] The most studied 5-LOX exerts an essential role in the production of LTs.[32] Therefore, LOXs are accepted as promising targets, which can be used for the development of LOX inhibitors for the treatment of relevant diseases such as inflammation, cancer, asthma, *etc.*

3. LOX inhibitors

In the past five years and more, many potent LOX inhibitors have been investigated. In order to facilitate their introduction, according to the mechanisms of action, these inhibitors were classified into direct LOX inhibitors and indirect FLAP inhibitors, and further subdivision was carried out according to the sources of these compounds, *e.g.*, modifications based on known structures, high throughput screening (HTS), virtual screening and rational design.

3.1 Inhibitors obtained by the modification of previously known structures

3.1.1 Indole derivatives. Based on the compounds **MK-886** (FLAP inhibitor, Fig. 1) and **MK-0591** (LT inhibitor) that have entered clinical studies, and the already listed drug **zileuton** (5-LOX inhibitor) with similar benzothiophene structure, scholars found that indole was a unique biological structure for drug research. Unfortunately, **MK-886** and **MK-0591** were terminated in phase II clinical studies. According to the three compounds mentioned above, compound **1** was synthesized by Singh *et al.* in 2013 and showed a higher potency with an IC_{50} value of 0.6 μM against 5-LOX than the reference drug **zileuton** (IC_{50} = 3.7 μM).[33] Mechanistic study indicated that the stoichiometry was 1 : 7 in the enzyme–compound complex. Molecular docking study showed that several hydrogen bonds were formed between oxygen of the carbonyl group and the F177 and Q413 residues of the 5-LOX active site. In 2014, Singh found more effective compounds **2a** and **2b**, with IC_{50} values of 0.0097 and 0.0086 μM, respectively, and without effect on cell viability.[34] In practice, compound **2a** was a methyl ester of compound **2b**. In molecular docking study, the methyl ester group and nitrogen atom of indole of compound **2a** were bound to Q554 and Q557 by H-bond interactions, while oxygen atom of the sulfonyl group, nitrogen atom of the indole, oxygen atom of the amide and carboxyl moieties of compound **2b** formed hydrogen bonds with Q554, Q557, V604 and N554, respectively. As a continuation of previous work, Singh combined indole and chromone groups to

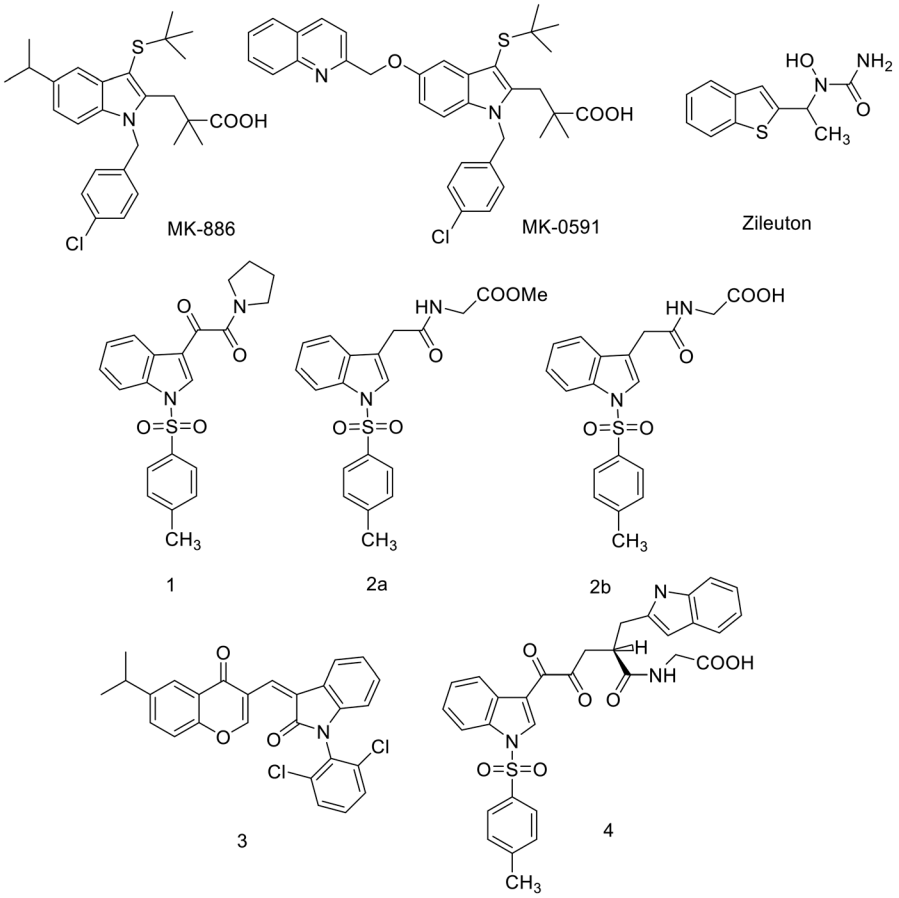


Fig. 1 Structures of compounds **MK-886**, **MK-0591**, **zileuton**, **1**, **2**, **3** and **4**.

design and synthesize compound **3** with an IC_{50} value of 0.02 μM against 5-LOX.[35] In another study of Singh in 2015 in which the number of indole derivatives had been expanded, compound **4**, an *S*-isomer, was found to display an amazing IC_{50} value of 0.002 μM.[36] However, its enantiomer exhibited a decreased potency of nearly 3000 times (IC_{50} = 5.61 μM). Molecular docking study revealed that this compound interacted with Phe177 and Asn554 by hydrogen bonds in the active site of 5-LOX. Compound **4** is highly likely to enter clinical studies due to its high activity and nontoxicity to animals.

In 2010, based on the structure of **tenidap** (Fig. 2), by replacing the thiophene group with the aminosulfonylphenyl or methylsulfonylphenyl group, known pharmacophoric groups, Lai *et al.* designed and synthesized a series of 3-[4-(amino/methylsulfonyl)phenyl]methylene-indolin-2-one derivatives, in which compound **5** was found to have a balanced

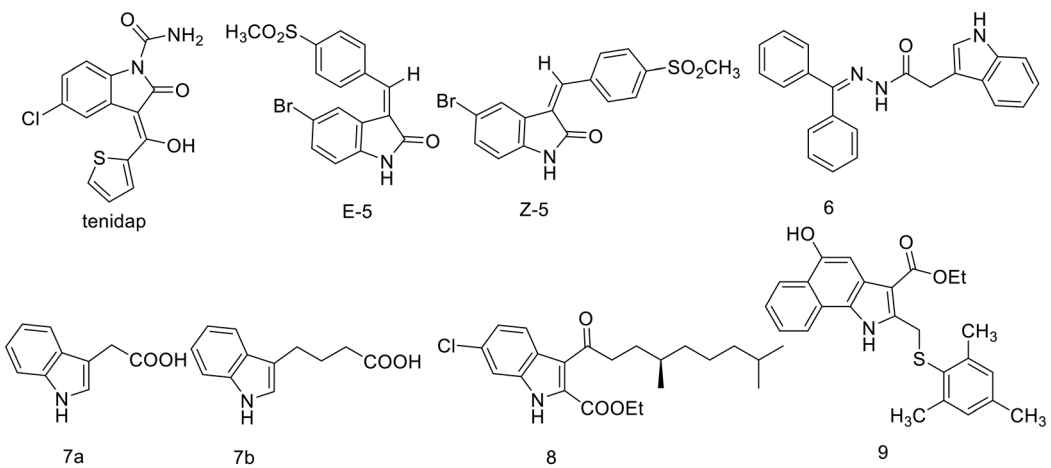


Fig. 2 Structures of compounds **tenidap**, **5**, **6**, **7**, **8** and **9**.

anti-inflammatory activity against both COX and LOX with IC_{50} values of 0.1 and 0.56 μM, respectively.[37] Practically, compound **5** was a mixture of *Z*-isomer and *E*-isomer. A SAR study indicated that if the bromine on the indole ring was replaced by a hydrogen, chlorine, fluorine, methyl or methoxy group, the inhibitory potency would be reduced by more than 100 times.

In 2014, Yar *et al.* designed and synthesized another series of (diphenylmethylene)-2-(1*H*-indole-3-yl)acetohydrazide derivatives, based on the indole acetic acid scaffold. In particular, compound **6** was found to show potent activity against LOX (IC_{50} = 53.61 μM).[38] Molecular docking study showed that the nitrogen atom of the indole group formed a hydrogen bond with LEU258. Additionally, the nitrogen atoms of the hydrazide formed hydrogen bonds with VAL256 and GLY265. In thermogravimetric analysis, compound **6** lost more than 80% of weight in the region of 450 °C.

Indoleacetic acid (IAA, compound **7a**) and indolebutyric acid (IBA, compound **7b**) were employed as LOX inhibitors by Dileep *et al.* in 2014 due to their activity with IC_{50} values of 42.98 and 17.82 μM, respectively.[39] Molecular docking study indicated that IAA and IBA competitively inhibited LOX, which suggested that they could have a similar inhibition mechanism against LOX. The carboxyl group of IAA and IBA interacted with Fe^{2+} ion in the active site of LOX. Besides, IAA and IBA also interacted with LOX by van der Waals force with constants of 69 and 80, respectively. In comparison, IBA had better binding affinity for LOX and showed higher potency than IAA.

In 2015, Dekker *et al.* obtained a series of novel anti-LOX inhibitors with high potency through acylating the 3-position of the indole ring, which were designed based on substitution oriented screening and SAR study. Among them, the isomers of chiral compound **8** possessed different activities. Its *S*-isomer (IC_{50} = 0.09 μM) had 3-fold higher activity against LOX than its *R*-isomer.[40] In molecular docking study, the indole, the ester carbonyl and the carbonyl groups could bind with Glu357, Gln548 and ferrous iron atom in the active site, respectively.

In 2016, in order to get more effective agents against LOX, Filosa *et al.* further modified the indole structure. Compound **9** was afforded by annexation of benzene and indole, which showed a satisfactory IC_{50} value of 0.2 μM.[41] In a carrageenan-induced mouse paw edema experiment, this compound performed well in reducing the inflammatory reactions. Systematical modifications at the *meta*-, *ortho*- or *ortho*/*para*-position of 2-phenylthiomethyl moiety in its structure indicated that 2,6-dichloro and 3,5-dichloro derivatives exhibited higher potency in inhibiting 5-LOX activity than 2,3-dichloro and 3,4-dichloro derivatives.

3.1.2 Coumarin derivatives. Compound **10** (Fig. 3) is a kind of coumarin derivative, with an inhibition of 34.75% at a concentration of 12.7 μM against 15-LOX.[42] In molecular docking study, its bond energy at the bonding site with 15-LOX and 5-LOX was −0.19 and 3.11 kcal mol^{-1}, respectively, which indicated that this compound has more affinity for human 5-LOX than 15-LOX. This phenomenon could result from the size of the catalytic cavity of 5-LOX being bigger than that of 15-LOX.[18] On account of the anti-inflammatory activity of coumarin derivatives, compound **11** was found by Roussaki *et al.* in 2014 to have an IC_{50} value against LOX of 65 μM. In the SAR study, the bromine atom at the 6-position and the methyl group at the 4-position were critical to the potency of LOX inhibition.[43] Another study conducted by Kumar *et al.* proved that 7,8-disubstituted-4-methylthiocoumarins also showed outstanding anti-inflammatory potency.[44] The above works indicated that 4-methylthiocoumarin could be a lead compound for the design of novel anti-inflammatory agents. In 2016, compounds **12a** and **12b**, types of 7-subsituted coumarin derivatives, were identified by Ghate *et al.* Their IC_{50} values against LOX were 2.8 and 2.1 μM, respectively.[45] The mechanism of **12b** against LOX could be either non-competitive inhibition or both non-competitive and competitive inhibition. Their structural difference was that **12a** has a chlorine atom at the C6-position of benzothiazole while **12b** has a methoxy group. Additionally, di-substituted benzothiazole ring would lead to a lower activity than mono-substituted one.

3.1.3 α,β-Unsaturated carbonyl compounds

Curcumin is isolated from turmeric, which is a traditional medicine from China and India. In 2011, according to the structure of curcumin, in a study conducted by Hadjipavlou-

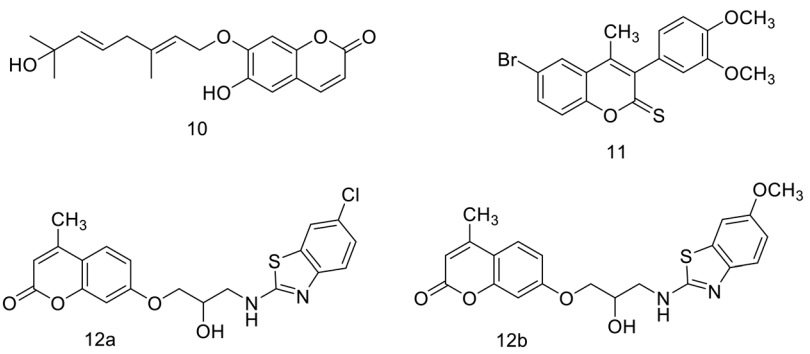


Fig. 3 Structures of compounds **10**, **11** and **12**.

Litina *et al.*, some α,β-unsaturated carbonyl compounds were synthesized and proved to have considerable potency against LOX. For example, compound **13** (Fig. 4) not only exhibited activity against LOX with an IC_{50} value of 37 μM, but also displayed a significant inhibitory activity against the growth of NCI-H460 (non-small cell lung cancer), MCF7 (breast cancer) and SF268 (central nervous system, glioma) cancer cell lines.[46] Additionally, the SAR study indicated that changing the naphthyl group to phenyl, thienyl, or indolyl group would decrease the inhibitory potency of LOX. In 2014, Bukhari *et al.* found that compound **14**, possessing an IC_{50} value of 29.28 μM against LOX, acted in a dose-dependent manner.[47] In molecular docking study, this compound could be placed in the binding cavity of LOX, forming several polar and hydrophobic interactions, and one of the nitro groups pointed to the iron cofactor. Therefore, the above compounds were regard as a fresh point to design new anti-inflammatory agents or to improve existing anti-inflammatory agents.

3.1.4 Triazole derivatives. In 2014, in the process of the structural modification of **tepoxalin**, a group of diaryl-1,2,4-triazole derivatives were synthesized and evaluated for their activity against 5-LOX by Wu *et al.* at China Pharmaceutical University. In this series, compound **15** (Fig. 5) presented ideal inhibitory activity with an IC_{50} value of 0.85 μM against 5-LOX.[48] Also, this compound revealed optimal anti-inflammatory activity with 54.1% inhibition at a dose of 30 mg kg^{-1} in xylene-induced ear edema of mice assay, which was slightly better than reference drug **celecoxib** with 46.7% inhibition activity. In albumen-induced rat paw edema and acetic acid-induced mouse vascular permeability experiments, compound **15** still exerted a high anti-inflammatory activity, equivalent to **celecoxib**. In 2015, as a continuation of previous work, the number of diaryl-1,2,4-triazole derivatives were expanded. Among them, compound **16** showed the most potent activity with an IC_{50} value of 0.71 μM against 5-LOX.[49] Surprisingly, this compound was also found to induce cell apoptosis and stagnation of cell G2/M phase in lung cancer A549. In another study conducted by Pelcman *et al.* in the same year, compound **17**, another triazole derivative, was selected as a clinical candidate. Unfortunately, this compound exhibited some non-target-associated effects in toxicological studies using mini-pigs, so that researchers had to stop in-depth study.[50]

3.1.5 Others. In 2012, Hansen *et al.* synthesized and evaluated some 3-hydroxybenzo[*b*]thiophene-2-carboxylic acid derivatives, which were designed based on the structure of **zileuton**. Among them, compounds **18a** and **18b** (Fig. 6) were found to show a significant activity against 5-LOX with IC_{50} values 0.97 and 0.51 μM, respectively. In particular, **18b** exhibited a higher activity than the reference drug **licofelone**.[51] In terms of the potency against COX-1, however, **18b** was weaker than **18a** with IC_{50} values of 0.41 and 2.5 μM, respectively. From the aspect of structural comparison, the only difference between the compounds was that **18a** had a chlorine substituent at the 6-position while **18b** possessed the chlorine substituent at the 7-position. Also, the isoxazolo[4,5-*d*]pyridazine derivatives had been proved to possess anti-inflammatory activity.[52,53] On the basis of this kind of structure, Velázquez-Martínez *et al.* designed, synthesized and evaluated a series of isoxazolo[4,5-*d*]pyridazin-4-(5*H*)-one derivatives for their anti-inflammatory activity. In this series, compound **19** exerted the best activity with an IC_{50} value of 6.3 μM against 5-LOX.[54] On the other hand, since this compound exhibited highest binding affinity in the active sites of both COX-2 and 5-LOX, it was also considered as a dual inhibitor with fairly good activity against COX-2. However, its potency against 5-LOX was 5- to 7-fold weaker than that of **zileuton**. The SAR study indicated that the methoxy group of compound **19** replaced by a nitro or hydrogen group would lead to decreased potency against 5-LOX. In another study, Wang *et al.* reported a 2- to 3-step semi-synthetic route for various kinds of azaphilone derivatives. Particularly, the azaphilone intermediate was excessively produced by *Aspergillus nidulans* engineered strain, which provided an easy synthetic route for the analogues of natural product. Azaphilone derivatives **20a** and **20b** were found to exhibit the best activity against 1-LOX, the IC_{50} values of which were 4.9 and 3.2 μM, respectively.[55]

In 2013, a dual COX/5-LOX inhibitor that was designed and synthesized based on the structure of di-*tert*-butyl phenol, such as **darbufelone** (Fig. 7), entered phase III clinical studies for the treatment of rheumatoid arthritis. Compound **21** was synthesized by Ghatak *et al.*, with an IC_{50} value of 5.1 μM against 5-LOX.[56] In a SAR study, the replacement of the

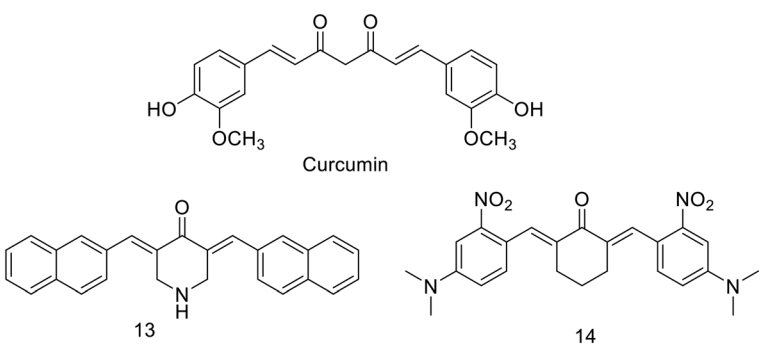


Fig. 4 Structures of compounds **curcumin**, **13** and **14**.

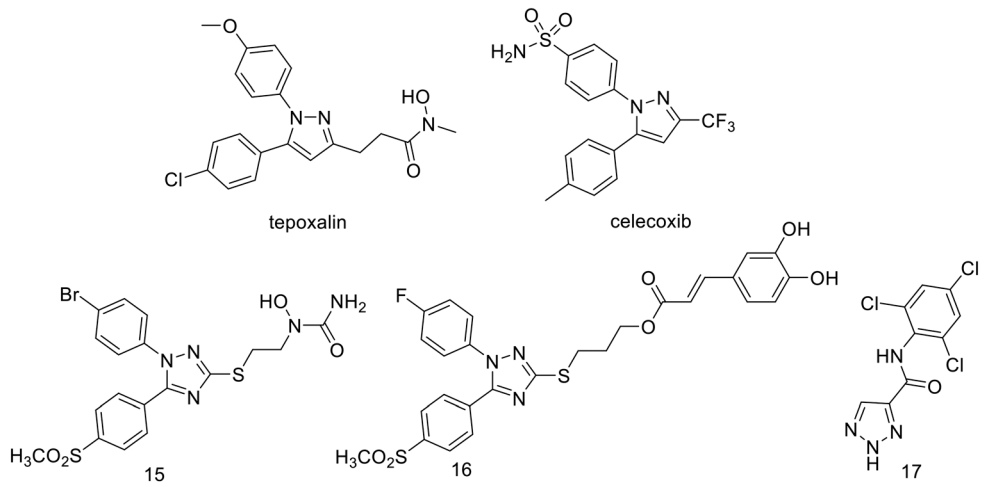


Fig. 5 Structures of compounds **tepoxalin**, **celecoxib**, **15**, **16** and **17**.

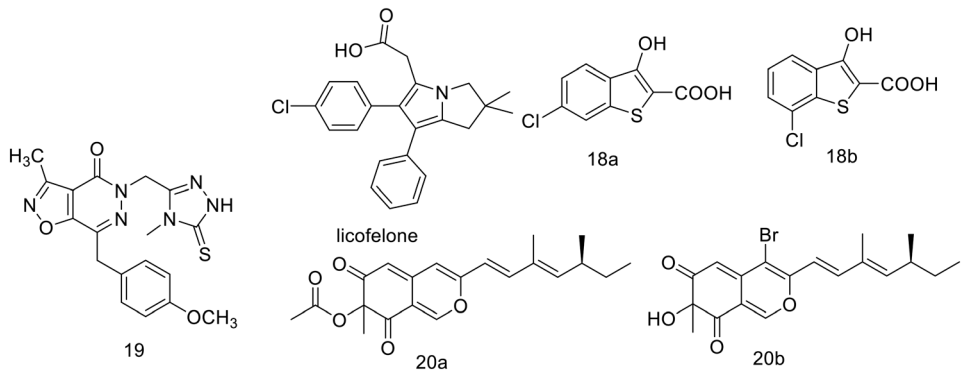


Fig. 6 Structures of compounds **licofelone**, **18**, **19** and **20**.

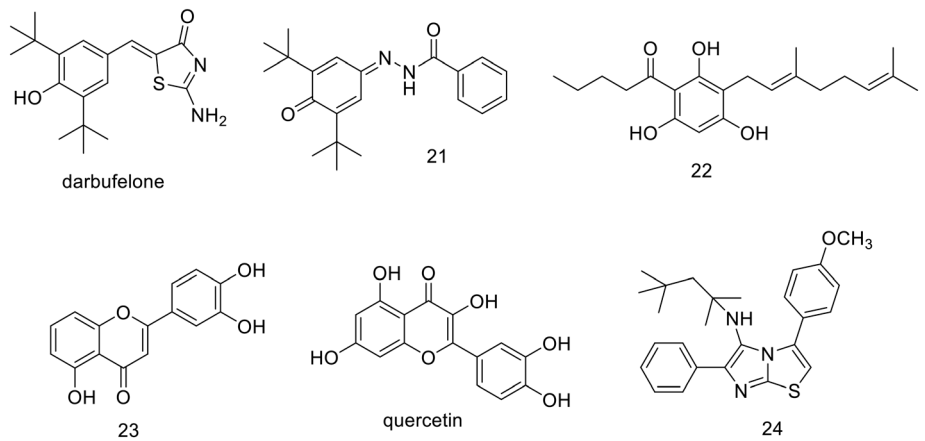


Fig. 7 Structures of compounds **darbufelone**, **21**, **22**, **23**, **quercetin** and **24**.

phenyl group by a pyridyl group would lead to a decrease in the inhibitory potency.

In 2014, the natural compound 2,4,6-trihydroxy-3-geranylacetophenone (tHGA) was shown to exhibit LOX inhibitory activity, it being isolated from *Melicope ptelefolia.* Shaari *et al.* synthesized a set of tHGA derivatives by using simple Friedel-Crafts acylation and alkylation reactions, in which compound 22 was found to show the highest potency against 15-LOX with an IC_{50} value of 10.32 μM,[57] two-fold better than that of the parent molecule tHGA that was similar to that of **zileuton** in an acute murine asthma model. In molecular docking experiment, this compound showed an optimal

binding interaction energy compared to other tHGAs, which was attributed to the strong hydrogen bond interactions between this compound and the amino acid residues in the active site of LOX. This study suggested that tHGA could be a parent molecule for the design of novel anti-inflammatory agents. In another study, conducted by Fernandes *et al.*, compound **23**, a type of flavonoid, had an inhibition constant against LOX and IC_{50} value against LTB4 produced by human neutrophils of 18 and 2.9 μM, respectively, better than the reference drug **quercetin** with values of 106.7 and 4.0 μM, respectively.[58] In Shafiee's study, a series of 3,6-diphenylimidazo[2,1-*b*]thiazol-5-amine analogues as inhibitors against 15-LOX were synthesized and evaluated.[59] Compound **24** was found to be the best one in this series with an IC_{50} value of 11.5 μM. Moreover, it could allow PC12 (rat neurons) cells to resist H_2O_2-induced cell death at a concentration of less than 10 μM. In molecular docking study, its main interaction forms were the CH-π interaction between the methoxyphenyl moiety and Phe576, the hydrophobic interaction between the aliphatic substituent and the hydrophobic pocket, the π-cation interaction between the phenyl ring and the catalytic Fe^{3+} in the active site, and the π–π interaction between the phenyl ring and His523. In the SAR study, on changing the methoxy group to other groups such as hydrogen or bromine group, the inhibitory potency for 15-LOX would decrease. Besides, introduction of some additional groups such as methoxy, nitryl or halogen group on another phenyl group would also lead to a decrease in the potency.

In 2015, compound **25** (Fig. 8) as a COX/5-LOX inhibitor was designed and synthesized based on the structure of licofelone, which exhibited stronger binding affinity for 5-LOX than other pyrrolizine derivatives.[60] Its anti-inflammatory potency was 1.24 times higher than that of the reference drug ibuprofen. On the other hand, in an acute ulcerogenicity study, compound **25** was tested at a dose of 0.48 mmol kg^{-1} by oral administration. The result showed that it was safer than ibuprofen. In a histopathological study, as expected, it did not show any damages in transverse sections of rat stomach while ibuprofen was found to show some damages. Hence, this compound should be a very promising candidate as a novel anti-inflammatory agent. In the same year, Dekker *et al.* investigated a series of 6-benzyloxysalicylate analogues.[61] Among them, compound **26** was proved to be a competitive inhibitor of 15-LOX with an IC_{50} value of 7.1 μM, while its *S*-isomer had 6-fold less activity than its *R*-isomer. Molecular docking study indicated that the interaction of the carbonyl group of its structure and Arg403 in the active site involved hydrogen bonding. In addition, in the active site of 5-LOX, a competitive inhibition relationship between *S*-isomer and *R*-isomer was exhibited as well.

In 2016, on the basis of four 2-arylbenzo[*b*]furan derivatives from *Artocarpus heterophyllus* exacted at East China University, a series of 2-arylbenzo[*b*]furan derivatives were designed, synthesized and evaluated for their activity against LOX. In this series, compound **27a** was found to exhibit the best potency against 12-LOX (IC_{50} = 0.21 μM), better than compound **27b** (IC_{50} = 1.3 μM).[62] On the other hand, in terms of the potency against 5-LOX and 15-LOX, compound **27a** (IC_{50} = 2.35 and 1.21 μM) was weaker than **27b** (IC_{50} = 1.67 and 0.84 μM). The difference in their structures is the position of a double bond. The SAR study showed that the non-substituted hydroxyl groups were indispensable for LOX inhibition activity, especially for 12-LOX inhibition activity. In another study, a series of 1,5-diarylpyrazole analogues were designed, synthesized and evaluated for their potency against LOX by Abdelall *et al.* In this series, compound **28** showed the highest potency against 15-LOX with an IC_{50} value of 3.98 μM, better than the reference **meclofenamate sodium** (IC_{50} = 5.64 μM).[63] In a carrageenan-induced rat foot paw edema assay, however, compound **28** was slightly weaker when compared to celecoxib. In Mojtahedi's study, a group of tacrine-based pyrano[2,3-*b*]pyrazole derivatives were synthesized and their potency against 15-LOX was evaluated. In this group, compound **29** exhibited the highest activity against 15-LOX with an IC_{50} value of 31 μM. Additionally, this compound also had potency to inhibit AChE (acetyl

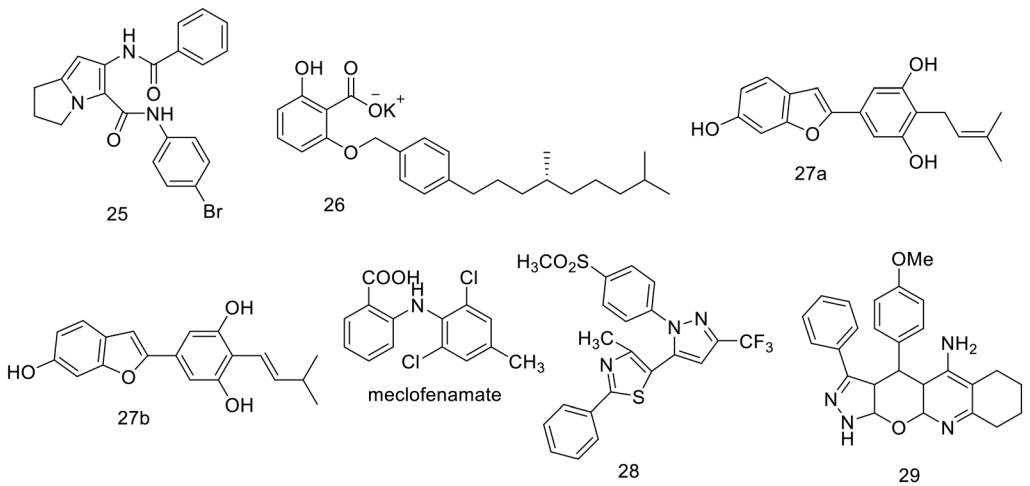


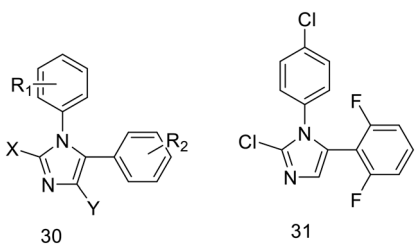
Fig. 8 Structures of compounds **25**, **26**, **27**, **28** and **29**.

cholinesterase) and BuChE (butyryl cholinesterase) (IC_{50} = 0.005–0.08 μM).[64]

Drugs that have only one target are gradually losing ground. More scholars are eager to find drugs that can match multiple targets simultaneously. In 2017, Ballatore *et al.* conducted a systematic SARs study based on the structures of 1,5-diarylimidazoles with microtubule-stabilizing activity and other anti-inflammatory agents targeting COX/5-LOX. Consequently, a prototypical structure **30** (Fig. 9) was obtained. On the basis of this, a series of 1,5-diarylimidazole derivatives were designed and synthesized.[65] Surprisingly, these derivatives preserved all the properties of the pro-structures, showing a multi-target feature. In particular, compound **31** inhibited the 5-LOX pathway, and displayed a 92% inhibition of LTB_4 at a concentration of 10 μM. In the SAR study, single substituted groups at the C-2 and C-4 positions of the imidazole ring were found to be less active than di-substituted, tri-substituted, or tetra-substituted ones. This study provided a good basis for discovery of active compounds with multiple targets.

3.2 Inhibitors obtained by rational design

In 2012, Shafiee *et al.* found that sulfur atoms enhanced the inhibitory activity for 15-LOX, especially heterocyclic thiones with N-heteroatom.[66] Based on this pharmacophore, Shafiee and co-workers designed a series of 4,5-diaryl-1*H*-imidazole-2(3*H*)-thione derivatives.[67] In particular, compound **32** (Fig. 10) showed an IC_{50} value of 4.7 μM against 15-LOX and free radical eliminating potency (IC_{50} = 14 μM). The SAR study showed that changing the thiol group to a methyl group at the C-2 position of the imidazole ring led to a dramatic decrease in the inhibitory activity against 15-LOX and radical eliminating potency. In molecular docking study, a suitable direction of the thiol group towards the Fe core in the active site of 15-LOX was observed, which indicated iron chelate formation between them. Another study conducted by Reddanna, by using site point connection and 3D quantitative structure–activity relationship (3D-QSAR) methods, resulted in the design and synthesis of a group of 4-(benzyloxy)-1-phenylbut-2-yn-1-ol analogues. Compound **33** showed the best potency against 5-LOX with an IC_{50} value of 8 μM in this group and also exhibited activity against various cancer cell lines without influence on normal cell lines.[68]



30 31

Fig. 9 Structures of compounds **30** and **31**.

In 2014, Rajitha designed and synthesized compound **34** (an *N*-(α-acetamidocinnamoyl)arylhydrazone derivative) by combining two pharmacophores of hydrazone and styrylcarbonyl groups, which was found to inhibit edema by 74%.[69] Molecular docking showed that the two main binding forms between this compound and 5-LOX were intermolecular hydrogen bonds and van der Waals interactions, and the docking energy was −10.95 kcal mol^{-1}, showing the best binding affinity to 5-LOX in this series. In another study conducted by Foroumadi *et al.*, based on the anti-inflammatory potency of thiourea and sulfonamide groups, a series of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives were synthesized and evaluated for their potency against 15-LOX.[70] Among them, compound **35** was found to be the most active derivative with an IC_{50} value of 1.8 μM. Molecular docking study indicated that the amino group of the sulfonamide moiety interacted with Ser510 and Gly720 by hydrogen bonds in the polar cavity of the active site. In addition, T-shaped π–π conjugation between the phenyl group and Phe576 also helped to form hydrogen bonds. In order to keep this structure stable, the lipophilic phenyl substituent occupied the hydrophobic active site and formed a π-cation in conjugation with Fe^{3+}. Additionally, introduction of a methyl group at the 2- or 4-position of the phenyl group would decrease the inhibitory activity.

In 2015, in a study conducted by Javed, the phthalazine group was found to be the pharmacophore of **vatalanib** (Fig. 11), which entered a phase III clinical study in the treatment of metastatic rectal cancer. In addition, the methanesulfonyl group was also the pharmacophore of **rofecoxib** (COX-2 inhibitor) and **etoricoxib** (COX-2 inhibitor). Combining these two pharmacophores, a series of phthalazinone derivatives with the methanesulfonyl group were designed, synthesized and evaluated for their potency against 5-LOX. Compound **36** was found to exhibit the best activity (IC_{50} = 6.2 μM) in this series in a carrageenan-induced rat paw edema model.[71]

In 2016, based on these three pharmacophores of sulfonyl group, thiazole ring and pyrazole ring, Abdelall and co-workers designed and synthesized a series of 1,5-diarylpyrazoline derivatives, and further evaluated their inhibitory potency against 15-LOX.[72] In this series, compound **37** showed the best potency with an IC_{50} value of 4.7 μM. Molecular docking study showed that the methoxy, sulfuryl and amino groups formed one or two hydrogen bonds with Ser510, Gln716 and Asp766 in the active site, respectively. Moreover, the removal of one or more methoxy groups could result in a decrease in the inhibitory potency against 15-LOX.

3.3 Inhibitors obtained by high throughput screening and virtual screening

In 2012, Young *et al.* investigated a series of 1,3-thiazole-2-amine analogues by HTS. Among them, compound **38** (Fig. 12), *N*-(3,5-dimethylphenyl)-4-(4-chlorophenyl)-1,3-thiazole-2-amine, exhibited the highest potency against 5-LOX

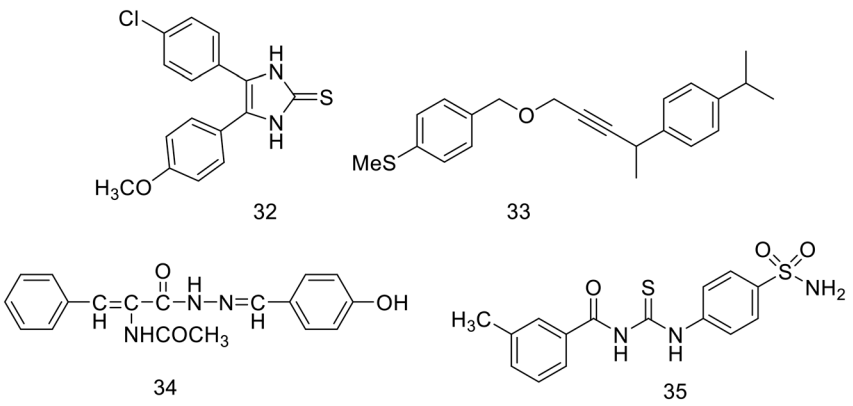


Fig. 10 Structures of compounds **32**, **33**, **34** and **35**.

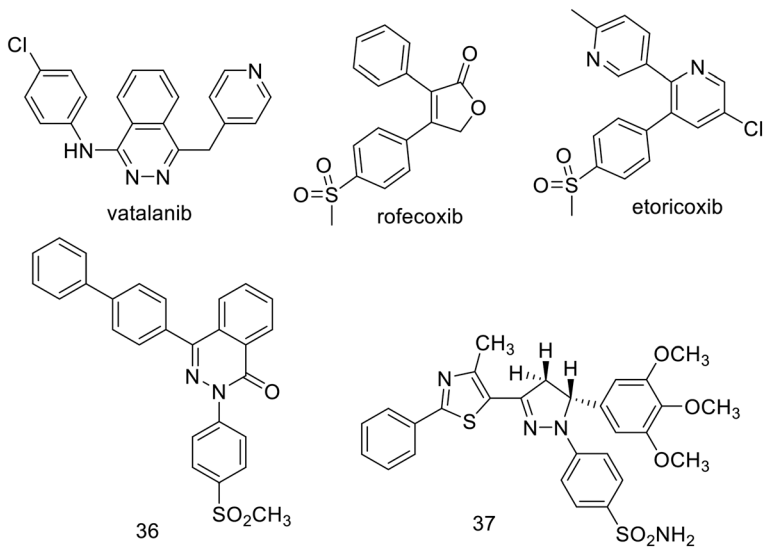


Fig. 11 Structures of compounds **vatalanib**, **rofecoxib**, **etoricoxib**, **36** and **37**.

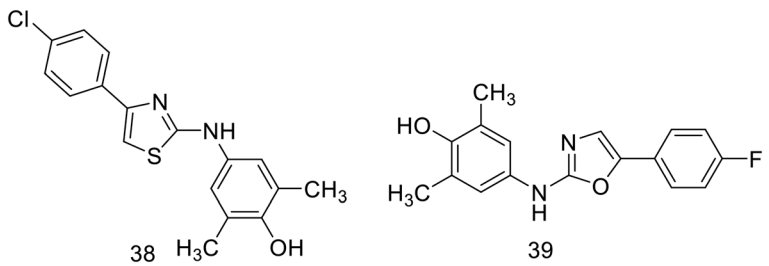


Fig. 12 Structures of compounds **38** and **39**.

with an IC_{50} value of 0.127 μM,[73] while that of the commercial drug zileuton was 0.18 μM. In the SAR study, the hydroxyl or amino group at the 2- or 4-position of the *N*-aryl moiety of the aminothiazole scaffold was found to be a key group for the inhibitory activity. In 2015, as a continuation of previous work, Young *et al.* designed and synthesized a series of *N*-aryl-5-aryloxazol-2-amine analogues by means of the principle of bioisostere, of which compound **39** was found to show higher potency against 5-LOX than **zileuton** by topical administration to cope with AA-induced ear edema.[74] The SAR study revealed that an amino group or a hydroxyl group at the *p*-position of the *N*-phenyl group was indispensable for the 5-LOX inhibitory potency, and additional halogen- or methyl group-substituted analogues affected the activity.

In 2014, based on the structure of **JMC-4** (Fig. 13), which was identified by virtual screening, a series of 3,5-dinitrobenzoate analogues were designed, synthesized and evaluated in cell-free and human whole blood assays for their

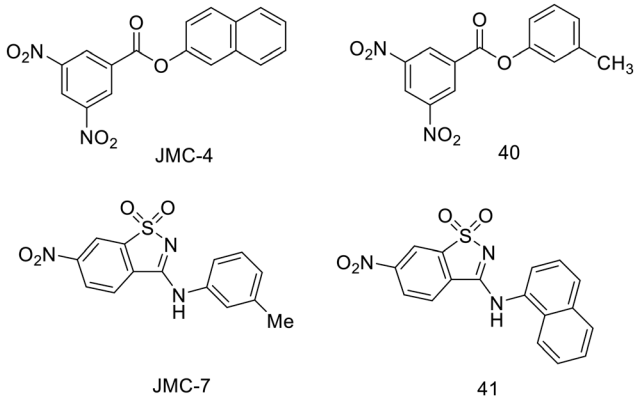


Fig. 13 Structures of compounds **JMC-4**, **40**, **JMC-7** and **41**.

5-LOX inhibition activity by Liu *et al.* at Peking University.[75] Among them, compound **40** was found to have good potency against 5-LOX with IC_{50} values of 0.006 and 0.5 μM in cell-free assay and human whole blood assay, respectively. The SAR study showed that substitution of the 3-phenyl group could enhance the activity while substitution of the 2,3-dichlorophenyl group could decrease the activity. Moreover, a single nitro substitution could significantly improve the potency and binding force as well. Additionally, compound **JMC-7** was also obtained by virtual screening. Based on this structure, another series of benzo[*d*]isothiazole 1,1-dioxide derivatives were synthesized and evaluated.[76] Consequently, **41** had higher anti-inflammatory potency with an IC_{50} value of 0.6 μM against LOX than **JMC-7** with an IC_{50} value of 1.9 μM. Molecular docking study showed that both compounds had a similar binding model, and especially, the hydrophobic interaction was crucial to enhance the potency of **41**.

In 2015, by means of virtual combinatorial library design and virtual screening, a series of 1,4-dihydropyrimidine derivatives were designed, synthesized and evaluated for their *in vitro* 5-LOX inhibitory activity. Among them, compound **42** (Fig. 14) was found to be a dual inhibitor, which exhibited the strongest potency with an inhibitory activity of 51.84% at a concentration of 100 μg mL^{-1}.[77] Binding mode revealed that this dual inhibitor should have a metal binding group, an electronegative group and a 'V' shape structure so as to bind with Fe^{2+} in the active site of 5-LOX, and electropositive amino acids in the active site of COX-1 and COX-2, respectively. In general, the decrease of the electron cloud density on the benzene ring would lead to a decrease in the inhibitory activity against 5-LOX.

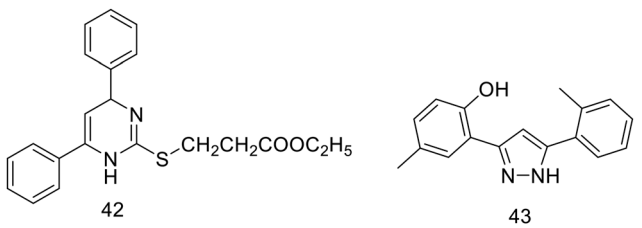


Fig. 14 Structures of compounds **42** and **43**.

In 2016, by means of HTS, cellular screening, SARs, and kinetic studies, Holman *et al.* found that compound **43** had high potency against human 12-/15-LOX with an IC_{50} value 3.4 μM *in vivo* and mouse neuronal cell line HT-22 with an IC_{50} value 10 μM *in vitro*.[78] Overall, the potency of diazole derivatives was lower than that of triazole derivatives in the aspect of inhibiting LOX activity. The SAR study indicated that changing of the 5-phenyl group to an *N*-benzylcarboxamide or a 4-methoxyphenylmethanone group would lead to a 10-fold lower activity. In contrast, modification of the 5-position was found to have a very small impact on the activity. In another study, pyrazole-3-carboxanilides found by HTS were proved to have perfect potency against 15-LOX. The SAR study indicated that *N*1-substituent was unnecessary for activity or selectivity, while an extra halogen substituent on the pyrazole ring could increase the potency.[50,79] Therefore, the *N*1-unsubstituted pyrazole-3-carboxanilides were developed as candidate drugs.

4. FLAP inhibitors

In recent years, FLAP inhibitors have also been developed, and some FLAP inhibitors entered clinical studies. However, they were terminated or made no progress for various reasons. Here we present several typical FLAP inhibitors.

4.1 FLAP inhibitors in preclinical studies

In 2012, Werz designed and synthesized a series of benzimidazole derivatives by combining structure- and ligand-based virtual screening and SAR studies. In this series, compound **44** (Fig. 15) showed the highest potency against LT formation (IC_{50} = 0.12 μM), while the precursor structure (**BRP-7**) exhibited an IC_{50} value of 0.31 μM against LT formation.[80] In molecular docking study, the methoxy group at the 5-position of the benzimidazole ring of compound **44** formed an extra H-bond with the C-terminal of the Asn57 in the active site of FLAP, while this phenomenon did not occur for compound **BRP-7**, which is probably the reason why compound **44** was much more active than **BRP-7**. In 2016, as a continuation of previous work, a great number of **BRP-7** derivatives were explored. Among them, compound **45** exhibited the most potent activity against LT biosynthesis in human neutrophils (IC_{50} = 0.07 μM) and monocytes (IC_{50} = 0.026 μM).[81] The SAR study indicated that the nitrile group at the C-5 position of the benzimidazole ring was the optimal group. In another series obtained using the same method, compound **46** was found to show inhibitory potency of LT biosynthesis (IC_{50} = 4.4 μM).[82] In contrast, its derivative **BRP-187** (4-(4-chlorophenyl)-5-[4-(quinolin-2-ylmethoxy)phenyl]isoxazol-3-carboxylic acid) showed a more potent activity in inhibiting LT biosynthesis (IC_{50} = 4.4 μM). Moreover, in another study of Werz, a series of 4,5-diarylisoxazole-3-carboxylic acid derivatives were designed and synthesized based on the structure of compound **46**. Among them, compounds **47** and **48** showed the best activity against LTs (IC_{50} = 0.24 μM).[83] In

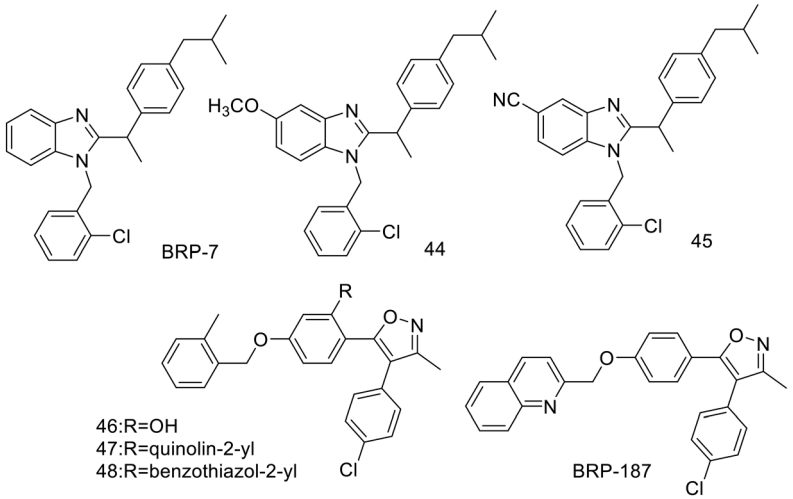


Fig. 15 Structures of compounds **BRP-7**, **44**–**48** and **BRP-187**.

molecular docking study, an extra H-bond was found with Lys116.

The same year, in another study conducted by Lin *et al.*, compound **49** (Fig. 16) was obtained by esterification on the basis of compound **50**.[84] Because of the introduction of an ester functional group, its IC_{50} value for FLAP binding was reduced to 2.1 nM, while that of compound **50** was 2.7 nM. In a leukotriene mediated human whole blood experiment, however, the IC_{50} value of compound **49** was 460 nM, while that of compound **50** was 36 nM. In SAR study, compound with a methoxy at the C-3 of the benzene ring exhibited the optimal activity of human whole blood experiment.

During the discovery of FLAP inhibitors, scholars focused on reducing the lipophilicity of FLAP inhibitors. In 2015, in a study conducted by Lemurell *et al.*, **AZD6642** was identified by rational design. The hydrophilic tetrahydrofuran (THF) ring introduced to its structure had a particular interaction with the target *via* the oxygen atom of the THF group.[85] Based on the pharmacokinetic data obtained from mice and dogs, the predicted dose of **AZD6642** in humans ranged from 15 to 25 μg per day.

In 2015, by means of structure guided design, compound **BI-665915** (Fig. 17) was designed and synthesized, and evaluated as a novel FLAP inhibitor at Boehringer Ingelheim Pharmaceuticals.[86] The IC_{50} values for FLAP binding and human whole blood LTB_4 production were 1.7 nM and 45 nM, respectively, and the aqueous equilibrium solubility at pH 6.8 was 48 μg mL^{-1}. Additionally, in terms of potential drug–drug interactions, **BI-665915** was predicted to have a low risk. The SAR study indicated that benzene was the best substitution on the oxadiazole ring, while amino or sulfonamide groups would reduce the activity.

In 2016, compound **51** was identified by Schuster *et al.* based virtual screening, displaying an IC_{50} value of 0.2 μM in FLAP test essay.[87] In practice, there are three main metabolic modes of AA: PGs produced by the COX pathway, LTs produced by the LOX pathway and EETs (epoxyeicosatrienoic acids), which are converted to hydroxyeicosatetraenoic acids by sEH, the soluble epoxide hydrolase produced by the cytochrome P450 pathway.[88] Blocking one pathway would amplify other pathways. Hence, multi-target compounds seemed particularly important for their activity. Most surprisingly, compound **51** not only exhibited anti-FLAP activity, but also inhibited the sEH action (IC_{50} = 0.02 μM).

4.2 FLAP inhibitors in clinical studies

Fiboflapon is now in phase II clinical study for the treatment of asthma, jointly developed by Bristol-Myers Squibb and GlaxoSmithKline. In FLAP binding test and human whole blood test, it showed an IC_{50} value of 2.9 and 76 nM, respectively.[89] In SAR study, the ethoxyl group replaced by the

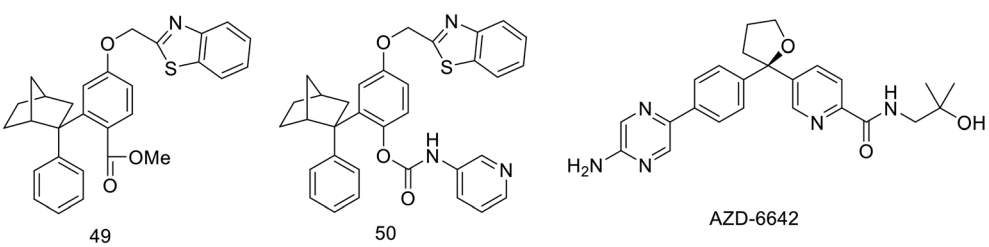


Fig. 16 Structures of compounds **49**, **50** and **AZD-6642**.

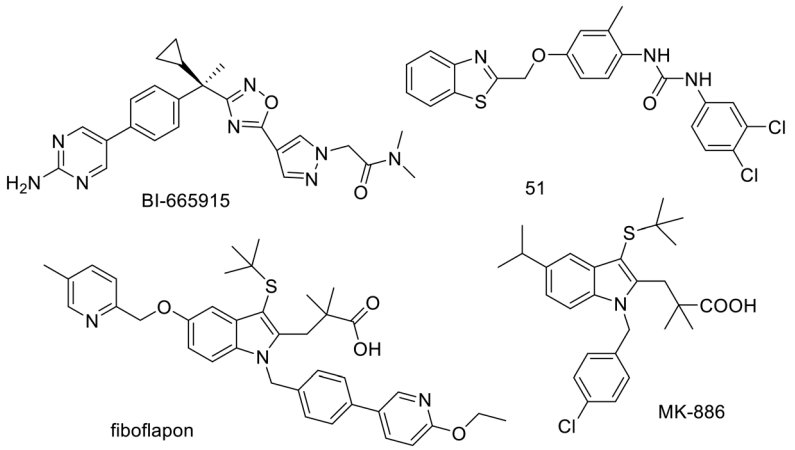


Fig. 17 Structures of compounds **BI-665915**, **51**, **fiboflapon** and **MK-886**.

trifluoromethyl group basically did not affect the activity of FLAP binding (IC_{50} = 2.1 nM), but decreased the activity in human whole blood test (IC_{50} = 180 nM). Additionally, compounds possessing the quinoline group showed the best performance.

MK-886 developed by Merck as a potent FLAP inhibitor, which exhibited an IC_{50} value of 102 nM in LT synthesis inhibition assay and had an effect on 5-LOX, was terminated in a phase II clinical study for the treatment of asthma, psoriasis and inflammatory bowel diseases.[90]

5. Conclusions

Inflammation is still a condition that is harmful to public health, which brings great pain to patients. LOX not only refers to oxidation of lipid, but also the involvement in producing LT, which mediates the occurrence of inflammation. Inhibiting LOX activity is a prospective method to treat inflammation, for the reason that many specific compounds were designed and synthesized as LOX inhibitors.

In this paper, the LOX and FLAP inhibitors reported recently are summarized, including their structures, activities, molecular docking and SAR studies. Of the compounds discussed, compound **4** displays the highest activity against 5-LOX with an IC_{50} value of 0.002 μM, better than that of the commercial drug zileuton with an IC_{50} value of 0.18 μM. Compounds **2a** and **2b** also show high activity, with IC_{50} values of 0.0097 and 0.0086 μM, respectively. The above three compounds were designed based on modification of an indole core. In general, the indole-based compounds exhibited relatively high activity, which indicates that more modifications of such a promising core should be investigated in depth. Besides, among the non-indole compounds, compound **40** shows the highest activity against 5-LOX (IC_{50} = 0.006 μM). In terms of FLAP inhibitors, compounds **50** and **BI-665915** exhibited high potency, with IC_{50} values both at an nM level.

In general, LOX inhibitors are developed more slowly than COX inhibitors due to a lack of specific structure of LOXs. Additionally, as a result of the coexistence of LOXs in the same organism, LOX inhibitors should be designed seriously so as to achieve the targeting effect. In particular, another regulatory domain that is different from the two known domains of the C-terminal catalytic domain and the N-terminal β-barrel domain has been found, which will provide new ideas for the design of new LOX inhibitors.[15] However, although a lot of LOX and FLAP inhibitors with high potency have been found so far, there is only one drug (zileuton) that has been put on the market, which can selectively inhibit 5-LOX in the treatment of asthma. Thus, it is believed that specific FLAP and LOX inhibitors with high selectively for 5-LOX as new anti-inflammatory agents will be discovered and applied in the near future.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

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