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Benzo[*d*]isothiazole 1,1-dioxide derivatives as dual functional inhibitors of 5-lipoxygenase and microsomal prostaglandin E_2 synthase-1

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ABSTRACT

A series of 6-nitro-3-(*m*-tolylamino) benzo[*d*]isothiazole 1,1-dioxide analogues were synthesized and evaluated for their inhibition activity against 5-lipoxygenase (5-LOX) and microsomal prostaglandin E_2 synthase (mPGES-1). These compounds can inhibit both enzymes with IC_{50} values ranging from 0.15 to 23.6 μM. One of the most potential compounds, **3g**, inhibits 5-LOX and mPGES-1 with IC_{50} values of 0.6 μM, 2.1 μM, respectively.

For inflammation and related disorders, leukotrienes and prostaglandins are two classes of important mediators produced from arachidonic acid (AA).[1] Leukotrienes are potent mediators of inflammatory and allergic reactions that are generated by the 5-lipoxygenase (5-LOX) pathway.[2] 5-LOX is a non-heme iron dioxygenase and catalyzes the transformation from AA to leuktriene A_4 (LTA_4), which is further mediated to other proinflammatory mediators such as leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4)[3,4] Thus 5-LOX inhibition represents an attractive strategy for therapeutic intervention. Though many 5-LOX inhibitors have been reported, currently only one compound with micromolar activity, zileuton, is on market for treating asthma.[5]

Prostaglandins are generated by the cyclooxygenase (COX) pathway. Among the prostaglandins, prostaglandin E_2 (PGE_2) is the most common inflammatory mediator and plays crucial roles in various biological events, such as neuronal functions, vascular hypertension, fever, pain hypersensitivity, and tumor genesis.[6,7] PGE_2 is synthesized from COX product prostaglandin H_2 (PGH_2) by terminal PGE_2 synthases (PGES). Three isoforms of PGES have been identified: cytosolic prostaglandin E_2 synthase (cPGES) and two microsomal prostaglandin E_2 synthases (mPGES-1 and mPGES-2). mPGES-1 is an inducible enzyme and responsible for the elevated levels of PGE_2 in inflammatory stimuli.[8] In recent years, mPGES-1 is identified as a novel anti-inflammatory therapeutic target.[7]

Traditional nonsteroidal anti-inflammatory drugs (NSAIDs, which inhibit both two isoforms of COX, COX-1 and COX-2) and COX-2 selective inhibitors (coxibs) are widely used to treat corresponding disorders. However, the use of NSAIDs is associated with gastrointestinal side effect and coxibs exert cardiovascular side effects.[9,10] According to the simulation result of the AA metabolic network dynamics, inhibiting multiple targets in the network may be effective in controlling the inflammation mediators and reduce the side effects.[11] Licofelone, a compound which can inhibit both 5-LOX and COX pathways, successfully completed phase III trials for treating osteoarthritis.[12] Furthermore, our recent study on the effects of inhibitor combinations on the AA network verified that the 5-LOX and mPGES-1 is a potential optimal dual target combination.[13]

Several 5-LOX and mPGES-1 dual functional inhibitors have been reported, such as pirinixic acid derivatives, arylpyrrolizines, modified acidic nonsteroidal anti-inflammatory drugs and 5-hydroxyindole-3-carboxylates.[14–17] However, there is no effective 5-LOX and mPGES-1 inhibitor used in clinic yet. Development

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of novel highly efficient 5-LOX and mPGES-1 dual functional inhibitors is highly demanded. In previous research, we identified a series of novel inhibitors for 5-LOX through virtual screening, and two of them appeared to be potential dual functional inhibitors of 5-LOX and mPGES-1. The two inhibitors provided new scaffolds to design multi-target anti-inflammatory drugs.[18] One of the dual functional inhibitors is 6-nitro-3-(*m*-tolylamino) benzo[*d*]isothiazole 1,1-dioxide (**JMC-7**) (IC_{50} values are 1.9 μM for 5-LOX and 6.7 μM for mPGES-1). No application has been reported for this compound. Herein we synthesized a series of **JMC-7** analogues and evaluated their inhibition activity toward 5-LOX and mPGES-1.

It is highly difficult to improve the potency of a dual-functional ligand to both targets simultaneously. To identify key interactions between **JMC-7** and the two targets, we docked the compound into the substrate binding sites of 5-LOX and mPGES-1. The crystal structure of 5-LOX (PDB entry: 3O8Y[19]) and a model of active conformation of mPGES-1 built by our group were used.[20] Molecular docking with flexible ligands and rigid receptor was performed with the program AutoDock 4.00.[21] Lamarckian genetic algorithm was used with following parameters: number of individuals in population, 300; maximum number of energy evaluations,

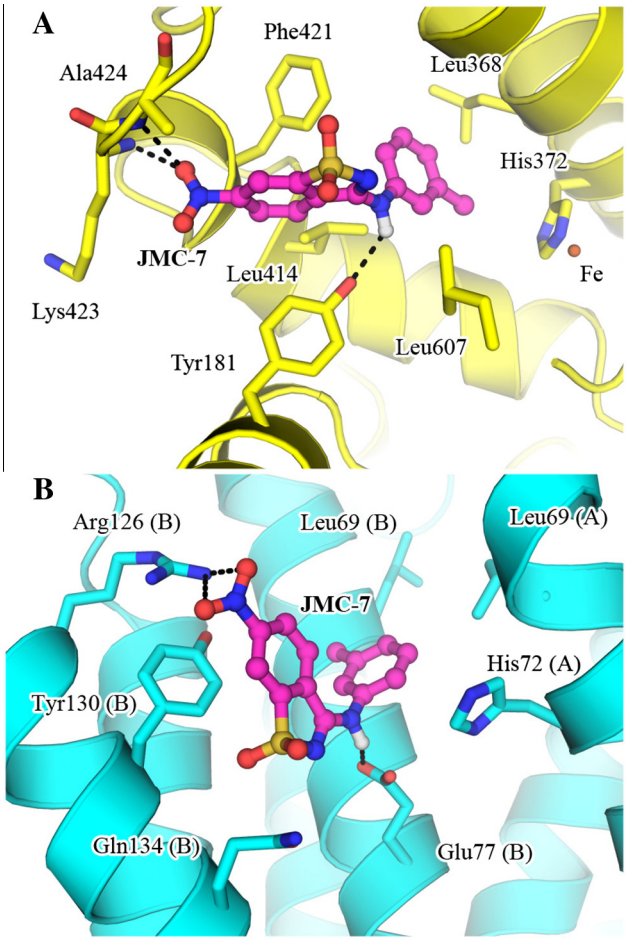
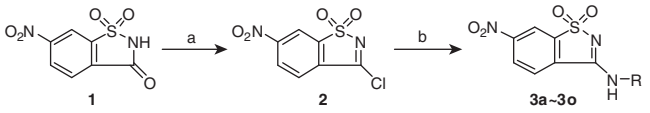
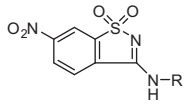


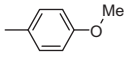
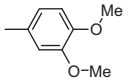
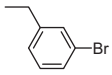
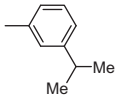
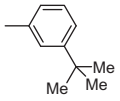
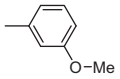
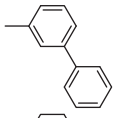
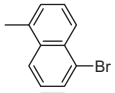
Figure 1. Key interactions of **JMC-7** (magenta) with 5-LOX (A, yellow) and mPGES-1 (B, cyan; letters following residue names stand for chain ID) (predicted by molecular docking).



Scheme 1. Regents and conditions: (a) $SOCl_2$, DMF (cat.), dioxane, 110 °C, 1.5 h; (b) RNH_2, NEt_3, THF, rt, 4~6 h.

Table 1
5-LOX and mPGES-1 inhibitory activity



Compd	R	In vitro IC_{50}[c] (μM)	
		5-LOX	mPGES-1
Zileuton[a]		1.1 ± 0.5	–
MF63[b]		–	0.0030 ± 0.0001
JMC-7		1.9 ± 0.1	6.7 ± 0.2
3a		8.6 ± 0.7	23.6 ± 4.9
3b		>50	5.7 ± 1.2
3c		3.4 ± 0.5	7.3 ± 1.5
3d		12.3 ± 3.8	16.9 ± 3.5
3e		23.5 ± 6.5	>50
3f		>50	>50
3g		0.6 ± 0.3	2.1 ± 0.4
3h		>50	>50
3i		0.25 ± 0.05	>50
3j		0.06 ± 0.02	>50
3k		0.24 ± 0.02	8.8 ± 1.3
3l		0.7 ± 0.9	>50
3m		>50	0.15 ± 0.06
3n		>50	>50
3o		14.9 ± 0.8	>50

[a] Positive control of 5-LOX IC_{50} values.
[b] Positive control of mPGES-1.
[c] Data are given as mean ± SE of $n \geqslant 3$ determinations.

25,000,000; maximum of generations, 27,000, number of runs, 256. Docked conformations of the 256 runs were clustered with a rms tolerance of 2.0 Å, and the lowest energy conformation from the largest cluster was taken as docking result.

Key interactions predicted by molecular docking are shown in Figure 1. For 5-LOX, **JMC-7** forms hydrogen bonds using the nitro group to the main-chain nitrogen atoms of Lys423 and Ala424 and using the amino nitrogen atom to the hydroxyl group of Tyr181. The aryl rings of **JMC-7** form hydrophobic interactions with the residues inside the cavity. For mPGES-1, hydrogen bonds form between the nitro group and Arg126, and the amino nitrogen atom and Glu77. There is also hydrophobic interaction between the aryl rings of **JMC-7** and residues inside the cavity. Thus both the 6-nitro group and the amino nitro atom of **JMC-7** are crucial for specific binding of the compound to the targets. The aryl rings of **JMC-7** participate in forming hydrophobic interaction. As there is extra space close to the *m*-tolyl group in both pockets, we optimized the compound at this moiety.

Analogues of **JMC-7** with *m*-tolyl replaced by different hydrophobic groups were synthesized. The synthetic route was shown in Scheme 1.[22] Commercially available 6-nitrosaccharin (**1**) was initially reacted with sulfuryl chloride to generate the intermediate 3-chloro-6-nitrobenzo[*d*]isothiazole 1,1-dioxide (**2**). Subsequent displacement of the chloride from this intermediate by a variety of aromatic amines gave the target compounds (**3a**∼**3o**) in moderate to high yields. ^{1}H NMR, ^{13}C NMR and HRMS data of all the synthesized compounds were in full agreement with the proposed structures (see Supporting Information).

All synthesized compounds were tested for inhibitory activities to 5-LOX in vitro. Protein expression and enzyme activity determination were performed using the same method as in our previous study.[18] The enzyme assay measures the fluorescent signal from the oxidation of an indicator, 2,7-dichlorodihydrofluorescein diacetate, to the highly fluorescent 2′,7′-dichlorofluorescein by the 5-LOX enzyme reaction product.[23] Zileuton was used as a positive control. The IC_{50} values of all the compounds are shown in Table 1. Most of **JMC-7** derivatives preserve 5-LOX inhibitory activities. Compound **3a** with phenyl group results in a little loss of potency (IC_{50} 8.6 μM). This may due to size decreasing of the hydrophobic group. Replacement of methyl group with bromine produces 3-((3-bromophenyl) amino)-6-nitrobenzo[*d*]isothiazole 1,1-dioxide (**3c**), which possesses similar inhibition activity (IC_{50} 3.4 μM) to **JMC-7**. The replacement of *m*-methyl with other large substituent, such as *i*-propyl (**3i**), *t*-butyl (**3j**), and methoxy (**3k**), show significant increasing of inhibition to 5-LOX, while **3j** is the strongest (0.06 μM). These results further confirm hydrophobic substituent in the *meta*-position enhance the interaction of the compounds with 5-LOX. However, the only exception is the chloride analogue **3b**, which is inactive. The introduction of methoxyl group into the *para*-position (**3d**) and dimethoxyl group into the *meta*- and *para*-positions (**3e**) reduces the activities of the compounds. In addition, substitution of *m*-tolyl by *m*-bromobenzyl (**3f**) cannot inhibit 5-LOX efficiently, indicating that flexible aryl group is not favored at the hydrophobic moiety. Compound **3h** with *o*-methyl group loses the inhibition activity to 5-LOX. Among the compounds with two-ring hydrophobic moiety, **3g** (with 1-naphthyl) and **3l** (with 3-biphenyl) afford 3-folds increase in potency, while **3m**, **3n**, and **3o** lose the activities. Thus larger multiple ring groups at this position may increase the interaction between the compound and 5-LOX.

The IC_{50} values of the compounds to inhibit mPGES-1 are also listed in Table 1. Protein expression and enzyme activity were measured using our reported protocols.[20] Assessment of PGE_2 conversion from PGH_2 was measured using the PGE_2 EIA kit (Cayman Chemical) for enzyme activity. A known mPGES-1 inhibitor, MF63, was used as the positive control. Compound with smaller hydrophobic group (phenyl, **3a**) has decreased inhibitory activity (IC_{50} 23.6 μM). For the *meta*-position, smaller substitution groups (halogen atom and methoxy) has similar activity to the parent compound with methyl at this position the activity of the compounds (**3b**, IC_{50} 5.7 μM; **3c**, IC_{50} 7.3 μM; **3k**, 8.8 μM), while larger substituent *i*-propyl (**3i**) or *t*-butyl (**3j**) reduce their activities. Compounds with methoxyl at the *para*-position (**3d**) or both *meta*- and *para*-positions (**3e**) are inactive, as well as methyl at the *ortho*-position (**3h**) and flexible aryl group (**3f**) at this moiety. In this test, the 1-naphthyl derivative (**3g**) is also 3-fold more active than **JMC-7**, while 5-quinolinyl derivative (**3m**) possesses 45-fold increasing activity. The rest compounds with two-ring hydrophobic moiety (**3n** and **3o)** lose the activities.

According to the inhibitory activities listed above, several compounds maintain comparable activity for inhibiting both enzymes. The optimized moiety affects the activity of the compounds to both 5-LOX and mPGES-1. To increase hydrophobic interaction, substitution group with proper size on phenyl is needed, and the *meta*-position is validated. Flexible aryl groups at this moiety decrease the inhibitory activities to both targets. Compounds with two-ring hydrophobic moiety may also be preferred. The strongest 5-LOX inhibitor is **3l**, however shows no inhibitory activity to mPGES-1. Also the strongest mPGES-1 inhibitor **3m** is inactive in inhibiting 5-LOX. The most balanced inhibitor, **3g**, has approximately 3-folder higher activity than **JMC-7** to both 5-LOX (IC_{50} 0.6 μM) and mPGES-1 (IC_{50} 2.1 μM). The dose-effect curves of **3g** are shown in Figure 2.

To analyze the interactions between **3g** and the targets, it was docked also into the inhibitor binding pockets. The docking results show that **3g** employs the similar binding modes as **JMC-7** does in the both targets. The interactions of **3g** to the targets were illustrated using LIGPLOT figures (Figure 3).[24] All the hydrogen bonds between **3g** and 5-LOX remained. For mPGES-1, the hydrogen bond formed with the amino nitro atom disappeared because of a slight position shift of **3g**, while the other two remained. Moreover, compared with the parent compound **JMC-7**, **3g** has stronger hydrophobic interaction to the pockets of 5-LOX and mPGES-1, the *ortho*-position may also be potential. Therefore, hydrophobic interaction is essential for the activity enhancing of **3g**. This may

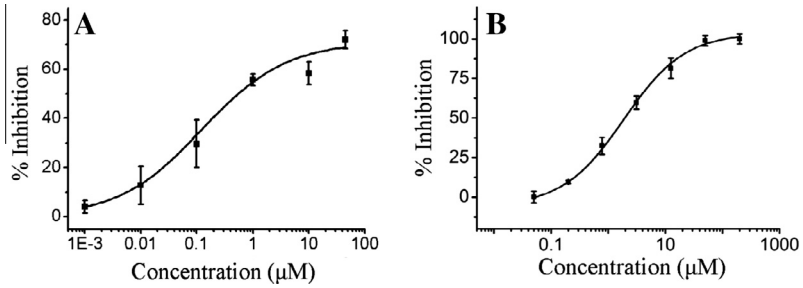


Figure 2. Dose-effect curves of compound **3g**. (A) Inhibiting 5-LOX; (B) inhibiting mPGES-1.

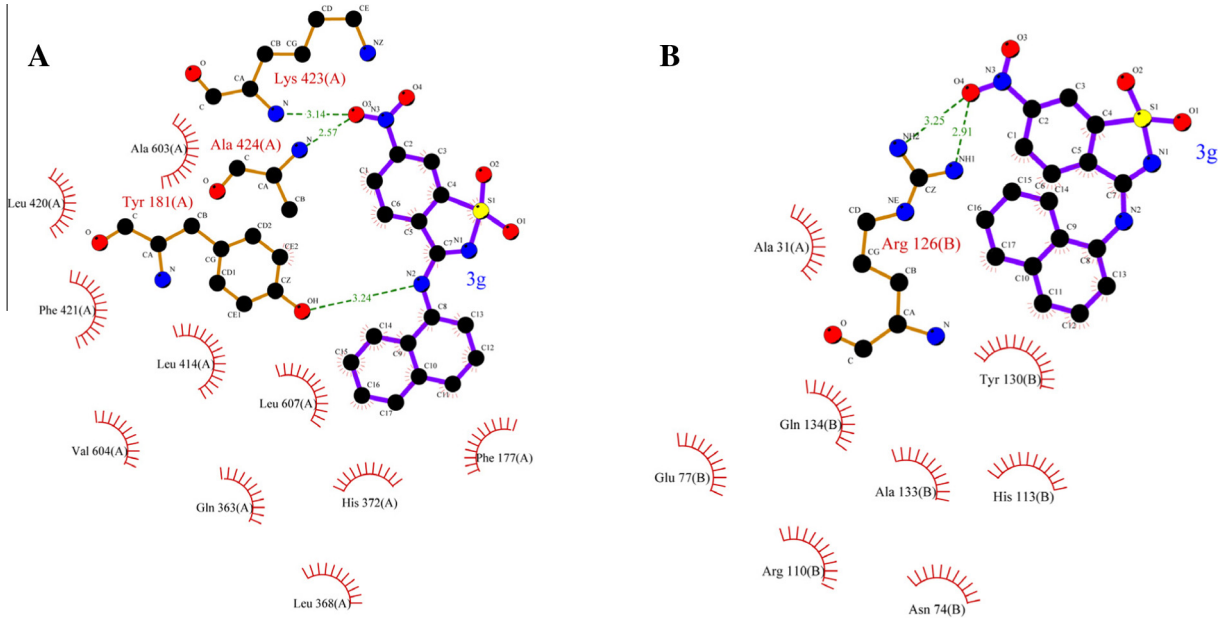


Figure 3. Interactions of compound **3g** with 5-LOX (A) and mPGES-1 (B) (generated using program LIGPLOT[24]).

provide useful guidance for the design of highly active 5-LOX and mPGES-1 dual functional inhibitors. Further studies are under investigation.

In conclusion, we synthesized a series of analogues of 6-nitro-3-(*m*-tolylamino) benzo[*d*]isothiazole 1,1-dioxide (**JMC-7**), as dual functional inhibitors of 5-LOX and mPGES-1. Through this optimization, we obtained several new dual functional inhibitors to 5-LOX and mPGES-1. One of the compounds, 3-(naphthalen-1-ylamino)-6-nitrobenzo[*d*]isothiazole 1,1-dioxide (**3g**), showed improved inhibition activities to both 5-LOX and mPGES-1. These compounds provide a new starting point for novel anti-inflammatory drug development.

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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.bmcl.2014.04.006. These data include MOL files and InChiKeys of the most important compounds described in this article.

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