



# Design, synthesis, and LFA-1/ICAM-1 antagonist activity evaluation of Lifitegrast analogues

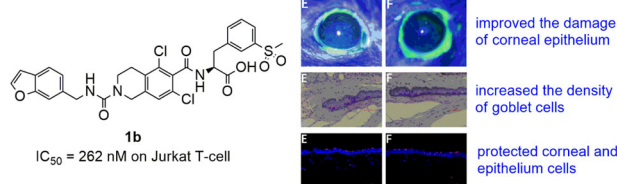
Guoxin Du<sup>1,2</sup> · Weiwei Du<sup>1,2</sup> · Yuanlong An<sup>1,2</sup> · Minnan Wang<sup>3</sup> · Feifei Hao<sup>2</sup> · Xiaochu Tong<sup>4</sup> · Qi Gong<sup>2,3</sup> · Xiangdong He<sup>1,2,3</sup> · Hualiang Jiang<sup>4</sup> · Wei He<sup>1</sup> · Mingyue Zheng<sup>4</sup> · Donglei Zhang<sup>1,2</sup>

Received: 25 October 2021 / Accepted: 18 January 2022 / Published online: 17 February 2022  
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

## Abstract

The interaction between Lymphocyte function-associated antigen 1 (LFA-1) and intercellular-adhesion molecule-1 (ICAM-1) plays important roles in the cell-mediated immune response and inflammation associated with dry eye disease. LFA-1/ICAM-1 antagonists can be used for the treatment of dry eye disease, such as Lifitegrast which has been approved by the FDA in 2016 as a new drug for the treatment of dry eye disease. In this study, we designed and synthesized some new structure compounds that are analogues to Lifitegrast, and their biological activities were evaluated by in vitro cell-based assay and also by in vivo mouse dry eye model. Our results demonstrated that one of these analogues of Lifitegrast (compound **1b**) showed good LFA-1/ICAM-1 antagonist activity in in vitro assay; meanwhile, it also significantly reduced ocular surface epithelial cells damage, increased goblet cell density in dry eye mouse and highly improved the symptoms of dry eye mouse.

## Graphical abstract



**Keywords** Lifitegrast · Analogues · LFA-1/ICAM-1 antagonist · Dry eye · Corneal epithelial injury

## Abbreviations

DED dry eye disease

LFA-1 lymphocyte function-associated antigen 1

ICAM-1 intercellular-adhesion molecule-1

THIQ Tetrahydroisoquinoline

SAR structure–activity relationship

DMF Dimethylformamide

HATU 2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetra-methyluronium hexafluorophosphate

Boc tert-Butyldicarbonate

TEA Triethylamine

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1007/s00044-022-02851-9>.

✉ Mingyue Zheng  
myzheng@simm.ac.cn

✉ Donglei Zhang  
zhangdonglei@huh.edu.cn

<sup>1</sup> The School of Pharmacy, He University, Shenyang 110163, PR China

<sup>2</sup> Shenyang Industrial Technology Institute of Ophthalmology, Shenyang 110163, PR China

<sup>3</sup> Shenyang He Vision Industrial Group Co., Ltd, Shenyang 110163, PR China

<sup>4</sup> Drug Discovery and Design Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

DCM	Dichloromethane
CDI	Carbonyldiimidazole
TMSOK	Potassium trimethylsilanolate
THF	Tetrahydrofuran
DIEA	Diisopropylethylamine
n-BuLi	n-Butyllithium
PPA	Polyphosphoric acid
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
DMAP	Dimethylaminopyridine
(Tf) <sub>2</sub> O	Trifluoroacetic anhydride
Ar	Aromatic groups
NMR	Nuclear Magnetic Resonance
TMS	Tetramethylsilane
ppm	parts per million
MS	mass spectrum
ESI	electrospray ionization
HRMS	high resolution mass spectrum
LCMS	liquid chromatography mass spectrometry
TLC	thin-layer chromatography

## Introduction

Dry eye disease (DED) is a complex, multifactorial condition characterized by inflammation of the ocular surface and lacrimal glands, as well as the reduction in the quality and/or quantity of tears [1]. DED can be categorized as “dry eye with reduced tear production (aqueous deficient) and dry eye with increased evaporation of the tear film known as the hyperevaporative type” [2]. Dry eye patients suffer from ocular-related symptoms, such as stinging, burning, itching, light sensitivity, and blurry vision which limit the quality of their life, as well as their ability to work [3]. The prevalence of DED is between 5% and 50% in the different countries, and this prevalence can be up to 75% in adults over age 40, with mostly female patients. In adults aged 18–40, 2.7% of these people have had an experience of DED [4].

Lymphocyte function-associated antigen 1 (LFA-1) is a leukocyte cell surface glycoprotein widely expressed on cells of a hematopoietic lineage [5]. Previous functional studies indicated that intercellular-adhesion molecule-1 functions as a ligand for LFA-1-dependent adhesion by a variety of leukocytes [6]. LFA-1 functions are extremely varied but play a critical role in facilitating effective immune responses. It has been reported that LFA-1 was implicated in numerous autoimmune and inflammatory conditions including inflammatory bowel disease, psoriasis, diabetes, and arthritis [7]. The previous study suggested that LFA-1/ICAM-1 interaction may play important roles in the cell-mediated immune response and inflammation associated with DED. Notably, inhibition

of LFA-1/ICAM-1 binding offers a novel and possible approach to reducing ocular surface inflammation in this condition [8].

The immunosuppressive mechanism of Lifitegrast is different from the first-generation immunosuppressor (cyclosporine A) for dry eye treatment. Lifitegrast (Fig. 1) is a small molecule that inhibits T-cell-mediated inflammation by blocking the binding of LFA-1 and ICAM-1, thus lessening overall inflammatory responses [9]. In vitro, Lifitegrast potently inhibited the attachment of LFA-1 to ICAM-1 in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC<sub>50</sub>) of 2.98 nmol/L [10]. The results from the phase 3 clinic trial of Lifitegrast have shown its ability to improve ocular surface epithelial health and associated symptoms of dry eye patients, thereby demonstrating its potential as a relatively fast-acting second-generation agent for the treatment of DED [11]. Lifitegrast became the second topical anti-inflammatory agent which has been approved by the FDA in 2016 for the treatment of DED [12].

Lifitegrast ophthalmic solution (Xiidra) is labeled for the treatment of DED in concentrations as high as 5.0%. Such high concentrations result in many side effects, such as ocular irritation, dysgeusia and reduced visual acuity, especially an unusual taste sensation in the mouth. The LFA-1/ICAM-1 antagonist activity of Lifitegrast is not high enough to overcome these side effects. Therefore, it is very interesting to find novel higher LFA-1/ICAM-1 antagonist activity compounds. The discovery process and structure–activity relationships (SARs) of Lifitegrast were reported in a previous report [13]. So far, no other medicinal chemistry studies about Lifitegrast analogues have been reported. In this study, a novel series of Lifitegrast analogues with systematic modifications were further designed, synthesized, and their activities as LFA-1/ICAM-1 antagonists were evaluated by in vitro and in vivo assays.

## Results and discussion

### Design of Lifitegrast analogues

Lifitegrast is used as a lead compound for designing analogues. It has been established that the structure of central tetrahydroisoquinoline (THIQ) scaffold (Fig. 2) is a pharmacophore that is essential for LFA-1/ICAM-1 antagonist activity [14].

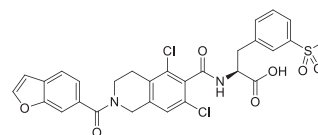
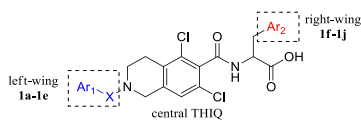


Fig. 1 Chemical structure of Lifitegrast



**Fig. 2** Design rationale of the target LFA-1/ICAM-1 antagonists

Previous SAR studies have shown that an aromatic group in the “left-wing” residue is necessary for LFA-1/ICAM-1 antagonist activity [15]. Therefore, we attempted to link tetrahydroisoquinoline and benzofuran with different groups (Fig. 2, **1a–1c**) to investigate the LFA-1/ICAM-1 antagonist activity. In order to investigate the effect of X groups with different electron-absorbing properties and different sizes on the activity, we selected sulfone group, N-methylene carbamoyl and methylene carbonyl as substituents. Meanwhile, the effects of substitution of benzofuran with different aromatic groups (Fig. 2, **1d–1e**) on the activity were investigated. Although benzotriazole and benzofuran have different atomic compositions, they are both benzo five-membered rings. From the activity results of benzotriazole, it can be seen whether there is a more suitable Ar<sub>1</sub> group. Ethynylbenzene was selected to investigate whether Ar<sub>1</sub> ring opening would have a significant effect on activity. At the same time, we incorporated different aromatic groups into “right-wing” amino acids (Fig. 2, **1f–1j**) to investigate the LFA-1/ICAM-1 antagonist activity. We cyclized methylsulfonyl with phenyl and converted methylsulfonylphenyl to benzene six-membered rings such as quinoline and 2-oxo-1,2,3,4-tetrahydroquinolin to investigate the effect of cyclization and different group size on the activity. According to the results of previous studies, Lifitegrast is located in the I-domain allosteric site of LFA-1/ICAM-1. Ar<sub>2</sub> group of Lifitegrast interact with the protein mainly through hydrogen-bonds with residues such as Tyr257 and Lys287 [16]. Therefore, methylsulfonyl group was converted to acetyl group and hydroxyl group to change the interaction mode of the hydrogen bond between Ar<sub>2</sub> with the protein to investigate the effect on the activity.

## Chemistry

Amide intermediate (**4**) was synthesized by condensation of carboxylic acid (**2**) with primary amine (**3**) in DMF in the presence of condensating agent HATU, which was deprotected Boc group to yield amide intermediate (**5**) (Scheme 1) [17]. The yield of condensation reaction is low due to steric size of compound **2** and **3**.

Analogues **1a–1e** were generated through the synthetic route outlined in Scheme 2. Amide intermediate (**5**) was treated with benzofuran-6-sulfonyl chloride (**10**), benzofuran-6-ylmethanamine (**14**), 2-(benzofuran-5-yl) acetic acid (**16**) [18], [1,2,4]triazolo[1,5-a]pyridine-6-carboxylic acid (**18**) [19] and 3-ethynylbenzoic acid **20** [20] in DCM in the presence of condensating agent HATU or CDI

to yield benzyl group analogues **11**, **15**, **17**, **19** and **21**, which provided analogues **1a–1e** in the presence of debenzoylation agent H<sub>2</sub> or TMSOK in MeOH or THF through removing the benzyl group [21]. The condensation activity of carboxyl with amino is stronger than that of amine with amino group, so the yield of compound **11**, **17**, **19**, **21** was higher than that of compound **15**.

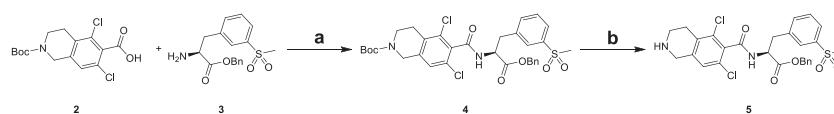
Williamson reaction of 2-bromo-1,1-diethoxyethane (**6**) with 3-bromophenol (**7**) yielded 1-bromo-3-(2,2-diethoxyethoxy)benzene (**8**), which was cyclized to the corresponding 6-bromobenzofuran (**9**) by heating in toluene containing cyclizing agent PPA. Sulfonylation of intermediate **9** by treatment with sulfonyl chloride in THF catalyzed by halogen-lithium exchange reagent n-BuLi gave the respective benzofuran-6-sulfonyl chloride (**10**) (Scheme 3) [22]. The sulfonation of intermediate **9** first involved the exchange of halogen atom to lithium atom, and then lithium atom was replaced by sulfonyl group. The complexity of the reaction mechanism led to the low yield of this reaction.

6-bromobenzofuran (**12**) was treated with zinc cyanide and cyanation catalyst tetrakis(triphenylphosphine)palladium to give benzofuran-6-carbonitrile (**13**), which was reduced by reducing agent lithium aluminum hydride yield benzofuran-6-ylmethanamine (**14**) (Scheme 4) [23]. The cyanidation reaction (**13**) and reduction reaction (**14**) were both classical reactions, the yields of this two reaction were normal.

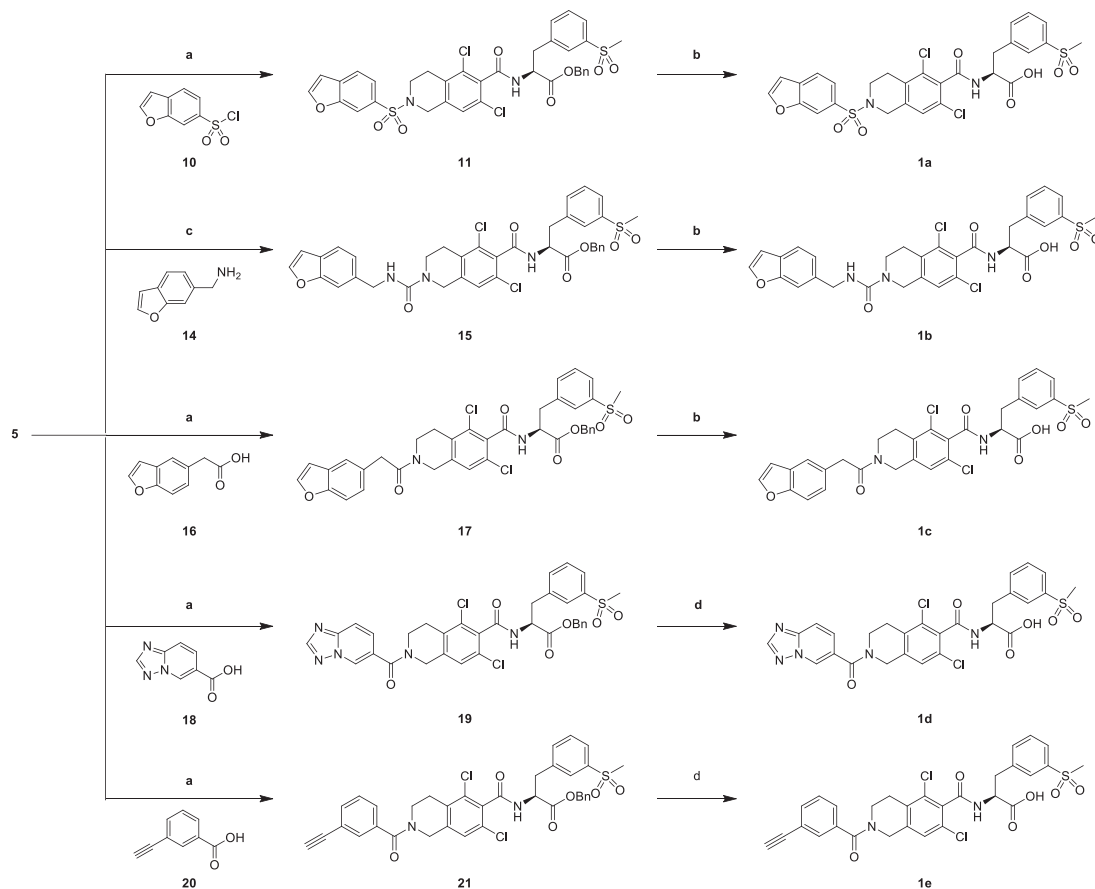
Condensation reaction of benzofuran-6-carboxylic acid (**22**) with methyl 5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (**23**) by condensating agent EDCI yielded carboxylic acid methyl ester intermediate (**24**), which was hydrolyzed to the corresponding carboxylic acid intermediate (**25**) by heating in pyridine containing hydrolysis reagent lithium iodide (Scheme 5) [24]. The hydrolysis of methyl carboxylate (**24**) involved the formation of methyl iodide and carboxylic acid lithium, then the reaction yield was slightly lower.

Analogues **1f–1h** were generated through the synthetic route outlined in Scheme 6. Carboxylic acid intermediate (**25**) was treated with ethyl 2-amino-3-(quinolin-6-yl)propanoate (**30**), ethyl 2-amino-3-(1,1-dioxido-2,3-dihydrobenzo[b]thiophen-6-yl)propanoate (**41**), ethyl 2-amino-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)propanoate (**50**) in DCM in the presence of condensating agent HATU to yield carboxylic acid ethyl ester analogues **31**, **42**, **51** which provided analogues **1f–1h** in the presence of hydrolysis reagent sodium hydroxide in EtOH, H<sub>2</sub>O and THF mixed solvent. The hydrolysis of ethyl carboxylate (**31**, **42**, **51**) is more difficult than that of methyl carboxylate (**24**), the yield is lower than that of methyl carboxylate.

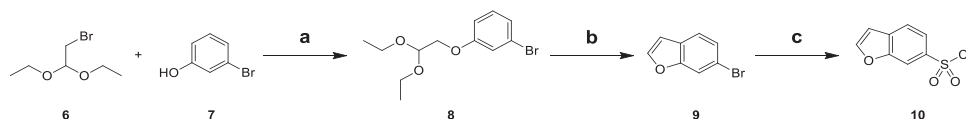
Quinoline-6-carbaldehyde (**26**) was reduced in methanol in the presence of reducing agent sodium borohydride to



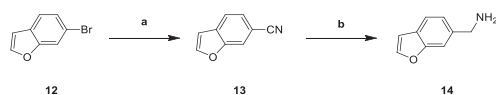
**Scheme 1** Reagents and conditions: a HATU, TEA, DMF, 20 °C, 16 h, 25.2%; b HCl, EtOAc, 20 °C, 3 h



**Scheme 2** Reagents and conditions: a TEA, HATU, DCM, 20 °C, 3 h, 69.8–87.2%; b H<sub>2</sub>, Raney-Ni, MeOH, 25 °C, 3 h, 9.42–33.4%; c CDI, DIEA, DCM, 0–25 °C, 1 h, 9.42%; d TMSOK, THF, 70 °C, 1 h, 22.0–22.2%



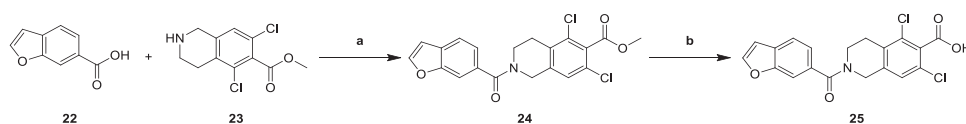
**Scheme 3** Reagents and conditions: a K<sub>2</sub>CO<sub>3</sub>, DMF, 100 °C, 10 h, 47.8%; b PPA, Toluene, 80 °C, 4 h, 40.3%; c n-BuLi, SO<sub>2</sub>, Sulfuryl Chloride, THF, –78 °C, 12 h, 22.7%



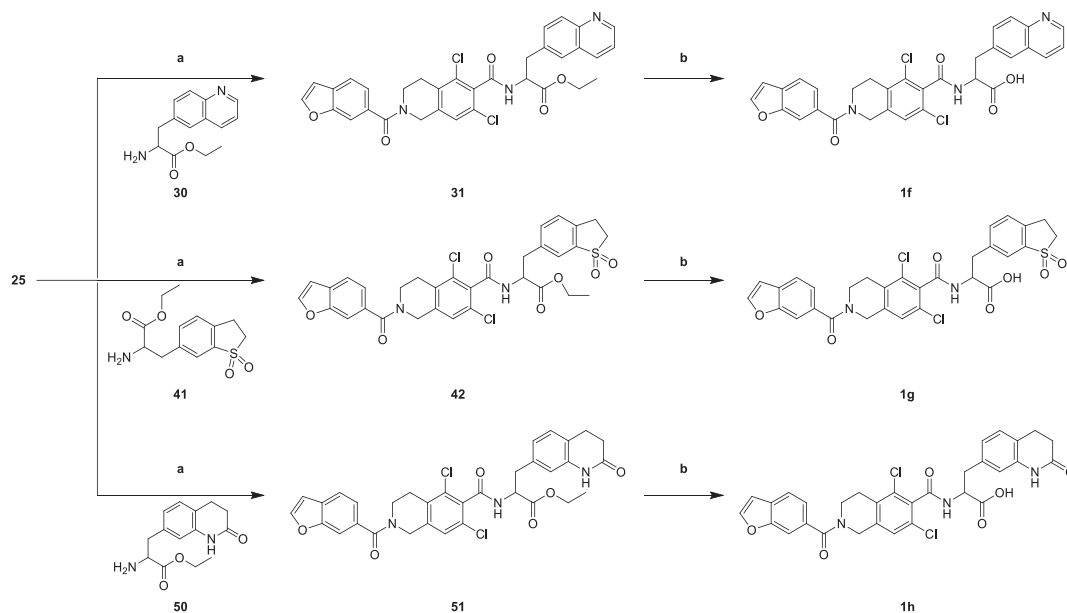
**Scheme 4** Reagents and conditions: a Zn(CN)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, DMF, 80 °C, 16 h, 68.8%; b LiAlH<sub>4</sub>, THF, 20 °C, 1 h, 48.6%

yield 6-Hydroxymethyl-quinoline (**27**), which provided 6-(bromomethyl)quinoline (**28**) in acetic acid in the presence of hydrogen bromide. The activity of hydrogen bromide as

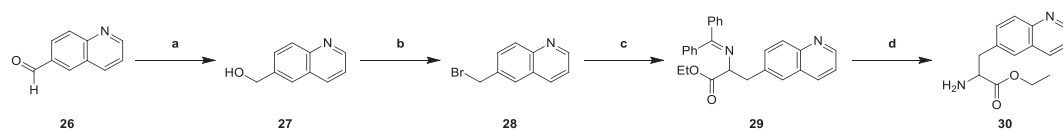
a halogenated agent generally resulted in a slightly lower yield of halogenated reaction (**28**). The intermediate (**28**) was treated with ethyl N-(diphenylmethylene)glycinate in THF in the presence of alkylation catalyst sodium hydride to yield ethyl 2-((diphenylmethylene)amino)-3-(quinolin-6-yl)propanoate (**29**), which was hydrolyzed in ethyl acetate in the presence of hydrolysis reagent hydrochloric acid to yield ethyl 2-amino-3-(quinolin-6-yl)propanoate (**30**)



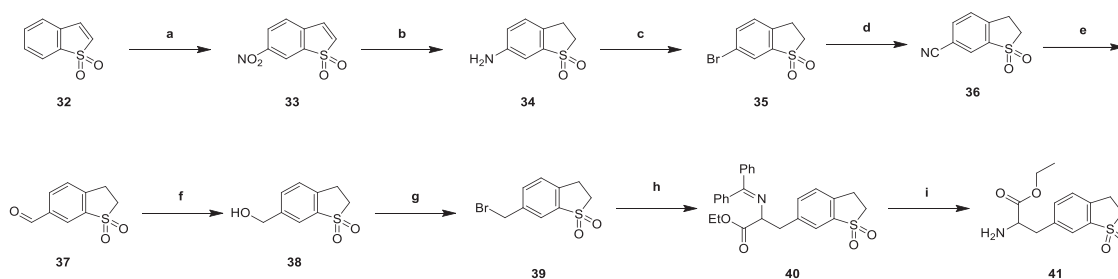
**Scheme 5** Reagents and conditions: a EDCI, DMAP, TEA, DMF, 25 °C, 15 h, 58.6%; b LiI, Pyridine, 100 °C, 3 h, 48.1%



**Scheme 6** Reagents and conditions: a HATU, TEA, DMAP, DCM, 20–30 °C, 20.5 h, 37.7–38.9%; b NaOH, EtOH/H<sub>2</sub>O/THF = 2/1/1, 20 °C, 2 h, 12.0–12.8%



**Scheme 7** Reagents and conditions: a NaBH<sub>4</sub>, MeOH, 0–15 °C, 3 h, 65.8%; b HBr, AcOH, 20 °C, 3 h, 37.5%; c Ethyl N-(diphenylmethylene)glycinate, NaH, THF, 5–20 °C, 20.5 h, 70.4%; d HCl, EtOAc, 20 °C, 0.5 h, 66.8%

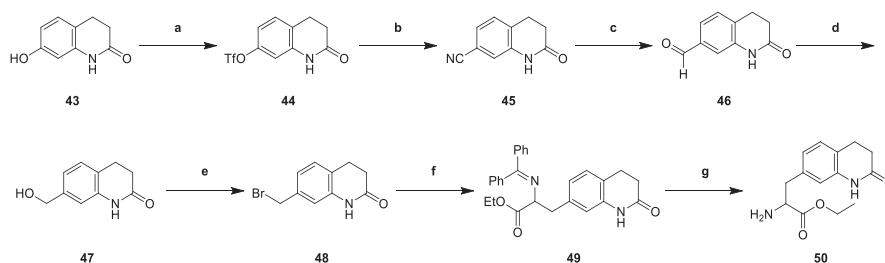


**Scheme 8** Reagents and conditions: a HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub> (1/1), 0 °C, 1 h, 70.8%; b Pd/C, H<sub>2</sub>, MeOH, 50 °C, 12 h, 64.0%; c NaNO<sub>2</sub>, CuBr, HBr, 0–70 °C, 1 h, 59.1%; d Zn(CN)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, DMF, 100 °C, 16 h, 63.9%; e Ni, HCOOH, 100 °C, 2 h, 88.6%; f NaBH<sub>4</sub>, MeOH, 0–15 °C, 3 h, 49.4%; g HBr, AcOH, 20 °C, 2 h, 60.7%; h Ethyl N-(diphenylmethylene)glycinate, NaH, THF, 0–20 °C, 16 h, 59.5%; i HCl, EtOAc, 20 °C, 1 h, 94.7%

(Scheme 7) [25]. Imine (**29**) was hydrolyzed to primary amine (**30**) under hydrochloric acid with normal yield.

2-amino-3-(1,1-dioxido-2,3-dihydrobenzo[*b*]thiophen-6-yl)ethyl propanoate (**41**) was generated from benzo[*b*]thiophene 1,1-dioxide (**32**) in Scheme 8. 6-nitrobenzo[*b*]

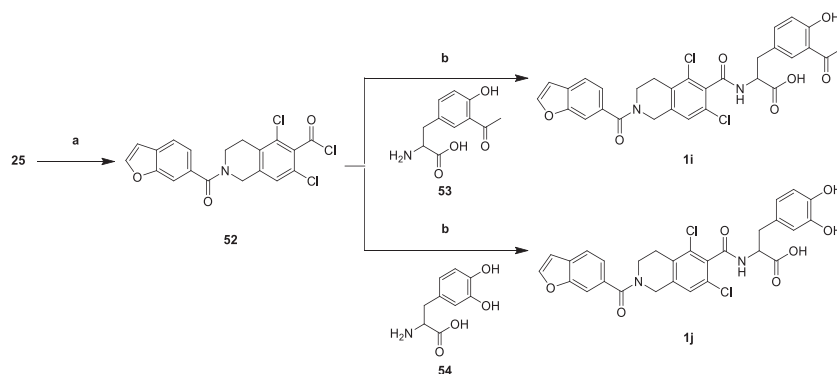
thiophene 1,1-dioxide (**33**) was obtained by treating compound (**32**) with nitric and sulfuric acid. This nitration reaction (**33**) was a classic reaction, and the yield is in normal range. The nitro group is reduced to an amine group to give 6-amino-2,3-dihydrobenzo[*b*]thiophene 1,1-dioxide



**Scheme 9** Reagents and conditions: a)  $(\text{Tf})_2\text{O}$ , Py,  $\text{CHCl}_3$ , 0–25 °C, 40 min, 55.2%; b)  $\text{Zn}(\text{CN})_2$ ,  $\text{Pd}(\text{PPh}_3)_4$ , DMF, 80 °C, 12 h, 55.2%; c) Ni,  $\text{HCOOH}$ , 100 °C, 2 h, 58.9%; d)  $\text{NaBH}_4$ , MeOH, 15 °C, 3 h, 49.4%; e)

HBr, AcOH, 25 °C, 0.5 h, 33.9%; f) Ethyl N-(diphenylmethylene)glycinate, NaH, THF, 0–25 °C, 5 h, 61.9%; g) HCl, EtOAc, 0–25 °C, 0.5 h, 88.6%

**Scheme 10** Reagents and conditions: a)  $\text{SOCl}_2$ , 70 °C, 2 h; b) TEA, DMF, 70 °C, 2 h, 5.19–5.43%



(**34**), which is replaced by the bromine atom to form 6-bromo-2,3-dihydrobenzo[*b*]thiophene 1,1-dioxide (**35**). The activity of substituting amino group (**34**) with hydrogen bromide as halogenated reagent was higher than that of substituting hydroxyl group (**27**). Zinc cyanide reacted with intermediate (**35**) to produce 2,3-dihydrobenzo[*b*]thiophene-6-carbonitrile 1,1-dioxide (**36**), which was reduced to give 2,3-dihydrobenzo[*b*]thiophene-6-carbaldehyde 1,1-dioxide (**37**). 2-amino-3-(1,1-dioxido-2,3-dihydrobenzo[*b*]thiophen-6-yl)ethyl propanoate (**41**) was synthesized from intermediate (**37**) through reduction reaction, halogenating reaction, hydrocarbonylation reaction and hydrolysis reaction [26]. These reactions belonged to the classical reactions, the yields were in the normal range.

2-amino-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)ethyl propanoate (**50**) was generated from 7-hydroxy-3,4-dihydroquinolin-2(1H)-one (**43**) in Scheme 9. The hydroxyl group of compound (**43**) was esterified by trifluoroacetic anhydride to give 2-oxo-1,2,3,4-tetrahydroquinolin-7-yl trifluoromethanesulfonate (**44**). Zinc cyanide reacted with intermediate (**44**) to produce 2-oxo-1,2,3,4-tetrahydroquinoline-7-carbonitrile (**45**), which was reduced to give 2-oxo-1,2,3,4-tetrahydroquinoline-7-carbaldehyde (**46**). Since trifluoroacetate was an excellent leaving group, the cyanidation reaction yield was over 50%. 2-amino-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)ethyl propanoate (**50**) was synthesized from intermediate (**46**) through reduction reaction, halogenating reaction,

hydrocarbonylation reaction and hydrolysis reaction [27]. These reactions belonged to the classical reactions, the yields were in the normal range.

Analogues **1i–1j** were generated through the synthetic route outlined in Scheme 10. Acyl chloride intermediate (**52**) was obtained by treating carboxylic acid intermediate (**25**) with dichlorosulfoxide. Compound **52** was directly used in the next reaction without purification. Acyl chloride intermediate (**52**) was acylated with compound 3-(3-acetyl-4-hydroxyphenyl)-2-aminopropanoic acid (**53**) and 2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (**54**) in the presence of TEA in DMF to yield analogues **1i** and **1j**, respectively. The yields of condensations (**1i**, **1j**) were low because the carboxyl group at the  $\alpha$  position reduces the nucleophilicity of the amino group (**53**, **54**).

A total of 5 target “left-wing” analogues, 5 target “right-wing” analogues and more than 30 intermediates were successfully synthesized. All the designed target analogues were confirmed correct structure by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, LCMS and HRMS, and the LCMS content reached more than 95%. The structures of analogues are shown in Table 1 and the detailed synthesis process of each compound can be found in Supplementary Materials of this article.

### Cell attachment assay

The  $\text{IC}_{50}$  value activity test results were shown in Fig. 3 and Table 1. The  $\text{IC}_{50}$  value of Lifitegrast was about 10.5 nM



**Table 1** LFA-1/ICAM-1 antagonist activities of Lifitegrast analogues

left-wing **1a-1e**      central THIQ      right-wing **1f-1j**

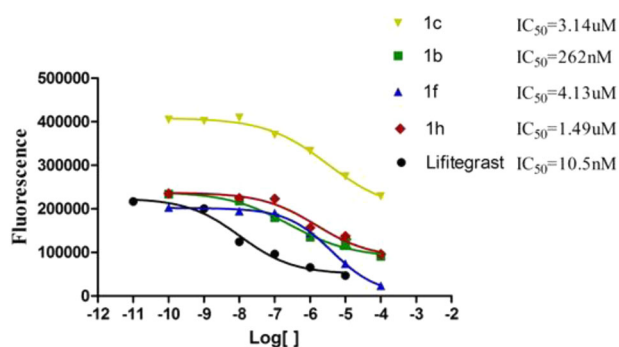
Compound	Ar <sub>1</sub>	X	Ar <sub>2</sub>	IC <sub>50</sub> (μM) <sup>1</sup>
<b>1a</b>				n.a.
<b>1b</b>				0.262
<b>1c</b>				3.14
<b>1d</b>				n.a.
<b>1e</b>				n.a.
<b>1f</b>				4.13
<b>1g</b>				n.a.
<b>1h</b>				1.49
<b>1i</b>				n.a.
<b>1j</b>				n.a.
Lifitegrast				0.011

n.a. no activity, Ar aromatic groups

<sup>1</sup>The IC<sub>50</sub> value is an average of three titrations with six concentration points

(Fig. 3) measured by our assay, this IC<sub>50</sub> value was very close to the previously reported IC<sub>50</sub> value of Lifitegrast (IC<sub>50</sub> = 2.98 nM and 9 nM in Jurkat or in HUT 78T cells, respectively) [9]. The activity test results showed that the IC<sub>50</sub> value of compound **1b** reached the level of 262 nM, and had a good LFA-1 inhibitory activity. The IC<sub>50</sub> of compounds **1c**, **1f** and **1h** also reached several micromol levels. Among them, **1f** and **1h** are racemes, and the activity of their S configuration will be higher.

From the results of Jurkat T-cell adhesion assay, we may summarize some rules about the relationship between the structures of the designed target compounds and LFA-1/ICAM-1 antagonist activities. With Lifitegrast as a lead compound, in the “left-wing” residue, changing carbonyl group in Lifitegrast to sulfone group (compound **1a**), or changing furyl to triazole (compound **1d**) or acetonyl (compound **1e**) decreased LFA-1/ICAM-1 antagonist activity obviously when compared with Lifitegrast. The



**Fig. 3** Titration curves of compounds **1c**, **1b**, **1f**, **1h** and Lifitegrast in the Jurkat T-cell adhesion assay. Compounds **1c**, **1b**, **1f** or **1h** inhibit the attachment of Jurkat cells to intercellular-adhesion molecule (ICAM)-1 in vitro, 1/10 serial dilutions of the compounds, and labeled Jurkat cells were incubated with either the compounds or Lifitegrast on plates containing captured ICAM-1. The solid lines are the fits of the data. The IC<sub>50</sub> values are provided in the legends

activity of compound **1a** showed that the carbonyl group could not be replaced by the more electron-absorbing sulfone group between benzofuran and tetrahydroisoquinoline, probably because the charge distribution of the whole molecule was affected. The charge distribution of triazole is quite different from that of furyl, which may contribute to the poor activity of compound **1d**. The ring structure of benzofuran was opened is the reason for the poor activity of compound **1e**, indicating that the ring structure plays an important role in the activity. While the carbonyl was changed to the N-methylene carbamoyl (compound **1b**) and methylene carbonyl (compound **1c**), the analogues show certain LFA-1/ICAM-1 antagonist activity. This result indicated that the length of the carbonyl between benzofuran and tetrahydroisoquinoline can be appropriately extended without adversely affecting the binding of the molecule to the target protein. The  $IC_{50}$  value of compound **1b** reached the level of 262 nM, this result suggested that the LFA-1/ICAM-1 antagonist activity can be maintained or even improved by only changing the X group to the other groups. In the “right-wing”  $Ar_2$  residue of the Lifitegrast, replacing methylsulfonyl benzene with quinoline (compound **1f**), 1-dioxido-2,3-dihydrobenzothiophen (compound **1g**), 2-oxo-1,2,3,4-tetrahydroquinolin (compound **1h**), 3-acetyl-4-hydroxyphenyl (compound **1i**) or 3,4-dihydroxyphenyl (compound **1j**) respectively decreased LFA-1/ICAM-1 antagonist activity. The cyclization of the sulfone group with benzene (compound **1g**) resulted in a loss of activity because of the influence on the binding mode of the sulfone group to the target protein. Meanwhile, the  $IC_{50}$  value of compounds **1f** and **1h** also reached several micromol levels. These results showed that replacing the  $Ar_2$  residue with benzene six-membered ring could not improve the LFA-1/ICAM-1 antagonist activity, this may be related to increasing the size of the  $AR_2$  group. Conversion of methylsulfonyl group to acetyl or hydroxyl groups on benzene ring also could not produce good activity. This result indicated that adding hydroxyl groups to the benzene ring did not increase the hydrogen bond interaction between the analogues and the target protein.

## Animal experiments

### Compound **1b** reduced the damage of corneal epithelium in dry eye mice model

After the 14 days of desiccating stress and drug-induction, we established a dry eye model that causes dry eye symptoms closer to the symptoms in patients with chronic DED [28]. Then we evaluated if compound **1b** could improve the permeability barrier integrity of the ocular surface in this animal model.

Fluorescein staining in Fig. 4 showed punctate staining of green on the corneal surfaces in the experimental and control groups at day 14. We can see that the fluorescence dyeing areas on the ocular surface were significantly increased in the dry eye model group (B, EDE group) compared with the blank group (A, control group). Treatment with 5% or 7% compound **1b** eye drops (E, F groups) improved the damage of corneal epithelium with less fluorescence dyeing areas, and treatment with 5% Lifitegrast (C group) was used as the positive control, which reveals the prominent therapeutically effects on DED.

### Compound **1b** enhanced the down-regulated goblet cells in dry eye mice model

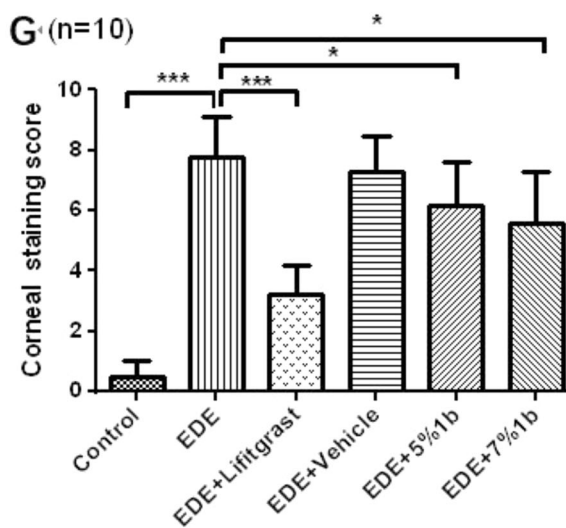
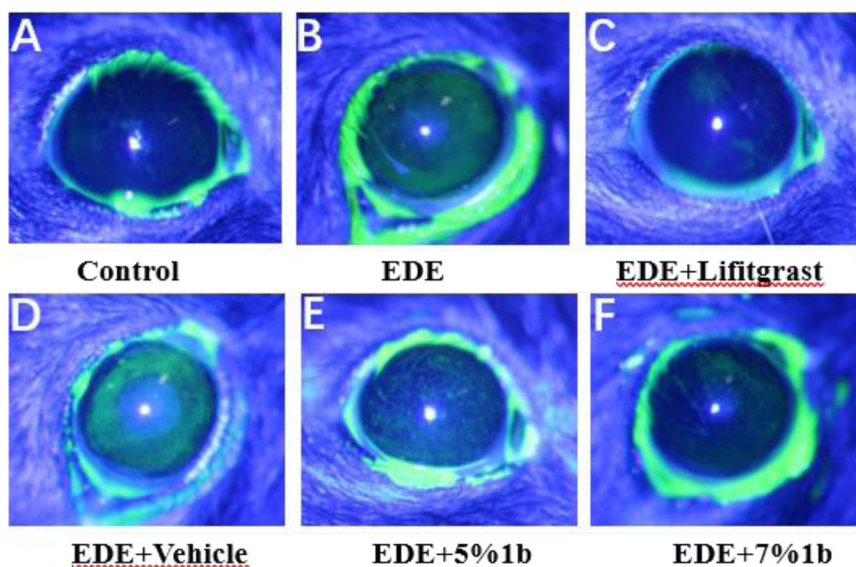
The conjunctival goblet cells were distributed mainly among the fornix epithelial cells. Goblet cells could secrete the mucin and keep the tear film stable. The symptoms of dry eye are usually accompanied by the loss of goblet cells [29]. Therefore, we assessed the effects of compound **1b** on goblet cells in dry eye mice model. As shown in Fig. 5, The number of goblet cells from the superior and inferior conjunctiva were markedly decreased in dry eye model group (B) compared with blank group (A, control group). Treatment with 5% or 7% compound **1b** (E and F groups) significantly increased the density of goblet cells compared with the dry eye model group (B). It is obvious that compound **1b** prevented the missing of the goblet cells in dry eye mice.

### Compound **1b** inhibited the apoptosis of corneal epithelial cells in dry eye mice model

In order to investigate if compound **1b** has anti-apoptotic effects to protect the ocular surface damage in dry eye mice, we detected the signals of apoptosis on corneal epithelium by using TUNEL staining method in dry eye mice model with or without compound **1b** treatment. TUNEL immunofluorescence staining is a method to analyze apoptosis at a late stage by detecting DNA fragmentation through labeling the terminal end of nucleic acids in the cells. The results of TUNEL immunofluorescence staining in Fig. 6 demonstrated that compared to control group, mean numbers of corneal epithelium cells which had the signals of TUNEL staining with Cy3 were significantly increased in the dry eye model groups (EDE, and EDE + Vehicle groups). In compound **1b** treatment group (EDE + 5% **1b**, or EDE + 7% **1b**), the amount of cells labeled with Cy3 were markedly decreased in cornea tissues when compared with that of dry eye model group, and the quantitative statistical analysis data of TUNEL positive cells in each group was shown in Fig. 6G. But the anti-apoptotic effect of compound **1b** was weaker than that of Lifitegrast treatment



**Fig. 4** Effect of compound **1b** on ocular surface damage in dry eye mice. After 14 days treatment on dry eye mice model, ocular surface of mice were stained with fluorescein sodium, the green fluorescence staining points represent the damaged area of ocular surface epithelium (A–F), and the corneal fluorescence staining score in graph A–F were calculated and shown in a bar graph (G). Data were shown as mean  $\pm$  SEM ( $n = 10$ , eyes/group). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Control: non-treated normal mice group; EDE: experimental dry eye mice group; EDE + Lifitgrast: experimental dry eye mice treated with Lifitgrast; EDE + Vehicle: experimental dry eye mice treated with control solvent; EDE + 5%**1b**: experimental dry eye mice treated with 5% compound **1b**; EDE + 7%**1b**: experimental dry eye mice treated with 7% compound **1b**



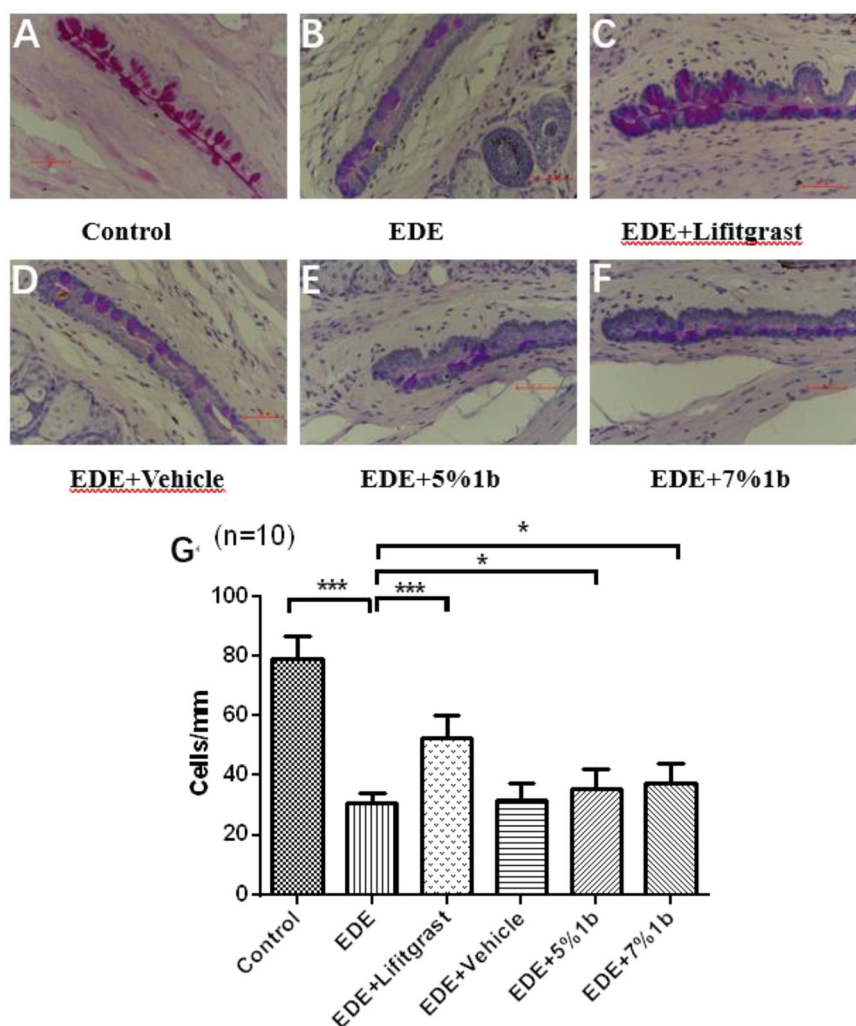
group. These results in Fig. 6 suggested that treatment with compound **1b** could protect corneal and conjunctival epithelium cells from apoptosis, maintained the integrity of ocular epithelium in dry eye mice.

The Lifitegrast eye drops showed a statistically significant reduction in ocular surface injury compared to the model control group, and the effect was stronger than compound **1b**. As shown in Fig. 4, 5% and 7% compound **1b** significantly reduced ocular surface epithelial cell damage in dry eye mice. 5% and 7% compound **1b** reduced the damage of corneal epithelium in dry eye mice model. The conjunctiva goblet cells secrete the mucin to maintain the stability of the tear film. The symptoms of dry eye are usually accompanied by a decreasing of the number or density of goblet cells. We evaluated the effect of 5% and 7% compound **1b** on goblet cells in dry eye mice model. A statistical histogram of goblet cell density is shown in

Fig. 5. 5% and 7% compound **1b** significantly increased goblet cell density and improved dry eye symptoms in mice with dry eye. As shown in Fig. 6, 5% and 7% compound **1b** has a protective effect on apoptosis of ocular surface cells in dry-eye mice, but the effect is weaker than that of Lifitegrast. The results from our in vivo animal model experiments showed that topical administration of 5% and 7% (W/V) compound **1b** improved the dry eye symptoms through reducing the ocular surface injuries, increasing the density of goblet cells on conjunctival epithelium and decreasing the apoptosis of cornea and conjunctiva. There was no statistical difference in the effect between 5% and 7% compound **1b**. There was no statistical difference in the effect between 5% and 7% compound **1b**. The in vitro assay showed the IC<sub>50</sub> value of compound **1b** reached the level of 262 nM which is lower than that of Lifitegrast (IC<sub>50</sub> value is about 11 nM). This result suggested that the LFA-1/ICAM-

**Fig. 5** Compound **1b** enhanced the density of goblet cells on conjunctival epithelium in dry eye mice after 14 days treatment. The results of histopathological evaluation of the goblet cells staining by periodic acid-schiff were shown in **A–F** images (size,  $\times 400$ ). The deep purple dyeing in conjunctiva represents goblet cells. The quantification of goblet cells densities in graph **A–F** was shown in **G** graph, data was shown as mean  $\pm$  SEM ( $n = 10$  eyes/group). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Control: non-treated normal mice group; EDE: experimental dry eye mice group; EDE + Lifitegrast: experimental dry eye mice treated with Lifitegrast; EDE + Vehicle: experimental dry eye mice treated with control solvent; EDE + 5%**1b**: experimental dry eye mice treated with 5% compound **1b**; EDE + 7%**1b**: experimental dry eye mice treated with 7% compound **1b**



**1** antagonist activity of compound **1b** may be weaker than that of Lifitegrast also in vivo, therefore, the anti-dry eye effects of compound **1b** is less as effective as Lifitegrast.

Although the effect of compound **1b** is weaker than Lifitegrast in vitro and in vivo, the structure of compound **1b** is different from Lifitegrast and circumvented the scope of Lifitegrast original patent. These results may serve as important milestone references for future structure optimization.

## Conclusions

In summary, in this study, 10 analogues with different groups in the “left-wing” or “right-wing” residues in Lifitegrast were designed according to molecular dynamics simulations and were synthesized through a variety of chemical methods. The Jurkat cell attachment assay was used to assess the LFA-1/ICAM-1 antagonist activity of these 10 analogues of Lifitegrast. Among them, the  $IC_{50}$  value of compound **1b** reached 262 nM. To further verify the biological activity of compound **1b** in vivo, dry eye mice model was developed to investigate

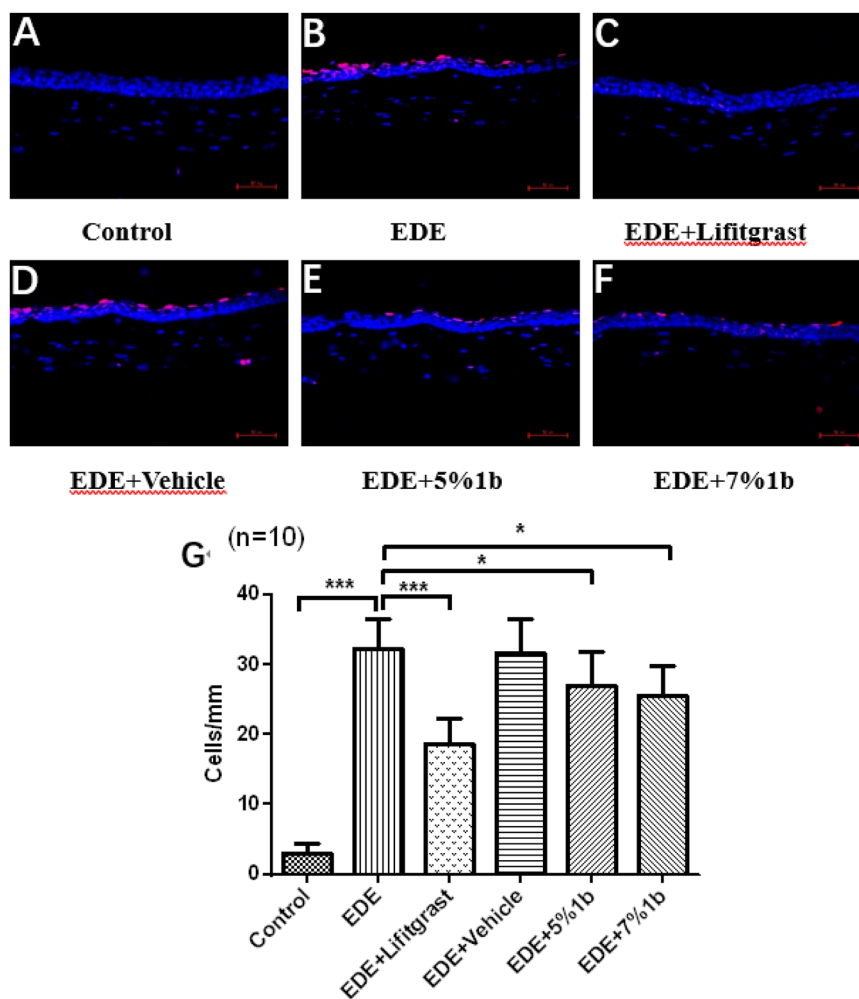
if compound **1b** could improve the symptoms of dry eye. The results of animal model demonstrated that 5%(W/V) compound **1b** significantly improved the symptoms of dry eye mice through reducing ocular surface epithelial cells damage, increasing goblet cell density and decreasing the apoptosis of cornea and conjunctiva in dry eye mice. The results of this study encourage us to further optimize the structure of compound **1b** in our future work, looking for a compound with a better LFA-1/ICAM-1 antagonist activity.

## Experimental

### Chemistry

All solvents and reagents were purchased from WuXi AppTec (Shanghai, China), Accela ChemBio Co., Ltd. (Shanghai, China), Titan Scientific Co.,Ltd. (Shanghai, China), and Hao Hong Pharma (Shanghai, China). Solvents and reagents were generally the best quality commercial-grade products and were used without further purification.  $^1H$  and  $^{13}C$  NMR spectra

**Fig. 6** Compound **1b** attenuated the apoptosis of corneal epithelium in dry eye mice model. The red fluorescence stain (TUNEL) represents apoptotic cells and the blue stain (DAPI) represents cell nucleus. The quantification of TUNEL positive staining cells in corneal epithelium were shown in **G** graphs, respectively. Data were shown as mean  $\pm$  SEM ( $n = 10$  eyes/group). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Control: non-treated normal mice group; EDE: experimental dry eye mice group; EDE + Lifitgrast: experimental dry eye mice treated with Lifitgrast; EDE + Vehicle: experimental dry eye mice treated with control solvent; EDE + 5%**1b**: experimental dry eye mice treated with 5% compound **1b**; EDE + 7%**1b**: experimental dry eye mice treated with 7% compound **1b**



were taken on Bruker Avance III 500 or 400 NMR spectrometers operating at 400 MHz for  $^1\text{H}$  NMR, and 100 MHz for  $^{13}\text{C}$  NMR, with tetramethylsilane (TMS) as the internal standard and  $\text{CDCl}_3$ , MeOD and  $\text{DMSO-d}_6$  as the solvent. Chemical shift values ( $\delta$ ) are reported in ppm relative to that of the internal TMS. The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and b = broad.  $^{13}\text{C}$  NMR spectra were recorded with complete proton decoupling. ESIMS data were recorded on Finnigan LCQ/DECA instruments. HRMS data were collected on Micromass QTOF Ultima (ESI) spectrometers. Silica gel  $\text{F}_{254}$  was used in analytical thin-layer chromatography (TLC), and silica gel was used in column chromatography; visualizations were accomplished with UV light (254 nm).

**(S)-2-(2-(tert-Butoxycarbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl) benzyl propanoate (4)**

A mixture of compound **2** (5.61 g, 16.2 mmol), compound **3** (4.50 g, 13.5 mmol), HATU (7.70 g, 20.2 mmol) and TEA (2.05 g, 20.25 mmol) in DMF (30 mL) was stirred at 20 °C

for 16 h. TLC showed new spot was formed.  $\text{H}_2\text{O}$  (30 mL) was added and the mixture was extracted with EtOAc (30 mL  $\times$  2). The organic layer was isolated and dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by flash silica gel chromatography. Compound **4** (2.20 g, 2.83 mmol, 25.2% yield) was obtained as light yellow gum.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.84 (s, 1H, CONH), 7.82–7.81 (m, 2H, Ar-H), 7.47–7.31 (m, 5H, Ar-H), 7.25–7.24 (s, 1H, Ar-H), 7.10–7.07 (m, 1H, Ar-H), 6.43–6.35 (m, 1H, Ar-H), 5.33–5.23 (m, 1H, CHCO), 5.22–5.16 (m, 2H,  $\text{CH}_2\text{N}$ ), 4.57 (s, 2H,  $\text{CH}_2$ , Bn), 3.64 (t,  $J = 6.0$  Hz, 2H,  $\text{CH}_2\text{N}$ ), 3.40 (m, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 3.34–3.24 (m, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 2.99 (s, 3H,  $\text{CH}_3\text{SO}_2$ ), 2.81–2.75 (m, 2H,  $\text{CH}_2\text{CH}$ ), 1.48 (s, 9H,  $\text{CH}_3$ , Boc).

**(S)-2-(5,7-Dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl) benzyl propanoate (5)**

To a solution of compound **4** (2.20 g, 3.33 mmol) in EtOAc (10.0 mL), HCl/EtOAc (4M, 15 mL) was added. The



mixture was stirred at 20 °C for 3 h. TLC showed new spot was formed. The residue was concentrated under reduced pressure. Compound **5** (1.80 g, crude, HCl) was obtained as white solid.  $^1\text{H NMR}$  (MeOD, 400 MHz)  $\delta$  7.91 (s, 1H, Ar-H), 7.82 (d,  $J = 7.8$  Hz, 1H, Ar-H), 7.64 (d,  $J = 7.8$  Hz, 1H, Ar-H), 7.58–7.51 (m, 1H, H-4, Ar-H), 7.42–7.29 (m, 5H, Ar-H), 7.31 (s, 1H, Ar-H), 5.22 (d,  $J = 4.4$  Hz, 2H,  $\text{CH}_2\text{N}$ ), 5.16 (m, 1H, CHCO), 4.36 (s, 2H,  $\text{CH}_2$ , Bn), 3.56–3.50 (m, 2H,  $\text{CH}_2\text{N}$ ), 3.49–3.41 (m, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 3.21–3.12 (m, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 3.07 (s, 3H,  $\text{CH}_3\text{SO}_2$ ), 3.01 (t,  $J = 6.4$  Hz, 2H,  $\text{CH}_2\text{CH}$ ), 2.01 (s, 1H,  $\text{CH}_2\text{NHCH}_2$ ).

**(S)-2-(2-(Benzofuran-6-ylsulfonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)benzyl propanoate (11)**

To a solution of compound **5** (0.300 g, 501  $\mu\text{mol}$ , HCl) in DCM (3.00 mL), TEA (152 mg, 1.51 mmol) and HATU (286 mg, 752  $\mu\text{mol}$ ) were added. The solution was stirred at 20 °C for 3 h, then compound **10** (108 mg, 501  $\mu\text{mol}$ ) was added via several portions. The reaction mixture was stirred at 20 °C for 2 h under  $\text{N}_2$  atmosphere. LCMS showed starting material was consumed completely. The reaction was quenched with water (3.00 mL), and the organic was extracted with DCM (3.00 mL  $\times$  3), then the organic layer was concentrated under reduced pressure. Compound **11** (0.260 g, 350  $\mu\text{mol}$ , 69.8% yield) was obtained as a brown solid.  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.01 (s, 1H, CONH), 7.81–7.85 (m, 1H,  $\text{O}^*\text{CH}=\text{CH}$ ), 7.81–7.85 (m, 1H, Ar-H), 7.77–7.81 (m, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.67–7.71 (m, 1H, Ar-H), 7.40–7.43 (m, 2H, Ar-H), 7.36–7.27 (m, 5H, Ar-H), 7.02 (s, 1H, Ar-H), 6.88 (d,  $J = 1.6$  Hz, 1H,  $^*\text{CH}=\text{CHO}$ ), 6.39 (d,  $J = 7.6$  Hz, 1H, Ar-H), 5.22–5.29 (m, 1H, CHCO), 5.19 (d,  $J = 6.0$  Hz, 2H,  $\text{CH}_2\text{N}$ ), 4.20 (s, 2H,  $\text{CH}_2$ , Bn), 3.35–3.39 (m, 2H,  $\text{CH}_2\text{N}$ ), 3.24–3.31 (m, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 2.93 (s, 3H,  $\text{CH}_3\text{SO}_2$ ), 2.86 (m, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 2.18 (m, 2H,  $\text{CH}_2\text{CH}$ ); LCMS  $m/z$  741.0  $[\text{M} + \text{H}]^+$ .

**(S)-2-(2-(Benzofuran-6-ylsulfonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)propanoic acid (1a)**

To a solution of compound **11** (0.260 g, 350  $\mu\text{mol}$ ) in MeOH (1.00 mL), Raney-Ni (30.0 mg, 350  $\mu\text{mol}$ ) was added under  $\text{N}_2$ . The suspension was degassed under vacuum and purged with  $\text{H}_2$  several times. Then the mixture was stirred under  $\text{H}_2$  (15 psi) at 25 °C for 3 h. TLC indicated starting material was consumed completely. After that, the suspension was filtered through a pad of Celite and the pad was washed with EtOH (2.00 mL  $\times$  3). The combined filtrates were concentrated to dryness to give product, and the

crude product was purified by prep-HPLC (HCl condition). Compound **1a** (0.022 g, 33.0  $\mu\text{mol}$ , 9.42% yield, 97.7% purity) was obtained as a white solid.  $^1\text{H NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  12.88 (s, 1H, COOH), 9.02 (d,  $J = 8.4$  Hz, 1H, CONH), 8.29 (d,  $J = 2.4$  Hz, 1H,  $\text{O}^*\text{CH}=\text{CH}$ ), 8.09 (s, 1H, Ar-H), 7.93 (d,  $J = 8.0$  Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.76 (d,  $J = 7.6$  Hz, 1H, Ar-H), 7.72 (m, 1H, Ar-H), 7.63 (m, 1H, Ar-H), 7.53–7.59 (m, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 7.15 (d,  $J = 1.2$  Hz, 1H,  $^*\text{CH}=\text{CHO}$ ), 4.72–4.81 (m, 1H, CHCO), 4.22 (s, 2H,  $\text{CH}_2\text{N}$ ), 3.75 (m, 2H,  $\text{CH}_2\text{N}$ ), 3.28 (dd,  $J = 14.4$ , 4.4 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 3.14 (s, 3H,  $\text{CH}_3\text{SO}_2$ ), 3.00 (dd,  $J = 14.0$ , 10.6 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 2.73 (m, 2H,  $\text{CH}_2\text{CH}$ ).  $^{13}\text{C NMR}$  ( $\text{DMSO}-d_6$ , 101 MHz)  $\delta$  172.514 (1C, COOH), 163.885 (1C, CONH), 153.768 (1C, Ar), 150.431 (1C,  $\text{O}^*\text{CH}=\text{CH}$ ) 141.065 (1C, Ar), 139.534 (1C, Ar), 136.104 (1C, Ar), 135.129 (1C, Ar), 134.905 (1C, Ar), 132.240 (1C, Ar), 131.886 (1C, Ar), 131.497 (1C, Ar), 130.991 (1C, Ar), 129.720 (1C, Ar), 128.731 (1C, Ar), 128.211 (1C, Ar), 126.218 (1C, Ar), 125.503 (1C, Ar), 122.824 (1C, Ar), 122.412 (1C, Ar), 111.710 (1C, Ar), 107.608 (1C,  $^*\text{CH}=\text{CHO}$ ), 53.490 (1C,  $^*\text{CHCO}$ ), 47.395 (1C,  $\text{CH}_2\text{N}$ ), 44.066 (1C,  $\text{CH}_2\text{N}$ ), 43.474 (1C,  $\text{CH}_3\text{SO}_2$ ), 36.765 (1C,  $^*\text{CH}_2\text{CH}$ ), 26.951 (1C,  $^*\text{CH}_2\text{CH}_2\text{N}$ ). LCMS  $m/z$  651.0  $[\text{M} + \text{H}]^+$ ; HR-MS  $m/z$  calcd. for  $\text{C}_{28}\text{H}_{24}\text{Cl}_2\text{N}_2\text{O}_8\text{S}_2$  (650.04) 651.0  $[\text{M} + \text{H}]^+$ , found: 651.0436  $[\text{M} + \text{H}]^+$ .

**(S)-2-(2-((Benzofuran-6-ylmethyl)carbamoyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)benzyl propanoate (15)**

CDI (220 mg, 1.36 mmol) was dissolved in DCM (3.00 mL) and cooled to 0 °C. A solution of compound **14** (0.20 g, 1.36 mmol) and DIEA (193 mg, 1.49 mmol) in DCM (3.00 mL) was added, then the reaction mixture was warmed to 25 °C for 0.5 h. A solution of compound **5** (763 mg, 1.28 mmol) and DIEA (176 mg, 1.36 mmol) in DCM (5.00 mL) was added and the reaction mixture was stirred for 0.5 h at 25 °C. LCMS showed the desired MS was detected. The reaction mixture was diluted with DCM (10.0 mL), washed with 1 M aq.  $\text{Na}_2\text{CO}_3$  (2  $\times$  25.0 mL), dried  $\text{MgSO}_4$  and concentrated in vacuum. Compound **15** (0.022 g, 33.0  $\mu\text{mol}$ , 9.42% yield) was obtained as a light yellow solid.  $^1\text{H NMR}$  (MeOD, 400 MHz)  $\delta$  9.01 (s, 1H, CONH), 7.93 (s, 1H, Ar-H), 7.84 (d,  $J = 7.8$  Hz, 1H, Ar-H), 7.72 (d,  $J = 2.0$  Hz, 1H,  $\text{O}^*\text{CH}=\text{CH}$ ), 7.67–7.63 (m, 1H, Ar-H), 7.58–7.52 (m, 2H, Ar-H), 7.44 (m, 1H, Ar-H), 7.42–7.34 (m, 5H, Ar-H), 7.23 (s, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 6.81 (d,  $J = 1.4$  Hz, 1H,  $^*\text{CH}=\text{CHO}$ ), 5.23 (d,  $J = 3.4$  Hz, 2H,  $\text{CH}_2\text{N}$ ), 5.20–5.12 (m, 1H, CHCO), 4.58 (s, 2H,  $\text{CH}_2$ , Bn), 4.50 (s, 2H,  $\text{CH}_2$ ,  $\text{CH}_2\text{NH}$ ), 3.70 (br t,  $J = 5.8$  Hz, 2H,  $\text{CH}_2\text{NH}$ ), 3.45 (dd,  $J = 5.4$ , 14.2 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ),

3.40–3.36 (m, 1H,  $^*CH_2CH_2N$ ), 3.22–3.11 (m, 1H, NHCH<sub>2</sub>), 3.08 (s, 3H, CH<sub>3</sub>SO<sub>2</sub>), 2.80 (t, J = 5.8 Hz, 2H, CH<sub>2</sub>CH); LCMS *m/z* 734.0[M + H]<sup>+</sup>.

**(S)-2-(2-((Benzofuran-6-ylmethyl)carbamoyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)propanoic acid (1b)**

To a solution of compound **15** (0.200 g, 272 μmol) in MeOH (5.00 mL), Raney-Ni (46.6 mg, 544 μmol) was added under N<sub>2</sub>. The suspension was degassed under vacuum and purged with H<sub>2</sub> several times. The mixture was stirred under H<sub>2</sub> (15 psi) at 25 °C for 1 h. LCMS indicated starting material was consumed completely and one major new peak with desired mass was detected. The suspension was filtered through a pad of Celite and the pad was washed with EtOH (2.00 mL × 3). The combined filtrates were concentrated to dryness to give product. The crude product was purified by prep-HPLC (HCl condition). Compound **1b** (0.025 g, 37.8 μmol, 13.8% yield) was obtained as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 9.02 (d, J = 8.4 Hz, 1H, CONH), 7.93 (d, J = 2.0 Hz, 1H, O<sup>\*</sup>CH=CH), 7.87 (s, 1H, Ar-H), 7.77 (d, J = 7.6 Hz, 1H, Ar-H), 7.67 (d, J = 7.6 Hz, 1H, Ar-H), 7.59 (m, 1H, Ar-H), 7.54 (m, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.35 (s, 1H, NHCH<sub>2</sub>), 7.26 (s, 1H, Ar-H), 7.17 (d, J = 8.0 Hz, 1H, Ar-H), 6.90 (d, J = 1.2 Hz, 1H,  $^*CH=CHO$ ), 4.74–4.83 (m, 1H, CHCO), 4.53 (s, 2H, CH<sub>2</sub>N), 4.35 (d, J = 4.0 Hz, 2H, CH<sub>2</sub>NH), 3.63 (m, 2H, CH<sub>2</sub>N), 3.29 (dd, J = 14.0, 4.2 Hz, 1H,  $^*CH_2CH_2N$ ), 3.15 (s, 3H, CH<sub>3</sub>SO<sub>2</sub>), 3.01 (dd, J = 13.6, 10.8 Hz, 1H,  $^*CH_2CH_2N$ ), 2.66 (m, 2H, CH<sub>2</sub>CH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 101 MHz) δ 172.518 (1C, COOH), 164.076 (1C, CONH), 157.670 (1C, NHCON), 154.940 (1C, Ar), 146.260 (1C, O<sup>\*</sup>CH=CH), 141.103 (1C, Ar), 139.558 (1C, Ar), 138.770 (1C, Ar), 138.106 (1C, Ar), 134.914 (1C, Ar), 134.770 (1C, Ar), 132.394 (1C, Ar), 131.614 (1C, Ar), 129.721 (1C, Ar), 128.595 (1C, Ar), 128.219 (1C, Ar), 126.183 (1C, Ar), 125.937 (1C, Ar), 125.518 (1C, Ar), 122.832 (1C, Ar), 121.243 (1C, Ar), 110.223 (1C, Ar), 106.987 (1C,  $^*CH=CHO$ ), 53.531 (1C,  $^*CHCO$ ), 49.075 (1C, CH<sub>2</sub>N), 45.739 (1C, CH<sub>2</sub>N), 44.092 (1C, CH<sub>3</sub>SO<sub>2</sub>), 41.225 (1C, CH<sub>2</sub>NH), 36.820 (1C,  $^*CH_2CH$ ), 26.420 (1C,  $^*CH_2CH_2N$ ). LCMS *m/z* 644.1 [M + H]<sup>+</sup>; HR-MS *m/z* calcd. for C<sub>30</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>7</sub>S (643.09) 644.0 [M + H]<sup>+</sup>, found: 644.1075 [M + H]<sup>+</sup>.

**(S)-2-(2-(2-(Benzofuran-5-yl)acetyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)benzyl propanoate (17)**

To a solution of compound **5** (0.200 g, 334 μmol) in DCM (2.00 mL), TEA (101 mg, 1.00 mmol, 139 μL) and HATU

(191 mg, 502 μmol) were added, the solution was stirred at 20 °C for 1 h, then compound **16** (58.9 mg, 334 μmol) was added via several portions. The reaction mixture was stirred at 20 °C for 2 h under N<sub>2</sub> atmosphere. LCMS showed starting material was consumed completely and one major new peak with desired mass was detected. The solution was quenched with water (2.00 mL) and the organic was extracted with DCM (2.00 mL × 3), then organic was concentrated under reduced pressure. Compound **17** (0.210 g, 292 μmol, 87.2% yield) was obtained as a brown solid; LCMS *m/z* 719.1 [M + H]<sup>+</sup>.

**(S)-2-(2-(2-(Benzofuran-5-yl)acetyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)propanoic acid (1c)**

To a solution of compound **17** (97.9 mg, 136 μmol) in MeOH (1.00 mL), Raney-Ni (11.6 mg, 136 μmol) was added under N<sub>2</sub>. The suspension was degassed under vacuum and purged with H<sub>2</sub> several times. The mixture was stirred under H<sub>2</sub> (15 psi) at 25 °C for 3 h. TLC indicated starting material was consumed completely. The suspension was filtered through a pad of Celite and the filter cake was washed with MeOH (2.00 mL × 3). The combined filtrates were concentrated to dryness to give product. The crude product was purified by prep-HPLC (HCl condition). Compound **1c** (0.030 g, 45.5 μmol, 33.4% yield) was obtained as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.79 (d, J = 7.6 Hz, 1H, CONH), 7.90 (d, J = 2.1 Hz, 1H, O<sup>\*</sup>CH=CH), 7.86 (s, 1H, Ar-H), 7.77 (d, J = 7.6 Hz, 1H, Ar-H), 7.65 (d, J = 7.7 Hz, 1H, Ar-H), 7.57 (d, J = 7.55 Hz, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 7.47 (d, J = 8.4 Hz, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.19 (d, J = 8.4 Hz, 1H, Ar-H), 6.88 (d, J = 2.2 Hz, 1H,  $^*CH=CHO$ ), 4.85–4.75 (m, 1H, CHCO), 4.62 (s, 2H, CH<sub>2</sub>N), 3.88 (s, 2H, CH<sub>2</sub>N), 3.77 (s, 2H, CH<sub>2</sub>CO), 3.30 (dd, J = 14.0, 4.7 Hz, 1H,  $^*CH_2CH_2N$ ), 3.12 (s, 3H, CH<sub>3</sub>SO<sub>2</sub>), 3.05 (dd, J = 14.3, 10.0 Hz, 1H,  $^*CH_2CH_2N$ ), 2.67 (s, 2H, CH<sub>2</sub>CH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 101 MHz) δ ppm 172.512 (1C, COOH), 170.113 (1C, CONH), 169.982 (1C, CH<sub>2</sub>CON), 164.002 (1C, Ar), 153.663 (1C, O<sup>\*</sup>CH=CH), 146.721 (1C, Ar), 141.099 (1C, Ar), 139.546 (1C, Ar), 137.643 (1C, Ar), 134.909 (1C, Ar), 134.843 (1C, Ar), 130.680 (1C, Ar), 129.724 (1C, Ar), 128.798 (1C, Ar), 127.814 (1C, Ar), 126.013 (1C, Ar), 125.889 (1C, Ar), 125.524 (1C, Ar), 122.003 (1C, Ar), 121.857 (1C, Ar), 111.473 (1C, Ar), 111.379 (1C, Ar), 107.062 (1C,  $^*CH=CHO$ ), 53.512 (1C, CHCO), 44.077 (1C, CH<sub>2</sub>N), 43.756 (1C, CH<sub>3</sub>SO<sub>2</sub>), 42.779 (1C, CH<sub>2</sub>N), 38.973 (1C, CH<sub>2</sub>CON), 36.807 (1C,  $^*CH_2CH$ ), 27.262 (1C,  $^*CH_2CH_2N$ ). LCMS *m/z* 629.1 [M + H]<sup>+</sup>; HR-MS *m/z* calcd. for C<sub>30</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>7</sub>S (628.08) 629.0 [M + H]<sup>+</sup>, found: 629.0922 [M + H]<sup>+</sup>.

**(S)-2-(2-([1,2,4]Triazolo[1,5-a]pyridine-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)benzyl propanoate (19)**

To a solution of compound **5** (0.200 g, 334  $\mu\text{mol}$ ) in DCM (2.00 mL), TEA (101 mg, 1.00 mmol) and HATU (190 mg, 502  $\mu\text{mol}$ ) were added, the solution was stirred at 20 °C for 1 h, then compound **18** (54.6 mg, 334  $\mu\text{mol}$ ) was added via several portions. The reaction mixture was stirred at 20 °C for 2 h under  $\text{N}_2$  atmosphere. LCMS showed starting material was consumed completely and one major new peak with desired mass was detected. The solution was quenched with water (2.00 mL) and the organic was extracted with DCM (2.00 mL  $\times$  3), then the organic was concentrated under reduced pressure. Compound **19** (0.220 g, crude) was obtained as a brown solid; LCMS  $m/z$  706.1  $[\text{M} + \text{H}]^+$ .

**(S)-2-(2-([1,2,4]Triazolo[1,5-a]pyridine-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl) propanoic acid (1d)**

To a solution of compound **19** (0.200 g, 283  $\mu\text{mol}$ ) in THF (2.00 mL), TMSOK (109 mg, 849  $\mu\text{mol}$ ) was added, then the mixture was stirred at 70 °C for 1 h. LCMS indicated starting material was consumed completely and one major new peak with desired mass was detected. The mixture was quenched with water (2.00 mL) and acidified with 1 N HCl to pH = 3, the mixture was extracted with EtOAc (2.00 mL  $\times$  3), then the organic layer was concentrated under reduced pressure. The crude product was purified by prep-HPLC (HCl condition). Compound **1d** (0.04 g, 62.4  $\mu\text{mol}$ , 22.0% yield) was obtained as a white solid.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  9.20 (s, 1H, Ar-H), 9.03 (d,  $J$  = 8.0 Hz, 1H, CONH), 8.62 (s, 1H, CH=N), 7.93 (d,  $J$  = 9.2 Hz, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 7.77 (d,  $J$  = 7.6 Hz, 1H, Ar-H), 7.72 (d,  $J$  = 7.6 Hz, 1H, Ar-H), 7.67 (d,  $J$  = 7.6 Hz, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.54–7.60 (m, 1H, Ar-H), 4.77–4.82 (m, 1H, CHCO), 4.74 (s, 2H,  $\text{CH}_2\text{N}$ ), 3.74 (m, 2H,  $\text{CH}_2\text{N}$ ), 3.30 (dd,  $J$  = 14.0, 4.3 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 3.15 (s, 3H,  $\text{CH}_3\text{SO}_2$ ), 3.02 (dd,  $J$  = 14.0, 10.6 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 2.80 (m, 2H,  $\text{CH}_2\text{CH}$ ).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 101 MHz)  $\delta$  172.526 (1C, COOH), 164.002 (1C, CONH), 155.184 (1C, CON), 155.034 (1C, CH=N), 150.221 (1C, Ar), 141.099 (1C, Ar), 139.561 (1C, Ar), 137.220 (1C, Ar), 135.018 (1C, Ar), 134.931 (1C, Ar), 132.109 (1C, Ar), 131.525 (1C, Ar), 130.133 (1C, Ar), 129.724 (1C, Ar), 129.061 (1C, Ar), 128.820 (1C, Ar), 128.193 (1C, Ar), 126.217 (1C, Ar), 125.524 (1C, Ar), 123.337 (1C, Ar), 116.634 (1C, Ar), 53.52 (1C,  $^*\text{CHCO}$ ), 44.08 (1C,  $\text{CH}_2\text{N}$ ), 40.65 (1C,  $\text{CH}_3\text{SO}_2$ ), 40.44 (1C,  $\text{CH}_2\text{N}$ ), 40.02 (1C,  $^*\text{CH}_2\text{CH}$ ), 36.80 (1C,  $^*\text{CH}_2\text{CH}_2\text{N}$ ). LCMS  $m/z$

616.0  $[\text{M} + \text{H}]^+$ ; HR-MS  $m/z$  calcd. for  $\text{C}_{27}\text{H}_{23}\text{Cl}_2\text{N}_5\text{O}_6\text{S}$  (615.07) 616.0  $[\text{M} + \text{H}]^+$ , found: 616.0825  $[\text{M} + \text{H}]^+$ .

**(S)-2-(2-(3-Ethynylbenzoyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)benzyl propanoate (21)**

To a solution of compound **5** (0.200 g, 334  $\mu\text{mol}$ ) in DCM (2.00 mL), TEA (101 mg, 1.00 mmol) and HATU (190 mg, 501  $\mu\text{mol}$ ) were added, the solution was stirred at 20 °C for 1 h, then compound **20** (48.9 mg, 334  $\mu\text{mol}$ ) was added via several portions. The reaction mixture was stirred at 20 °C for 2 h under  $\text{N}_2$  atmosphere. LCMS showed starting material was consumed completely and one major new peak with desired mass was detected. The reaction was quenched with water (2.00 mL) and the organic was extracted DCM (2.00 mL  $\times$  3), then the organic was concentrated under reduced pressure. Compound **21** (0.200 g, 290  $\mu\text{mol}$ , 86.7% yield) was obtained as a brown solid; LCMS  $m/z$  689.1  $[\text{M} + \text{H}]^+$ .

**(S)-2-(2-(3-Ethynylbenzoyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)propanoic acid (1e)**

To a solution of compound **21** (0.200 g, 290  $\mu\text{mol}$ ) in THF (2.00 mL), TMSOK (111 mg, 870  $\mu\text{mol}$ ) was added, then the mixture was stirred at 70 °C for 1 h. LCMS indicated starting material was consumed completely, and new peak with desired mass was detected. The mixture was quenched with water (2.00 mL) and acidified with 1 N HCl to pH = 3, the mixture was extracted with EtOAc (2.00 mL  $\times$  3), then the organic layer was concentrated under reduced pressure. The crude product was purified by prep-HPLC (HCl condition). Compound **1e** (0.040 g, 64.4  $\mu\text{mol}$ , 22.2% yield) was obtained as a white solid.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  9.04 (d,  $J$  = 7.6 Hz, 1H, CONH), 7.86 (s, 1H, Ar-H), 7.77 (d,  $J$  = 7.6 Hz, 1H, Ar-H), 7.67 (d,  $J$  = 7.6 Hz, 1H, Ar-H), 7.54–7.61 (m, 2H, Ar-H), 7.49 (s, 1H, Ar-H), 7.45–7.54 (m, 1H, Ar-H), 7.40–7.45 (m, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 4.73–4.82 (m, 1H, CHCO), 4.68 (s, 2H,  $\text{CH}_2\text{N}$ ), 4.12 (s, 1H,  $\text{CH}\equiv\text{C}$ ), 3.69 (m, 2H,  $\text{CH}_2\text{N}$ ), 3.29 (dd,  $J$  = 14.0, 4.5 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 3.15 (s, 3H,  $\text{CH}_3\text{SO}_2$ ), 3.02 (dd,  $J$  = 14.0, 10.6 Hz, 1H,  $^*\text{CH}_2\text{CH}_2\text{N}$ ), 2.75 (m, 2H,  $\text{CH}_2\text{CH}$ ).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 101 MHz)  $\delta$  172.534 (1C, COOH), 164.010 (1C, CONH), 141.114 (1C, CON), 139.554 (1C, Ar), 137.300 (1C, Ar), 136.659 (1C, Ar), 135.00 (1C, Ar), 134.923 (1C, Ar), 133.458 (1C, Ar), 132.072 (1C, Ar), 131.576 (1C, Ar), 130.50 (1C, Ar), 130.410 (1C, Ar), 129.732 (1C, Ar), 129.564 (1C, Ar), 128.879 (1C, Ar), 128.193 (1C, Ar), 127.821 (1C, Ar), 125.786 (1C, Ar), 125.532 (1C, Ar), 122.549 (1C, Ar), 83.196 (1C,  $^*\text{C}\equiv\text{CH}$ ), 82.183 (1C,  $^*\text{CH}\equiv\text{C}$ ), 53.512 (1C,



\*CHCO), 44.084 (1C, CH<sub>2</sub>N), 40.671 (1C, CH<sub>3</sub>SO<sub>2</sub>), 40.462 (1C, CH<sub>2</sub>N), 40.253 (1C, \*CH<sub>2</sub>CH), 36.814 (1C, \*CH<sub>2</sub>CH<sub>2</sub>N). **LCMS** *m/z* 599.1 [M + H]<sup>+</sup>; **HR-MS** *m/z* calcd. for C<sub>29</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>6</sub>S (598.07) 599.0 [M + H]<sup>+</sup>, found: 599.0801 [M + H]<sup>+</sup>.

### 1-Bromo-3-(2,2-diethoxyethoxy)benzene (8)

To a solution of compound **6** (10.0 g, 57.8 mmol) and 2-bromo-1,1-diethoxy-ethane **7** (22.8 g, 115 mmol) in DMF (60.0 mL), K<sub>2</sub>CO<sub>3</sub> (7.99 g, 57.8 mmol) was added, then air in the reaction system was replaced with nitrogen three times, and the mixture was stirred at 100 °C for 10 h. TLC indicated starting material was consumed completely. After that, the reaction solution was diluted with water (200 mL) and then extracted with ethyl acetate (60 mL × 3). The extract was dried over anhydrous sodium sulfate and concentrated. Then the oil was purified by silica gel column chromatography. Compound **8** (8.00 g, 27.6 mmol, 47.8% yield) was obtained as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.07–7.17 (m, 3H, Ar–H), 6.83–6.90 (m, 1H, Ar–H), 4.82 (t, J = 5.2 Hz, 1H, CHO<sub>2</sub>), 3.99 (d, J = 5.2 Hz, 2H, CH<sub>2</sub>O), 3.71–3.83 (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.55–3.70 (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), 1.22–1.29 (m, 6H, CH<sub>3</sub>CH<sub>2</sub>).

### 6-Bromobenzofuran (9)

PPA (2.27 g, 83.0 mmol) was added to a solution of compound **8** (8.00 g, 27.6 mmol) in toluene (40.0 mL), the mixture was stirred at 80 °C for 4 h. TLC indicated starting material was consumed completely. The solution was cooled to room temperature, quenched with water (60.0 mL), extracted with EtOAc (40 mL × 3), the organic layer was washed with brine (40.0 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filter. Then the organic layer was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography. Compound **9** (4.40 g, 11.1 mmol, 40.3% yield, ~50% purity) was obtained as brown oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.70 (s, 1H, H-7), 7.61 (d, J = 2.4 Hz, 1H, H-2), 7.47–7.50 (m, 1H, H-4), 7.37 (dd, J = 8.4, 1.8 Hz, 1H, H-5), 6.76 (dd, J = 2.4, 0.8 Hz, 1H, H-3).

### Benzofuran-6-sulfonyl chloride (10)

To a solution of compound **9** (0.400 g, 2.03 mmol) in THF (4.00 mL), n-BuLi (0.130 g, 812 μL) was added at –78 °C with atmosphere of nitrogen, the solution was stirred at –78 °C for 0.5 h. Then to the solution was added SO<sub>2</sub> (1.30 g, 20.3 mmol) in THF (2.00 mL), the solution was stirred at 20 °C for another 1.5 h. The solution was concentrated under vacuum. The residue was dissolved in hexane, then the mixture was added sulfonyl chloride (548 mg, 4.06 mmol) at 0 °C. The resulting solution was

stirred for 10 h at 20 °C. TLC indicated starting material was consumed completely. After that, the mixture reaction was filter, and the organic layer was concentrated under reduced pressure to left a residue. The residue was purified by prep-TLC. Compound **10** (0.200 g, 923 μmol, 22.7% yield) was obtained as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.24 (s, 1H, H-7), 7.92–7.99 (m, 2H, H-2 H-4), 7.83 (d, J = 8.4 Hz, 1H, H-5), 6.95 (s, 1H, H-3).

### Benzofuran-6-carbonitrile (13)

To a solution of compound **12** (2.00 g, 10.2 mmol) in DMF (12.0 mL), Zn(CN)<sub>2</sub> (19.6 g, 167 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (1.41 g, 1.22 mmol) were added under a nitrogen atmosphere, the reaction mixture was stirred at 80 °C for 16 h. TLC indicated starting material was consumed completely, and two new spots were detected. The reaction mixture was diluted with toluene (10.0 mL) and the phases were separated. The aqueous phase was extracted twice with toluene (10.0 mL × 2). The combined organic was washed with brine (100 mL) and saturated aqueous ammonium hydroxide (20 mL), then dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether/Ethyl acetate = 100/1 to 10/1). Compound **13** (1.00 g, 6.99 mmol 68.8% yield) was obtained as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ = 7.84 (s, 1H, H-7), 7.82 (d, J = 2.0 Hz, 1H, H-2), 7.70 (d, J = 8.0 Hz, 1H, H-4), 7.52 (d, J = 8.0 Hz, 1H, H-5), 6.87 (d, J = 1.3 Hz, 1H, H-3).

### Benzofuran-6-ylmethanamine (14)

To a mixture of LiAlH<sub>4</sub> (265 mg, 6.99 mmol) in THF (10.0 mL), compound **13** (1.00 g, 6.99 mmol) was added in one portion at 20 °C. The mixture was stirred at 20 °C for 1 h. TLC indicated one new spot was detected. 1 mL of saturated Na<sub>2</sub>SO<sub>4</sub> was added to the reaction mixture carefully, and then filtered. The filtration was concentrated to give compound **14** (0.5 g, 3.40 mmol, 48.60% yield) as a light yellow oil. <sup>1</sup>H NMR (MeOD, 400 MHz) δ 7.71 (d, J = 2.0 Hz, 1H, H-2), 7.55 (d, J = 8.0 Hz, 1H, H-4), 7.49 (s, 1H, H-7), 7.21 (d, J = 8.0 Hz, 1H, H-5), 6.79–6.80 (m, 1H, H-3), 3.88 (s, 2H, CH<sub>2</sub>, CH<sub>2</sub>NH<sub>2</sub>); **LCMS** *m/z* 148.1 [M + H]<sup>+</sup>.

### 2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-methyl carboxylate (24)

To a mixture of compound **23** (5.00 g, 16.8 mmol), ECDI (3.56 g, 18.5 mmol), DMAP (164 mg, 1.35 mmol) and TEA (5.12 g, 50.58 mmol) in DMF (30 mL), compound **22** (2.73 g, 16.8 mmol) was added. Then the mixture was

stirred at 25 °C for 16 h. TLC indicated reactant **23** was consumed completely, and the reaction was clean according to TLC. The reaction mixture was poured into H<sub>2</sub>O (120 mL). The mixture was diluted with DCM 10.0 mL and washed with H<sub>2</sub>O (50.0 mL), aq. citric acid (50.0 mL), aq. NaHCO<sub>3</sub> (50.0 mL). The combined organic layers were washed with brine 100 mL dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. Compound **24** (4.00 g, 9.90 mmol, 58.6% yield) was obtained as yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.12 (d, J = 2.4 Hz, 1H, O\*CH=CH), 7.78–7.71 (m, 1H, Ar–H), 7.74 (s, 1H, Ar–H), 7.68–7.47 (m, 1H, Ar–H), 7.35 (br d, J = 7.6 Hz, 1H, Ar–H), 7.05 (d, J = 1.6 Hz, 1H, \*CH=CHO), 4.79 (s, 2H, CH<sub>2</sub>N), 3.91 (s, 3H, CH<sub>3</sub>, CH<sub>3</sub>O), 3.79–3.55 (m, 2H, CH<sub>2</sub>N), 2.93–2.79 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N).

### 2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid (**25**))

To a solution of compound **24** (4.00 g, 9.90 mmol) in pyridine (24.0 mL), LiI (5.30 g, 39.5 mmol) was added. Then the mixture was stirred at 100 °C for 16 h. TLC indicated reactant **24** was consumed completely and the reaction was clean according to TLC. The reaction mixture was concentrated under reduced pressure to remove pyridine. The reaction mixture was adjusted the pH to 2 by adding HCl (3 M, 40.0 mL), solid was precipitate out, then the mixture was filtered and the filter cake was washed with H<sub>2</sub>O (50.0 mL), dried in vacuum to give residue. The crude product was purified by re-crystallization from Petroleum ether: Ethyl acetate = 1: 1 (20.0 mL) at 15 °C. Compound **25** (2.00 g, 4.77 mmol, 48.1% yield) was obtained as brown solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.12 (d, J = 2.4 Hz, 1H, O\*CH=CH), 7.78 (s, 1H, Ar–H), 7.70 (s, 1H, Ar–H), 7.64–7.45 (m, 1H, Ar–H), 7.35 (d, J = 7.2 Hz, 1H, Ar–H), 7.05 (d, J = 1.6 Hz, 1H, \*CH=CHO), 4.78 (d, J = 1.2 Hz, 2H, CH<sub>2</sub>N), 4.01–3.71 (m, 2H, CH<sub>2</sub>N), 2.85 (t, J = 5.6 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>N); LCMS *m/z* 390.1 [M + H]<sup>+</sup>.

### 2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(quinolin-6-yl)ethyl propanoate (**31**)

To a solution of compound **25** (0.300 g, 768 μmol) in DCM (3.00 mL), TEA (23.3 mg, 2.31 mmol) and HATU (438 mg, 1.15 mmol) were added, the mixture was stirred at 20 °C for 0.5 h, then compound **30** (187 mg, 768 μmol) and DMAP (75.1 mg, 615 μmol) were added, the mixture was stirred for 2 h at 20 °C. TLC indicated reactant **25** was consumed completely. The mixture poured into H<sub>2</sub>O (10 mL),

extracted with EtOAc (10 mL × 2), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give crude product. The residue was purified by column chromatography. Compound **31** (0.200 g, 291 μmol, 37.9% yield, 90.0% purity) was obtained as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.88 (d, J = 3.2 Hz, 1H, CONH), 8.10 (d, J = 8.0 Hz, 1H, Ar–H), 8.02 (d, J = 8.8 Hz, 1H, Ar–H), 7.73 (d, J = 1.6 Hz, 1H, O\*CH=CH), 7.72 (s, 1H, Ar–H), 7.65 (d, J = 8.0 Hz, 1H, Ar–H), 7.62–7.56 (m, 3H, Ar–H), 7.40 (dd, J = 4.0, 8.3 Hz, 1H, Ar–H), 7.31 (d, J = 7.6 Hz, 1H, Ar–H), 6.83 (d, J = 1.2 Hz, 1H, \*CH=CHO), 6.59 (s, 1H, Ar–H), 5.34–5.19 (m, 1H, CHCO), 4.75 (s, 2H, CH<sub>2</sub>N), 4.22 (q, J = 7.2 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.89–3.58 (m, 2H, CH<sub>2</sub>N), 3.49 (d, J = 5.8 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>N), 2.98–2.83 (m, 2H, CH<sub>2</sub>CH), 1.31–1.17 (m, 3H, CH<sub>3</sub>, CH<sub>3</sub>CH<sub>2</sub>); LCMS *m/z* 616.3 [M + H]<sup>+</sup>.

### 2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(quinolin-6-yl)propanoic acid (**1f**)

To a solution of compound **31** (200 mg, 324 μmol) in EtOH (2.00 mL) and THF (1.00 mL), NaOH (38.9 mg, 973 μmol) in H<sub>2</sub>O (1.00 mL) were added, the mixture was stirred at 20 °C for 2 h. TLC indicated reactant **31** was consumed completely. EtOH and THF was removed, the mixture was adjusted pH = 4, then solid was formed, filtered and the filter cake concentrated to give crude product. The crude product was triturated with Petroleum ether: Ethyl acetate = 10: 1 (20 mL) at 30 °C for 30 min. Compound **1f** (25.0 mg, 41.6 μmol, 12.8% yield) was obtained as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.89 (s, 1H, CONH), 8.82 (d, J = 8.8 Hz, 1H, Ar–H), 8.32 (d, J = 8.8 Hz, 1H, Ar–H), 8.06 (s, 1H, Ar–H), 7.97 (d, J = 9.2 Hz, 1H, Ar–H), 7.89 (s, 1H, O\*CH=CH), 7.75 (d, J = 8.8 Hz, 1H, Ar–H), 7.74 (d, J = 8.8 Hz, 1H, Ar–H), 7.66 (s, 1H, Ar–H), 7.54 (dd, J = 4.8, 8.2 Hz, 1H, Ar–H), 7.31 (d, J = 10.0 Hz, 1H, Ar–H), 7.30 (d, J = 10.0 Hz, 1H, Ar–H), 7.02 (s, 1H, \*CH=CHO), 4.88 (m, 1H, CHCO), 4.72 (s, 2H, CH<sub>2</sub>N), 3.74 (m, 2H, CH<sub>2</sub>N), 3.46–3.33 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 3.27–3.20 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 2.78 (m, 2H, CH<sub>2</sub>CH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 101 MHz) δ 172.698 (1C, COOH), 169.912 (1C, CONH), 164.022 (1C, CON), 154.114 (1C, Ar), 149.783 (1C, Ar), 148.209 (1C, Ar), 145.927 (1C, O\*CH=CH), 137.477 (1C, Ar), 137.456 (1C, Ar), 136.806 (1C, Ar), 135.027 (1C, Ar), 132.461 (1C, Ar), 132.133 (1C, Ar), 132.046 (1C, Ar), 131.623 (1C, Ar), 129.151 (1C, Ar), 128.852 (1C, Ar), 128.495 (1C, Ar), 128.262 (1C, Ar), 128.028 (1C, Ar), 126.206 (1C, Ar), 122.480 (1C, Ar), 121.992 (1C, Ar), 121.897 (1C, Ar), 110.801 (1C, Ar), 107.316 (1C, \*CH=CHO), 53.701 (1C, \*CHCO), 49.072 (1C, CH<sub>2</sub>N), 40.654 (1C, CH<sub>2</sub>N), 40.246 (1C, \*CH<sub>2</sub>CH), 37.137 (1C, \*CH<sub>2</sub>CH<sub>2</sub>N). LCMS *m/z* 588.1 [M + H]<sup>+</sup>; HR-MS *m/z* calcd. for C<sub>31</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>5</sub> (587.10) 588.1 [M + H]<sup>+</sup>; found: 588.1070 [M + H]<sup>+</sup>.

**2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(1,1-dioxido-2,3-dihydrobenzo[b]thiophen-6-yl)ethyl propanoate (42)**

To a solution of compound **25** (300 mg, 768  $\mu\text{mol}$ ) in DCM (5.00 mL), TEA (233 mg, 2.31 mmol) and HATU (438 mg, 1.15 mmol) were added. The mixture was stirred for 30 min, then compound **41** (217 mg, 768  $\mu\text{mol}$ ), and DMAP (75.1 mg, 615  $\mu\text{mol}$ ) were added. The mixture was stirred for 20 h at 30 °C. TLC indicated reactant **25** was consumed completely. The mixture was poured into H<sub>2</sub>O (10 mL), extracted with EtOAc (20 mL), washed with 0.5 M HCl (10 mL), NaHCO<sub>3</sub> (10 mL), brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give crude product. The residue was purified by column chromatography. Compound **42** (0.200 g, 289  $\mu\text{mol}$ , 37.7% yield) was obtained as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.73 (d, *J* = 2.4 Hz, 1H, O\*CH=CH), 7.69–7.59 (m, 3H, Ar–H), 7.59–7.49 (m, 1H, Ar–H), 7.32 (d, *J* = 8.0 Hz, 2H, Ar–H), 6.83 (s, 1H, Ar–H), 6.57–6.44 (m, 1H, \*CH=CHO), 5.18–5.09 (m, 1H, CHCO), 4.89–4.62 (s, 2H, CH<sub>2</sub>N), 4.29–4.16 (m, 2H, CH<sub>2</sub>, CH<sub>2</sub>CH<sub>3</sub>), 3.88–3.62 (m, 2H, CH<sub>2</sub>N), 3.52–3.39 (m, 2H, \*CH<sub>2</sub>CH<sub>2</sub>N), 3.39–3.28 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>), 2.90 (m, 2H, CH<sub>2</sub>CH), 1.31–1.27 (m, 3H, CH<sub>3</sub>CH<sub>2</sub>); LCMS *m/z* 655.3 [M + H]<sup>+</sup>.

**2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(1,1-dioxido-2,3-dihydrobenzo[b]thiophen-6-yl)propanoic acid (1g)**

To a solution of compound **42** (0.200 g, 305  $\mu\text{mol}$ ) in EtOH (2.00 mL) and THF (1.00 mL), NaOH (36.6 mg, 915  $\mu\text{mol}$ ) in H<sub>2</sub>O (1.00 mL) was added. The mixture was stirred at 20 °C for 20 h. TLC indicated reactant **42** was consumed completely. EtOH and THF was removed, the mixture was adjusted pH = 4, then solid was formed, filtered and the filter cake concentrated to give crude product. The crude product was triturated with Petroleum ether: Ethyl acetate = 10: 1 (20 mL) at 30 °C for 30 min. Compound **1g** (24.0 mg, 36.6  $\mu\text{mol}$ , 12.0% yield) was obtained as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  12.85 (s, 1H, COOH), 9.02 (d, *J* = 8.8 Hz, 1H, CONH), 8.11 (d, *J* = 2.0 Hz, 1H, O\*CH=CH), 7.81–7.66 (m, 2H, Ar–H), 7.62 (s, 1H, Ar–H), 7.64–7.55 (m, 1H, Ar–H), 7.43 (d, *J* = 7.6 Hz, 2H, Ar–H), 7.32 (s, 1H, Ar–H), 7.03 (s, 1H, \*CH=CHO), 4.69–4.70 (m, 1H, CHCO), 4.69 (s, 2H, CH<sub>2</sub>N), 3.87–3.59 (m, 2H, CH<sub>2</sub>N), 3.54 (t, *J* = 6.8 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>), 3.30–3.16 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 3.25–3.16 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 3.07–2.94 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 2.77 (m, 2H, CH<sub>2</sub>CH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 101 MHz)  $\delta$  172.522 (1C, COOH), 170.011 (1C, CON), 164.116 (1C, CONH), 154.114 (1C, Ar), 148.221 (1C, O\*CH=CH), 139.209 (1C, Ar), 139.029 (1C, Ar), 137.505

(1C, Ar), 136.682 (1C, Ar), 134.999 (1C, Ar), 134.892 (1C, Ar), 132.146 (1C, Ar), 132.081 (1C, Ar), 131.735 (1C, Ar), 129.142 (1C, Ar), 128.940 (1C, Ar), 127.857 (1C, Ar), 126.225 (1C, Ar), 122.484 (1C, Ar), 121.906 (1C, Ar), 121.668 (1C, Ar), 110.800 (1C, Ar), 107.319 (1C, \*CH=CHO), 53.750 (1C, \*CHCO), 51.056 (1C, \*CH<sub>2</sub>CH<sub>2</sub>), 44.440 (1C, CH<sub>2</sub>N), 40.711 (1C, CH<sub>2</sub>N), 40.385 (1C, \*CH<sub>2</sub>CH), 36.512 (1C, \*CH<sub>2</sub>CH<sub>2</sub>), 25.081 (1C, \*CH<sub>2</sub>CH<sub>2</sub>N). LCMS *m/z* 627.0 [M + H]<sup>+</sup>; HR-MS *m/z* calcd. for C<sub>30</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>7</sub>S (626.07) 627.0 [M + H]<sup>+</sup>; found: 627.0760 [M + H]<sup>+</sup>.

**2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)ethyl propanoate (51)**

To a solution of compound **25** (0.300 g, 768  $\mu\text{mol}$ ) in DCM (5.00 mL), TEA (233 mg, 2.31 mmol) and HATU (438 mg, 1.15 mmol) were added. The mixture was stirred at 20 °C for 0.5 h, then compound **50** (201 mg, 768  $\mu\text{mol}$ ) and DMAP (75.1 mg, 615  $\mu\text{mol}$ ) were added. The mixture was stirred at 30 °C for 20 h. TLC indicated reactant **25** was consumed completely. The mixture was poured into H<sub>2</sub>O (10 mL), extracted with EtOAc (20 mL), washed with 0.5 M HCl (10 mL), NaHCO<sub>3</sub> (10 mL), brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give crude product. The residue was purified by column chromatography. Compound **51** (0.200 g, 299  $\mu\text{mol}$ , 38.9% yield) was obtained as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.13 (s, 1H, CONH), 7.74 (d, *J* = 2.0 Hz, 1H, O\*CH=CH), 7.67 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.63 (s, 1H, Ar–H), 7.33 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.08 (d, *J* = 7.6 Hz, 1H, Ar–H), 6.89–6.82 (m, 2H, Ar–H), 6.65 (s, 1H, \*CH=CHO), 6.38 (d, *J* = 8.0 Hz, 1H, Ar–H), 5.23–5.09 (m, 1H, CHCO), 4.80 (s, 2H, CH<sub>2</sub>N), 4.23 (q, *J* = 7.2 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.94–3.69 (m, 2H, CH<sub>2</sub>N), 3.29–3.14 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 3.29–3.14 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 3.00–2.95 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 2.95–2.85 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 2.69–2.55 (m, 2H, CH<sub>2</sub>CH), 1.36–1.20 (m, 3H, CH<sub>3</sub>, CH<sub>3</sub>CH<sub>2</sub>); LCMS *m/z* 634.4 [M + H]<sup>+</sup>.

**2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)propanoic acid (1h)**

To a solution of compound **51** (0.200 g, 315  $\mu\text{mol}$ ) in MeOH (2.00 mL) and THF (1.00 mL), NaOH (37.8 mg, 945  $\mu\text{mol}$ ) in H<sub>2</sub>O (1.00 mL) were added, the mixture was stirred at 20 °C for 2 h. TLC indicated reactant **51** was consumed completely. EtOH and THF was removed, the mixture was adjusted pH = 4, then solid was formed, filtered and the filter cake concentrated to give crude product. The crude product was triturated with Petroleum ether: Ethyl acetate = 10: 1 (20 mL) at

30 °C for 30 min. Compound **1h** (0.024 g, 37.9 μmol, 12.0% yield) was obtained as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 12.76 (s, 1H, COOH), 10.05 (s, 1H, CH<sub>2</sub>CONH), 9.00 (d, J = 8.4 Hz, 1H, CONHCH), 8.13 (d, J = 2.0 Hz, 1H, O<sup>\*</sup>CH=CH), 7.83–7.70 (m, 1H, Ar–H), 7.70–7.65 (m, 1H, Ar–H), 7.50–7.36 (m, 1H, Ar–H), 7.36–7.26 (m, 1H, Ar–H), 7.14–7.10 (m, 1H, Ar–H), 7.10–7.00 (m, 1H, Ar–H), 6.83 (d, J = 7.6 Hz, 1H, Ar–H), 6.75 (s, 1H, <sup>\*</sup>CH=CHO), 4.75 (s, 2H, CH<sub>2</sub>N), 4.66–4.57 (m, 1H, CHCO), 3.65 (m, 2H, CH<sub>2</sub>N), 3.04 (dd, J = 5.2, 13.6 Hz, 1H, <sup>\*</sup>CH<sub>2</sub>CH<sub>2</sub>N), 2.89–2.82 (m, 2H, CH<sub>2</sub>CH), 2.80 (m, 1H, <sup>\*</sup>CH<sub>2</sub>CH<sub>2</sub>N), 2.82–2.74 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 2.39 (t, J = 7.6 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 101 MHz) δ 172.731 (1C, COOH), 170.724 (1C, CONH), 170.322 (1C, CH<sub>2</sub><sup>\*</sup>CONH), 163.957 (1C, CONH), 154.121 (1C, Ar), 148.214 (1C, O<sup>\*</sup>CH=CH), 138.574 (1C, Ar), 137.447 (1C, Ar), 136.739 (1C, Ar), 135.114 (1C, Ar), 132.110 (1C, Ar), 132.074 (1C, Ar), 131.713 (1C, Ar), 129.157 (1C, Ar), 128.962 (1C, Ar), 127.907 (1C, Ar), 126.196 (1C, Ar), 123.358 (1C, Ar), 122.499 (1C, Ar), 122.159 (1C, Ar), 121.906 (1C, Ar), 116.274 (1C, Ar), 110.807 (1C, Ar), 107.319 (1C, <sup>\*</sup>CH=CHO), 59.719 (1C, <sup>\*</sup>CHCO), 54.017 (1C, CH<sub>2</sub>N), 44.550 (1C, CH<sub>2</sub>N), 37.155 (1C, <sup>\*</sup>CH<sub>2</sub>CH), 31.067 (1C, <sup>\*</sup>CH<sub>2</sub>CH<sub>2</sub>), 27.123 (1C, CH<sub>2</sub><sup>\*</sup>CH<sub>2</sub>), 24.973 (1C, <sup>\*</sup>CH<sub>2</sub>CH<sub>2</sub>N). LCMS *m/z* 606.0 [M + H]<sup>+</sup>; HR-MS *m/z* calcd. for C<sub>31</sub>H<sub>25</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>6</sub> (605.11) 606.0 [M + H]<sup>+</sup>; found: 606.1202 [M + H]<sup>+</sup>.

### 6-Hydroxymethyl-quinoline (27)

To a solution of compound **26** (3.00 g, 19.0 mmol) in MeOH (15.0 mL), NaBH<sub>4</sub> (2.20 g, 58.1 mmol) was added in portions at 0 °C. Then the mixture was stirred at 15 °C for 3 h. TLC showed the starting material was consumed completely. The reaction mixture was partitioned between H<sub>2</sub>O (100 mL) and EtOAc (60.0 mL, 40.0 mL, 20.0 mL). The organic phase was separated, washed with brine 50.0 mL, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. Compound **27** (2.00 g, 12.5 mmol, 65.8% yield) was obtained as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.80 (dd, J = 1.6, 4.0 Hz, 1H, H-2), 8.08 (d, J = 8.0 Hz, 1H, H-4), 8.02 (d, J = 8.4 Hz, 1H, H-8), 7.77 (s, 1H, H-5), 7.65 (dd, J = 1.6, 8.8 Hz, 1H, H-7), 7.34 (dd, J = 4.4, 8.4 Hz, 1H, H-3), 4.89 (s, 2H, CH<sub>2</sub>).

### 6-(Bromomethyl)quinoline (28)

To a solution of compound **27** (0.700 g, 4.40 mmol) in HBr/AcOH (5.00 mL), the mixture was stirred at 20 °C for 3 h. TLC indicated reactant **27** was consumed completely. The mixture was concentrated to give product. The crude product was triturated with Petroleum ether: Ethyl acetate = 5: 1 (10 mL) at

20 °C for 30 min. Compound **28** (0.500 g, 1.65 mmol, 37.5% yield) was obtained as a yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 9.39–9.28 (m, 1H, H-2), 9.12 (d, J = 8.4 Hz, 1H, H-4), 8.41 (s, 1H, H-5), 8.34–8.25 (m, 1H, H-8), 8.24–8.14 (m, 1H, H-7), 8.08 (dd, J = 5.2, 8.3 Hz, 1H, H-3), 4.99 (s, 2H, CH<sub>2</sub>); LCMS *m/z* 222.2 [M + H]<sup>+</sup>.

### 2-((Diphenylmethylene)amino)-3-(quinolin-6-yl)ethyl propanoate (29)

To a solution of ethyl N-(diphenylmethylene)glycinate (882 mg, 3.30 mmol) in THF (10 mL), NaH (132 mg, 3.30 mmol) was added at 5 °C, the mixture was stirred for 0.5 h, then compound **28** (0.500 g, 1.65 mmol) was added, the mixture was stirred at 20 °C for 2 h. TLC indicated reactant **28** was consumed completely. The mixture was poured into NH<sub>4</sub>Cl (10 mL), extracted with EtOAc (20 mL), brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give crude product. The residue was purified by column chromatography. Compound **29** (0.500 g, 1.16 mmol, 70.4% yield, 95.0% purity) was obtained as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.87 (dd, J = 1.6, 4.3 Hz, 1H, Ar–H), 8.01 (d, J = 8.0 Hz, 1H, Ar–H), 7.94 (d, J = 8.8 Hz, 1H, Ar–H), 7.59–7.53 (m, 2H, Ar–H), 7.51 (s, 1H, Ar–H), 7.45–6.56 (m, 10H, Ar–H), 4.38 (dd, J = 4.4, 9.3 Hz, 1H, CHCO), 4.29–4.12 (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.54–3.44 (m, 1H, <sup>\*</sup>CH<sub>2</sub>CHCO), 3.43–3.32 (m, 1H, <sup>\*</sup>CH<sub>2</sub>CHCO), 1.27 (t, J = 7.2 Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>); LCMS *m/z* 409.4 [M + H]<sup>+</sup>.

### 2-Amino-3-(quinolin-6-yl)ethyl propanoate (30)

To a solution of compound **29** (0.500 g, 1.22 mmol) in EtOAc (3 mL) and HCl (1.00 M, 2 mL), the mixture was stirred at 20 °C for 0.5 h. TLC indicated reactant **29** was consumed completely. The mixture was extracted with EtOAc (10 mL), the aqueous phase adjusted pH = 8 with aq. NaCO<sub>3</sub>, and extracted with EtOAc (10 mL × 2), brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give product. Compound **30** (0.200 g, 818 μmol, 66.8% yield) was obtained as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.89 (dd, J = 1.6, 4.2 Hz, 1H, H-2), 8.11 (d, J = 8.4 Hz, 1H, H-4), 8.06 (d, J = 8.8 Hz, 1H, H-8), 7.65 (s, 1H, H-5), 7.59 (dd, J = 1.6, 8.7 Hz, 1H, H-7), 7.39 (dd, J = 4.4, 8.3 Hz, 1H, H-3), 4.18 (q, J = 7.2 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.82 (dd, J = 5.2, 7.8 Hz, 1H, CHCO), 3.28 (dd, J = 5.2, 13.6 Hz, 1H, <sup>\*</sup>CH<sub>2</sub>CHCO), 3.06 (dd, J = 8.0, 13.6 Hz, 1H, <sup>\*</sup>CH<sub>2</sub>CHCO), 1.23 (t, J = 7.2 Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>).

### 6-Nitrobenzo[b]thiophene 1,1-dioxide (33)

To a solution of HNO<sub>3</sub> (35.0 g, 333 mmol) in H<sub>2</sub>SO<sub>4</sub> (46.0 g, 460 mmol) at 0 °C, then compound **32** (10.0 g, 60.2 mmol) was added at 0 °C for 1 h. TLC showed the



starting material was consumed completely. The mixture was poured into ice water (300 mL) and the mixture was partitioned with EtOAc (200 mL) and the organic layer was washed with aq. NaHCO<sub>3</sub> (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness. The residue was purified by column chromatography. Compound **33** (9.00 g, 42.6 mmol, 70.8% yield) was obtained as light yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.57 (d, J = 1.6 Hz, 1H, H-7), 8.48 (dd, J = 2.0, 8.3 Hz, 1H, H-5), 7.59 (d, J = 8.0 Hz, 1H, H-4), 7.34 (dd, J = 0.8, 7.0 Hz, 1H, H-3), 7.01 (d, J = 6.8 Hz, 1H, H-2).

### 6-Amino-2,3-dihydrobenzo[b]thiophene 1,1-dioxide (34)

To a solution of compound **33** (9.00 g, 42.6 mmol) in MeOH (90.0 mL), Pd/C (0.90 g, 10% purity) was added under N<sub>2</sub>. The suspension was degassed under vacuum and purged with H<sub>2</sub> several times. The mixture was stirred under H<sub>2</sub> (50 Psi) at 50 °C for 12 h. TLC showed the starting material was consumed completely. The suspension was filtered through a pad of Celite and the filter cake was washed with MeOH (100 mL × 3). The combined filtrates were concentrated to dryness. The crude product was triturated with EtOAc (20 mL) at 25 °C for 30 min. Compound **34** (5.00 g, 27.3 mmol, 64.0% yield) was obtained as light yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 7.13 (d, J = 8.4 Hz, 1H, H-4), 6.83 (dd, J = 2.4, 8.3 Hz, 1H, H-5), 6.75 (d, J = 2.0 Hz, 1H, H-7), 5.58 (s, 2H, NH<sub>2</sub>), 3.52–3.40 (m, 2H, H-2), 3.12 (t, J = 6.8 Hz, 2H, H-3); LCMS *m/z* 184.1 [M + H]<sup>+</sup>.

### 6-Bromo-2,3-dihydrobenzo[b]thiophene 1,1-dioxide (35)

To a solution of compound **34** (5.00 g, 27.3 mmol) in HBr (50.0 mL, 40 % purity) at 0 °C, NaNO<sub>2</sub> (2.45 g, 35.5 mmol) was added portion wise. The temperature was kept BELOW 8 °C during this addition. The resulting mixture was then added to a suspension of CuBr (2.74 g, 19.1 mmol) in HBr (10.0 mL) at 70 °C. The resulting mixture was stirred at 70 °C for 1 h. TLC showed the starting material was consumed completely. The mixture was poured into ice water (200 mL) and then was partitioned with EtOAc (100 mL × 3), the organic layer was washed with H<sub>2</sub>O (150 mL) dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness. The residue was purified by column chromatography. Compound **35** (4.00 g, 16.2 mmol, 59.1% yield) was obtained as light yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.85 (d, J = 1.2 Hz, 1H, H-7), 7.67 (dd, J = 1.6, 8.2 Hz, 1H, H-5), 7.27 (s, 1H, H-4),

3.55–3.47 (m, 2H, H-2), 3.36–3.30 (m, 2H, H-3); LCMS *m/z* 246.9 [M + H]<sup>+</sup>.

### 2,3-Dihydrobenzo[b]thiophene-6-carbonitrile 1,1-dioxide (36)

To a solution of compound **35** (4.00 g, 16.2 mmol) in DMF (24.0 mL), Zn(CN)<sub>2</sub> (3.04 g, 25.9 mmol) and Pd (PPh<sub>3</sub>)<sub>4</sub> (2.24 g, 1.95 mmol) were added under a nitrogen atmosphere, the reaction mixture was stirred at 80 °C for 16 h. TLC showed the starting material was consumed completely. The mixture was partitioned between EtOAc (100 mL × 3) and H<sub>2</sub>O (100 mL), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness. The crude product was triturated with EtOAc (10.0 mL) for 1 h at 25 °C. Compound **36** (2.00 g, 10.4 mmol, 63.9% yield) was obtained as light yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.41 (d, J = 0.8 Hz, 1H, H-7), 8.11 (dd, J = 1.2, 8.0 Hz, 1H, H-5), 7.76 (dd, J = 0.8, 8.0 Hz, 1H, H-4), 3.71–3.63 (m, 2H, H-2), 3.49–3.41 (m, 2H, H-3).

### 2,3-Dihydrobenzo[b]thiophene-6-carbaldehyde 1,1-dioxide (37)

Compound **36** (0.500 g, 2.59 mmol) was added into a solution of Raney-Ni (221.70 mg, 2.59 mmol) in HCOOH (5.00 mL) in one portion at 25 °C under N<sub>2</sub>. The mixture was stirred at 100 °C for 2 h. Two reactions were combined for work up. The mixture was cooled to 20 °C and filter by Celite. The filtration was concentrated in reduced pressure to give compound **37** (0.900 g, 4.59 mmol, 88.6% yield) as a light yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 10.10 (s, 1H, CHO), 8.28 (s, 1H, H-7), 8.16 (dd, J = 1.4, 7.8 Hz, 1H, H-5), 7.61 (d, J = 7.8 Hz, 1H, H-4), 3.65–3.59 (m, 2H, H-2), 3.56–3.50 (m, 2H, H-3).

### 6-(Hydroxymethyl)-2,3-dihydrobenzo[b]thiophene 1,1-dioxide (38)

To a solution of compound **37** (2.00 g, 10.1 mmol) in MeOH (10.0 mL), NaBH<sub>4</sub> (1.16 g, 30.5 mmol) was added in portions at 0 °C. Then the mixture was stirred at 15 °C for 3 h. TLC showed the starting material was consumed completely. The reaction mixture was partitioned between H<sub>2</sub>O (100 mL) and EtOAc (60.0 mL, 40.0 mL, 20.0 mL). The organic phase was separated, washed with brine 50.0 mL, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. Compound **38** (1.00 g, 5.04 mmol, 49.4% yield) was obtained as yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.74 (s, 1H, H-7),

7.62–7.56 (m, 1H, H-5), 7.38 (d,  $J = 7.8$  Hz, 1H, H-4), 4.77 (s, 2H, CH<sub>2</sub>, CH<sub>2</sub>OH), 3.56–3.46 (m, 2H, H-2), 3.43–3.34 (m, 2H, H-3), 2.01 (s, 1H, OH).

### 6-(Bromomethyl)-2,3-dihydrobenzo[b]thiophene 1,1-dioxide (39)

A mixture of compound **38** (500 mg, 2.52 mmol) in HBr (3.00 mL) was stirred at 20 °C for 2 h. TLC showed new spots were formed. The residue was poured into H<sub>2</sub>O (10 mL) and extracted with EtOAc (10 mL × 2). The organic layer was isolated, dried (MgSO<sub>4</sub>) and concentrated in vacuo. Compound **39** (500 mg, 1.53 mmol, 60.7% yield) was obtained as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta$ ) 7.76 (s, 1H, H-7), 7.71 (d,  $J = 8.0$  Hz, 1H, H-5), 7.48 (d,  $J = 8.0$  Hz, 1H, H-4), 4.66 (s, 2H, CH<sub>2</sub>, CH<sub>2</sub>Br), 3.61–3.50 (m, 2H, H-2), 3.40 (d,  $J = 7.2$  Hz, 2H, H-3); LCMS  $m/z$  261.1 [M + H]<sup>+</sup>.

### 3-(1,1-Dioxido-2,3-dihydrobenzo[b]thiophen-6-yl)-2-(diphenylmethylene)aminoethyl propanoate (40)

A mixture of compound **39** (500 mg, 1.91 mmol), ethyl N-(diphenylmethylene)glycinate (767 mg, 2.87 mmol), NaH (114 mg, 2.87 mmol, 60% purity) in THF (3.00 mL) was stirred at 0–20 °C for 16 h. TLC showed new spots were formed. The residue was poured into H<sub>2</sub>O (15 mL) and the mixture was extracted with EtOAc (15 mL × 2). The organic layer was isolated, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by flash silica gel chromatography. Compound **40** (600 mg, 1.14 mmol, 59.5% yield) was obtained as yellow gum. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta$ ) 7.58 (d,  $J = 7.6$  Hz, 1H, Ar-H), 7.45–29 (m, 10H, Ar-H), 7.22 (d,  $J = 8.0$  Hz, 1H, Ar-H), 6.75 (d,  $J = 4.8$  Hz, 1H, Ar-H), 4.33–4.26 (m, 1H, CHCO), 4.13 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.52–3.43 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 3.40–3.36 (m, 2H, CH<sub>2</sub>CHCO), 3.36–3.26 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 1.27 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>); LCMS  $m/z$  448.3 [M + H]<sup>+</sup>.

### 2-Amino-3-(1,1-dioxido-2,3-dihydrobenzo[b]thiophen-6-yl)ethyl propanoate (41)

To a solution of compound **40** (500 mg, 1.12 mmol) in EtOAc (1.00 mL), HCl (1 M, 2.50 mL) was added. The mixture was stirred at 20 °C for 1 h. TLC showed new spots were formed. The residue was poured into H<sub>2</sub>O (5 mL) and extracted with EtOAc (5 mL). The aqueous phase was basified by NaHCO<sub>3</sub> to pH = ~8 and extracted with DCM (5 mL × 3). The organic phase was concentrated under reduced pressure. Compound **41** (300 mg, 1.06 mmol, 94.7% yield) was obtained as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta$ ) 7.60 (s, 1H, H-7), 7.45 (dd,  $J = 1.2, 7.9$  Hz, 1H, H-5), 7.32 (d,  $J = 8.0$  Hz, 1H, H-4), 4.18 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.75–3.66 (m, 1H, CHCO), 3.55–3.44 (m,

2H, CH<sub>2</sub>CH<sub>2</sub>), 3.41–3.32 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 3.15 (dd,  $J = 5.2, 13.7$  Hz, 1H, \*CH<sub>2</sub>CHCO), 2.93 (dd,  $J = 8.0, 13.7$  Hz, 1H, \*CH<sub>2</sub>CHCO), 1.26 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>).

### 2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl trifluoromethanesulfonate (44)

To a solution of compound **43** (5.00 g, 30.6 mmol) in CHCl<sub>3</sub> (50.0 mL), pyridine (5.28 g, 66.8 mmol, 5.39 mL) was added. The mixture was stirred at 25 °C for 10 min and then cooled to 0 °C. (Tf)<sub>2</sub>O (8.60 g, 30.6 mmol) was slowly added drop wise under 0 °C followed by stirring for 10 min. The reaction was stirred at 25 °C for 20 min. TLC showed the reaction was complete. The solution was poured into water (20.0 mL) and DCM (10.0 mL) was added. The organic phase was separated and the aqueous was extracted with DCM (2 × 10.0 mL). The organic phase were dried over magnesium sulfate, filtered and concentrated in vacuum. The crude product was purified by column chromatography. Compound **44** (5.00 g, 16.9 mmol, 55.2% yield) was obtained as white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta$ ) 9.44 (s, 1H, CONH), 7.23 (d,  $J = 8.4$  Hz, 1H, H-8), 6.90 (dd,  $J = 2.4, 8.3$  Hz, 1H, H-5), 6.80 (d,  $J = 2.4$  Hz, 1H, H-6), 3.01 (t,  $J = 7.6$  Hz, 2H, H-4), 2.80–2.57 (m, 2H, H-3); LCMS  $m/z$  296.0 [M + H]<sup>+</sup>.

### 2-Oxo-1,2,3,4-tetrahydroquinoline-7-carbonitrile (45)

To a solution of compound **44** (5.00 g, 16.9 mmol) in DMF (30.0 mL), Zn(CN)<sub>2</sub> (1.59 g, 13.5 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (1.96 g, 1.69 mmol) were added, then the reaction was stirred at 80 °C for 12 h. TLC showed the reaction was complete. The solution was poured into water (20.0 mL) and DCM (10.0 mL) was added. The organic phase was separated and the aqueous was extracted with DCM (2 × 10.0 mL). The organic phase were dried over magnesium sulfate, filtered and concentrated in vacuum. Compound **45** (2.00 g, 9.35 mmol, 55.2% yield) was obtained as off-white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz,  $\delta$ ) 10.34 (s, 1H, CONH), 7.39–7.36 (m, 2H, H-8 H-5), 7.15 (s, 1H, H-6), 2.96 (t,  $J = 7.6$  Hz, 2H, H-4), 2.49–2.46 (m, 2H, H-3); LCMS  $m/z$  173.1 [M + H]<sup>+</sup>.

### 2-Oxo-1,2,3,4-tetrahydroquinoline-7-carbaldehyde (46)

To a solution of compound **45** (2.00 g, 11.6 mmol) in HCOOH (20.0 mL), Ni (2.00 g, 34.0 mmol) was added, then the reaction was stirred at 100 °C for 2 h. TLC showed the reaction was complete. The mixture was filtered, the



filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography. Compound **46** (1.20 g, 6.85 mmol, 58.9% yield) was obtained as brown solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 10.34 (s, 1H, CHO), 9.90 (s, 1H, CONH), 7.49 (dd, *J* = 1.6, 7.6 Hz, 1H, H-8), 7.44–7.39 (m, 1H, H-5), 7.33 (d, *J* = 1.6 Hz, 1H, H-6), 2.97 (t, *J* = 7.6 Hz, 2H, H-4), 2.49–2.45 (m, 2H, H-3); LCMS *m/z* 176.1 [M + H]<sup>+</sup>.

### 7-(Hydroxymethyl)-3,4-dihydroquinolin-2(1H)-one (47)

To a solution of compound **46** (2.00 g, 11.4 mmol) in MeOH (10.0 mL), NaBH<sub>4</sub> (0.86 g, 22.0 mmol) was added in portions at 0 °C. Then the mixture was stirred at 15 °C for 3 h. TLC showed the starting material was consumed completely. The reaction mixture was partitioned between H<sub>2</sub>O (100 mL) and EtOAc (60 mL, 40 mL, 20 mL). The organic phase was separated, washed with brine 50.0 mL, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. Compound **47** (1.00 g, 5.64 mmol, 49.4% yield) was obtained as yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 10.05 (s, 1H, CONH), 7.08 (d, *J* = 8.2 Hz, 1H, H-8), 6.85–6.80 (m, 2H, H-5 H-6), 4.40 (d, *J* = 4.6 Hz, 2H, CH<sub>2</sub>, CH<sub>2</sub>OH), 2.83 (t, *J* = 7.4 Hz, 2H, H-4), 2.45–2.37 (m, 2H, H-3); LCMS *m/z* 178.0 [M + H]<sup>+</sup>.

### 7-(Bromomethyl)-3,4-dihydroquinolin-2(1H)-one (48)

A solution of compound **47** (1.00 g, 6.13 mmol) in HBr (6.00 mL), then the mixture was stirred at 25 °C for 0.5 h. TLC showed the reaction was complete. The solution was poured into water (10.0 mL) and DCM (10.0 mL) was added. The organic phase was separated and the aqueous was extracted with DCM (2 × 10.0 mL). The organic phase were dried over magnesium sulfate, filtered and concentrated in vacuum. The crude product was triturated with EtOAc (10.0 mL) at 25 °C for 10 min. Compound **48** (0.500 g, 2.08 mmol, 33.9% yield) was obtained as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 10.15 (s, 1H, CONH), 7.14 (d, *J* = 7.6 Hz, 1H, H-8), 6.98 (dd, *J* = 1.6, 7.6 Hz, 1H, H-5), 6.90 (s, 1H, H-6), 4.64 (s, 2H, CH<sub>2</sub>, CH<sub>2</sub>Br), 2.85 (t, *J* = 7.6 Hz, 2H, H-4), 2.46–2.40 (m, 2H, H-3); LCMS *m/z* 240.0 [M + H]<sup>+</sup>.

### 2-((Diphenylmethylene)amino)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)ethyl propanoate (49)

To a solution of compound **48** (0.500 g, 2.08 mmol) and ethyl N-(diphenylmethylene)glycinate (1.11 g, 4.16 mmol) in THF (3.00 mL), NaH (166 mg, 4.16 mmol, 60% purity)

was added at 0 °C, then the mixture was stirred at 25 °C for 5 h. TLC showed the reaction was complete. The solution was poured into water (5.00 mL) and DCM (5.00 mL) was added. The organic phase was separated and the aqueous was extracted with DCM (2 × 5.00 mL). The organic phase were dried over magnesium sulfate, filtered and concentrated in vacuum. The crude product was purified by column chromatography. Compound **49** (0.550 g, 1.29 mmol, 61.9% yield). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 7.74–6.67 (m, 10H, Ar-H), 6.99 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.75–6.67 (m, 1H, Ar-H), 6.39 (d, *J* = 1.2 Hz, 1H, Ar-H), 4.27–4.22 (m, 1H, CHCO), 4.22–4.12 (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.28–3.18 (m, 1H, \*CH<sub>2</sub>CHCO), 3.17–3.07 (m, 1H, \*CH<sub>2</sub>CHCO), 2.91 (t, *J* = 7.6 Hz, CH<sub>2</sub>CH<sub>2</sub>), 2.64–2.55 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 1.27 (t, *J* = 7.2 Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>); LCMS *m/z* 427.2 [M + H]<sup>+</sup>.

### 2-Amino-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)ethyl propanoate (50)

To a solution of **49** (0.550 g, 1.29 mmol) in EtOAc (3.00 mL), HCl (2 M, 2.58 mL) was added at 0 °C, then the mixture was stirred at 25 °C for 0.5 h. TLC showed the reaction was complete. The mixture extracted with EtOAc (5.00 mL). The organic phase was adjusted to pH = 7–8 with NaHCO<sub>3</sub>, extracted with DCM (10 × 5.00 mL). The combined organic phase was washed with brine (20.0 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in vacuum. Compound **50** (0.300 g, 1.14 mmol, 88.6% yield) was obtained as colorless oil. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.28 (s, 1H, CONH), 7.10 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.83 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.63 (s, 1H, Ar-H), 4.20 (q, *J* = 7.2 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.70 (dd, *J* = 5.2, 7.9 Hz, 1H, CHCO), 3.05 (dd, *J* = 5.2, 13.6 Hz, 1H, \*CH<sub>2</sub>CHCO), 2.94 (t, *J* = 7.6 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>), 2.81 (dd, *J* = 8.0, 13.6 Hz, 1H, \*CH<sub>2</sub>CHCO), 2.63 (t, *J* = 7.6 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>), 1.29–1.25 (m, 3H, CH<sub>3</sub>CH<sub>2</sub>); LCMS *m/z* 263.1 [M + H]<sup>+</sup>.

### 3-(3-Acetyl-4-hydroxyphenyl)-2-(2-(benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)propanoic acid (1i)

To a solution of compound **25** (0.300 g, 768 μmol) in SOCl<sub>2</sub> (3.00 mL), was stirred at 70 °C for 2 h, until the mixture was concentrated. A solution of 3-(3-acetyl-4-hydroxyphenyl)-2-aminopropanoic acid **53** (188 mg, 845 μmol) in DMF (3 mL) and TEA (233 mg, 2.31 mmol) were added, and the reaction mixture was stirred at 20 °C for 2 h. TLC indicated reactant **25** was consumed completely. The mixture was poured into citric acid (10 mL), extracted with EtOAc (20 mL), concentrated to give crude product. The residue was purified by prep-HPLC. Compound **1i** (0.025 g, 39.8 μmol, 5.19% yield) was obtained as a yellow solid.

**<sup>1</sup>H NMR** (DMSO-*d*<sub>6</sub>, 400 MHz) δ 11.73 (s, 1H, COOH), 8.77 (d, *J* = 7.6 Hz, 1H, CONH), 8.07 (d, *J* = 2.0 Hz, 1H, O\*CH=CH), 7.81 (s, 1H, Ar-H), 7.74 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.50–7.42 (m, 1H, Ar-H), 7.37–7.28 (m, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.05–7.00 (m, 1H, \*CH=CHO), 6.87 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.80–4.65 (m, 1H, CHCO), 4.75 (s, 2H, CH<sub>2</sub>N), 4.28 (s, 1H, Ar-OH), 3.76 (s, 2H, CH<sub>2</sub>N), 3.16 (d, *J* = 4.8 Hz, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 2.95 (dd, *J* = 9.6, 13.8 Hz, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 2.81 (m, 2H, CH<sub>2</sub>CH), 2.61 (s, 3H, CH<sub>3</sub>CO). **<sup>13</sup>C NMR** (DMSO-*d*<sub>6</sub>, 101 MHz) δ 205.022 (1C, COCH<sub>3</sub>), 172.752 (1C, COOH), 167.452 (1C, CON), 164.025 (1C, CONH), 160.030 (1C, Ar), 154.124 (1C, Ar), 148.211 (1C, O\*CH=CH), 137.843 (1C, Ar), 137.501 (1C, Ar), 135.109 (1C, Ar), 132.630 (1C, Ar), 132.193 (1C, Ar), 132.157 (1C, Ar), 132.084 (1C, Ar), 131.646 (1C, Ar), 129.153 (1C, Ar), 128.883 (1C, Ar), 128.373 (1C, Ar), 122.482 (1C, Ar), 121.906 (1C, Ar), 120.331 (1C, Ar), 117.779 (1C, Ar), 110.794 (1C, Ar), 107.316 (1C, \*CH=CHO), 53.838 (1C, CHCO), 36.208 (1C, CH<sub>2</sub>N), 30.273 (1C, CH<sub>2</sub>N), 28.830 (1C, \*CH<sub>2</sub>CH), 27.933 (1C, \*CH<sub>3</sub>CO), 22.858 (1C, \*CH<sub>2</sub>CH<sub>2</sub>N). **LCMS** *m/z* 595.0 [M + H]<sup>+</sup>; **HR-MS** *m/z* calcd. for C<sub>30</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>7</sub> (594.10) 595.0 [M + H]<sup>+</sup>; found: 595.0844 [M + H]<sup>+</sup>.

## 2-(2-(Benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3,4-dihydroxyphenyl)propanoic acid (**1j**)

To a solution of compound **25** (0.300 g, 768 μmol) in SOCl<sub>2</sub> (3 mL), was stirred at 70 °C for 2 h, until the mixture was concentrated. A solution of 2-amino-3-(3,4-dihydroxyphenyl)propanoic acid **54** (166 mg, 845 μmol) in DMF (3 mL) and TEA (233 mg, 2.31 mmol) were added, and the reaction mixture was stirred at 20 °C for 2 h. TLC indicated reactant **25** was consumed completely. The mixture was poured into citric acid (10 mL), extracted with EtOAc (20 mL), concentrated to give crude product. The residue was purified by prep-HPLC. Compound **1j** (0.025 g, 41.7 μmol, 5.43% yield) was obtained as a off-white solid. **<sup>1</sup>H NMR** (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.64 (s, 1H, CONH), 8.38 (s, 2H, Ar-OH), 8.07 (d, *J* = 1.6 Hz, 1H, O\*CH=CH), 7.75 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 7.40–7.29 (m, 1H, Ar-H), 7.34 (s, 1H, Ar-H) 7.02 (s, 1H, Ar-H), 6.68 (s, 1H, \*CH=CHO), 6.62 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.54 (d, *J* = 7.6 Hz, 1H, Ar-H), 4.74 (s, 2H, CH<sub>2</sub>N), 4.65–4.55 (m, 1H, CHCO), 3.76 (s, 2H, CH<sub>2</sub>N), 2.95 (dd, *J* = 5.6, 14.0 Hz, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 2.89–2.82 (m, 1H, \*CH<sub>2</sub>CH<sub>2</sub>N), 2.82–2.78 (m, 2H, CH<sub>2</sub>CH). **<sup>13</sup>C NMR** (DMSO-*d*<sub>6</sub>, 101 MHz) δ 172.935 (1C, COOH), 163.974 (1C, CONH), 154.131 (1C, CON), 148.211 (1C, Ar), 145.412 (1C, O\*CH=CH), 145.339 (1C, Ar), 144.252 (1C, Ar), 137.391 (1C, Ar), 135.201 (1C, Ar), 132.110 (1C, Ar),

132.084 (1C, Ar), 131.756 (1C, Ar), 129.145 (1C, Ar), 129.001 (1C, Ar), 128.489 (1C, Ar), 126.200 (1C, Ar), 122.496 (1C, Ar), 121.913 (1C, Ar), 120.425 (1C, Ar), 116.999 (1C, Ar), 115.701 (1C, Ar), 110.801 (1C, Ar), 107.316 (1C, \*CH=CHO), 54.421 (1C, \*CHCO), 40.687 (1C, CH<sub>2</sub>N), 40.475 (1C, CH<sub>2</sub>N), 40.311 (1C, \*CH<sub>2</sub>CH), 36.872 (1C, \*CH<sub>2</sub>CH<sub>2</sub>N). **LCMS** *m/z* 569.2 [M + H]<sup>+</sup>; **HR-MS** *m/z* calcd. for C<sub>28</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>7</sub> (568.08) 569.2 [M + H]<sup>+</sup>; found: 569.0673 [M + H]<sup>+</sup>.

## Jurkat T-cell adhesion assay

Cells and Cell Culture: Lifitegrast (Selleck, S3714, Shanghai, China), Human leukemia T lymphocytes (Jurkat, Clone E6-1, the catalog number is SPSC-513, was obtained from the cell bank of Chinese Academy of Sciences, Shanghai, China) were cultured in RPMI 1640 Medium (Invitrogen, 11875-093) supplemented with 5% fetal bovine serum at 37 °C with 5% CO<sub>2</sub> in a humidified atmosphere.

To evaluate the ability of compounds **1a–1j** to inhibit the attachment of Jurkat cells to intercellular-adhesion molecule ICAM-1 in vitro, 100 mM stock solutions of the compounds and a positive control (Lifitegrast) were prepared in an aqueous solution of dimethylsulfoxide (1:1 (v/v)), respectively, and then diluted by adding assay medium to achieve and maintain the desired concentration throughout the assay. The Jurkat cells were labeled with an 8 μM solution of 2', 7'-bis-(2-carboxyethyl)-5-(and-6)- carboxyfluorescein in growth medium at room temperature for 15 min. Labeled cells were incubated in 70 μL of assay medium in each well of a 96-well plate at 5 × 10<sup>5</sup> cells per well with 70 μL of compound or the positive control in assay medium at 37 °C for 30 min. A 100 μL aliquot of this fluorescence-labeled Jurkat cell suspension was allowed to settle in the presence of compound or the positive control in wells of a 96-well plate coated with recombinant human ICAM-1 expressed as an Fc chimera (R&D Systems, Minneapolis, MN) at 37 °C for 1 h. A dose titration of each test compound was run (10<sup>-10</sup>–10<sup>-4</sup> M) in three separate lanes on a single plate, non-adherent cells were removed by washing with the medium. The adherent cells were quantitated by measuring the intensity of fluorescence from adherent cells, and the IC<sub>50</sub> values were calculated by using a standard four-parameter logistic nonlinear regression analysis of the data (GraphPad Software Inc., San Diego, CA) [13].

## Animal experiments

### Dry eye mice model

Drugs and instruments: Scopolamine (0.5 mg/0.2 mL, Sigma-Aldrich, S-098-1g, St. Louis, MO); 5% (W/V) or 7% (W/V) compound **1b** eye drops (containing 2.6%

glycerinum and 0.01% benzalkonium chloride 0.01%, pH range from 8.0 to 8.5). Lifitegrast (5%, Shire, 07548, Columbia, USA); OCT (VWR, 8551-00, Suwanee, USA); TUNEL (Beyotime Institute of Biotechnology, C1090, Haimen, China); (Nikon Eclipse Ni-U, Japan); Stereographic fluorescence microscope (Nikon SMZ1500, Japan); Frozen slicing machine (LEIKA CM1860UV, Germany); Intelligent drying oven (ZD-890C; Ouyi Company, Hangzhou, China). In this study, the animal experimental procedures were approved by the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006), and adhered to the standards in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

All experimental procedures related to animals' welfare in this research were performed according to the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006), and the standards in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animal experimental protocols in this study were also approved by the animal ethics committee of He University. Thirty female C57/BL6 mice (age range, 6–8 weeks) were purchased from Liaoning Changsheng Biotechnology Co., LTD. The animals were divided into 6 groups (each group,  $n = 5$ ): blank control group, model control group, two compound **1b** treatment groups (concentrations: 5% and 7% W/V), vehicle control group treated with the vehicle of compound **1b** solution and positive control group treated with 5% Lifitegrast. The dry eye model of mice was created by intelligent environment control system combined with drug induction. Two-step dehumidification method was used to control the environmental humidity, The relative humidity in the room was reduced to  $40 \pm 5\%$  using the temperature dehumidifier, and the intelligent dehumidifier (dehumidification range RH 10–80%) is used to further reduce the humidity of the cabinet. At the same time, the air flow in the cabinet were maintained at  $2.1 \pm 0.2$  m/s by the ventilator located 20 cm from the mouse cages in the cabinet. Through the above measures, humidity, temperature and air flow are effectively regulated. C57/BL6 mice were exposed to dry conditions (RH = 15; WV = 2 m/s; T = 21–23 °C) for 14 days, subcutaneously were injected to hindquarters alternately with scopolamine hydrobromide (0.5 mg/0.2 ml) three times a day (9:00 am, 1:00 PM, 4:00 pm) for 14 days to induce dry eye symptoms [30]. At the same time of dry eye development, the treatment groups mice were administrated on the surface of the both eyeball with 5  $\mu$ L compound **1b** eye drops, or treated with Lifitegrast (5%, USA) individually, and treatment with the vehicle of compound **1b** solution was used as vehicle control group. There were 5 mice in each group, and twice a day for topical administration of drugs (at 9:00 am and 3:00 pm, respectively).

### Frozen section preparation

Periodic acid-Schiff (PAS) and TUNEL staining were performed on frozen section in this study. The animals were sacrificed, and the eyeballs and appendages ( $n = 10$  eyes/group) were surgically excised and then embedded by OCT (VWR, Suwanee, GA, USA). The central sagittal plane of the eyeball was sectioned with 6  $\mu$ m thickness and stored at  $-80$  °C [31].

### Corneal fluorescein staining

The mice were in an unanesthetized state, 2  $\mu$ L of 1% fluorescein sodium solution was dropped into the conjunctival sac of mice using a micropipette. The cornea was examined using slit-lamp microscopy in cobalt blue light 2 min after fluorescein instillation. The damaged areas of corneal epithelium were stained in green, the stained areas were assessed according to a standardized grading system ranging from 0 to 3, with the corneal surface divided into five regions, 0 score: no dyeing; 1 score: few punctate dyeing; 2 score: diffuse punctate dyeing; 3 score: flake dyeing [32]. Then the total score from the five regions was analyzed.

### Measurement of goblet cell density

Ocular sections were cut at the center of the eye, where the lens has its maximum diameter. Sections (6  $\mu$ m) were stained by periodic-Schiff (PAS) reagent for measuring goblet cell density. The sections were observed and taken for counting with a digital camera (Eclipse Ni-U; Nikon). Goblet cell density was measured in the superior and inferior conjunctival of the eyes of each group. And using image-analysis software (NIS Elements Software, Nikon) and expressed as number of goblet cells per mm [33].

### TUNEL staining

Frozen slices of 6  $\mu$ m were used for the terminal deoxynucleotidyl transferase-mediated FITC-dUTP nick end labeling (TUNEL) apoptosis Assay kit (C1089, Beyotime Institute of Biotechnology, Haimen, China) detection. According to the manufacture's protocol, sections were fixed in 4% paraformaldehyde for 15 min at room temperature, permeabilized in 0.3% Triton X-100 for 3 min at 4 °C. Then the frozen slices were incubated with TUNEL assay at room temperature for 60 min, and the slices were then stained with DAPI (0.5  $\mu$ g/ml, staining the nucleus) for 5 min. After washing with PBS, the sections were visualized, and the images were captured on an inverted fluorescent microscope ( $\times 400$  amplification, Nikon, ECLIPSE Ni, Japan). The central corneal epithelial field of 200  $\mu$ m

was selected, and the number of TUNEL positive cells was counted and recorded [34].

### Statistical analysis

Data are expressed as the mean  $\pm$  standard error of the mean (SEM) of at least three independent experiments. The data were analyzed by one-way analysis of variance (ANOVA) with SPSS 20.0 (IBM). The histograms were plotted with Graphpad prism 8. Image J was used to show the image and calculate the gray value of protein bands in western blot.

**Acknowledgements** This research was funded by Technology Program of Liaoning, grant number 2019JH2/10300011.

### Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Consent for publication** All authors have seen the manuscript and agree to its publication.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

### References

- Bartlett J, Keith M, Sudharshan L, Snedecor S. Associations between signs and symptoms of dry eye disease: a systematic review. *Clin Ophthalmol*. 2015;9:1719–30. <https://doi.org/10.2147/OPTH.S89700>.
- Rouen PA, White ML. Dry eye disease: prevalence, assessment, and management. *Home Healthc Now*. 2018;36:74–83. <https://doi.org/10.1097/NHH.0000000000000652>.
- Pult H, Purslow C, Murphy P. The relationship between clinical signs and dry eye symptoms. *Eye*. 2011;25:502–10. <https://doi.org/10.1038/eye.2010.228>.
- Potvin R, Makari S, Rapuano CJ. Tear film osmolarity and dry eye disease: a review of the literature. *Clin Ophthalmol*. 2015;9:2039–47. <https://doi.org/10.2147/OPTH.S95242>.
- Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell*. 1987;51:813–9. [https://doi.org/10.1016/0092-8674\(87\)90104-8](https://doi.org/10.1016/0092-8674(87)90104-8).
- Makgoba MW, Sanders ME, Luce GEG, Dustin ML, Springer TA, Clark EA. et al. ICAM-1 a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. *Nature*. 1988;331:86–88. <https://doi.org/10.1038/331086a0>.
- Walling BL, Kim M. LFA-1 in T Cell Migration and Differentiation. *Front Immunol*. 2018;9:952. <https://doi.org/10.3389/fimmu.2018.00952>.
- Pflugfelder SC, Stern M, Zhang S, Shojaei A. LFA-1/ICAM-1 Interaction as a Therapeutic Target in Dry Eye Disease. *J Ocul Pharmacol Therap*. 2017;33:5–12. <https://doi.org/10.1089/jop.2016.0105>.
- Perez VL, Pflugfelder SC, Zhang S, Shojaei A, Haque R. Lifitegrast, a Novel Integrin Antagonist for Treatment of Dry Eye Disease. *Ocul Surf*. 2016;14:207–15. <https://doi.org/10.1016/j.jtos.2016.01.001>.
- Keating GM. Lifitegrast Ophthalmic Solution 5%: A Review in Dry Eye Disease. *Drugs*. 2017;77:201–8. <https://doi.org/10.1007/s40265-016-0681-1>.
- Sheppard JD, Torkildsen GL, Lonsdale JD, D'Ambrosio FA, McLaurin EB, Eiferman RA. et al. Lifitegrast Ophthalmic Solution 5.0% for Treatment of Dry Eye Disease: Results of the OPUS-1 Phase 3 Study. *Ophthalmology*. 2014;121:475–83. <https://doi.org/10.1016/j.ophtha.2013.09.015>.
- Lollett IV, Galor A. Dry eye syndrome: developments and Lifitegrast in perspective. *Clin Ophthalmol*. 2018;12:125–39. <https://doi.org/10.2147/OPTH.S126668>.
- Zhong M, Gadek TR, Bui M, Shen W, Bumier J, Barr KJ. et al. Discovery and Development of Potent LFA-1/ICAM-1 Antagonist SAR 1118 as an Ophthalmic Solution for Treating Dry Eye. *ACS Med Chem Lett*. 2012;3:203–6. <https://doi.org/10.1021/ml2002482>.
- Zhong M, Shen W, Barr KJ, Arbitrario JP, Arkin MR, Bui M. et al. Discovery of tetrahydroisoquinoline (THIQ) derivatives as potent and orally bioavailable LFA-1/ICAM-1 antagonists. *Bioorg Med Chem Lett*. 2020;20:5269–73. <https://doi.org/10.1016/j.bmcl.2010.06.145>.
- Zhong M, Hanan EJ, Shen W, Bui M, Arkin MR, Barr KJ. et al. Structure–activity relationship (SAR) of the  $\alpha$ -amino acid residue of potent tetrahydroisoquinoline (THIQ)-derived LFA-1/ICAM-1 antagonists. *Bioorg Med Chem Lett*. 2011;21:307–10. <https://doi.org/10.1016/j.bmcl.2010.11.014>.
- Abdullahi M, Olotu FA, Soliman ME. Allosteric inhibition abrogates dysregulated LFA-1 activation: Structural insight into mechanisms of diminished immunologic disease. *Comput Biol Chem*. 2018;73:49–56. <https://doi.org/10.1016/j.compbiolchem.2018.02.002>.
- Vuković G, Marinković A, Obradović M, Radmilović V, Čolić M, Aleksić R. et al. Synthesis, characterization and cytotoxicity of surface amino-functionalized water-dispersible multi-walled carbon nanotubes. *Appl Surf Sci*. 2009;255:8057–75. <https://doi.org/10.1016/j.apsusc.2009.05.016>.
- Michael DM, Arthur AH, Karin T, Kevin BS, Rajnandan P, David MS. et al. Structure–Activity Studies for a Novel Series of N-(Arylethyl)-N-(1,2,3,4-tetrahydronaphthalen-1-ylmethyl)-N-methylamines Possessing Dual 5-HT Uptake Inhibiting and  $\alpha$ 2-Antagonistic Activities. *J Med Chem*. 1997;40:1049–62. <https://doi.org/10.1021/jm960723m>.
- Wolfgang G, Matthias N, Bernd P, Claus R, Sébastien S. Comparison of inhibitory activity of isomeric triazolopyridine derivatives towards adenosine receptor subtypes or do similar structures reveal similar bioactivities?. *Bioorg Med Chem Lett*. 2004;14:3307–12. <https://doi.org/10.1016/j.bmcl.2004.03.104>.
- Takayoshi S, Yosuke O, Masaki R, Masashige B, Aogu G, Yukihiko I. et al. Rapid Discovery of Highly Potent and Selective Inhibitors of Histone Deacetylase 8 Using Click Chemistry to Generate Candidate Libraries. *J Med Chem*. 2012;55:9562–75. <https://doi.org/10.1021/jm300837y>.
- Bajwa JS. Chemoselective deprotection of benzyl esters in the presence of benzyl ethers, benzyloxymethyl ethers and n-benzyl groups by catalytic transfer hydrogenation. *Tetrahedron Lett*. 1992;33:2299–302. <https://doi.org/10.1002/chin.199243125>.
- Wang Y, Chen F, Di H, Xu Y, Xiao Q, Wang X. et al. Discovery of Potent Benzofuran Derived Diapophytoene Desaturase (CrtN) Inhibitors with Enhanced Oral Bioavailability for the Treatment of Methicillin-Resistant *S. aureus* (MRSA) Infections. *J Med Chem*. 2016;59:3215–30. <https://doi.org/10.1021/acs.jmedchem.5b01984>.
- Wang Y, Huang W, Xin M, Chen P, Gui L, Zhao X. et al. Identification of 4-(2-furanyl)pyrimidin-2-amines as Janus kinase 2 inhibitors. *Bioorg Med Chem*. 2017;25:75–83. <https://doi.org/10.1016/j.bmc.2016.10.011>.



24. Ozaki Y, Hosoya A, Okamura K, Kim SW. A Convenient Synthesis of 2-Alkylated 1,4-Benzenediols. *Synlett*. 1997;4:365–6. <https://doi.org/10.1055/s-1997-806>.
25. Kilbourn MR, Dischino DD, Welch MJ. Synthesis of DL-[3-11C] phenylalanine. *Int J Appl Radiat Isotopes*. 1984;35:603–5. [https://doi.org/10.1016/0020-708X\(84\)90103-0](https://doi.org/10.1016/0020-708X(84)90103-0).
26. Wang XF, Tian XT, Ohkoshi E, Qin B, Liu YN, Wu PC. et al. Design and synthesis of diarylamines and diarylethers as cytotoxic antitumor agents. *Bioorg Med Chem Lett*. 2012;22:6224–8. <https://doi.org/10.1016/j.bmcl.2012.08.014>.
27. Kubota H, Rice KC. Palladium-catalyzed cyanation of hindered, electron-rich aryl triflates by zinc cyanide. *Tetrahedron Lett*. 1998;39:2907–10. <https://doi.org/10.1002/chin.199830049>.
28. Zhang Y, An Y, He X, Zhang D, He W. Esculetin protects human corneal epithelial cells from oxidative stress through Nrf-2 signaling pathway. *Exp Eye Res*. 2020;202:108360. <https://doi.org/10.1016/j.exer.2020.108360>.
29. Gipson IK. Goblet cells of the conjunctiva: A review of recent findings. *Prog Retinal Eye Res*. 2016;54:49–63. <https://doi.org/10.1016/j.preteyeres.2016.04.005>.
30. Zhang X, Chen W, De Paiva CS, Corrales RM, Volpe EA, McClellan AJ. et al. Interferon- $\gamma$  Exacerbates Dry Eye-Induced Apoptosis in Conjunctiva through Dual Apoptotic Pathways. *Investig Ophthalmol Vis Sci*. 2011;52:6279–85. <https://doi.org/10.1167/iovs.10-7081>.
31. Ma N, Siegfried C, Kubota M, Huang J, Liu Y, Liu M. et al. Expression profiling of ascorbic Acid-Related transporters in human and mouse eyes. *Investig Ophthalmol Vis Sci*. 2016;57:3440–50. <https://doi.org/10.1167/iovs.16-19162>.
32. Lemp MA. Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. *Clao J*. 1995;21:221–32. <https://doi.org/10.1016/j.susc.2013.06.001>.
33. Chen W, Zhang X, Zhang J, Chen J, Wang S, Wang Q. et al. A Murine Model of Dry Eye Induced by an Intelligently Controlled Environmental System. *Investig Ophthalmol Vis Sci*. 2008;49:1386–91. <https://doi.org/10.1167/iovs.07-0744>.
34. Li W, Fang Q, Zhong P, Chen L, Wang L, Zhang Y. et al. EGFR Inhibition Blocks Palmitic Acid-induced inflammation in cardiomyocytes and Prevents Hyperlipidemia-induced Cardiac Injury in Mice. *Sci Rep*. 2016;6:24580. <https://doi.org/10.1038/srep24580>.