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Discovery and SAR study of piperidine-based derivatives as novel influenza virus inhibitors

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Abstract

A series of piperidine-based derivatives were identified as novel and potent inhibitors of influenza virus through structural modification of the original compound that was selected from a high-throughput screen. Various analogues were synthesized and confirmed as inhibitors. The structure-activity relationship (SAR) studies suggested that the ether linkage between the quinoline and piperidine is critical to the inhibitory activity. Optimized compound *tert*-Butyl 4-(quinolin-4-yloxy)piperidine-1-carboxylate **11e** had an excellent inhibitory activity of influenza virus infection against a variety of influenza virus strains with EC₅₀ values as low as 0.05 μM. The selectivity index value (SI = MLD₅₀/EC₅₀) of **11e** is over 160,000 based on cytotoxicity measured by MTT assays of three cell lines. We carried out the time-of-addition experiment to delineate the mechanism of inhibition. The result indicates that **11e** interferes with an early to middle stage of influenza virus replication.

Introduction

Emergence of influenza A H1N1 virus of swine origin in 2009 serves as a warning once again that new strains of influenza viruses may spread worldwide at an unexpected moment.¹⁻² In 2011, patients infected by avian influenza virus were again identified in Hong Kong and Shenzhen of China.³ Since March, 2013, a novel avian influenza A H7N9 virus that infects humans has been uncovered in China.⁴ Therefore, prevention and treatment of influenza virus infection is a constant demand for the public health. The most effective way to control newly emerged influenza virus may be anti-influenza drugs because production and delivery of suitable vaccines could lag behind virus spread. Currently, the popular treatment of influenza virus infection is by neuraminidase inhibitors (NAIs), such as oseltamivir, zanamivir and peramivir.⁵⁻⁹ NAIs bind to the active site of neuraminidase that is a major surface antigen of influenza virus type A and B, and inhibit the release of newly

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produced influenza virus from infected cells.¹⁰⁻¹¹ However, the efficacy of NAIs may be diminished when mutations occur in the active site of neuraminidase. One of the mutations that occur fairly often in clinical isolates of influenza virus is at residue His274.¹²⁻¹⁵ When His274 was changed to a tyrosine or other residues, NAIs could no longer bind to neuraminidase tightly so that the mutant influenza virus becomes drug-resistant. If drug-resistant influenza virus begins to circulate in humans, NAI drugs would become obsolete. It is therefore necessary to develop novel inhibitors of influenza virus that target different viral proteins. If novel inhibitors may be developed as anti-influenza drugs, they may be used effectively to treat infection of NAI-resistant influenza virus or be included in combination therapy with NAIs to reduce the chance of emergence of drug-resistant influenza virus.

In the replication cycle of influenza virus in the host cell, there are a number of critical steps that may be blocked by inhibitors to stop virus infection. These steps include virus attachment to cell surface receptors (sialomolecules on cell surface), membrane fusion during virus entry, virus replication, virus assembly at the plasma membrane, and release.¹⁶ Some of the steps occur outside the host cell, such as virus attachment and release, whereas other steps take place inside the host cell. Since NAIs act on neuraminidase outside the host cell, we focus on finding inhibitors that act inside the host cell. It is likely that inhibitors that work inside the host cell would target influenza virus proteins other than neuraminidase. A high throughput screen was developed for identifying such inhibitors among a library of 10,000 compounds.¹⁷ A novel piperidine-based derivative was found to inhibit influenza virus infection effectively. Virological studies confirmed that this series of compounds inhibit influenza virus replication after virus entry into the host cell. One of the optimized piperidine-based derivatives has a potent anti-influenza activity in nanomolar range. We report here our strategy for inhibitor identification and structure-activity relationship (SAR) studies of this series of piperidine-based derivatives with regard to anti-influenza activities against influenza virus infection.

Results and discussion

HTS leading hit discovery

In order to identify anti-influenza virus agents that can inhibit virus replication inside the host cell, we developed a cell-based assay for influenza virus infection as a high throughput screen (HTS). MDCK cells¹⁸ were plated in 96-well trays and allowed to grow for 24 hours in an incubator set at 37°C under 5% CO₂. When cells became 90% confluent, each well was inoculated with 300 PFU of influenza virus A/Udm/72 (H3N2). Virus infection was allowed to proceed for 48 hours and the cells were fixed and stained. Without inhibitors, infected cells would be cleared as shown in Fig. 1a. Ribavirin and a fusion inhibitor F136¹⁹ that acts outside the host cell were used as controls. To identify inhibitors that act only inside the host cell, compounds were added in the growth media at 100 µg/mL concentration after the virus inoculum was incubated with the cells for one hour. Using this HTS strategy, we have discovered a novel piperidine-based derivative, ethyl 4-(quinolin-4-ylthio)piperidine-1-carboxylate (Fig. 1b), (labelled here as P114F5) from a library of 10,000 compounds (MyriaScreen from Sigma-Aldrich). The EC₅₀ value of P114F5 is 13.9 µM based on plaque assay (Fig. 1c). There is no literature report of this compound as an anti-

influenza agent based on our literature survey. A time-of-addition experiment was carried out to confirm that P114F5 indeed inhibits influenza virus infection after it enters the host cell. As shown in Fig. 1d, P114F5 was most effective as an inhibitor of influenza virus when it was added to the host cell during virus inoculation and one hour after inoculation. When it was added later, no significant anti-influenza activities were observed, suggesting that P114F5 does not interfere with influenza virus release as NAIs.

Hit optimization and biological evaluation

In order to gain more insights on the mechanism of action by P114F5, we systematically altered the chemical structure of P114F5 to probe the contribution of P114F5 substructures to the anti-influenza activity. The structure-activity relationship (SAR) studies would help to explore the subunits in P114F5 pertained to its anti-influenza activity. For the convenience of SAR discussion, P114F5 can be readily divided into four subunits, a quinolinyl core (A), a sulfur core (B), a piperidinyl core (C), and a carbamate core (D), leading to a study design where one subunit can be independently varied in the presence of three other fixed subunits (Fig. 2).

We initially tested the influence of subunit A (quinolinylgroup) on the anti-influenza activity by replacing quinolinylgroup in P114F5 with substituted quinolinyl (**1a** and **1b**), pyridinyl (**1c**), substituted phenyl (**1d** and **1e**), naphthyl (**1f**), substituted 2-oxo-2H-chromen-4-yl (**1g** and **1h**), and 2-oxo-1,2-dihydroquinolin-4-yl (**1i**) groups, and compounds **1a-i** (Table 1) were made by nucleophilic replacement of a halo compound with a thiol compound produced the corresponding compounds **1a-i**.²⁰ Thioether **1a-c** and **1g-i** were directly obtained from the commercially available 4-chloro compound **2a-b** and **2g-i** in very good yields by treatment of **3**²¹ with slightly more than one equivalent of sodium hydride in DMF at 50 °C. In addition, commercially available benzenethiol **2d-e** or naphthalenethiol derivatives **2f** were coupled with bromo compound **4**²² by the treatment of sodium hydride to generate the desired compound **1d-f** (Scheme 1).

As shown in Table 1, the anti-influenza activity of these compounds with different modifications of subunit A varied greatly, such as compounds **1a**, **1c** and **1g-i** that did not show any inhibition to influenza virus at a concentration of 100 μM, while other compounds, such as **1b**, **1d**, **1e** and **1f**, have moderate inhibitory activities, though they were usually less potent than the original HTS hit P114F5 (EC₅₀ = 13.9 μM). These results indicated that the modification of subunit A didn't dramatically improve compound's inhibitory potency.

Next, we switched our focus on subunit B, a sulfur subunit in compound P114F5. Compounds **6a** and **6b** were prepared from **5a** and **5b**²³ (Table 2), in which the sulfur subunit in compound P114F5 was replaced with O and NH subunit, respectively. The synthesis of compounds **6a-b** was similar to that of compounds **1a-i** (Scheme 2). The nucleophilic displacement of 4-chloroquinoline occurs readily either by an oxygen or amine nucleophile to deliver the corresponding compounds **6a-b**.

As we can see from Table 2, the analogue **6a** with oxygen as subunit B showed a good inhibitory activity with a 6-fold improvement over the HTS hit P114F5, while analogue **6b** with amine as subunit B showed moderate inhibitory activity. The modification of subunit B

can clearly affect the anti-influenza activity. Compounds with oxygen as subunit B showed a better inhibitory activity, compared to the HTS hit P114F5. This result implied that using oxygen as subunit B is very important for the anti-influenza activity.

Thirdly an investigation of the substitutions of the piperidinyl ring (subunit C) was carried out, in which a phenyl ring or a cyclohexyl ring was used. The synthesis of **8a-f** was also similar to that of compounds **1a-i** by use of the nucleophilic displacement reaction from 4-chloroquinoline and **7a-f** (Scheme 3).

The data for compounds **8a-f** identified the role of subunit C on its anti-influenza activity, as summarized in Table 3. The activity of compounds **8a-f** varied greatly. Using a phenyl ring as subunit C will result in the loss of inhibitory activity, no matter that sulfur or amine or oxygen was used as subunit B, such as compounds **8a-c**. However, compounds with a cyclohexyl ring as subunit C showed a good inhibitory activity with the EC₅₀ value from 10 μM to 36 μM, such as compounds **8d-f**. These results indicated that an non-planar ring used as subunit C can present an important conformation in the inhibitor structure for its interactions with the target, then exhibited an excellent inhibitory activity.

Finally, we explored the role of subunit D (carbamate) of P114F5 by fixing subunit B as S or O and subunit C as piperidine moieties. Ethyl carbamate was firstly converted to other alkyl carbamates, such as methyl carbamate **11a** and **11d**, *tert*-butyl carbamate **11b** and **11e**, *n*-hexyl carbamate **11c** and **11f**. These compounds can be prepared following the synthetic route below (Scheme 4): the nucleophilic displacement reaction between 4-chloroquinoline and *tert*-butyl 4-mercaptopiperidine-1-carboxylate **9a** or *tert*-butyl 4-hydroxypiperidine-1-carboxylate **9b** easily offered compound **11b** or **11c**, following the treatment with TFA to generate compound **11g** or **11h**, which was treated with *N,N'*-carbonyldiimidazole (CDI), then R-OH was added to produce the desired compounds **11c** and **11f**.²⁴⁻²⁵ The synthesis of **11i-j** was prepared via the nucleophilic displacement reaction according to the published procedure from 4-chloroquinoline and tetrahydrofuran-3-ol or tetrahydro-2H-pyran-4-ol.

Table 4 revealed that alkyl carbamate analogues with sulfur as subunit B did not show clear enhancement in its inhibitory activity compared to P114F5. However, alkyl carbamate analogues with oxygen as subunit B showed a dramatic improvement in the inhibitory activity, such as compound **11e** with *tert*-butyl carbamate group has a 100-fold better activity compared to HTS hit. Removal of the carbamate group significantly reduced the activity, such as **11h**. The in-house synthesized compounds without carbamate motif, such as compounds **11i-j** with tetrahydrofuran or tetrahydro-2H-pyran as subunit C and subunit D, led to loss of activity. These results indicated that carbamate group may play an important role on the biological activity by specific interactions with acceptors by forming hydrogen bond. Compound with the bulky alkyl (*tert*-butyl) in carbamate motif showed an excellent anti-influenza activity. This result can be explained that the bulky alkyl in carbamate motif may form close integration during compound being bound to its target.²⁶

A follow up-focused library changing the carbamate group to an amide group by introducing an amino acid moiety was then synthesized. In this library, we kept sulfur or oxygen as

subunit B and quinoline as subunit A. The synthesis of compound **12a-g** in Table 5 was outlined in Scheme 5. The classic amide bond formation can be easily achieved using 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) as a coupling reagent between amine compound **11g** or **11h** and Boc-protected amino acid to generate the corresponding compound **12a-g**.²⁷

The anti-influenza activities of compounds with amino acid as subunit D are summarized in Table 5. The introduction of an amino acid did not clearly improve the inhibitory activity with sulfur or oxygen as subunit B. Usually, these compounds showed a clear inhibitory activity with the EC₅₀ value from 10 μM to 100 μM. Introducing amino acid motif as subunit D to increase the ability of forming hydrogen bond with acceptors did not clearly improve the anti-influenza activity. We believe that the substantial steric effect of amino acid side chains in **12a-g** resulted in their worse anti-influenza activities.

Cytotoxic activity of the most potent compound **11e** in different cell lines

To exclude that the observed anti-influenza activity could be due to non-specific cytotoxic activity, MTT assays of the most potent inhibitor **11e** were performed by incubating with **11e** three cell lines, i.e. Madin-Darby canine kidney (MDCK) cells, human embryonic kidney cells (HEK293 cells) and human epithelial carcinoma cell line (HeLa cells).²⁸ As shown in Table 6, **11e** did not exhibit cytotoxicity in any of the cell lines up to a concentration of millimolar (mM) range, and its selectivity index value (SI = MLD₅₀/EC₅₀) was over 160,000. This result indicated that the excellent anti-influenza activity of the piperidine-based derivatives was not due to non-specific cytotoxic activity.

In vitro anti-influenza activity of the most potent compound **11e** against various strains

A good anti-influenza drug should have an excellent inhibitory activity against different strains of influenza viruses. After evaluating anti-influenza activities using influenza virus A/Udorn/72 (H3N2), we were interested in investigating the potent inhibitor **11e** with other strains of influenza viruses. For this purpose, we selected four strains of influenza A virus (two H3N2 and two H1N1 strains) and one strain of influenza B (Table 7). The results showed that **11e** also effectively inhibited all the influenza virus strains tested with varied EC₅₀ values that could be as low as 0.05 μM. These results indicated that **11e** is a broad spectrum anti-influenza inhibitor and could potentially provide an effective coverage against infection over different strains of influenza virus.

Time-of-addition studies of the most potent compound **11e**

To explore the mechanism of action, time-of-addition studies (Figure 3) were carried out to determine the effectiveness of **11e** to reduce the viral yield with regard to the time when the compound was added to the culture medium during the course of the infection.²⁹ As shown in Figure 3, significant inhibition of virus replication was observed when compound **11e** was added to the cells within 6 h after virus inoculation. However, compound **11e** did not show obvious inhibition of influenza virus infection when incubated with the virus inoculum prior to infection, in contrast to the fusion inhibitor that was used as a control. The studies suggested that **11e** could act at early stages of influenza virus replication cycle, including entry and/or an early replication phase.

Conclusions

The threat of a pandemic influenza is a burden that will remain for a long period of time. To date, vaccination is considered as an effective preventive measure to control influenza. However, vaccine development for millions of people, especially with antigenic shift and drift of influenza viruses, is still a multifaceted challenge. To find an alternative, a global trend is to stockpile effective anti-influenza medications. Considering the emergence of resistant strains to current medications it is necessary to develop new therapeutic agents that are effective against emerging drug-resistant influenza viruses. A piperidine-based compound was identified as a novel influenza virus inhibitor from the HTS campaign. Chemistry-driven optimization of the hit compound has resulted in the discovery of the optimized compound **11e** with a high potency in inhibiting influenza virus replication, and a clear SAR has been obtained. Substitutions with different aromatic rings were tolerated for subunit A, but compounds with quinolinyl groups usually offered a better inhibitory activity. Oxygen as subunit B was a game changing replacement for sulfur found in P114F5. Rigid rings as subunit C will lead to a substantial loss of inhibitory activity, most likely due to conformational specificities in interactions with the potential target. The carbamate group as subunit D resulted in compound **11e** with an excellent inhibitory potency ($EC_{50} = 0.10 \mu M$). Compound **11e** inhibits the activity of both influenza A and B strains *in vitro*, suggesting that it could act as a starting compound for further anti-influenza drug development. We did not observe cytotoxicity of **11e** in any of the cell lines up to a concentration of millimolar (mM) range, and its selectivity index value was over 160,000. The preliminary mechanistic studies of compound **11e** suggest that this type of piperidine-based derivatives interferes with an early stage of influenza virus replication. The precise target with which compound **11e** interacts remains to be uncovered. The optimized leading compound identified here could open up new opportunities for anti-influenza drug discovery. Work is continued to optimize the selectivity and explore the detailed mechanism of action for this novel inhibitor.

Experimental section

Chemistry

Anhydrous THF and dioxane were distilled from sodium-benzophenone, and dichloromethane was distilled from calcium hydride. All starting materials were purchased from either J&K Chemicals Co. or Sigma Aldrich Chemical Co.. Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Tsingdao silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and potassiumpermanganate, and heat as developing agents. Tsingdao silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography to purified compounds. Yields refer to chromatographically and spectroscopically (1H NMR) homogeneous materials, unless otherwise stated. Melting points were measured in SGW X-4B microscopic melting point apparatus, and were uncorrected. All compounds were characterized by 1H and ^{13}C NMR

using Bruker 300 MHz NMR and/or Bruker 400 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform δ 7.26 and δ 77.5, DMSO- d_6 δ 2.54 and δ 39.5, and coupling constants (J) are reported in Hertz (Hz) (where, s = singlet, b = broad, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of doublet, t = triplet, td = double triplet, q = quartet, m = multiplet) and analyzed using ACD NMR data processing. High resolution mass spectra were taken using Bruker Apex IV RTMS using electrospray ionisation (ESI) technique. Mass spectra values were reported as m/z .

For the preparation of 1a-c and 1g-i

To a degassed solution of ethyl 4-mercaptopiperidine-1-carboxylate **3**²¹ (227mg, 1.20 mmol) in 5 mL of anhydrous DMF was added NaH (60% in mineral oil, 48mg, 1.20 mmol) at 0°C. The suspension mixture was stirred for additional 15 min at room temperature. Then, a solution of the commercial 4-chloro-quinoline derivatives **2a-c** or other aromatic halo compounds **2g-i** (1.00 mmol) in 5 mL of anhydrous DMF was added to the above suspension mixture, and the mixture was stirred at 50°C until halo compounds disappeared. The reaction mixture was then poured in ice-water and extracted with ethyl acetate, dried over MgSO₄, and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **1a-e** and **1g-i** with the 60-85% yield. **For the preparation of 1d-f:** To a degassed solution of benzenethiol **2d-e** or naphthalene-1-thiol derivatives **2f** (1.20 mmol) in 5 mL of degassed DMF was added NaH (60% in mineral oil, 48mg, 1.20 mmol) at 0°C. The suspension mixture was stirred for additional 15 min at room temperature. Then, a solution of the commercial ethyl 4-bromopiperidine-1-carboxylate **4**²² (236mg, 1.00 mmol) in 5 mL of anhydrous DMF was added to the above suspension mixture, and the mixture was stirred for 5 h at 50°C until **4** disappeared. The reaction mixture was then poured in ice-water and extracted with ethyl acetate, dried over MgSO₄, and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **1d-f** with the 75-90% yield.

Ethyl 4-((2-phenylquinolin-4-yl)thio)piperidine-1-carboxylate 1a

was prepared from 4-chloro-2-phenylquinoline and ethyl 4-mercaptopiperidine-1-carboxylate in 65% yield as pale yellow solid. Mp: 110-112 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.22 (dd, J = 8.4, 3.0, 2H, Ar-*H*), 8.11 (J = 8.4, 1.8, 2H, Ar-*H*), 7.78 (s, 1H, Ar-*H*), 7.73 (t, J = 6.9, 1H, Ar-*H*), 7.56-7.47 (m, 4H, Ar-*H*), 4.12 (q, J = 6.0, 2H, OCH₂), 4.09-4.00 (m, 2H, NCH₂), 3.66-3.62 (m, 1H, SCH), 3.20-3.11 (m, 2H, NCH₂), 2.13-2.10 (m, 2H, NCH₂CH₂), 1.78-1.72 (m, 2H, NCH₂CH₂), 1.26 (t, J = 6.9, 3H, OCH₂CH₃). ¹³C NMR (75.5 MHz, CDCl₃): δ 156.48, 155.33, 147.96, 145.14, 139.46, 130.26, 130.04, 129.44, 128.87, 127.52, 126.53, 126.36, 123.96, 117.74, 61.44, 42.97, 42.30, 31.90, 14.64. HRMS (ESI) calcd for C₂₃H₂₅N₂O₂S [M+H]⁺ 393.1637, found 393.1630.

Ethyl 4-((2-chloroquinolin-4-yl)thio)piperidine-1-carboxylate 1b

was prepared from 2,4-dichloroquinoline and ethyl 4-mercaptopiperidine-1-carboxylate in 60% yield as off-white solid. Mp: 98-100 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.14 (dd, J =

7.5, 3.0, 1H, Ar-*H*), 7.92 (d, *J* = 8.1, 1H, Ar-*H*), 7.73 (dt, *J* = 6.9, 1.5, 1H, Ar-*H*), 7.53 (dt, *J* = 8.1, 1.2, 1H, Ar-*H*), 7.30 (s, 1H, Ar-*H*), 4.32-4.26 (m, 1H, SCH), 4.15 (q, *J* = 7.0, 2H, OCH₂), 4.10-3.90 (m, 2H, NCH₂), 3.27-3.18 (m, 1H, NCH₂), 2.97-2.89 (m, 1H, NCH₂CH₂), 2.20-2.14 (m, 1H, NCH₂CH₂), 2.02-1.96 (m, 1H, NCH₂CH₂), 1.80-1.56 (m, 1H, NCH₂CH₂), 1.26 (t, *J* = 7.0, 3H, OCH₂CH₃). ¹³C NMR (75.5 MHz, CDCl₃): δ 158.92, 155.01, 154.97, 148.61, 141.41, 131.71, 128.43, 127.28, 124.18, 123.73, 120.94, 61.18, 43.47, 36.33, 35.42, 31.75, 21.14, 14.96. HRMS (ESI) calcd for C₁₇H₁₉N₂O₂NaSCl [M+Na]⁺, 373.0753, found 373.0746.

Ethyl 4-(pyridin-4-ylthio)piperidine-1-carboxylate 1c

was prepared from 4-chloropyridine and ethyl 4-mercaptopiperidine-1-carboxylate in 85% yield as gray solid. Mp: 88-90 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.62 (d, *J* = 6.6, 2H, Ar-*H*), 7.90 (d, *J* = 6.6, 2H, Ar-*H*), 4.06-3.99 (m, 3H, OCH₂ and SCH), 3.92-3.87 (m, 2H, NCH₂), 3.15-3.07 (m, 2H, NCH₂), 2.05-2.00 (m, 2H, NCH₂CH₂), 1.56-1.51 (m, 2H, NCH₂CH₂), 1.17 (t, *J* = 6.9, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 161.39, 154.88, 140.32, 122.97, 61.22, 43.11, 40.73, 31.17, 14.98. HRMS (ESI) calcd for C₁₃H₁₉N₂O₂S [M+H]⁺ 267.1167, found 267.1161.

Ethyl 4-(p-tolylthio)piperidine-1-carboxylate 1d

was prepared from 4-methylbenzenethiol and ethyl 4-bromopiperidine-1-carboxylate in 75% yield as pale yellow solid. Mp: 82-85 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.34 (d, *J* = 8.1, 2H, Ar-*H*), 7.12 (d, *J* = 8.1, 2H, Ar-*H*), 4.12 (q, *J* = 7.2, 2H, OCH₂), 4.03-3.99 (m, 2H, NCH₂), 3.15-3.10 (m, 1H, SCH), 2.99-2.90 (m, 2H, NCH₂), 2.34 (s, 3H, Ar-CH₃), 1.94-1.88 (m, 2H, NCH₂CH₂), 1.58-1.49 (m, 2H, NCH₂CH₂), 1.25 (t, *J* = 7.2, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 154.89, 137.15, 132.60, 130.07, 61.07, 43.59, 43.20, 32.14, 20.93, 14.85. HRMS (ESI) calcd for C₁₅H₂₂NO₂S [M+H]⁺ 280.1371, found 280.1367.

Ethyl 4-((4-methoxyphenyl)thio)piperidine-1-carboxylate 1e

was prepared from 4-methoxybenzenethiol and ethyl 4-bromopiperidine-1-carboxylate in 80% yield as pale yellow solid. Mp: 90-92 °C. ¹H NMR (300 MHz, CDCl₃): δ 7.39 (d, *J* = 9.0, 2H, Ar-*H*), 6.84 (d, *J* = 9.0, 2H, Ar-*H*), 4.10 (q, *J* = 7.2, 2H, OCH₂), 4.04-3.95 (m, 2H, NCH₂), 3.80 (s, 3H, OCH₃), 3.05-2.96 (m, 1H, SCH), 2.96-2.86 (m, 2H, NCH₂), 1.90-1.84 (m, 2H, NCH₂CH₂), 1.53-1.41 (m, 2H, NCH₂CH₂), 1.23 (t, *J* = 7.0, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 159.56, 154.93, 135.67, 123.43, 115.04, 61.10, 55.55, 44.44, 43.25, 32.15, 14.89. HRMS (ESI) calcd for C₁₅H₂₂NO₃S [M+H]⁺ 296.1320 found 296.1309.

Ethyl 4-(naphthalen-1-ylthio)piperidine-1-carboxylate 1f

was prepared from naphthalene-1-thiol and ethyl 4-bromopiperidine-1-carboxylate in 78% yield as pale yellow solid. Mp: 95-97 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.54 (dd, *J* = 6.9, 1.2, 1H, Ar-*H*), 7.89-7.82 (m, 2H, Ar-*H*), 7.75 (dd, *J* = 8.0, 1.2, 1H, Ar-*H*), 7.42 (dd, *J* = 8.4, 1.2, 1H, Ar-*H*), 4.12 (q, *J* = 7.2, 2H, OCH₂), 4.03-3.99 (m, 2H, NCH₂), 3.28-3.21 (m, 1H, SCH), 2.98-2.89 (m, 2H, NCH₂), 1.93-1.88 (m, 2H, NCH₂CH₂), 1.62-1.58 (m, 2H,

NCH₂CH₂), 1.26 (t, *J* = 7.2, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 154.93, 134.06, 133.76, 131.71, 130.86, 129.02, 128.60, 127.23, 126.78, 126.19, 125.31, 61.12, 43.77, 43.20, 32.24, 14.91. HRMS (ESI) calcd for C₁₈H₂₂NO₂S [M+H]⁺ 316.1371, found 316.1363.

Ethyl 4-((7-methyl-2-oxo-2H-chromen-4-yl)thio)piperidine-1-carboxylate 1g

was prepared from 4-chloro-7-methyl-2H-chromen-2-one and ethyl 4-mercaptopiperidine-1-carboxylate in 85% yield as off-white solid. Mp: 120-122 °C. ¹H NMR (300 MHz, CDCl₃): δ 7.61 (d, *J* = 8.4, 2H, Ar-*H*), 7.14-7.08 (m, 2H, Ar-*H*), 6.15 (s, 1H, Ar-*H*), 4.16 (q, *J* = 7.2, 2H, OCH₂), 4.11-4.02 (m, 2H, NCH₂), 3.59-3.53 (m, 1H, SCH), 3.21-3.13 (m, 2H, NCH₂), 2.45 (s, 3H, Ar-CH₃), 2.19-2.14 (m, 2H, NCH₂CH₂), 1.81-1.76 (m, 2H, NCH₂CH₂), 1.26 (q, *J* = 7.2, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 158.68, 154.90, 154.25, 152.17, 143.87, 125.87, 123.83, 117.24, 115.49, 106.81, 70.54, 61.19, 31.38, 21.38, 14.96. HRMS (ESI) calcd for C₁₈H₂₂NO₄S [M+H]⁺ 348.1270, found 348.1265.

Ethyl 4-((7-methoxy-2-oxo-2H-chromen-4-yl)thio)piperidine-1-carboxylate 1h

was prepared from 4-chloro-7-methoxy-2H-chromen-2-one and ethyl 4-mercaptopiperidine-1-carboxylate in 80% yield as off-white solid. Mp: 125-127 °C. ¹H NMR (300 MHz, CDCl₃): δ 7.63 (d, *J* = 9.0, 1H, Ar-*H*), 6.86-6.81 (m, 2H, Ar-*H*), 6.06 (s, 1H, Ar-*H*), 4.16 (q, *J* = 7.2, 2H, OCH₂), 4.10-4.03 (m, 2H, NCH₂), 3.88 (s, 3H, Ar-OCH₃), 3.58-3.55 (m, 1H, SCH), 3.21-3.12 (m, 2H, NCH₂), 2.19-2.23 (m, 2H, NCH₂CH₂), 1.79-1.72 (m, 2H, NCH₂CH₂), 1.28 (t, *J* = 6.9, 3H, OCH₂CH₃). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 163.10, 158.87, 154.90, 154.40, 154.05, 125.32, 112.74, 111.31, 104.78, 101.36, 61.18, 56.40, 43.16, 31.45, 14.98. HRMS (ESI) calcd for C₁₈H₂₂NO₅S [M+H]⁺ 364.1219, found 364.1209.

Ethyl 4-((2-oxo-1,2-dihydroquinolin-4-yl)thio)piperidine-1-carboxylate 1i

was prepared from 4-chloroquinolin-2(1H)-one and ethyl 4-mercaptopiperidine-1-carboxylate in 72% yield as off-white solid. Mp: 132-135 °C. ¹H NMR (300 MHz, CDCl₃): δ 12.24 (s, 1H, CONH), 7.81 (d, *J* = 7.2, 1H, Ar-*H*), 7.47-7.45 (m, 1H, Ar-*H*), 7.37-7.34 (m, 1H, Ar-*H*), 7.17-7.15 (m, 1H, Ar-*H*), 6.49 (s, 1H, Ar-*H*), 4.10 (q, *J* = 7.2, 2H, OCH₂), 4.03-3.98 (m, 2H, NCH₂), 3.56-3.49 (m, 1H, SCH), 2.15-2.09 (m, 2H, NCH₂CH₂), 1.71-1.67 (m, 2H, NCH₂CH₂), 1.22 (t, *J* = 7.2, 3H, OCH₂CH₃). ¹³CNMR (100 MHz, CDCl₃): δ 163.02, 155.35, 150.73, 137.61, 131.06, 123.95, 122.46, 118.81, 116.77, 114.24, 61.50, 43.15, 40.95, 31.40, 14.65. HRMS (ESI) calcd for C₁₇H₂₁N₂O₃S [M+H]⁺ 333.1273, found 333.1272.

For the preparation of 6a-b

To a degassed solution of ethyl 4-hydroxypiperidine-1-carboxylate **5a** or ethyl 4-aminopiperidine-1-carboxylate **5b**²⁴ (1.20 mmol) in 5 mL of anhydrous DMF was added NaH (60% in mineral oil, 48mg, 1.20 mmol) at 0°C. The suspension mixture was stirred for additional 15 min at room temperature. Then, a solution of the commercial 4-chloro-quinoline (163mg, 1.00 mmol) in 5 mL of anhydrous DMF was added to the above suspension mixture, and the mixture was stirred at 90°C until 4-chloro-quinoline

disappeared. The reaction mixture was then poured in ice-water and extracted with ethyl acetate, dried over MgSO_4 , and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **6a-b** with the 75-90% yield

Ethyl 4-(quinolin-4-yloxy)piperidine-1-carboxylate **6a**

was prepared from 4-chloroquinoline and ethyl 4-hydroxypiperidine-1-carboxylate in 90% yield as pale yellow solid. Mp: 95-97°C. ^1H NMR (300 MHz, CDCl_3): δ 8.73 (d, $J = 5.0$, 1H, Ar-*H*), 8.23 (dd, $J = 8.4$, 0.9, 1H, Ar-*H*), 8.03 (d, $J = 8.7$, 1H, Ar-*H*), 7.70 (dd, $J = 9.9$, 1.5, 1H, Ar-*H*), 7.51 (dd, $J = 8.4$, 0.9, 1H, Ar-*H*), 6.74 (d, $J = 5.4$, 1H, Ar-*H*), 4.82-4.68 (m, 1H, OCH), 4.17 (q, $J = 7.2$, 2H, OCH_2), 3.78-3.70 (m, 2H, NCH_2), 3.64-3.56 (m, 2H, NCH_2), 2.04-1.93 (m, 4H, NCH_2CH_2), 1.26 (m, $J = 7.2$, 3H, OCH_2CH_3). ^{13}C NMR (100 MHz, CDCl_3): δ 159.77, 155.47, 151.07, 149.45, 129.83, 128.88, 125.63, 121.78, 101.39, 72.12, 61.42, 40.40, 29.97, 14.66. HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{21}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$ 301.1552, found 301.1544.

Ethyl 4-(quinolin-4-ylamino)piperidine-1-carboxylate **6b**

was prepared from 4-chloroquinoline and ethyl 4-aminopiperidine-1-carboxylate in 75% yield as white solid. Mp: 88-90 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.16 (d, $J = 7.8$, 1H, Ar-*H*), 7.73 (dd, $J = 8.1$, 1.8, 1H, Ar-*H*), 7.42-7.35 (m, 2H, Ar-*H*), 7.26(t, $J = 8.1$, 1H, Ar-*H*), 7.07 (d, $J = 8.1$, 1H, Ar-*H*), 6.61 (d, $J = 7.8$, 1H, Ar-*H*), 5.79 (d, $J = 7.8$, 1H, Ar-NH-CH), 4.13-3.94 (m, 4H, NCH_2 and OCH_2CH_3), 3.75-3.68 (m, 1H, Ar-NH-CH), 3.03-2.92 (m, 2H, NCH_2), 2.01-1.97 (m, 2H, NCH_2CH_2), 1.50-1.38 (m, 2H, NCH_2CH_2), 1.18(t, $J = 6.9$, 3H, OCH_2CH_3). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 155.17, 143.07, 134.59, 128.33, 127.16, 126.00, 124.30, 123.53, 122.10, 115.88, 104.15, 61.11, 49.45, 43.06, 31.58, 15.01. HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_2$ $[\text{M}]^+$ 299.1634, found 299.1750.

The synthetic procedure of **8a-f** is similar to that of synthetic procedure of **6a-b**, respectively.

Ethyl 4-(quinolin-4-ylthio)benzoate **8a**

was prepared from 4-chloroquinoline and ethyl 4-mercaptobenzoate in 85% yield as white solid. Mp: 115-117 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.68 (d, $J = 9.4$, 1H, Ar-*H*), 8.23 (d, $J = 0.9$, 1H, Ar-*H*), 8.20 (d, $J = 0.9$, 1H, Ar-*H*), 8.10 (d, $J = 8.4$, 2H, Ar-*H*), 7.77 (dt, $J = 5.7$, 1.5, 1H, Ar-*H*), 7.60 (dt, $J = 6.2$, 1.2, 1H, Ar-*H*), 7.53 (dd, $J = 6.6$, 1.2, 1H, Ar-*H*), 7.03 (d, $J = 4.5$, 1H, Ar-*H*), 4.40 (q, $J = 7.2$, 2H, OCH_2), 1.40 (t, $J = 7.2$, 3H, OCH_2CH_3). ^{13}C NMR (75.5 MHz, $\text{DMSO}-d_6$): δ 165.46, 150.50, 148.02, 144.15, 137.37, 132.91, 130.92, 130.71, 130.29, 130.26, 127.85, 126.38, 124.10, 121.70, 61.41, 14.50. HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{16}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$ 310.0902, found 310.0891.

Ethyl 4-(quinolin-4-yloxy)benzoate **8b**

was prepared from 4-chloroquinoline and ethyl 4-hydroxybenzoate in 80% yield as pale white solid. Mp: 106-108 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.74 (d, $J = 4.2$, 1H, Ar-*H*), 8.32 (d, $J = 1.0$, 1H), 8.17 (m, 3H, Ar-*H*), 7.80 (dt, $J = 5.1$, 0.9, 1H, Ar-*H*), 7.60 (dt, $J = 6.3$, 0.9, 1H, Ar-*H*), 7.23 (d, $J = 3.6$, 2H, Ar-*H*), 6.67 (d, $J = 3.9$, 1H, Ar-*H*), 4.41 (d, $J = 5.1$, 2H,

OCH₂), 1.42 (t, *J* = 5.4, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, CDCl₃): δ 165.39, 159.99, 158.73, 151.89, 149.81, 132.16, 130.83, 129.32, 127.12, 121.78, 121.28, 120.67, 106.71, 61.24, 40.65, 38.99, 14.54. HRMS (ESI) calcd for C₁₈H₁₆NO₃ [M+H]⁺ 294.1130, found 294.1126.

Ethyl 4-(quinolin-4-ylamino)benzoate 8c

was prepared from 4-chloroquinoline and ethyl 4-aminobenzoate in 50% yield as pale white solid. Mp: 98-100 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.32 (s, 1H, Ar-*H*), 8.59 (d, *J* = 4.0, 1H, Ar-*H*), 8.33 (d, *J* = 6.0, 1H, Ar-*NH*-Ar), 7.93 (m, 3H, Ar-*H*), 7.73 (t, *J* = 1.0, 1H, Ar-*H*), 7.59 (t, *J* = 1.0, 1H, Ar-*H*), 7.44 (d, *J* = 6.0, 2H, Ar-*H*), 7.26 (d, *J* = 4.0, 1H, Ar-*H*), 4.29 (q, *J* = 6.0, 2H, OCH₂), 1.31 (t, *J* = 6.0, 3H, OCH₂CH₃). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 165.78, 151.08, 149.41, 146.50, 146.17, 131.15, 129.96, 129.63, 125.59, 123.33, 122.77, 121.10, 119.35, 105.18, 60.74, 14.64. HRMS (ESI) calcd for C₁₈H₁₇N₂O₂ [M+H]⁺ 293.1290, found 293.1287.

Ethyl 4-(quinolin-4-yloxy)cyclohexanecarboxylate 8d

as *cis/trans* mixture was prepared from 4-chloroquinoline and ethyl 4-hydroxycyclohexanecarboxylate (*cis/trans* isomers) in 65% yield as pale white solid. Mp: 91-96 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.73 (d, *J* = 5.4, 1H, Ar-*H*), 8.20 (d, *J* = 7.5, 0.9, 1H, Ar-*H*), 8.03 (d, *J* = 8.4, 1H, Ar-*H*), 7.70 (dt, *J* = 8.1, 1.5, 1H, Ar-*H*), 7.50 (dd, *J* = 8.1, 0.9, 1H, Ar-*H*), 6.74 (d, *J* = 5.4, 1H, Ar-*H*), 4.56-4.52 (m, 1H, Ar-OCH), 4.18 (q, *J* = 6.9, 2H, OCH₂), 2.51-2.49 (m, 1H, CHCO₂Et), 2.32-2.28 (m, 2H, CH₂CH₂), 2.20-2.14 (m, 2H, CH₂CH₂), 1.74-1.66 (m, 4H, CH₂CH₂), 1.29 (t, *J* = 6.9, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 174.91, 159.83, 151.84, 149.29, 130.04, 128.90, 125.90, 122.03, 121.62, 102.56, 75.27, 60.25, 55.23, 41.37, 30.04, 26.46, 14.46. HRMS (ESI) calcd for C₁₈H₂₃NO₃ [M+H]⁺ 300.1600, found 300.1591.

Ethyl 4-(quinolin-4-ylthio)cyclohexanecarboxylate 8e

as *cis/trans* mixture was prepared from 4-chloroquinoline and ethyl 4-mercaptocyclohexanecarboxylate (*cis/trans* isomers) in 80% yield as pale white solid. Mp: 94-99 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.72 (d, *J* = 4.8, 1H, Ar-*H*), 8.22 (d, *J* = 8.4, 1H, Ar-*H*), 8.09 (d, *J* = 8.4, 1H, Ar-*H*), 7.73 (dt, *J* = 8.1, 0.9, 1H, Ar-*H*), 7.59 (dt, *J* = 8.1, 0.9, 1H, Ar-*H*), 7.26 (s, 1H, Ar-*H*), 4.17 (q, *J* = 7.2, 2H, OCH₂), 3.82-3.6 (m, 1H, Ar-SCH), 2.53-2.48 (m, 1H, CHCO₂Et), 2.14-2.05 (m, 2H, CH₂CH₂), 2.01-1.96 (m, 4H, CH₂CH₂), 1.86-1.79 (m, 2H, CH₂CH), 1.28 (t, *J* = 6.8, 3H, OCH₂CH₃). ¹³CNMR (75.5 MHz, DMSO-*d*₆): δ 174.66, 149.95, 147.50, 145.83, 130.23, 130.05, 127.02, 126.49, 123.83, 117.99, 70.54, 60.24, 41.53, 29.40, 25.50, 14.50. HRMS (ESI) calcd for C₁₈H₂₂NO₂S [M+H]⁺ 316.1371, found 316.1365.

Ethyl 4-(quinolin-4-ylamino)cyclohexanecarboxylate 8f

as *cis/trans* mixture was prepared from 4-chloroquinoline and ethyl 4-aminocyclohexanecarboxylate (*cis/trans* isomers) in 55% yield as pale white solid. Mp: 87-92 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.54 (t, *J* = 5.2, 1H, Ar-*H*), 7.98 (d, *J* = 8.4, 1H, Ar-*H*), 7.71 (t, *J* = 7.6, 1H, Ar-*H*), 7.63 (dt, *J* = 6.8, 0.9, 1H, Ar-*H*), 7.43 (t, *J* = 7.2, 1H, Ar-

H), 6.44 (t, *J* = 5.6, 1H, Ar-*H*), 5.01 (d, *J* = 6.8, 0.6H, Ar-NH-CH), 4.90-4.80 (m, 0.4H, Ar-NH-CH), 4.20-4.11 (m, 2H, OCH₂), 3.72 (b, 0.6H Ar-NH-CH), 3.54-3.50 (m, 0.4H, Ar-NH-CH), 2.60-2.56 (m, 0.6H, CHCO₂Et), 2.39-2.31 (m, 0.4H, CHCO₂Et), 2.16-2.08 (m, 1H, CH₂CH₂), 2.03-1.92 (m, 2H, CH₂CH₂), 1.84-1.79 (m, 2H, CH₂CH₂), 1.72-1.62 (m, 1H, CH₂CH₂), 1.39-1.32 (m, 1H, CH₂CH₂), 1.28 (t, *J* = 6.8, 3H, OCH₂CH₃). ¹³C NMR (75.5 MHz, CDCl₃): δ 175.23, 174.57, 151.00, 149.36, 149.31, 148.86, 129.33, 129.29, 129.05, 123.94, 122.46, 122.35, 119.30, 119.25, 99.00, 70.55, 60.15, 50.60, 49.84, 42.34, 31.09, 28.65, 28.00, 25.66, 14.58, 14.52. HRMS (ESI) calcd for C₁₈H₂₃N₂O₂ [M+H]⁺ 299.1741.

For the preparation of **11a-b** and **11d-e**

To a degassed solution of alkyl 4-mercaptopiperidine-1-carboxylate **9a-b** or alkyl 4-hydroxypiperidine-1-carboxylate **9d-e** (1.20 mmol) in 5 mL of anhydrous DMF was added NaH (60% in mineral oil, 48mg, 299.1760, found 1.20 mmol) at 0°C. The suspension mixture was stirred for additional 15 min at room temperature. Then, a solution of 4-chloro-quinoline (1.00 mmol) in 5 mL of anhydrous DMF was added to the above suspension mixture, and the mixture was stirred for 5 h at 50°C until 4-chloro-quinoline disappeared. The reaction mixture was then poured in ice-water and extracted with ethyl acetate, dried over MgSO₄, and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **11a-b** and **11d-e** with the 70-90% yield.

For the preparation of **11g-h**

To a solution of compound **11b** or **11e** (1.00 mmol) in 5 mL of CH₂Cl₂ was added trifluoroacetic acid (1.0 mL, 1.00 mmol) at 0°C. Then, the mixture was stirred at room temperature until **11b** or **11e** disappeared. The reaction mixture was concentrated under reduced pressure. Then the residue was resolved in EtOAc, washed with saturated NaHCO₃ solution, dried over MgSO₄, and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **11g-h** with the 90-95% yield.

For the preparation of **11c** and **11f**

To a degassed solution of *n*-hexyl alcohol (1.00 mmol) in 5 mL of degassed THF was added *N,N'*-carbonyldiimidazole (162mg, 1.00 mmol) at 0°C. The mixture was stirred for additional 15 min at room temperature. Then, a solution of the **11g** or **11h** (1.00 mmol) in 5 mL of anhydrous THF was added to the above mixture, and the mixture was stirred at room temperature for 12h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate, washed with 10% HCl and saturated NaCl solution, dried over MgSO₄, and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **11c** and **11f** with the 55-70% yield.

For the preparation of **11i-j**

To a degassed solution of tetrahydrofuran-3-ol or tetrahydro-2H-pyran-4-ol (1.20 mmol) in 5 mL of anhydrous DMF was added NaH (60% in mineral oil, 48mg, 1.20 mmol) at 0°C. The suspension mixture was stirred for additional 15 min at room temperature. Then, a

solution of 4-chloroquinoline (1.00 mmol) in 5 mL of anhydrous DMF was added to the above suspension mixture, and the mixture was stirred for 5 h at 50°C until 4-chloroquinoline disappeared. The reaction mixture was then poured in ice-water and extracted with ethyl acetate, dried over MgSO_4 , and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **11i-j** with the 90-95% yield.

Methyl 4-(quinolin-4-ylthio)piperidine-1-carboxylate **11a**

was prepared from 4-chloro-quinoline and methyl 4-mercaptopiperidine-1-carboxylate in 90% yield as yellow solid. Mp: 105-107 °C. ^1H NMR (400 MHz, CDCl_3): δ 8.74 (d, $J = 4.8$, 1H, Ar-*H*), 8.19 (d, $J = 8.4$, 1H, Ar-*H*), 8.09 (d, $J = 8.4$, 1H, Ar-*H*), 7.73 (dt, $J = 8.4$, 4.8, 1H, Ar-*H*), 7.56 (dt, $J = 8.4$, 4.8, 1H, Ar-*H*), 7.29 (d, $J = 4.8$, 1H, Ar-*H*), 4.10-3.95 (b, 2H, NCH_2), 3.70 (s, 3H, CO_2CH_3), 3.64-3.59 (m, 1H, Ar-*SCH*), 3.19-3.12 (m, 2H, NCH_2), 2.16-2.05 (m, 2H, NCH_2CH_2), 1.80-1.65 (m, 2H, NCH_2CH_2). ^{13}C NMR (100 MHz, CDCl_3): δ 155.93, 149.16, 147.76, 145.69, 130.16, 130.03, 127.46, 126.83, 124.15, 118.62, 52.89, 43.29, 41.95, 31.77. HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 303.1167, found 303.1160.

tert-Butyl 4-(quinolin-4-ylthio)piperidine-1-carboxylate **11b**

was prepared from 4-chloro-quinoline and *tert*-butyl 4-mercapto-piperidine-1-carboxylate **9a** in 80% yield as yellow solid. Mp: 100-102 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.69 (d, $J = 4.8$, 1H, Ar-*H*), 8.15 (dd, $J = 8.7$, 0.9, 1H, Ar-*H*), 8.03 (d, $J = 8.4$, 1H, Ar-*H*), 7.67 (dt, $J = 6.9$, 1.5, 1H, Ar-*H*), 7.51 (dt, $J = 5.7$, 1.5, 1, Ar-*H*), 7.24 (d, $J = 4.8$, 1H, Ar-*H*), 3.95-3.88 (m, 2H, NCH_2), 3.58-3.52 (m, 1H, *SCH*), 3.08-2.99 (m, 2H, NCH_2), 2.03-1.99 (m, 2H, NCH_2CH_2), 1.68-1.58 (m, 2H, NCH_2CH_2), 1.41 (s, 9H, OC_4H_9). ^{13}C NMR (75.5 MHz, CDCl_3): δ 154.52, 149.10, 147.69, 145.34, 129.91, 129.80, 127.19, 126.48, 123.91, 118.21, 79.77, 41.79, 31.60, 28.28. HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{25}\text{N}_2\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 345.1637, found 345.1626.

n-Hexyl 4-(quinolin-4-ylthio)piperidine-1-carboxylate **11c**

was prepared from **11g** and *n*-hexyl alcohol in 55% yield as pale yellow solid. Mp: 96-99°C. ^1H -NMR (400 MHz, CDCl_3): δ 8.75 (d, $J = 4.8$, 1H, Ar-*H*), 8.20 (d, $J = 8.4$, 1H, Ar-*H*), 8.13 (d, $J = 8.4$, 1H, Ar-*H*), 7.75 (dt, $J = 8.4$, 1.2, 1H, Ar-*H*), 7.60 (d, $J = 7.2$, 1H, Ar-*H*), 7.31 (d, $J = 4.8$, 1H, Ar-*H*), 4.10-3.90 (m, 4H, $\text{OCH}_2\text{C}_5\text{H}_{11}$ and NCH_2), 3.65-3.63 (m, 1H, *SCH*), 3.20-3.13 (m, 2H, NCH_2), 2.21-2.11 (m, 2H, NCH_2CH_2), 1.80-1.70 (m, 2H, NCH_2CH_2), 1.65-1.55 (m, 2H, $\text{OC}_2\text{H}_4\text{CH}_2\text{C}_3\text{H}_7$), 1.37-1.25 (m, 6H, $\text{OC}_2\text{H}_4\text{C}_3\text{H}_6\text{CH}_3$), 0.91 (t, $J = 3.6$, 3H, $\text{OC}_5\text{H}_{10}\text{CH}_3$). ^{13}C NMR (100 MHz, CDCl_3): δ 155.61, 150.02, 146.83, 145.42, 132.93, 129.95, 128.85, 128.34, 117.63, 68.07, 45.43, 40.12, 31.56, 31.27, 30.45, 28.86, 25.52, 22.40, 14.16, 149.17, 147.77, 145.69, 130.12, 130.03, 127.45, 126.80, 124.14, 118.60, 65.90, 43.22, 42.00, 31.79, 31.59, 29.08, 25.75, 22.68, 14.16. HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{29}\text{N}_2\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 373.1950 found 373.1945.

Methyl 4-(quinolin-4-yloxy)piperidine-1-carboxylate 11d

was prepared from 4-chloroquinoline and methyl 4-hydroxypiperidine-1-carboxylate in 82% yield as orange colour solid. Mp: 102-104 °C. ¹H-NMR (300 MHz, CDCl₃): δ 8.73 (d, *J* = 5.1, 1H, Ar-*H*), 8.22 (dd, *J* = 8.4, 1H, Ar-*H*), 8.04 (d, *J* = 8.4, 1H, Ar-*H*), 7.70 (dt, *J* = 8.1, 0.9, 1H, Ar-*H*), 7.50 (dt, *J* = 8.4, 0.9, 1H, Ar-*H*), 6.74 (d, *J* = 5.1, 1H, Ar-*H*), 4.87-4.75 (m, 1H, OCH), 3.79-3.68 (m, 5H, CO₂CH₃ and, NCH₂), 3.64-3.56 (m, 2H, NCH₂), 2.08-1.95 (m, 4H, NCH₂CH₂). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 159.61, 155.52, 151.76, 149.13, 130.23, 128.78, 126.12, 122.12, 121.61, 102.74, 72.51, 52.78, 40.82, 30.05. HRMS (ESI) calcd for C₁₆H₁₉N₂O₃ [M+H]⁺ 287.1396, found 287.1400.

tert-Butyl 4-(quinolin-4-yloxy)piperidine-1-carboxylate 11e

was prepared from 4-chloroquinoline and *tert*-butyl 4-hydroxypiperidine-1-carboxylate **9b** in 75% yield as pale yellow solid. Mp: 95-97°C. ¹H NMR (400 MHz, CDCl₃): δ 8.74 (d, *J* = 5.1, 1H, Ar-*H*), 8.23 (dd, *J* = 6.3, 0.9, 1H, Ar-*H*), 8.03 (d, *J* = 8.4, 1H, Ar-*H*), 7.71 (t, *J* = 6.6, 1H, Ar-*H*), 7.51 (t, *J* = 6.6, 1H, Ar-*H*), 6.74 (d, *J* = 4.2, 1H, Ar-*H*), 4.84-4.81 (m, 1H, OCH), 3.74-3.68 (m, 2H, NCH₂), 3.56-3.50 (m, 2H, NCH₂), 2.07-1.94 (m, 4H, NCH₂CH₂), 1.29 (s, 9H, OC₄H₉). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 159.48, 154.32, 151.84, 149.37, 130.10, 128.97, 126.01, 122.11, 121.64, 102.69, 79.20, 72.62, 30.20, 28.68. HRMS (ESI) calcd for C₁₉H₂₆N₂O₃ [M+H]⁺ 329.1865, found 329.1855.

***n*-Hexyl 4-(quinolin-4-yloxy)piperidine-1-carboxylate 11f**

was prepared from **11h** and *n*-hexyl alcohol in 65% yield as pale yellow solid. Mp: 88-90 °C. ¹H-NMR (400 MHz, CDCl₃): δ 8.73 (d, *J* = 5.4, 1H, Ar-*H*), 8.21 (dd, *J* = 8.4, 1.2, 1H, Ar-*H*), 8.06 (d, *J* = 8.4, 1H, Ar-*H*), 7.70 (dt, *J* = 5.4, 1.2, 1H, Ar-*H*), 7.52 (dd, *J* = 8.0, 0.8, 1H, Ar-*H*), 6.74 (d, *J* = 5.6, 1H, Ar-*H*), 4.90-4.80 (m, 1H, OCH), 4.09 (d, *J* = 6.7, 2HOCH₂C₅H₁₁), 3.80-3.70 (m, 2H), 3.65-3.51 (m, 2H, NCH₂), 2.10-1.90 (m, 4H, NCH₂CH₂), 1.70-1.60 (m, 2H, OC₂H₄CH₂C₃H₇), 1.40-1.30 (m, 6H, OC₂H₄C₃H₆CH₃), 1.28 (t, *J* = 6.3, 3H, OC₃H₁₀CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 160.22, 155.76, 151.01, 149.25, 130.23, 128.82, 125.97, 122.02, 121.98, 101.53, 72.42, 65.92, 40.58, 31.61, 30.16, 29.11, 25.77, 22.69, 14.16. HRMS (ESI) calcd for C₂₁H₂₉N₂O₂S [M+H]⁺ 373.1950 373.1948.

4-(Piperidin-4-ylthio)quinoline hydrochloride 11g

was prepared from **11b** by the treatment of TFA in 90% yield, which was converted its hydrochloride salt as yellow solid. Mp: found 99-103 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.20 (b, 2H, (CH₂)NH·HCl), 8.99 (d, *J* = 6.0, 1H, Ar-*H*), 8.27 (d, *J* = 9.0, 2H, Ar-*H*), 8.10 (t, *J* = 6.9, 1H, Ar-*H*), 7.96 (d, *J* = 6.6, 1H, Ar-*H*), 7.85 (t, *J* = 7.2, 1H, Ar-*H*), 4.40-4.15 (m, 1H, SCH), 3.40-3.31 (m, 2H, NCH₂), 3.15-3.10 (m, 2H, NCH₂), 2.31-2.25 (m, 2H, NCH₂CH₂), 1.96-1.93 (m, 2H, NCH₂CH₂). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 157.75, 143.67, 137.86, 134.47, 129.69, 125.65, 124.30, 122.99, 116.96, 42.87, 40.66, 28.15. HRMS (ESI) calcd for C₁₄H₁₇N₂S [M+H-HCl]⁺ 245.1112, found 245.1111.

4-(Piperidin-4-yloxy)quinolone 11h

was prepared from **11e** by the treatment of TFA in 95% yield as off-yellow solid. Mp: 93-95°C. ¹H NMR (400 MHz, CDCl₃): δ 8.72 (d, *J* = 5.2, 1H, Ar-*H*), 8.26 (d, *J* = 8.4, 1H, Ar-*H*), 8.02 (d, *J* = 8.4, 1H, Ar-*H*), 7.70 (dt, *J* = 6.0, 1.2, 1H, Ar-*H*), 7.51 (t, *J* = 7.6, 1H, Ar-*H*), 6.74 (d, *J* = 5.2, 1H, Ar-*H*), 5.69-5.31 (b, 1H, (CH₂)NH), 4.47-4.71 (m, 1H, OCH), 3.25-3.19 (m, 2H, NCH₂), 2.88-2.81 (m, 2H, NCH₂), 2.15-2.10 (m, 2H, NCH₂CH₂), 1.91-1.75 (m, 2H, NCH₂CH₂). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 159.63, 151.87, 149.38, 130.04, 129.00, 125.93, 122.07, 121.74, 102.70, 74.37, 43.62, 40.69, 32.00. HRMS (ESI) calcd for C₁₄H₁₇N₂O [M+H]⁺ 229.1341, found 229.1338.

4-((Tetrahydrofuran-3-yl)oxy)quinolone 11i

was prepared from 4-chloroquinoline and tetrahydrofuran-3-ol in 90% yield as pale yellow solid. Mp: 87-90 °C. ¹H NMR (300 MHz, CDCl₃): δ 9.07 (b, 1H, Ar-*H*), 8.63 (d, *J* = 6.3 1H, Ar-*H*), 8.34 (d, *J* = 6.3, 1H, Ar-*H*), 7.99 (t, *J* = 6.0 1H, Ar-*H*), 7.78 (t, *J* = 6.3 1H, Ar-*H*), 7.34 (b, 1H, Ar-*H*), 5.52 (s, 1H, OCH), 4.26-4.11 (m, 3H, OCH₂CHCH₂ and CH₂OCH₂CH₂), 4.05-3.95 (m, 1H, CH₂OCH₂CH₂), 2.33 (b, 1H, CH₂OCH₂CH₂), 2.20 (b, 1H, CH₂OCH₂CH₂). ¹³C NMR (75.5 MHz, CDCl₃): δ 166.91, 146.81, 139.27, 134.84, 129.12, 123.46, 120.86, 103.80, 82.05, 72.35, 70.54, 66.98, 32.69. HRMS (ESI) calcd for C₁₃H₁₄NO₂ [M+H]⁺ 216.1025, found 216.1022.

4-((Tetrahydro-2H-pyran-4-yl)oxy)quinolone 11j

was prepared from 4-chloroquinoline and tetrahydro-2H-pyran-4-ol in 95% yield as yellow solid. Mp: 87-91 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.97 (b, 1H, Ar-*H*), 8.69 (d, *J* = 6.3, 1H, Ar-*H*), 8.38 (d, *J* = 6.3, 1H, Ar-*H*), 8.01 (t, *J* = 6.0, 1H, Ar-*H*), 7.80 (t, *J* = 5.7, 1H, Ar-*H*), 7.26-7.16 (m, 1H, Ar-*H*), 5.12 (b, 1H, OCH), 4.10-4.07 (m, 2H, CH₂OCH₂CH₂), 3.77-3.72 (m, 2H, CH₂OCH₂CH₂), 2.35-2.25 (m, 2H, CH₂OCH₂CH), 2.06-2.04 (m, 2H, CH₂OCH₂CH₂). ¹³C NMR (75.5 MHz, CDCl₃): δ 166.80, 146.72, 139.30, 134.81, 129.06, 123.47, 120.94, 120.78, 103.63, 75.82, 64.48, 31.21. HRMS (ESI) calcd for C₁₄H₁₆NO₂ [M+H]⁺ 230.1181, found 230.1180.

For the preparation of 12a-g

To a degassed solution of **11g-h** (1.00 mmol) and Boc-AA-CO₂H (1.10 mmol), and HOBT (1.10 mmol) in 10 mL of anhydrous DCM was added EDC*HCl (230 mg, 1.20 mmol) at 0°C. The mixture was stirred over night at room temperature. Then, the reaction was quenched with H₂O (15.00 mL). The mixture was extracted with DCM (20.0 mL), and organic phase was sequentially washed with 10% HCl, saturated NaCl solution, saturated NaHCO₃ solution, and saturated NaCl solution, dried over MgSO₄, and concentrated. The residue was purified by flash chromatograph (silica, EtOAc/hexane as eluent) to give the title compound **12a-g** with the 65-85% yield.

(S)-tert-Bbutyl (1-oxo-3-phenyl-1-(4-(quinolin-4-ylthio)piperidin-1-yl)propan-2-yl)carbamate 12a

was prepared from **11g** and Boc-Phe-OH in 85% yield as pale yellow solid. Mp: 180-182 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.71(d, *J* = 4.8, 1H, Ar-*H*), 8.15 (d, *J* = 6.0, 1H, Ar-*H*),

8.09-8.06 (m, 1H, Ar-*H*), 7.73-7.70 (m, 1H, Ar-*H*), 7.60-7.54 (m, 1H, Ar-*H*), 7.35-7.12 (m, 6H, Ar-*H*), 5.50-5.44 (m, 1H, *NHBoc*), 4.87-4.84 (m, 1H, *CHNHBoc*), 4.32-4.10 (m, 1H, *SCH*), 3.63-3.57 (m, 1H, *NCH₂*), 3.51-3.45 (m, 1H, *NCH₂*), 3.20-3.01 (m, 1H, *NCH₂*), 2.99-2.90 (m, 2.5H, *PhCH₂CH* and *NCH₂*), 2.75-2.60 (m, 0.5H, *PhCH₂CH*), 2.10-1.92 (m, 2H, *NCH₂CH₂*), 1.80-1.53 (m, 2H, *NCH₂CH₂*), 1.40 (s, 9H, *NHCO₂C₄H₉*). ¹³C NMR (100 MHz, CDCl₃): δ 170.35, 155.22, 149.26, 147.89, 145.32, 136.64, 130.12, 129.73, 128.86, 127.49, 127.34, 127.11, 126.86, 124.16, 118.92, 79.94, 51.12, 45.13, 44.49, 41.49, 40.62, 31.84, 28.50. HRMS (ESI) calcd for C₂₈H₃₄N₃O₃S [M+H]⁺ 492.2321, found 492.2316.

(S)-tert-Butyl (4-amino-1,4-dioxo-1-(4-(quinolin-4-ylthio)piperidin-1-yl)butan-2-yl)carbamate 12b

was prepared from **11g** and Boc-Asn-OH in 73% yield as pale yellow solid. Mp: 118-120 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.75 (d, *J* = 4.0, 1H, Ar-*H*), 8.18 (d, *J* = 8.0, 1H, Ar-*H*), 8.10 (d, *J* = 8.4, 1H, Ar-*H*), 7.73 (t, *J* = 7.2, 1H, Ar-*H*), 7.56 (d, *J* = 7.2, 1H, Ar-*H*), 7.29 (d, *J* = 3.6, 1H, Ar-*H*), 6.20-6.18 (m, 1H, *CONH₂*), 5.66-5.63 (m, 1H, *NHBoc*), 5.42-5.39 (m, 1H, *CONH₂*), 4.94-4.89 (m, 1H, *CHNHBoc*), 4.26-3.96 (m, 1H, *NCH₂*), 3.66-3.60 (m, 1H, *NCH₂*), 3.35-3.08 (m, 2H, *NCH₂CH₂*), 2.65-2.47 (m, 2H, *CH₂CONH₂*), 2.15-2.06 (m, 2H, *NCH₂CH₂*), 1.37 (s, 9H, *NHCO₂C₄H₉*). ¹³C NMR (100 MHz, CDCl₃): 167.30, 154.81, 149.35, 147.95, 144.99, 144.86, 130.18, 127.47, 126.94, 124.15, 119.04, 116.88, 81.12, 47.04, 45.32, 44.69, 42.07, 41.69, 41.57, 41.47, 32.35, 32.08, 31.57, 31.27, 28.40. HRMS (ESI) calcd for C₂₃H₃₁N₄O₄S [M+H]⁺ 459.2066, found 459.2068.

(S)-tert-Butyl (2-oxo-1-phenyl-2-(4-(quinolin-4-ylthio)piperidin-1-yl)ethyl)carbamate 12c

was prepared from **11g** and *L*-2-((*tert*-butoxycarbonyl)amino)-2-phenylacetic acid 65% yield as pale yellow solid. Mp: 90-92 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.70 (t, *J* = 2.8, 1H, Ar-*H*), 8.20 (d, *J* = 5.2, 1H, Ar-*H*), 8.10-8.05 (m, 1H, Ar-*H*), 7.73-7.70 (m, 1H, Ar-*H*), 7.58-7.50 (m, 1H, Ar-*H*), 7.35-7.26 (m, 5H, Ar-*H*), 7.20 (d, *J* = 4.8, 1H, Ar-*H*), 6.08-6.03 (m, 1H, *NHBoc*), 5.57 (t, *J* = 6.8, 1H, *PhCHNHBoc*), 4.48-4.30 (m, 0.5H, *SCH*), 4.20-4.10 (m, 0.5H, *SCH*), 3.77-3.71 (m, 1H, *NCH₂*), 3.60-3.54 (m, 1H, *NCH₂*), 3.48-3.46 (m, 0.5H, *NCH₂*), 3.40-3.08 (m, 1.5H, *NCH₂*), 2.10-2.00 (m, 2H, *NCH₂CH₂*), 1.90-1.50 (m, 2H, *NCH₂CH₂*), 1.42 (s, 9H, *NHCO₂C₄H₉*). ¹³C NMR (100 MHz, CDCl₃): δ 168.48, 155.21, 149.23, 147.90, 144.92, 138.26, 130.14, 129.34, 128.54, 127.76, 126.90, 124.13, 118.94, 79.93, 55.44, 44.78, 41.74, 31.43, 28.51. HRMS (ESI) calcd for C₂₇H₃₂N₃O₃S [M+H]⁺ 478.2164, found 478.2145.

(S)-tert-Butyl 2-(4-(quinolin-4-ylthio)piperidine-1-carbonyl)-pyrrolidine-1-carboxylate 12d as tautomer was prepared from 11g

and Boc-Pro-OH in 72% yield as white solid. Mp: 168-171 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.69 (s, 1H, Ar-*H*), 8.12 (s, 1H, Ar-*H*), 8.03 (s, 1H, Ar-*H*), 7.67 (s, 1H, Ar-*H*), 7.51 (s, 1H, Ar-*H*), 7.24 (s, 1H, Ar-*H*), 4.62-4.49 (m, 1H, *COCHNHBocCH₂*), 4.40-4.04 (m, 1H, *SCH*), 3.96-3.00 (m, 6H, *NCH₂* and *CH₂CH₂NBoc*), 2.40-1.50 (m, 8H, *NCH₂CH₂*, *CH₂CH₂NBoc* and *CHCH₂CH₂CH₂NBoc*), 1.41 (s, 9H, *NHCO₂C₄H₉*). ¹³C NMR (100 MHz, CDCl₃): δ 170.82, 170.73, 154.60, 154.03, 149.20, 147.81, 147.72, 147.62, 145.43, 145.13, 130.15, 130.06, 129.87, 127.38, 126.86, 124.08, 118.93, 118.63, 79.64, 57.06,

56.84, 56.46, 46.84, 46.61, 44.68, 44.46, 44.09, 42.03, 41.88, 41.77, 41.59, 41.43, 41.10, 32.58, 32.31, 32.12, 32.02, 31.49, 30.76, 30.57, 30.01, 29.77, 28.64, 28.54, 24.32, 23.68, 23.45. HRMS (ESI) calcd for $C_{24}H_{32}N_3O_3S$ $[M+H]^+$ 442.2164, found 442.2158.

(S)-tert-Butyl (1-oxo-3-phenyl-1-(4-(quinolin-4-yloxy)piperidin-1-yl)propan-2-yl)carbamate 12e

was prepared from **11h** and Boc-Phe-OH in 85% yield as white solid. Mp: 132-135 °C. 1H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H, Ar-H), 8.10 (d, J = 5.4, 1H, Ar-H), 8.01 (t, J = 9.2, 1H, Ar-H), 7.70-7.60 (m, 1H, Ar-H), 7.51 (td, J = 28.2, 6.9, 1H, Ar-H), 7.26-7.10 (m, 5H, Ar-H), 6.60 (dd, J = 10.5, 5.2, 1H, Ar-H), 5.59 (d, J = 6.0, 1H, $NHBoc$), 4.87 (d, J = 6.8, 1H, $CHNHBoc$), 4.65 (d, J = 40.0, 1H, OCH), 3.80 (dd, J = 50.2, 13.2, 1H, $PhCH_2CH$), 3.65-3.40 (m, 2H, $PhCH_2CH$ and NCH_2), 3.35-3.10 (m, 1H, NCH_2), 3.05-2.90 (m, 2H, $PhCH_2CH$ and NCH_2), 2.00-1.60 (m, 2H, NCH_2CH_2), 1.39 (s, 9H, $NHCO_2C_4H_9$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 159.82, 155.28, 151.13, 149.34, 136.84, 130.15, 129.80, 129.71, 128.98, 128.90, 128.70, 127.11, 125.94, 121.85, 101.53, 79.86, 71.62, 51.12, 42.13, 40.64, 38.59, 30.21, 28.50. HRMS (ESI) calcd for $C_{28}H_{34}N_3O_4$ $[M+H]^+$ 476.2549, found 476.2540.

(S)-tert-Butyl 2-(4-(quinolin-4-yloxy)piperidine-1-carbonyl)-pyrrolidine-1-carboxylate 12f

as tautomer was prepared from **11h** and Boc-Pro-OH in 77% yield as white solid. Mp: 106-108 °C. 1H NMR (400 MHz, $CDCl_3$): δ 8.72 (d, J = 5.2, 1H, Ar-H), 8.18 (t, J = 8.0, 1Hv), 8.03 (d, J = 8.4, 1H, Ar-H), 7.69 (t, J = 7.6, 1H, Ar-H), 7.50 (d, J = 6.8, 1H, Ar-H), 6.73 (d, J = 4.4, 1H, Ar-H), 4.90-4.80 (m, 1H, $COCHNHBocCH_2$), 4.60 (dd, J = 54.5, 6.8, 1H, OCH), 4.01-3.40 (m, 6H, NCH_2 and CH_2CH_2NHBoc), 2.47-1.75 (m, 8H, NCH_2CH_2 , CH_2CH_2NHBoc and $CHCH_2CH_2CH_2NHBoc$), 1.44 (s, 4.5H, $NHCO_2C_4H_9$), 1.41 (s, 4.5H, $NHCO_2C_4H_9$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.82, 170.70, 159.82, 159.65, 154.46, 153.83, 150.94, 149.24, 149.12, 129.99, 128.81, 128.72, 128.56, 125.71, 121.68, 121.59, 101.32, 79.48, 79.43, 71.89, 56.89, 56.73, 56.31, 56.21, 46.68, 41.93, 41.69, 41.44, 38.93, 38.76, 38.53, 38.43, 30.78, 30.47, 30.37, 30.06, 29.83, 29.68, 28.44, 28.40, 24.13, 23.44, 23.33. HRMS (ESI) calcd for $C_{24}H_{32}N_3O_4$ $[M+H]^+$ 426.2393, found 426.2386.

(S)-tert-Butyl (4-amino-1,4-dioxo-1-(4-(quinolin-4-yloxy)-piperidin-1-yl)butan-2-yl)carbamate 12g was prepared from 11h

and Boc-Asn-OH in 85% yield as white solid. Mp: 108-110 °C. 1H NMR (400 MHz, $CDCl_3$): δ 8.75 (d, J = 5.6, 1H, Ar-H), 8.22 (dd, J = 8.4, 2.9, 1H, Ar-H), 8.06 (d, J = 8.4, 1H, Ar-H), 7.72 (dt, J = 6.9, 1.6, 1H, Ar-H), 7.52 (d, J = 7.5, 1H, Ar-H), 6.75 (dd, J = 4.8, 3.2, 1H, Ar-H), 6.36 (b, 1H, $CONH_2$), 5.76 (dd, J = 38.0, 9.0, 1H, OCH), 5.58 (b, 1H, $CONH_2$), 5.03 (q, J = 8.0, 1H, $CHNHBoc$), 4.92 (d, J = 3.6, 1H, $NHBoc$), 4.08-3.61 (m, 4H, NCH_2), 2.75-2.68 (m, 1H, CH_2CONH_2), 2.65-2.55 (m, 1H, CH_2CONH_2), 2.30-2.00 (m, 4H, NCH_2CH_2), 1.48 (s, 9H, $NHCO_2C_4H_9$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.56, 170.05, 160.68, 155.48, 150.52, 148.17, 130.73, 128.16, 126.34, 122.01, 121.87, 101.67, 80.47, 72.49, 72.07, 39.23, 30.69, 29.92, 28.47. HRMS (ESI) calcd for $C_{23}H_{31}N_4O_5$ $[M+H]^+$ 443.2294, found 443.2290.

Cells and viruses

Madin-Darby canine kidney cell (MDCK), HEK 293 cells and Hela cells were obtained from China Center for Type Culture Collection. Influenza A/udorn/72 (H3N2), A/PR/8/34 (H1N1), A₁/Jingfang 86-1(H1N1), and A₃/Lufang93-1(H3N2)China Center for Type Culture Collection. B/Shenzhen/747 were obtained from Shenzhen Centers for Disease Control and Prevention (China).

MDCK cell-based anti-influenza assay for the high-throughput screening protocol in 96-well plate

The flat-bottomed 96-well plate (plate A) was added 110 μ L of DMEM with 0.1% TCPK-trypsin and 1% antibiotics in each well. Then, 1 μ L of 5mM of the positive control solution (Ribavirin) was added to wells 12-A, B, C and D, and 1 μ L of 1 μ M of negative control solution (F136) was added to 12-E, F, G and H in plate A. The remaining wells was added 1 μ L of compound solution (each well contains a compound at 10 μ g/100 μ L in 1% DMSO, from MyriaScreen from Sigma-Aldrich). Another 96-well plates, named as plate B, with MDCK cells growing in monolayer was washed with PBS, and infected with 100 PFU A/Udorn/72 influenza viruses in each well except for well 1-A, B, C and D as blank. After infection for one hour, plate B was washed with PBS. Then, 100 μ L of compound-medium in plate A was added to plate B. The resulting plate was incubated for 48h in the incubator at 37°C under 5% CO₂. The plate were fixed and stained with 0.5% crystal violet solution. Wells as blank control and Ribavirin control will be colour as deep purple. Wells in which cytopathic effects occurred will be clear or partially clear. Positive inhibition of virus infection by the screening compound is identified when the well is stained the same as the ribavirin control.

Determination of EC₅₀ of piperidine-based derivatives by MDCK cell-based anti-influenza assay

The anti-influenza virus activities of piperidine-based derivatives were measured by the EC₅₀ values. MDCK cells were seeded (1.2×10^5 cells/well in DMEM with 10% FBS) in 12-well tissue culture plates and incubated for 48 hours at 37 °C in a humidified 5% CO₂ incubator. The cells were infected with virus (100PFU, Udorn-4) in the incubator for 1 h. After adsorption of the virus, the virus suspension was removed and the cells were washed with DMEM, The cell monolayer were then overlaid with DMEM medium containing 1% agar and serial compounds dilutions (2-fold serial dilutions for 6 gradients, each concentration measured 2 wells) in the presence of 1% TCPK-trypsin and 1% antibiotics. After infection for 48 hours, the monolayer were fixed and stained with 0.5% crystal violet solution. The number of virus-induced plaques was counted.

in vitro Cytotoxic studies of 11e

Cytotoxicity of **11e** was assessed by MTT assays. 5×10^4 cells/well were seeded on a flat-bottomed 96-well tissue culture plates, and incubated for 18 h at 37 °C for cell adherence. Then the medium was removed and replenished with 100 μ L of DMEM containing different concentrations of **11e** ranging from 32 μ M to 10000 μ M for 24 h in an atmosphere of 5% CO₂ at 37 °C. For the MTT assay, which evaluates mitochondrial viability, 20 μ L of MTT

solution (5 mg/mL) was added, and the plates were incubated for an additional 4 h. After incubation, the supernatant was carefully removed from the wells followed by the addition of 150 μ L DMSO with thorough mixing. The optical density at 570 and 630 nm (background) was determined on an ELISA reader. The cell viability was expressed as the percentage of control absorbance obtained in untreated cells after subtracting the absorbance from an appropriate background. Last, the minimum lethal dose for 50% of the cells (MLD₅₀) was determined.

Time-of-addition assay

MDCK cells (1.2×10^5 cells/well) were seeded in 12-well tissue culture plates and incubated for 48 hours. The cells were infected with virus (100PFU) in the incubator for 1 h. After adsorption of the virus, the virus suspension was removed and the cells were washed with DMEM, then replenished with fresh virus medium (DMEM with 1% antibiotics and 1%thou TPCK-trypsin). The test medium containing the test compound solution was added during the periods -2 to 0 h (adsorption), 0-2, 1-3, 2-4, 4-6, 6-8, and 18-20 h. After each incubation period, the monolayer was washed with DMEM and incubated with fresh virus medium. After 24 hours, the supernatant was collected and the viral yield was measured. Statistical analysis was analysed by ANOVA followed by Tukey's multiple comparison test using Prism 5.0b.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. HTS leading hit P114F5. a) A HTS for inhibitors of influenza A virus infection. Wells A1-D1 was uninfected, whereas wells E1-H1 were infected with influenza virus inoculum incubated with the placebo solution. Ribavirin (50 μ M, 1% DMSO) in wells A12-D12 and fusion inhibitor F136 in wells E12-H12 were selected as controls. Well F5 was selected as positive result. b) Structural information of HTS leading hit. c) EC₅₀ measurement. d) Time of addition using P114F5 (30 μ M, 1% DMSO).

Fig. 2. Diversity-oriented synthesis of derivatives of P114F5 by modifications of four subunits: aquinoliny core (A), a sulfur core (B), a piperidinyl core (C), and a carbamate core (D).

Fig. 3.

Time-of-addition effect of **11e** (1.0 μM, 1% DMSO) on influenza A/udorn/72 (H3N2) infection in MDCK cells (Viral yields obtained with respect to different exposure period of **11e** to the culture medium during influenza infection).*: $P < 0.05$.

Scheme 1.

Reaction conditions. (a) NaH, DMF, 50 °C, 5h, 60-85%.

Scheme 2.

Reaction conditions. (a) NaH, DMF, 50 °C, 5h, 75-90%.

Scheme 3.

Reaction conditions. (a) NaH, DMF, 50 °C, 5h, 50-85%.

Scheme 4.

Reaction conditions. (a) NaH, DMF, 50 °C, 5h, 70-90%; (b) TFA, DCM, room temperature, 2h, 90-95%; (c) *N,N'*-carbonyldiimidazole(CDI), then *n*-C₆H₁₃OH, r. t., THF, 12h, 55-70%.

Scheme 5.

Reaction conditions. (i) Boc-AA-CO₂ H, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride(EDC·HCl), HOBt, DCM, room temperature, 12h, 65-85%.

Table 1Optimization of subunit A. In vitro anti-influenza A virus activities of compounds 1a-i produced via Scheme 1^a

Compd.	Structure	EC ₅₀ (μM)
P114F5		13.9
1a		>100
1b		47.0
1c		>100
1d		67.5
1e		52.9
1f		23.1
1g		>100
1h		>100
1i		>100

^aUsing influenza virus A/Udorn/72 strain (H3N2).

Table 2Optimization of subunit B. In vitro anti-influenza A virus activities of compounds **6a-b**.^a

Compd.	Structure	EC ₅₀ (μM)
P114F5		13.9
6a		2.33
6b		10.0

^aUsing influenza virus A/Udorn/72 strain (H3N2).

Table 3Optimization of subunit C. In vitro anti-influenza A virus activities of compounds 8a-f.^a

Compd.	Structure	EC ₅₀ (μM)
P114F5		13.9
8a		>100
8b		>100
8c		79.0
8d		36.2
8e		19.5
8f		10.0

^aUsing influenza virus A/Udorn/72 strain (H3N2).

Table 4Optimization of subunit D. In vitro anti-influenza A virus activities of compounds 11a-j.^a

Compd.	Structure	EC ₅₀ (μM)
P114F5		13.9
11a		30.0
11b		64.5
11c		>100
11d		10.0
11e		0.10
11f		10.0
11g		91.0
11h		>100
11i		>100
11j		>100

^aUsing influenza virus A/Udorn/72 strain (H3N2).

Table 5Further optimization of subunit D. In vitro anti-influenza A virus activities of compounds **12a-g**.^a

Compd.	Structure	EC ₅₀ (μM)
12a		15.5
12b		>100
12c		>100
12d		38.5
12e		17.8
12f		29.1
12g		>100

^aUsing influenza virus A/Udorn/72 strain (H3N2).

Table 6

Cytotoxic study of **11e** in Madin-Darby canine kidney (MDCK) cells, human embryonic kidney cells (HEK293 cells) and human epithelial carcinoma cell line (HeLa cells).^a

MDL₅₀ (μM)			
mean ± SD			
	MDCK cells	HEK 293 cells	Hela cells
11e	10061.92 ±235.86	8101.97 ±473.16	8850.21 ±406.99

^aThe cytotoxicity of **11e** is expressed by MDL₅₀ in μM, calculated for three cells lines. Data are expressed as the mean ± SD of triplicates for each concentration.

Table 7

EC₅₀ values (μM) against various influenza virus strains of compound **11e** and HTS hit P114F5.^a

Virus.	11e	P114F5
Influenza A/udorn/72 (H3N2)	0.10	13.9
Influenza A/PR/8/34 (H1N1)	0.07	8.78
Influenza A ₁ /Jingfang 86-1(H1N1)	0.10	9.05
Influenza A ₃ /Lufang 93-1(H3N2)	0.06	11.7
Influenza B/Shenzhen/747	0.05	10.9

^aEC₅₀ values are the compound concentrations for 50% reduction of plaques.