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Short communication

Imidazole-based pinanamine derivatives: Discovery of dual inhibitors of the wild-type and drug-resistant mutant of the influenza A virus

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ABSTRACT

We previously reported potent hit compound **4** inhibiting the wild-type influenza A virus A/HK/68 (H3N2) and A/M2-S31N mutant viruses A/WS/33 (H1N1), with its latter activity quite weak. To further increase its potency, a structure-activity relationship study of a series of imidazole-linked pinanamine derivatives was conducted by modifying the imidazole ring of this compound. Several compounds of this series inhibited the amantadine-sensitive virus at low micromolar concentrations. Among them, **33** was the most potent compound, which was identified as being active on an amantadine-sensitive virus through blocking of the viral M2 ion channel. Furthermore, **33** markedly inhibited the amantadine-resistant virus (IC_{50} = 3.4 μM) and its activity increased by almost 24-fold compared to initial compound, with its action mechanism being not M2 channel mediated.

1. Introduction

Influenza A virus is one of the major human pathogens that has caused two of the four documented occasional pandemics and is responsible for seasonal epidemic outbreaks, posing a continuous threat to public health and socioeconomic development. Available anti-influenza medications applied in clinical treatment mainly focus on only a few influenza protein targets: the vaccines for hemagglutinin (HA), antivirals for M2 ion channels (amantadine and rimantadine) and neuraminidase (NA) (oseltamivir, zanamivir, peramivir, and laninamivir) [1]. However, prophylactic vaccines are not completely effective against a rapidly spreading influenza pandemic because of the substantial lead time for vaccine production [2]. In addition, currently circulating influenza A virus strains exhibit strong resistance to the adamantane class of antiviral drugs, which undermine their utility [3–7]. Moreover, rapid emergence of oseltamivir-resistant seasonal and pandemic strains has diminished the use of NA inhibitors [8–10]. Clearly, there is urgent need for discovery of a novel generation of anti-influenza agents, especially after the recent 2009 H1N1 (swine flu) and 2013 H7N9 outbreaks [11,12].

M2 proton channel of the influenza A virus is the target of amantadine and rimantadine (Fig. 1), which is a yet validated homotetrameric proton-selective ion channel. This modular protein serves multiple functions in the viral life cycle, including acidification and uncoating of the endosome-entrapped virus [13–15], mediation of the viral assembly and budding [16]. The M2 protein has been extensively studied because of its medical importance, particularly after the emergence of widespread amantadine-resistant mutants [17]. Comprehensive functional studies of point mutations in the pore-lining residues of M2 have been carried out to identify additional sites that might impart amantadine resistance [18]. Indeed, large-scale sequencing of transmissible viruses from 1918 to 2010 show that mutations to pore-lining residues are allowed only within the TM helix at positions 26, 27, and 31. In this regard, S31N represents the dominant amantadine-resistant mutation in M2, which accounted for 98–99.9% of the transmissible amantadine-resistant H1N1, H5N1, and H7N9 strains isolated from humans, birds, and swine in the last decade [19–21].

Extensive efforts have been devoted to the development of

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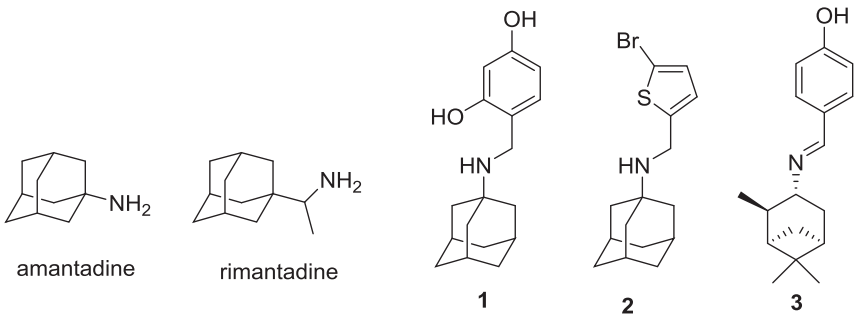


Fig. 1. Structure of Amantadine, Rimantadine and some reported M2 inhibitors.

compounds that target drug-resistant A/M2 mutants. In general, the designed hydrophobic scaffold of M2 inhibitors includes adamantane-based groups (expanded or contracted bones) and several types of novel scaffolds (alkyl rings, aromatic rings, extended or constricted adamantane groups and other cage structures of various sizes) [22,23]. In addition, numerous small molecules have been observed that target the L26F, V27A [24–27] and S31N mutants of the M2 channel [28]. For instance, some adamantane-based M2 inhibitors, such as compounds **1** and **2** (Fig. 1), have been reported to block both the wild-type and the S31N mutant of M2 [29,30]. Interestingly, a series of 2-adamantanamine-based compounds [31] and some polycyclic amines [32] and azapropellanes [33] that were initially intended to be developed as M2 inhibitors were observed to inhibit the amantadine-resistant influenza A virus through an alternative mechanism. Therefore, exploring novel non-adamantane-based M2 inhibitors that target the amantadine-resistant influenza A virus is of great interest.

3-Pinanamine (Fig. 2) was identified by our research group as a novel M2 inhibitor which was more active only against the wild-type M2 channel than amantadine [34]. Later on, the attachment of 4-hydroxyphenyl group to the amine led to pinanamine-based imine derivative **3** (Fig. 2), which was a highly potent M2 inhibitor inhibiting wild-type influenza A virus [35]. Interestingly, compound **4** (Fig. 2) was an imidazole derivative of pinanamine that affected the M2-S31N ion channel and its relevant virus strain [36]. Based on these preliminary results, in this research, we have designed and synthesized a series of imidazole-linked pinanamine derivatives and investigated as inhibitors of M2 blockers. Among them, the most potent inhibitor **33** exhibited dual inhibitory activity against amantadine-sensitive, as well as amantadine-resistant influenza A viruses at low micromolar concentrations.

2. Results and discussion

The synthetic route of the 4-substituted imidazole-based aldehyde derivative **8a**–**e** is outlined in Scheme 1. Briefly, various substituted β-ketoester **5** was chlorinated with sulfuryl chloride to yield chlorinated β-ketoester **6**, which was subsequently refluxed with formamide in the presence of water to give 4-substituted imidazole-5-carboxylate **7** [37]. Compound 7 was then reduced with $LiAlH_4$ to give the corresponding alcohol, and active MnO_2 was used for the oxidation to accomplish imidazole carboxaldehyde derivatives **8a**–**e**.

Reagents and conditions: (i) SO_2Cl_2, $CHCl_3$, reflux, 2.5 h; (ii) NH_2CHO, water, 180 °C, 3.5 h; (iii) $LiAlH_4$, 0–25 °C, 4 h; (iv) active MnO_2, acetone, r.t., 12 h.

For the synthesis of 2-substituted imidazole aldehyde derivatives, alternative methods were used (Scheme 2). Several 2-substituted imidazoles (**17a-j)** were prepared via Radziszewski reaction or modified conditions [38–40] despite the low yield (approximately 20–40%). At first, the 2-substituted imidazoles **12a**–**g** were prepared by the condensation of the α-dicarbonyl compound **9** and the appropriate aldehyde **11a**–**g** with an ammonia source (aqueous ammonia or ammonium carbonate) in alcohol. And then **12a**–**g** and compounds **14a-c** (commercially available) were protected by the ortho-directing *N,N*-dimethylsulfamoyl group using dimethylsulfamoyl chloride and meantime sodium hydride as base in moderate yield [41]. Finally, lithiation of **15a-j** with *n*-BuLi followed by the addition of DMF and acidic aqueous workup afforded the 3-substituted imidazole aldehydes **17a**–**j** [42]. The same route was used to synthesize the 4-methyl-2-substituted imidazole aldehydes **18a**–**b** using pyruvaldehydes **10** and **11h**–**i** as the starting materials.

Compound **20** was obtained using the Pinner reaction via reaction of a thiophene-2-carbonitrile **19** with sodium methoxide in methanol at room temperature, subse-quently treated with ammonium chloride at the same temperature for 72 h [43]. The corresponding imidine **20** was then condensed with 1,3-dihydroxyacetone dimer in the presence of ammonium chloride in aqueous ammonia at 80 °C for 6 h to afford the intermediate **21**. This intermediate was converted into imidazolecarbaldehyde **22** by MnO_2 oxidation in acetone [44].

Reagents and conditions: (i) NH_4HCO_3, CH_3OH; (ii) 60% NaH, DMF, 0 °C, 0.5 h; Me_2NSO_2Cl, 0–25 °C, 2 h; (iii) *n*-BuLi, DMF, −78–50 °C, 2 h; 1 M HCl, 0–25 °C, 2 h; (iv) CH_3ONa, MeOH; (v) NH_4Cl, rt, 72 h; (vi) 1,3-dihydroxyacetone dimer, NH_4Cl, $NH_3.H_2O$, 80 °C, 6 h; (vii) active MnO_2, acetone, r.t., 12 h.[a] These materials were commercially available.

For the synthesis of compounds **29a-c** (Scheme 3), the β-keto

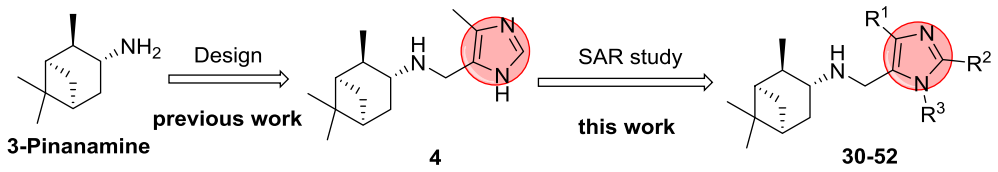
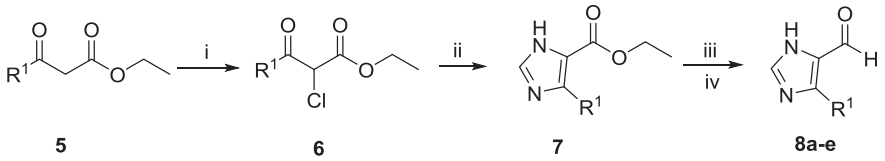
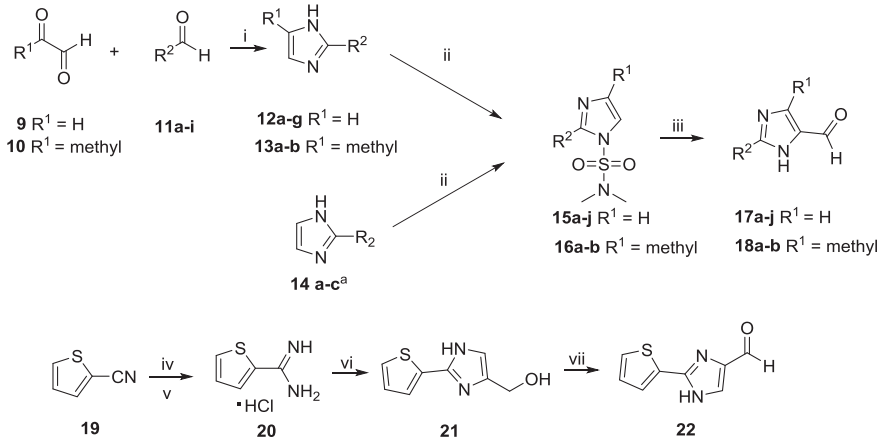


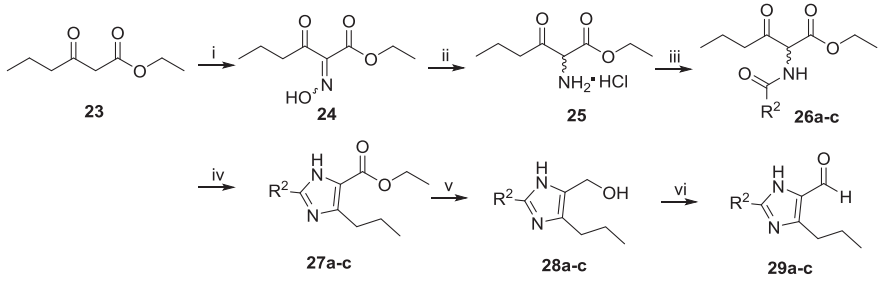
Fig. 2. Content of this research.



Scheme 1. Preparation of 4-substituted imidazole aldehyde derivatives.



Scheme 2. Preparation of 2-substituted or 4-methyl-2-substituted imidazole aldehyde derivatives.

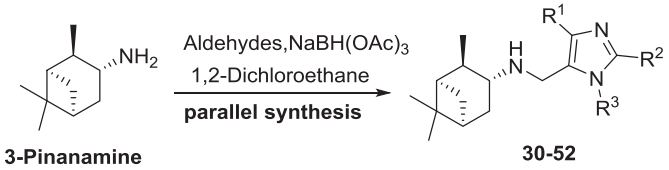


Scheme 3. Synthesis of 4-*n*-propyl-2-substituted imidazole aldehyde derivatives.

ester **23** was conveniently transformed into the oxime **24** by treatment with sodium nitrite in the presence of acetic acid. The oxime **24** was then reduced with Pd/C and an alcoholic solution of hydrochloric acid under atmospheric pressure of hydrogen to afford the requisite α-amino β-ketoester hydrochloride **25** in quantitative yield [45]. The compound **25** was then coupled with corresponding carboxylic acid with HATU/triethyl-amine as coupling reagents to afford the desired amide **26a-c**, which was treated with ammonium acetate in acetic acid glacial by refluxing overnight to accomplish the intermediates **27a-c** [46]. And then exposure of a solution of compounds **27a-c** in THF to reducing agent lithium aluminum hydride resulted in **28a-c**, which easily underwent conversion to the desired imidazolecarboxaldehydes **29a-c** via active MnO_2 oxidation [44].

With the aforementioned aldehydes in hand, a series of parallel classical reductive amination reactions [47] were subsequently used to synthesize the imidazole-based pinanamine derivatives **30**–**52** (Scheme 4).

Reagents and conditions: (i) $NaNO_2$, $AcOH/H_2O$, 0–25 °C; (ii) H_2, 10% Pd/C, 30–40% ethanolic HCl solution, r.t., 24 h; 89% for two steps; (iii) Corresponding carboxylic acid, HATU, NEt_3, CH_3CN, r.t., 1.5 h; (iv) NH_4OAc, AcOH, r.t. → reflux, overnight; (v) $LiAlH_4$, 0–25 °C, 4 h; (vi) active MnO_2, acetone, r.t., 12 h.

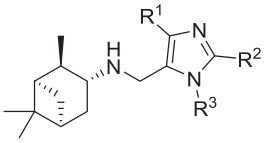


Scheme 4. Synthesis of imidazole-based pinanamine derivatives.

2.1. Viral inhibition assay

Antiviral activity of all the compounds was evaluated using the cytopathic effect (CPE) assay and MTT cell viability test. Compounds **30**–**52** were initially tested by microscopic scoring of the CPE. The A/HK/68 strain containing an A/M2-WT protein, and the A/WSN/33 strain with the amantadine-resistant A/M2-S31N mutant were used. The inhibition activities obtained by the CPE method were confirmed by the MTT cell viability assay. In parallel, the synthesized compounds were exposed to uninfected MDCK cells to assess the cytotoxicity by MTT assay, and the data were recorded as the CC_{50} value. If the effective antiviral concentration of the compounds was greater than the CC_{50}, the compounds were identified as exhibiting no activity (NA). The results are shown in Table 1.

Table 1
In vitro Inhibition Efficiency on influenza A Viruses.[a]



Compound				Antiviral IC_{50}[b] (μM)				Cytotoxicity (CC_{50},[c] μM)
				A/HK/68		A/WSN/33		
	R^1	R^2	R^3	CPE	MTT	CPE	MTT	
30	Et	H	H	5.8 ± 0.6	6.7 ± 1.1[d]	NA[e]	–[f]	180.1
31	Cyclopropyl	H	H	3.4 ± 0.4	3.8 ± 0.7	NA	–	162.5
32	t-Bu	H	H	8.3 ± 1.3	12.9 ± 2.2	NA	–	72.3
33	n-Pr	H	H	2.5 ± 0.2	3.3 ± 0.6	3.4 ± 0.5	7.3 ± 1.5	200.2
34	Ph	H	H	NA	–	NA	–	39.7
35	H	Me	H	NA	–	NA	–	202.0
36	H	Et	H	NA	–	9.5 ± 2.1	–	137.4
37	H	Cyclopropyl	H	NA	–	29.4 ± 4.6	–	94.5
38	H	i-Pr	H	NA	–	NA	–	113.8
39	H	t-Bu	H	8.8 ± 0.6	12.4 ± 1.1	9.7 ± 0.7	11.6 ± 0.4	107.4
40	H	2-isobutyl	H	8.7 ± 0.4	15.2 ± 1.4	8.9 ± 0.5	18.6 ± 1.8	74.8
41	H	n-Bu	H	NA	–	NA	–	70.4
42	H	cyclopentyl	H	NA	–	NA	–	37.2
43	H	cyclohexyl	H	NA	–	NA	–	33.5
44	H	Ph	H	7.6 ± 0.3	10.7 ± 0.9	9.0 ± 0.5	11.0 ± 0.7	79.9
45	H	thiophen-2-yl	H	NA	–	NA	–	75.6
46	Me	Et	H	4.1 ± 0.2	4.5 ± 0.3	NA	–	135.5
47	Me	i-Pr	H	NA	–	NA	–	73.1
48	Me	Cyclopropyl	H	15.3 ± 1.4	21.2 ± 1.6	9.4 ± 1.0	21.4 ± 1.3	74.9
49	n-Pr	i-Pr	H	10.2 ± 1.1	13.5 ± 1.8	8.8 ± 1.3	11.3 ± 0.9	156.4
50	n-Pr	Cyclopropyl	H	6.9 ± 0.7	10.8 ± 2.0	7.3 ± 0.8	9.7 ± 1.2	112.1
51	n-Pr	Ph	H	13.5 ± 1.6	15.2 ± 1.7	12.3 ± 1.1	16.1 ± 2.1	143.4
52	H	H	Me	12.0 ± 1.1	–	NA	–	
4	Me	H	H	3.2 ± 0.9	–	95.5 ± 5.4	–	251.5
amantadine		3.9 ± 0.2	11.6 ± 1.0	>100	–	337.2		

[a] The effects of the compounds were determined by measuring the survival of MDCK cells infected with the influenza A virus using the CPE assay and MTT cell viability test.
[b] IC_{50} represents the 50% effective concentration.
[c] CC_{50} represents 50% cytotoxic concentration, as determined by the MTT cell viability test.
[d] Mean ± SD.
[e] NA, no activity.
[f] The symbol "–" indicates "not tested." Values shown are the mean of 2–3 determinations.

2.2. Structure-activity relationship (SAR) studies

On the basis of our previous data [36], the (4-methyl-1*H*-imidazol-5-yl)methyl pinanamine **4** inhibited the amantadine-sensitive strain A/HK/68 in the low micromolar range and was marginally more effective than amantadine in inhibiting A/WSN/33. Therefore, the 4-position of imidazole was modified initially, resulting in the synthesis of compounds **30**–**34**. The 4-position of imidazole was substituted by ethyl, which led to inhibitor **30** of the A/HK/68 virus. The ethyl was changed to a larger group (cyclopropyl, **31**), which increased the inhibition (IC_{50} = 3.4 μM). However, compound **32**, with an even larger hydrophobic aliphatic group (*tert*-butyl), clearly weakened the activity. Compound **33**, with *n*-propyl as a medium-size group, exhibited potent effectiveness (IC_{50} = 2.5 μM). Surprisingly, **33** effectively inhibited the amantadine-resistant strain A/WSN/33, with IC_{50} = 3.4 μM; meanwhile, compound **30**, **31** and **32** exhibited no activity. An aromatic group such as phenyl in this 4-position gave the inactive compound **34**. The information above in hand revealed that a medium size, hydrophobic, aliphatic group such as *n*-propyl introduced at the 4-position of imidazole would be beneficial for inhibition activity of the amantadine-sensitive influenza virus. With the exception of the case of **34**, other aromatic groups were not attempted.

To fill the blank 2-position of imidazole, the 2-substituted imidazole derivatives **35**–**45** were synthesized. Unfortunately, the compounds with small-sized groups such as methyl (**35**), ethyl (**36**), cyclopropyl (**37**) and isopropyl (**38**) and those with large-sized groups such as *n*-butyl (**41**), cyclopentyl (**42**) and cyclohexyl (**43**) were all inactive. Similar to the case of the 4-position-substituted compounds, the compounds with medium-sized groups *tert*-butyl (**39**) and isobutyl (**40**) exhibited moderate effectiveness against the A/HK/68 (IC_{50} = 8.8 μM and 8.7 μM, respectively) virus strains. Moving the phenyl of **34** to the 2-position of imidazole afforded compound **44**, which exhibited moderate activity against the two virus strains. However, replacing the phenyl of **34** with its bioisostere thiophen-2-yl brought about a total loss of activity.

We also explored the 2,4-disubstituted imidazole derivatives **46**–**51**. Compound **46** with R^1 = methyl and R^2 = ethyl exhibited favorable activity (IC_{50} = 4.1 μM) against the A/HK/68 strain but no activity against the A/WSN/33 strain. Replacing the ethyl of **46** with cyclopropyl afforded the less active compound **48**, of which the latter activity was recovered, while the effectiveness of 2-isopropyl substituted derivative **47** was lost. These data above in hand clearly indicated some compounds containing R^1 = *n*-Pr and several R^2 medium-size substituents could be more potent compound with favorable antiviral activity, hereby compounds **49**–**51** were prepared. Just as expected, all these compounds retained dual inhibitory activity against wild-type and mutant virus strain while their activities decreased slightly compared to compound **33**. In addition,

the analog **51** containing R^1 = *n*-propyl and R^2 = phenyl only exhibited moderate activity against the two virus strains (13.5 μM and 12.3 μM).

The 1-methyl-substituted compound **52** exhibited decreased activity compared with the 1-free imidazole compound (IC_{50} = 6.6 μM) [36]. For this reason, other 1-substi-tuted compounds were not attempted.

In summary, the SAR study demonstrated that most of the synthesized compounds exhibited more or less antiviral effectiveness. Among them, compound **33** was the most active against both amantadine-sensitive and amantadine-resistant virus strains, with low cytotoxicity.

2.3. Two-electrode voltage clamp assay

Compounds **33** and **46** were selected for measurement of their inhibitory properties against A/M2-WT and A/M2-S31N ion channels using two-electrode voltage clamp (TEVC) assays. The compounds were tested at a concentration of 100 μM and their inhibition of the electric current through the A/M2-WT and A/M2-S31N ion channels was recorded in Table 2. In consistent with the result of the cell-based phenotypic method, the A/M2-WT ion channel activity was blocked by **33** and **46** in high percentage (97.9 ± 0.2% and 91.7 ± 2.1%, respectively). However, the inhibition of **33** on the A/M2-S31N channel exhibited an inconsistency. Compound **33** was marginally more potent than compound **4** toward the A/WSN/33 virus, as we reported previously; however, its ion-channel inhibition of M2/S31N was less effective than that of **4** (3% vs. 27%) [35]. This result suggests that **33** may inhibit the A/WSN/33 virus strain through an alternative mechanism, perhaps just the same as target metastasis of several reported compounds [31–33]. Further biological evaluation and mechanism are ongoing.

3. Conclusion

We explored the structure-activity relationship of imidazole-based pinanamine derivatives and demonstrated that this series of compounds inhibited the amantadine-sensitive virus at a micromolar concentration by blocking the A/M2-WT ion channel. Among them, the most potent compound **33** remarkably exhibited dual inhibitory activity against the A/M2 wild-type virus A/HK/68 and the amantadine-resistant strain A/WSN/33, with IC_{50} values 2.5 μM and 3.4 μM respectively. In addition, the A/M2-WT ion channel activity blocked by **33** was in high percentage, while the related mechanism of action in inhibiting amantadine-resistant strains is currently exploring. These results may be of great significance to design novel non-adamantane-based antiviral agents.

4. Experimental procedure

4.1. Chemistry

Commercially available compounds and solvents were analytical reagent grades and were used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang GF254 silica gel plates (5 × 10 cm) and Zones were detected visually under ultraviolet irradiation (254 nm) by either spraying with an ethanol solution of 2,4-dinitrophenylhydrazine or by being fumed with iodine steam. Compounds were purified silica gel column chromatography which performed on silica gel (200–300 mesh) from Qing Dao Hai Yang. All final products were characterized by ^{1}H NMR, ^{13}C NMR and HRMS, selected intermediates were confirmed by ^{1}H NMR, possibly also MS. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker NMR AVANCE 500 (500 MHz), and the NMR reagents $CDCl_3$ and DMSO-d_6 were used as internal standards. High resolu-tion mass spectra were performed on an Agilent 6210 LC/MSD TOF spectrograph. Low resolution mass spectra were measured on an Agilent MSD-1200 ESI-MS system. All test compounds were given as its hydrochloride and confirmed to be ≥ 95% in purity determined by analytical HPLC method on Dionex Summit HPLC. Melting points were determined using an X6 melting point apparatus (Beijing Fukai instrument Co. Ltd.) and were reported uncorrected.

4.1.1. General procedure for synthesis of imidazolecarbaldehydes *8a–e*

Using the synthetic procedure of 4-*tert*-butyl-1*H*-imidazole-5-carbaldehyde (**8c**) as an example.

Sulfuryl chloride (5.3 mL, 9.0 g, 66 mmol) was added dropwise to ethyl pivaloylacetate (10.2 mL, 5.2 g, 55 mmol) in 50 mL of $CHCl_3$ at 0 °C. The resulting mixture was allowed to warm to room temperature and was stirred for 30 min, after which it was heated under reflux for 2.5 h. After cooling to room temperature, the reaction mixture was diluted with chloroform, washed with sodium bicarbonate, water and then brine successively. The organic phase was dried and evaporated to afford 2-chloro-4,4-dimethyl-3-oxopentanoic acid ethyl ester as a clear oil, of sufficient purity to be used directly in the next step (10.6 g, 92%).

A solution of 2-chloro-4,4-dimethyl-3-oxopentanoic acid ethyl ester (10.6 g, 51.44 mmol) in formamide (17.8 ml, 23.2 g, 51.44 mmol) and water (1.85 ml, 102.9 mmol) was heated at 180 °C for 3.5 h. The mixture was allowed to cool to room temperature, then water (20 ml) was added and the mixture was exhaustively extracted with chloroform. The crude product was purified by flash chromatography over silica gel to give ethyl 4-(*tert*-butyl)-1*H*-imidazole-5-carboxylate (2.22 g, 22%)

To a solution of 5-*tert*-butyl-3-imidazole-4-carboxylic acid ethyl ester (2.20 g, 11.21 mmol) in THF (60 mL) was added $LiAlH_4$ (553 mg, 14.57 mmol) in portions under argon atmosphere at 0 °C, and then the mixture was stirred for 4 h at room temperature. To this solution was added moist Na_2SO_4 solid slowly at 0 °C, and the resulting precipitate was removed by Celite filtration and washed by THF several times. The solvent was removed by evaporation and the residual oil was purified by silica gel chromatography using CH_2Cl_2/MeOH (200:1) as eluent to give an oil of (4-(*tert*-butyl)-1*H*-

Table 2
Inhibitory properties of compounds 33 and 46 on A/M2-WT and S31N ion-channel Functions.[a]

Compound	A/M2-WT Inhibition by 100 μM after 2 min (%)	A/M2-S31N Inhibition by 100 μM after 2 min (%)
amantadine	93.8 ± 2.1[b]	24.3 ± 2.8
4[36]	95.8	27
33	97.9 ± 0.2	3.47 ± 3.1
46	91.7 ± 2.1	1.73 ± 3.0

[a] The inhibitory properties of the compounds were determined on A/M2 proteins expressed in *Xenopus* oocytes by TEVC assay.
[b] Mean ± SD.

imidazol-5-yl)methanol (1.23 g, 71%):

To a solution of alcohol **6** (1.23 g, 7.97 mmol) in acetone (20 mL) was added active MnO_2 (16.2 g, 186.2 mmol), and the mixture was stirred at room temperature for 12 h. After filtration through a pad of Celite to remove MnO_2, the solvent was removed by evaporation, and the residual white powder was purified by silica gel column chromatography using CH_2Cl_2/MeOH (200:1) as eluent to give 958.2 mg (79%) 4-(*tert*-butyl)-1*H*-imidazole-5-carbaldehyde of off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 7.67 (s, 1H), 10.06 (s, 1H).

Other 4-substituted imidazolealdehydes was prepared using the same standard procedure.

4.1.2. General procedure for synthesis of 2-substituted imidazole **12 a-g**

4.1.2.1. General procedure for synthesis of 2-substituted imidazole, method A. To a solution of appropriate aldehyde (50 mmol) in ethanol (160 mL) at 0 °C was added a solution of 40% oxalaldehyde in water (6.25 mL, 55 mmol) and a solution of 25–28% ammonium hydroxide in water (500 mmol, 40 mL). After stirring for 96 h at room temperature, the reaction mixture was concentrated and water was added. The product was extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography with dichloromethane as eluent to yield the title compound.

The following compounds were synthesized using method A: 2-isopropyl-1*H*-imidazole (**12a**), 2-*tert*-butyl-1*H*-imidazole (**12b**), 2-phenyl-1*H*-imid-azole (12g).

4.1.2.2. General procedure for synthesis of 2-substituted imidazole, method B. A suspension of NH_4HCO_3 (13.8 g, 174.6 mmol) and 40% glyoxal solution (10.9 mL,87.3 mmol) in water (9 mL) was treated dropwise with appropriate aldehyde (9.4 mL, 87.3 mmol) at 0 °C. The mixture was stirred at 25 °C for 24 h. The mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The product was purified by column chromatography (CH_2Cl_2/MeOH = 25:1) to obtain the title compound.

The following compound was synthesized using method B: 2-isobutyl-1*H*-imidazole (**12c**), 2-cyclopropyl-1*H*-imidazole (**12d**), 2-cyclopentyl-1*H*-imidazole (**12e**), 2-cyclohexyl-1*H*-imidazole (**12f**).

4.1.3. General procedure for synthesis of 2-isopropyl-4-methyl-1H-imidazole (**13a**) and 2-cyclopropyl-4-methyl-1H-imidazole (**13b**)

2-isopropyl-4-methyl-1*H*-imidazole (**13a**) A solution of isobutyraldehyde (50 mmol) in 25 mL ethanol was treated with ammonium hydroxide (28%, 25 mL) at 55 °C, Methylglyoxal (63 mmol) was added dropwise. The resulting solution was stirred for 16 h at 60 °C, the reaction mixture was concentrated and the residue was partitioned between ethyl acetate (50 mL) and water (30 mL). The organic phase was washed by brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography with dichloromethane as eluent to yield the title compound (4.65 g, 75%) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.29 (br, 1H), 6.55 (s, 1H), 3.03–2.91 (m, 1H), 2.22 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H). LC/MS (ESI) *m/z*: $[M + 1]^+$ calcd. for $C_7H_{12}N_2$, 125.10; found, 125.1.

2-cyclopropyl-4-methyl-1*H*-imidazole (**13b**) was prepared using standard proce-duer similar to the preparation of **13a**. Brown liquid (3.34 g, 45.5%), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (br, 1H), 6.57 (s, 1H), 2.18 (s, 3H), 1.94–1.86 (m, 1H), 0.94–0.86 (m, 4H). LC/MS (ESI) m/z: $[M + 1]^+$ calcd for $C_7H_{10}N_2$, 122.08; found, 122.1.

4.1.4. General procedure for synthesis of imidazolecarbaldehydes **17a-j** and **18 a-b**

Using the synthetic procedure of 2-phenyl-1*H*-imidazole-5-carbaldehyde (**17j**) as an example.

2-Phenyl-1*H*-imidazole (2.8 g, 19.42 mmol) was added to a suspension of 60% NaH (2.33 g, 58.26 mmol) in DMF 20 ml at 0 °C and the mixture was stirred for 0.5 h. Then N,N-dimethylsulfamoyl chloride (3.34 g, 23.3 mmol) were added dropwise and the reaction mixture was stirred for 2 h at room temperature. Saturated NH_4Cl solution was added and the resulting mixture as extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel chromatography with ethyl acetate/petroleum ether (1:4) as eluent to furnish (2.4 g, 49.2%) of the title compound as a dark yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72–7.66 (m, 2H), 7.48–7.40 (m, 4H), 7.10 (d, J = 1.6 Hz, 1H), 2.48 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 147.63, 130.39, 130.12, 129.87, 127.94, 127.74, 122.08, 37.52.

In a 250 ml three neck round-bottomed flask, which was purged and maintained with an inert atmosphere of nitrogen, was placed a solution of N,N-dimethyl-2-phenyl-1*H*-imidazole-1-sulfonamide (2.3 g, 9.16 mmol) in THF 30 ml. This was followed by the addition of *n*-butyllithium (4.4 mL, 10.99 mmol) dropwise at −78 °C over a period of 30 min. To this mixture was added DMF (4.58 mL, 59.54 mmol). The resulting solution was stirred at −50 °C for 30 min, then hydrogen chloride (23 mL, 22.9 mmol, 1M) was added.The reaction mixture was stirred for 2 h at room temperature. The pH of the solution was adjusted to 7–8 with saturated sodium bicarbonate solution. The resulting solution was extracted with 3 × 15 ml of ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel chromatography (ethyl acetate/petroleum ether = 1:4) to furnish the title compound (1.37 g, 87.3%) as yellow solid. Total yield: 43.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.34 (s, 1H), 9.76 (s, 1H), 8.02 (s, 2H), 7.93 (s, 1H), 7.46 (s, 3H). LC/MS (ESI) *m/z*: $[M + 1]^+$ calcd for $C_{10}H_8N_2O$, 173.06; found, 173.1.

Other imidazolecarbaldehydes were prepared as described in the procedure for synthesizing **17j**.

4.1.5. Synthesis of 2-(thiophen-2-yl)-1H-imidazole-4-carbaldehyde (**22**)

A dry 250 mL round-bottomed flask, equipped for magnetic stirring, was charged with dry methanol 40 mL and sodium methoxide (5.4 M solution in MeOH) (14.78 mL, 79.8 mmol) at N_2 atmosphere. The mixture was cooled to 0 °C. Thiophene-2-carbonitrile (4.12 mL, 53.2 mmol) was added dropwise. And then the reaction mixture was stirred at room temperature for 3 h. To the resulting solution was slowly added ammonium chloride (5.69 g, 106.4 mmol) and the mixture was stirred at room temperature for 68 h. The resulting suspension was filtered and the solvent was removed under reduced pressure. The solid obtained was washed with ethyl ether (3 × 25 mL) to give (4.2 g, 48.5%) of 2-thiophenecarboxamidine (HCl).

A mixture of 2-thiophenecarboxamidine (2.44 g, 15 mmol), 1,3-dihydroxyacetone dimer (4.05 g, 22.5 mmol), and NH_4Cl (4.81 g, 90 mmol) in ammonium hydroxide (25 mL) was heated to 80 °C for 6 h. The mixture was poured into ice-cold water, extracted with CH_2Cl_2, and dried over Na_2SO_4. The solvent was removed and the product was recrystallized from ethyl acetate to give the title compound (700 mg, 25.6%) as brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.42 (s, 1H), 7.53–7.46 (m, 2H), 7.10–7.03 (m, 1H), 5.13–4.85 (m, 1H), 4.45–4.35 (m, 2H). LC/MS (ESI) m/z: $[M + 1]^+$

calcd for $C_{12}H_{23}N$: 181.04, found: 181.0.

A suspension of alcohol (500 mg, 2.77 mmol), active MnO_2 (2.4 g, 27.7 mmol) in acetone 20 ml was vigorously shaken at room temperature till the alcohol was fully oxidized. The reaction mixture was filtered using a Celite pad. The MnO_2 was washed with plenty of acetone and the collected organic phases were concentrated to give the crude product. Purification by silica gel chromatography (ethyl acetate/petroleum ether = 1:1) yielded the desired aldehyde (295 mg, 59.7%) as red solid. Compound **22**, total yield: 7.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.42 (br, 1H), 9.71 (s, 1H), 8.04 (s, 1H), 7.78 (s, 1H), 7.67 (d, J = 4.7 Hz, 1H), 7.17 (dd, J = 4.8, 3.6 Hz, 1H).

4.1.6. General procedure for synthesis 4-n-propyl 2-substituted imidazolecarbalde-hydes ***29a-c***

4.1.6.1. Synthesis of ethyl 2-amino-3-oxohexanoate hydrochloride. A solution of ethyl 3-oxo-hexanoate **23** (4.91 g, 5 mL, 31 mmol) in acetic acid (20 mL) was cooled to 0 °C, and a solution of sodium nitrite (5.35 g, 77.5 mmol) in water (17 mL) was added dropwise, with the temperature of the reaction mixture being kept below 5 °C. The stirring mixture was maintained at 0 °C for 2 h and at room temper-ature for 2.5 h. The reaction mixture was then diluted and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution, dried with magnesium sulfate and concentrated under reduced pressure to give the α-hydroxyimino β-ketoester **24** quantitatively (5.16 g, 89%) as a clear oil.

A dry 100 ml round-bottomed flask was charged with 10% Pd/C (1.54 g, 33%), α-hydroxyimino β-ketoester **24** (4.68 g, 25 mmol) and absolute ethanol 20 ml. The mixture was degased and refilled with hydrogen. 30–40% ethanolic HCl solution 10 ml was added dropwise to the resulting mixture. The reaction mixture was stirred at room temperature under atmospheric pressure of hydrogen for 24 h. The suspension was then filtered through a Celite pad and washed with ethanol several times. The filtrate was concentrated under reduced pressure to give the desired β-ketoester hydrochloride (5.24 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.94 (s, 3 H), 5.24 (s, 1 H), 2.83–2.69 (m, 2 H), 1.54 (sextuplet, J = 7.3 Hz, 2 H), 1.24 (t, J = 7.1 Hz, 3 H), 0.86 (t, J = 7.4 Hz, 3 H). LC/MS (ESI) m/z: $[M-HCl + H]^+$ calcd for $C_8H_{16}NO_3$, 174.11; found, 174.1.

*4.1.6.2. Preparation of 2-isopropyl-4-propyl-1H-imidazole-5-carbaldehyde **29a**.* Isobutyric acid (704.9 mg, 8 mmol), amine (1.68 g, 8 mmol) and triethylamine (1.62 g, 2.21 ml, 16 mmol) were dissolved in acetonitrile (20 mL), and O-(7-Aza-benzotriazol-1-yl)-N,N,N′,N'-tetraMethyluronium hexafluorophosphate (HATU) (3.35 g, 8.8 mmol) was added to the solution in portions. The mixture was stirred at room temperature for 1.5 h, the reaction was completed. The reaction was quenched with 100 mL brine and the product extracted with ethyl acetate. The combined organic layers were washed with 2N HCl solution, water, 5% $NaHCO_3$ solution, and then brine successively. The organic layers were dried over MgSO4, filtered, and concentrated in *vacuo* and the crude product was purified by silica gel chromato-graphy to give the amide (1.62 g, 83.3%) as a white solid.

Ammonium acetate (2.31 g, 30 mmol) was added to a solution of ethyl 2-isobutyramido-3-oxohexanoate (1.46 g, 6 mmol) in acetic acid (15 mL). The mixture was heated to reflux overnight. After cooling, the solvent was evaporated under reduced pressure and the residue was taken up in saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography with ethyl acetate/petroleum ether (1:1) as eluent to give the ethyl 2-isopropyl-4-propyl-1*H*-imidazole-5-carboxylate (834.4 mg, 63%) as a white solid. ^{1}H NMR (400 MHz,$CDCl_3$) δ: 4.32 (q, J = 7.1 Hz, 2H) 3.11–3.01 (m, 1H), 2.83 (t, J = 7.7 Hz, 2H), 1.38–1.32 (9H, m), 0.94 (t, J = 7.4 Hz, 3H). LC/MS (ESI) m/z: $[M + 1]^+$ calcd for $C_{12}H_{20}N_2O_2$, 225.15; found, 225.1.

To a solution of ethyl 2-isopropyl-4-propyl-1*H*-imidazole-5-carboxylate **27a** (788.6 mg, 3.5 mmol) in THF (20 mL) was added $LiAlH_4$ (265.6 mg, 7 mmol) portionwise under argon atmosphere at 0 °C, and then the mixture was stirred for 4 h at room temperature. To this solution was added moist Na_2SO_4 solid slowly at 0 °C, and the resulting precipitate was removed by Celite filtration and washed with THF several times. The solvent was evaporated to give the corresponding crude alcohol **28a,** which was used next step without purification.

To a solution of **28a** in acetone (20 mL) was added active MnO_2 (6.1 g, 70 mmol), and the mixture was stirred at room temperature for 12 h. After filtration through a pad of Celite to remove solid MnO_2, the solvent was removed by evaporation, and the residual white powder was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 1:1) to give the desired aldehyde **29a** (480.7 mg, 76.2%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.24 (br, 1H), 9.65 (s, 1H), 3.15–3.05 (m, 1H), 2.80 (t, J = 7.6 Hz, 2H), 1.79–1.70 (m, 2H), 1.36 (s, 3H), 1.34 (s, 3H), 0.97 (t, J = 7.3 Hz, 3H). LC/MS (ESI) m/z: $[M + 1]^+$ calcd for $C_{10}H_{16}N_2O$, 181.13; found, 181.1.

2-cyclopropyl-4-propyl-1*H*-imidazole-5-carbaldehyde (**29b**) was prepared using standard procedure similar to the preparation of **29a**. White powder, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.64 (br, 1H), 9.57 (s, 1H), 2.76 (t, J = 7.6 Hz, 2H), 2.02–1.97 (m, 1H), 1.77–1.67 (m, 3H), 1.13–1.05 (m, 4H), 0.96 (t, J = 7.3 Hz, 3H). LC/MS (ESI) m/z: $[M + 1]^+$ calcd for $C_{10}H_{14}N_2O$, 179.11; found, 179.1.

2-phenyl-4-propyl-1*H*-imidazole-5-carbaldehyde (**29c**), light powder, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.93 (br, 1H), 9.77 (s, 1H), 8.01–7.91 (s, 2H), 7.51–7.40 (m, 3H), 2.90 (t, J = 7.6 Hz, 2H), 1.85–1.80 (m, 2H), 1.01 (t, J = 7.3 Hz, 3H). LC/MS (ESI) m/z: $[M + 1]^+$ calcd for $C_{13}H_{14}N_2O$, 215.11; found, 215.1.

4.1.7. General procedure of the synthesis of imidazole-based pinanamine derivatives ***30–52***

A dry 25 mL round-bottomed flask, equipped for magnetic stirring, was charged with (1R,2R,3R,5S)-(−)-isopinocampheylamine (3.85 mmol, 1.1 eq) and the aldehyde (3.5 mmol, 1 eq) in 1,2-dichloroethane (10 mL). And then sodium triacetoxyborohydride (7 mmol, 2 eq) was added in portions. The mixture was stirred at room temperature under a N_2 atmosphere for 1.5 h. The reaction mixture was quenched by adding aqueous saturated $NaHCO_3$ solution, and the product was extracted with dichloromethane. The organic layers were dried over anhydrous $MgSO_4$, filtered and evapor-ated to give the crude free base. Purification by silica gel chromatography eluted with dichloromethane/Methanol (20:1–10:1) to give the free base. Corresponding salt was achieved by addition of saturated hydrogen chloride in ethyl acetate solution.

*4.1.7.1. (1R,2R,3R,5S)-N-((4-ethyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo-[3.1.1]heptan-3-amine hydrochloride (**30**).* Compound **30** was synthesized according to the general procedure. White power, yield: 11.9%, mp 187–189 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.73 (br, 2H), 10.04 (s, 1H), 9.50 (s, 1H), 9.09 (s, 1H), 4.30 (s, 2H), 2.78 (q, J = 7.2 Hz, 2H), 2.41–2.32 (m, 1H), 2.31–2.25 (m, 1H), 2.23–2.15 (m, 1H), 2.11–1.87 (m, 3H), 1.85–1.77 (m, 1H), 1.45 (d, J = 10 Hz, 1H), 1.28–1.15 (m, 9H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 134.85, 133.75, 119.47, 55.91, 46.97, 40.29, 38.39, 37.18, 31.80, 30.59, 27.22, 23.22, 20.72, 16.63, 13.41. HRMS calcd for $C_{16}H_{27}N_3$ $[M + H]^+$, 262.2278, found, 262.2278.

*4.1.7.2. (1R,2R,3R,5S)-N-((4-cyclopropyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**31**).* White powder, yield: 15.7%, mp 202–204 °C. ^{1}H NMR (400 MHz,

DMSO-d_6) δ: 14.54 (br, 2H), 9.98 (s, 1H), 9.49 (s, 1H), 8.98 (s, 1H), 4.35 (s, 2H), 3.53 (s, 1H), 2.44–2.35 (m, 1H), 2.32–2.18 (m, 3H), 2.06–1.97 (m, 2H), 1.81 (s, 1H), 1.42 (d, J = 10 Hz, 1H), 1.26–1.15 (m, 7H), 1.03 (d, J = 7.6 Hz, 2H), 0.93 (s, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 135.35, 133.83, 121.04, 56.25, 47.41, 40.76, 38.83, 32.29, 31.03, 27.68, 23.66, 21.13, 7.93, 7.77, 5.60. HRMS calcd for $C_{17}H_{27}N_3$ $[M + H]^+$, 274.2278, found, 274.2278.

*4.1.7.3. (1R,2R,3R,5S)-N-((4-(tert-butyl)-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride(**32**).* Light yellow powder, yield: 10.1%, mp 130–132 °C. ^{1}H NMR (400 MHz, DMSO) δ: 14.65 (br, 2H), 10.14 (s, 1H), 9.48 (s, 1H), 9.16 (s, 1H), 8.19 (s, 1H), 4.39 (s, 2H), 2.42 (t, J = 11.2 Hz, 1H), 2.33–2.19 (m, 2H), 2.09–1.95 (m, 2H), 1.81 (t, J = 5.2 Hz, 1H), 1.50 (d, J = 10 Hz, 1H), 1.43 (s, 9H), 1.23–1.19 (m, 6H), 0.95 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 140.15, 134.14, 119.14, 57.02, 47.48, 40.82, 38.90, 32.59, 32.24, 31.03, 30.08, 27.73, 23.68, 21.17. HRMS calcd for $C_{18}H_{31}N_3$ $[M + H]^+$, 290.2590; found, 290.2591.

*4.1.7.4. (1R,2R,3R,5S)-N-((4-propyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicycle[3.1.1]heptan-3-amine hydrochloride (**33**).* Pale yellow powder, yield: 15.3%, mp 217–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.62 (br, 1H), 9.93 (s, 1H), 9.44 (s, 1H), 9.02 (s, 1H), 4.30 (s, 2H), 3.53 (s, 1H), 2.73 (t, J = 7.2 Hz, 2H), 2.37 (t, J = 11.2 Hz, 1H), 2.32–2.25 (m, 1H), 2.20 (t, J = 6.4 Hz, 1H), 2.07–2.02 (m, 1H), 1.97 (s, 1H), 1.81 (d, J = 5.6 Hz, 1H), 1.69–1.60 (m, 2H), 1.42 (d, J = 10.0 Hz, 1H), 1.23–1.15 (m, 7H), 0.92–0.89 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 134.25, 133.79, 120.71, 56.39, 47.46, 40.77, 38.81, 37.81, 32.26, 31.04, 27.67, 25.31, 23.64, 22.26, 21.17, 13.75. HRMS calcd for $C_{17}H_{29}N_3$ $[M + H]^+$, 276.2434; found, 276.2432.

*4.1.7.5. (1R,2R,3R,5S)-N-((4-phenyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicycle[3.1.1]heptan-3-amine hydrochloride (**34**).* White powder, yield: 13.6%, mp 156–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.12 (s, 1H), 9.57 (s, 1H), 9.16 (s, 1H), 7.79–7.68 (m, 2H), 7.60–7.52 (m, 3H), 4.41 (s, 2H), 3.53 (s, 1H), 2.32–2.10 (m, 3H), 1.98–1.92 (m, 2H), 1.83–1.72 (m, 1H), 1.42 (d, J = 10 Hz, 1H), 1.18 (s, 3H), 1.12 (d, J = 7.2 Hz, 3H), 0.84 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 135.19, 132.30, 130.04, 129.55, 129.03, 126.91, 121.44, 56.31, 47.41, 40.78, 40.59, 38.81, 38.67, 32.25, 30.79, 27.68, 23.61, 21.05. HRMS calcd for $C_{20}H_{27}N_3$ $[M + H]^+$, 310.2278; found, 310.2276.

*4.1.7.6. (1R,2R,3R,5S)-N-((2-methyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicycle[3.1.1]heptan-3-amine hydrochloride (**35**).* White powder, yield: 51.2%, mp 201–203 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.77 (br, 2H), 10.11 (s, 1H), 9.71 (s, 1H), 7.75 (s, 1H), 4.44–4.07 (m, 2H), 2.60 (s, 3H), 2.43–2.36 (m, 1H), 2.31–2.23 (m, 1H), 2.22–2.12 (m, 1H), 2.01 (ddd, J = 14.0, 6.0, 2.4 Hz, 1H), 1.96 (s, 1H), 1.79 (t, J = 5.2 Hz, 1H), 1.44 (d, J = 10.0 Hz, 1H), 1.20 (s, 3H), 1.17 (d, J = 7.2 Hz, 3H), 0.91 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 144.24, 124.13, 120.16, 55.90, 47.45, 40.76, 38.84, 38.49, 32.17, 30.99, 27.69, 23.76, 21.16, 11.56. HRMS calcd for $C_{15}H_{25}N_3$ $[M + H]^+$, 248.2121; found, 248.2120.

*4.1.7.7. (1R,2R,3R,5S)-N-((2-ethyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**36**).* Light yellow powder, yield: 53.6%, mp 227–229 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.77 (br, 2H), 10.04 (s, 1H), 9.66 (s, 1H), 7.75 (s, 1H), 4.40–4.15 (m, 2H), 2.96 (q, J = 7.6 Hz, 2H), 2.39 (t, J = 12 Hz, 1H), 2.32–2.23 (m, 1H), 2.22–2.13 (m, 1H), 2.06–1.98 (m, 1H), 1.96 (s, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.42 (d, J = 10.0 Hz, 1H), 1.33 (t, J = 7.6 Hz, 3H), 1.20 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 148.54, 123.77, 119.63, 55.57, 46.97, 40.28, 38.36, 38.12, 31.76, 30.61, 27.21, 23.27, 20.69, 18.89, 11.20. HRMS calcd for $C_{16}H_{27}N_3$ $[M + H]^+$, 262.2278; found, 262.2278.

*4.1.7.8. (1R,2R,3R,5S)-N-((2-cyclopropyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**37**).* White powder, yield: 51.3%, mp 260–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.61 (br, 2H), 9.90 (s, 1H), 9.55 (s, 1H), 7.65 (s, 1H), 4.26 (s, 2H), 3.43–3.32 (m Hz, 1H), 2.44–2.24 (m, 3H), 2.21–2.12 (m, 1H), 2.05–1.93 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.39 (d, J = 10 Hz, 1H), 1.28–1.23 (m, 2H), 1.23–1.19 (m, 5H), 1.18 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 149.38, 123.42, 119.31, 55.59, 46.98, 40.28, 38.35, 38.07, 31.75, 30.61, 27.21, 23.27, 20.71, 9.39, 6.90. HRMS calcd for $C_{17}H_{27}N_3$ $[M + H]^+$, 274.2278; found, 274.2276.

*4.1.7.9. (1R,2R,3R,5S)-N-((2-isopropyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**38**).* Whiter powder, yield: 56.7%, mp 178–180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.78 (br, 2H), 10.00 (s, 1H), 9.65 (s, 1H), 7.75 (s, 1H), 4.32 (s, 2H), 3.50–3.41 (m, 1H), 3.40–3.28 (m, 1H), 2.39 (t, J = 12.0 Hz, 1H), 2.33–2.24 (m, 1H), 2.23–2.14 (m, 1H), 2.07–1.99 (m, 1H), 1.96 (s, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.40 (d, J = 10.0 Hz, 1H), 1.38 (s, 3H), 1.36 (s, 3H), 1.21 (s, 3H), 1.19 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.37, 124.27, 120.06, 56.08, 47.45, 40.74, 38.82, 38.59, 32.23, 31.07, 27.67, 26.66, 23.72, 21.18, 20.75. HRMS calcd for $C_{17}H_{29}N_3$ $[M + H]^+$, 276.2434; found, 276.2435.

*4.1.7.10. (1R,2R,3R,5S)-N-((2-(tert-butyl)-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**39**).* White powder, yield: 44.6% mp 189–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.76 (br, 2H), 10.04 (s, 1H), 9.66 (s, 1H), 7.75 (s, 1H), 4.35 (s, 2H), 3.44–3.30 (m, 1H), 2.39–2.27 (m, 2H), 2.23–2.13 (m, 1H), 2.04–1.97 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.45 (s, 9H), 1.38 (d, J = 10.0 Hz, 1H), 1.21 (s, 3H), 1.19 (d, J = 5.0 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 155.22, 120.78, 56.68, 55.80, 49.63, 47.91, 47.81, 42.26, 41.32, 41.20, 39.27, 39.07, 33.86, 33.71, 33.41, 32.74, 31.57, 29.13, 28.34, 28.14, 24.17, 24.06, 21.66, 21.06. HRMS calcd for $C_{18}H_{31}N_3$ $[M + H]^+$, 290.2591; found, 290.2593.

*4.1.7.11. (1R,2R,3R,5S)-N-((2-isobutyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**40**).* White powder, yield: 63.5%, mp 159–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.82 (br, 2H), 10.04 (s, 1H), 9.66 (s, 1H), 7.77 (s, 1H), 4.34–4.24 (m, 2H), 2.82 (d, J = 7.2 Hz, 2H), 2.40 (t, J = 12.4 Hz, 1H), 2.28 (s, 1H), 2.19–2.14 (m, 2H), 2.02–1.91 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.40 (s, 1H), 1.20 (s, 3H), 1.16 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H), 0.91 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 146.94, 124.38, 120.21, 55.88, 47.43, 40.75, 38.84, 38.49, 34.11, 32.26, 31.01, 27.87, 27.69, 23.72, 22.25, 22.22, 21.12. HRMS calcd for $C_{18}H_{31}N_3$ $[M + H]^+$, 290.2591, found, 290.2591.

*4.1.7.12. (1R,2R,3R,5S)-N-((2-butyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**41**).* White powder, yield: 44.0%, mp 188–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.73 (br, 2H), 10.01 (s, 1H), 9.63 (s, 1H), 7.74 (s, 1H), 4.41–4.09 (m, 2H), 2.94 (t, J = 7.2 Hz, 2H), 2.40 (t, J = 11.2 Hz, 1H), 2.31–2.26 (m, 1H), 2.22–2.13 (m, 1H), 2.02–1.96 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.74 (m, (t, J = 7.2 Hz, 2H), 1.40 (d, J = 10 Hz, 1H), 1.36–1.24 (m, 2H), 1.20 (s, 3H), 1.17 (d, J = 7.2 Hz, 3H), 0.95–0.85 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 150.03, 126.37, 122.34, 58.12, 49.65, 42.96, 41.03, 40.66, 34.43, 33.20, 31.21, 29.88, 27.30, 25.92, 23.91, 23.34, 15.95. HRMS calcd for $C_{18}H_{31}N_3$ $[M + H]^+$, 290.2591; found, 290.2592.

*4.1.7.13. (1R,2R,3R,5S)-N-((2-cyclopentyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**42**).* White solid, yield: 46.1%, mp 173–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.74 (br, 2H), 9.95 (s, 1H), 9.61 (s, 1H), 7.72 (s, 1H), 4.30

(s, 2H), 3.54–3.30 (m, 1H), 2.39 (t, J = 11.2 Hz, 1H), 2.33–2.24 (m, 1H), 2.19–2.16 (m, 3H), 2.05–1.92 (m, 2H), 1.87–1.80 (m, 5H), 1.73–1.59 (m, 2H), 1.40 (d, J = 10.0 Hz, 1H), 1.21 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 150.70, 123.88, 119.65, 55.68, 46.98, 40.28, 38.35, 38.17, 36.07, 31.80, 31.45, 30.64, 27.21, 24.81, 23.26, 20.71. HRMS calcd for $C_{19}H_{31}N_3$ $[M + H]^+$, 302.2590; found, 302.2591.

*4.1.7.14. (1R,2R,3R,5S)-N-((2-cyclohexyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**43**).* White powder, yield: 59.3%, mp 179–181 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.72 (br, 2H), 9.92 (s, 1H), 9.60 (s, 1H), 7.74 (s, 1H), 4.32 (s, 2H), 3.45 (s, 1H), 3.04 (tt, J = 11.6, 3.2 Hz, 1H), 2.39 (t, J = 11.2 Hz, 1H), 2.34–2.24 (m, 1H), 2.22–2.12 (m, 1H), 2.09–1.92 (m, 4H), 1.86–1.75 (m, 3H), 1.73–1.66 (m, 1H), 1.65–1.52 (m, 2H), 1.44–1.31 (m, 3H), 1.26 (dt, J = 12.4, 3.1 Hz, 1H), 1.21 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.36, 124.27, 120.08, 56.14, 47.43, 40.74, 38.81, 38.62, 35.38, 32.27, 31.10, 30.53, 30.51, 27.67, 25.31, 23.71, 21.16. HRMS calcd for $C_{20}H_{33}N_3$ $[M + H]^+$, 316.2747; found, 316.2748.

*4.1.7.15. (1R,2R,3R,5S)-N-((2-phenyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**44**).* White powder, yield: 75.3%, mp 188–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.86 (s, 1H), 9.53 (s, 1H), 8.19 (s, 2H), 7.88 (s, 1H), 7.64 (d, J = 6.0 Hz, 3H), 4.40 (s, 2H), 2.49–2.38 (m, 1H), 2.35–2.26 (m, 1H), 2.24–2.13 (m, 1H), 2.09–1.94 (m, 2H), 1.81 (t, J = 5.2 Hz, 1H), 1.38 (d, J = 10 Hz, 1H), 1.21–1.19 (m, 6H), 0.93 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 143.94, 132.45, 130.01, 127.04, 126.39, 123.86, 121.56, 56.09, 47.47, 40.78, 40.60, 38.84, 32.32, 31.17, 27.71, 23.77, 21.20. HRMS calcd for $C_{20}H_{27}N_3$ $[M + H]^+$, 310.2278; found, 310.2279.

*4.1.7.16. (1R,2R,3R,5S)-N-((2-(thiophen-2-yl)-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**45**).* Light yellow powder, yield: 72.3%, mp 207–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.80 (s, 1H), 9.46 (s, 1H), 8.02 (s, 1H), 7.88 (d, J = 4.8 Hz, 1H), 7.75 (s, 1H), 7.29 (dd, J = 4.8, 4.0 Hz, 1H), 4.35–4.25 (m, 2H), 2.42 (t, J = 11.2 Hz, 1H), 2.34–2.25 (m, 1H), 2.22–2.12 (m, 1H), 2.04–1.97 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.38 (d, J = 10.0 Hz, 1H), 1.21 (s, 3H), 1.17 (d, J = 7.2 Hz, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 139.06, 131.47, 130.40, 128.64, 120.56, 55.49, 47.00, 40.31, 40.06, 38.57, 38.36, 31.81, 30.65, 27.23, 23.27, 20.71. HRMS calcd for $C_{18}H_{25}N_3S$ $[M + H]^+$, 316.1842; found, 316.1843.

*4.1.7.17. (1R,2R,5S)-N-((2-ethyl-4-methyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**46**).* White powder, yield:68.2%, mp 193–195 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.63 (br, 2H), 9.99 (s, 1H), 9.47 (s, 1H), 4.25 (s, 2H), 3.53 (s, 1H), 2.92 (q, J = 7.6 Hz, 2H), 2.43–2.16 (m, 6H), 2.09–2.02 (m, 1H), 2.02–1.88 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.45 (d, J = 10.0 Hz, 1H), 1.32 (t, J = 7.6 Hz, 3H), 1.21 (s, 3H), 1.19 (d, J = 3.0 Hz, 3H), 0.93 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 147.30, 128.89, 119.02, 55.93, 46.98, 40.29, 39.97, 38.35, 37.33, 31.76, 30.62, 27.20, 23.21, 20.73, 18.86, 11.03, 9.03. HRMS calcd for $C_{17}H_{29}N_3$ $[M + H]^+$, 276.2434; found, 276.2436.

*4.1.7.18. (1R,2R,3R,5S)-N-((2-isopropyl-4-methyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**47**).* White powder, yield: 57.4%, mp 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.57 (br, 2H), 9.92 (s, 1H), 9.43 (s, 1H), 4.27 (s, 2H), 3.54 (s, 1H), 3.34–3.20 (m, 1H), 2.42–2.32 (m, 4H), 2.31–2.16 (m, 2H), 2.05 (ddd, J = 14.0, 5.6, 2.4 Hz, 1H), 1.97 (s, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.44 (d, J = 10.0 Hz, 1H), 1.37 (s, 3H), 1.36 (s, 3H), 1.21–1.19 (m, 6H), 0.93 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 150.66, 129.01, 118.94, 55.93, 47.01, 40.30, 39.97, 38.36, 37.34, 31.75, 30.56, 27.22, 26.14, 23.22, 20.75, 20.19, 20.17, 9.01. HRMS calcd for $C_{18}H_{31}N_3$ $[M + H]^+$, 290.2591; found, 290.2590.

*4.1.7.19. (1R,2R,3R,5S)-N-((2-cyclopropyl-4-methyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride (**48**).* Light yellow powder, yield: 49.4%, mp 248–250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.45 (br, 2H), 9.86 (s, 1H), 9.36 (s, 1H), 4.22 (s, 2H), 3.52 (s, 1H), 2.37 (t, J = 11.2 Hz, 1H), 2.32–2.13 (m, 6H), 2.09–1.99 (m, 1H), 1.96 (s, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.43 (d, J = 10.0 Hz, 1H), 1.25 (t, J = 2.4 Hz, 1H), 1.24–1.16 (m, 9H), 0.93 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 148.17, 128.67, 118.64, 55.95, 47.00, 40.30, 39.97, 38.38, 31.75, 30.61, 27.23, 23.25, 20.77, 9.24, 9.20, 9.02, 6.85. HRMS calcd for $C_{18}H_{29}N_3$ $[M + H]^+$, 288.2434; found, 288.2436.

*4.1.7.20. (1R,2R,3R,5S)-N-((2-isopropyl-4-propyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride(**49**).* White powder, yield: 39.5%, mp 182–184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.52 (br, 2H), 9.88 (s, 1H), 9.43 (s, 1H), 4.29 (s, 2H), 3.55 (s, 1H), 3.41–3.26 (m, 1H), 2.69 (t, J = 7.6 Hz, 2H), 2.42–2.26 (m, 2H), 2.25–2.16 (m, 1H), 2.10–1.93 (m, 2H), 1.81 (t, J = 5.2 Hz, 1H), 1.69–1.60 (m, 2H), 1.41 (d, J = 10.0 Hz, 1H), 1.38 (s, 3H), 1.36 (s, 3H), 1.21 (s, 3H), 1.19 (d, J = 7.2 Hz, 3H), 0.96–0.86 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.83, 133.26, 119.46, 56.44, 47.45, 40.76, 38.81, 37.87, 32.36, 31.13, 27.67, 26.69, 25.28, 23.63, 22.40, 21.16, 20.70, 20.68, 13.79. HRMS calcd for $C_{20}H_{35}N_3$ $[M + H]^+$, 318.2904; found, 318.2903.

*4.1.7.21. (1R,2R,3R,5S)-N-((2-cyclopropyl-4-propyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride(**50**).* White powder, yield: 46.2%, mp 176–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.52 (s, 1H), 14.36 (s, 1H), 9.83 (s, 1H), 9.35 (s, 1H), 4.24 (s, 2H), 3.53 (s, 1H), 2.65 (t, J = 7.6 Hz, 2H), 2.41–2.24 (m, 3H), 2.23–2.15 (m, 1H), 2.08–1.92 (m, 2H), 1.80 (t, J = 5.2 Hz, 1H), 1.69–1.59 (m, 2H), 1.41 (d, J = 10 Hz, 1H), 1.28–1.15 (m, 10H), 0.94–0.87 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 149.23, 132.89, 119.08, 56.42, 47.45, 40.75, 38.81, 37.83, 32.32, 31.13, 27.67, 25.28, 23.65, 22.27, 21.18, 13.77, 9.78, 9.75, 7.36. HRMS calcd for $C_{20}H_{33}N_3$ $[M + H]^+$, 316.2747; found, 316.2747.

*4.1.7.22. (1R,2R,3R,5S)-N-((2-phenyl-4-propyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-amine hydrochloride(**51**).* White powder, yield: 43.7%, mp 196–198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.90 (s, 1H), 9.43 (s, 1H), 8.27–8.24 (m, 2H), 7.69–7.65 (m, 3H), 4.40 (s, 2H), 3.64 (s, 1H), 2.80 (t, J = 7.6 Hz, 2H), 2.40 (t, J = 12.0 Hz, 1H), 2.35–2.18 (m, 2H), 2.12–2.06 (m, 1H), 2.03–1.95 (m, 1H), 1.82 (t, J = 5.2 Hz, 1H), 1.78–1.69 (m, 2H), 1.42 (d, J = 10.0 Hz, 1H), 1.22–1.20 (m, 6H), 1.00–0.88 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 143.07, 135.08, 132.48, 129.96, 126.97, 123.49, 121.47, 56.49, 47.48, 40.79, 40.60, 38.81, 38.00, 32.38, 31.11, 27.68, 25.43, 23.66, 22.68, 21.19, 13.81. HRMS calcd for $C_{23}H_{33}N_3$ $[M + H]^+$, 352.2747; found, 352.2748.

*4.1.7.23. (1R,2R,3R,5S)-N-((1-methyl-1H-imidazol-5-yl)methyl)-2,6,6-trimethylbicycle[3.1.1]heptan-3-amine hydrochloride (**52**).* White powder, yield: 89.7%, mp 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.32 (br, 1H), 9.77 (s, 1H), 9.13 (s, 1H), 7.94 (s, 1H), 4.33 (s, 2H), 4.00 (s, 3H), 3.59 (s, 1H), 2.39 (t, J = 12.2 Hz, 1H), 2.32–2.20 (m, 2H), 2.05 (ddd, J = 13.6, 5.8, 2.4 Hz, 1H), 1.99–1.96 (m, 1H), 1.80 (td, J = 5.2, 1.6 Hz, 1H), 1.56 (d, J = 10.0 Hz, 1H), 1.25–1.17 (m, 6H), 0.95 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 136.09, 126.25, 121.99, 56.21, 47.10, 40.35, 38.48, 36.38, 33.92, 31.64, 30.71, 27.27, 23.25, 20.82. HRMS: calculated for $C_{15}H_{25}N_3$ $[M + H]^+$, 248.2048; found, 248.2049.

4.2. Cytopathic effect (CPE) assay Protocol

In CPE assay, MDCK cells were grown to a confluent monolayer in a 96-well culture plate at a concentration of 5 × 104 for 24 h. The medium was removed, and the cells were rinsed twice. An infectious virus at 100 $TCID_{50}$ was inoculated into the MDCK cells, which were then incubated for 2 h at 37 °C in 5% CO_2. The virus supernatant was then removed, followed by the addition of serial two-fold dilutions of antiviral compounds in DMEM containing 1.5 μg/mL trypsin. After being incubated at 34 °C in 5% CO_2 for 48 h, the infected cells displayed 100% CPE under the microscope, and the CPE percentages in the antiviral compound-treated groups were recorded. The IC_{50} values were calculated using a non-linear regression model in GraphPad Prism.

4.3. MTT cell viability test

MDCK cells in 96-well plate were left untreated or treated with various concentration of compounds for 48 h, and treated with 20 μL of MTT stock solution (5 mg/mL) at 37 °C for 4 h. Reaction was blocked by DMSO and absorbance was read at 490 nm using Thermo multiskan MK3 Spectrum spectro-photometer. The IC_{50} values were calculated using Reed–Muench method.

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