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Design, synthesis and *in vitro* biological evaluation of isoxazol-4-carboxa piperidyl derivatives as new anti-influenza A agents targeting virus nucleoprotein†

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Influenza infection is a major cause of morbidity and mortality during seasonal epidemics and sporadic pandemics. It is important and urgent to develop new anti-influenza agents with a new mechanism of action. Nucleozin has been reported as a potent antagonist of nucleoprotein accumulation in the nucleus. In this study, a new series of isoxazol-4-carboxa piperidyl derivatives **1a–j** were synthesized and their chemical structures were confirmed by ^{1}H, ^{13}C NMR and mass spectral data. Furthermore, all the synthesized compounds were evaluated for *in vitro* anti-influenza virus activity against influenza virus (A/PR/8/34 H1N1). Among all the compounds, **1a**, **1b**, **1c**, **1f** and **1g** exhibited more potent activity than the standard drug, and compound **1b** has showed most promising anti-influenza virus activity. These results are also consistent with the docking study results in terms of the design of compounds targeting influenza A *via* viral nucleoprotein.

Introduction

Seasonal and pandemic influenza represent one of the major threats to public health. The annual influenza epidemic results in 250 000–500 000 deaths worldwide.[1] During the past century, the 1918 Spanish flu, 1957 Asian flu and 1968 Hongkong flu pandemics caused millions of fatalities.[2] Influenza infection is a major cause of morbidity and mortality during seasonal epidemics and sporadic pandemics. Nucleoproteins (NP) that encapsulate viral RNA forming ribonucleoprotein (RNP) particles in influenza viruses are responsible for vital functions of transcription, assembly and packaging.[3,4] More importantly, the viral genetic shift may eventually cause a breakthrough of interspecies and inter-human transmission barriers and raise the possibility of transmission from animals to human beings or *vice versa* (human beings to animals), which would be disastrous to public health.[5–7] Two classes of anti-influenza drugs are available in the market, M2 inhibitors and neuraminidase inhibitors. However, the efficacies of these drugs are limited due to the rapid emergence of mutated viral strains and side effects.[8,9] Thereby, it is important and urgent to develop new anti-influenza agents with a new mechanism of action.[10,11]

Influenza virus is a single-stranded, negative-sense, segmented RNA virus of the Orthomyxovirus family. As such, it uses a heterotrimeric RNA-dependent RNA polymerase in complex with its RNA genome and associated nucleoprotein (NP) to synthesize the requisite viral RNAs (vRNAs), mRNAs, and cRNAs needed for replication.[12,13] NP plays several roles during the viral life cycle, including ribonucleoprotein (RNP) formation, transport of vRNA-polymerase complex to the nucleus, viral replication, and virion assembly.[14–17] Consequently, inhibitors of NP function may affect multiple stages of the life cycle.

Nucleozin has been reported as a "potent antagonist of NP accumulation in the nucleus".[18] Nucleozin induces the formation of NP aggregates and antagonizes its nuclear accumulation, leading to cessation of viral replication. It was reported that a series of nucleozin derivatives showed a good antiviral activity *in vivo*.[19] In previous work, we designed and synthesized a series of nucleozin derivatives with prominent antiviral activities against influenza A H1N1.[20] In the present study, target compounds of isoxazol-4-carboxa piperidyl derivatives were designed and synthesized. All the newly synthesized compounds were evaluated in cell toxicity assays and viral inhibition assays.

Results

Chemistry

Nucleozin was the lead compound of a series of derivatives selected by Kao and coworkers,[18] and it was comprised of four components: aryl group, isoxazole, piperazinyl and 2-chloro-4-

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nitrophenyl. The authors studied the effect of the substitution on the aryl portion of phenyl with different groups such as Cl-, MeO-, both on anti-influenza virus activity and cytotoxicity. The work highlighted the importance of the substitution on the aromatic ring, in particular they found nucleozin impeded influenza A virus replication *in vitro* with a nanomolar median effective concentration (EC_{50}) and protected mice challenged with lethal doses of avian influenza A H5N1.

In order to assess the relevance of the piperazinyl group and its importance for anti-influenza virus activity, we planned to synthesize nucleozin piperidyl derivatives and test them against influenza virus. The aim was to evaluate modifications such as the introduction of substituents on the aromatic ring and the piperidyl group substituted, as depicted in Fig. 1.

The synthetic route to prepare all the isoxazol-4-carboxa piperidyl derivatives is depicted in Scheme 1. 5-Methyl-isoxazole-4-carboxylic acid **4a–j** and 4-(2-chloro-4-nitrophenyl) piperidine **9** are key intermediates for the preparation of the desired compound **1a–j**. Firstly, ethyl acetoacetate was treated with different substituted aromatic nitriles in toluene and $TiCl_4$ was added. Then the mixture was refluxed for 2 h to afford compounds **2a–j**.[21,22] The intermediates **2a–j** were reacted with hydroxylamine hydrochloride in ethanol and the reaction mixture was stirred at reflux for 2 h to obtain compounds **3a–j.** Further, these compounds **3a–j** were hydrolyzed under basic condition to afford pure compounds **4a–j**.

On the other hand, the synthesis of 4-(2-chloro-4-nitrophenyl) piperidine **9** was carried out *via* a 5-step reaction. The formation of the intermediate 3-(2-chlorophenyl) pentanedioic acid **6** was obtained by the reaction of 2-chlorobenzaldehyde and ethyl acetoacetate with piperidine in ethanol at reflux temperature for 3 h. The subsequent hydrolyzation with NaOH in 50% ethanol at reflux temperature provided the compound **6**. The intermediate **6** reacted with ammonia at 200 °C to obtain compound **7**. The intermediate **9** was synthesized by the reduction and nitration of compound **7**. Finally, these acid intermediates **4a–j** were subjected to coupling reaction with intermediate **9** in the presence of EDCI, DIEA as coupling reagents and stirred at room temperature for 2 h in anhydrous DCM as the solvent to afford pure corresponding products **1a–j** as shown in Scheme 1. The synthesized piperidyl derivatives **1a–j** were characterized by $^1H/^{13}C$ NMR and mass spectral analysis.

Anti-influenza virus activity and cytotoxicity measurement

Cells and viruses. Madin–Darby canine kidney (MDCK) cells (purchase from Thermo Fisher Scientific, catalog# H1398) were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum in a humidified 5% CO_2 incubator at 37 °C. Influenza A/PR/8/34 (H1N1) was propagated in 10 day-old chicken egg embryos at 37 °C and harvested for 48 h after inoculation in pooled allantoic fluid. After a brief centrifugation (3000 rpm at room temperature for 20 min) and a virus titer measurement by hemagglutination test, the virus was aliquot and stored at a −80 °C freezer.

MDCK cell based viral inhibition assays. In brief, MDCK cells were added to 96-well plates at a density of 4000 cells per well. After 24 h, the plates were infected by the influenza A (A/PR/8/34 H1N1 strain) virus for 2 h. The newly synthesized compounds were dissolved in DMSO and diluted to 6 consecutive 3-fold dilutions. Then the compounds were added to the above wells and all the plates were allowed to culture for 48 h. Free DMSO with the same concentration was used as negative control while ribavirin was utilized as positive control. At the end of the 48 h incubation, the cytopathic effect (CPE) of cells was observed. Cell viability was determined by the 4-methylumbelliferyl-α-*N*-acetyl-neuraminate method.

MDCK cell based cell toxicity assays. In brief, MDCK cells were added to 96-well plates at a density of 4000 cells per well and cultured for 24 h. The newly synthesized compounds were dissolved in DMSO and diluted to 6 consecutive 3-fold dilutions. Then the compounds were added to the above wells and all the plates were allowed to culture for 72 h. Free DMSO with the same concentration was used as negative control and ribavirin was served as positive controls. At the end of the 72 h incubation, the cytopathic effect (CPE) of cells was observed. Cell viability was determined by the alamarBlue® method.

Data processing and molecular docking study. The CC_{50} and EC_{50} values were calculated *via* nonlinear regression using GraphPad Prism 5. Schrodinger 2013 was used to perform the docking simulations. From the RCSB Protein Data Bank, the crystal structure of influenza A virus nucleoprotein (PDB ID: 3RO5) in complex with ligands was retrieved. Afterwards, "Protein Preparation Wizard" in Maestro-8.5 (Schrodinger's 2013) was used to remove the water molecules and add the polar hydrogens atoms. The protocol was generated based on the ligand in the crystal structure. Other parameter referring the default values was set. Ten poses were generated for each ligand and the lowest energy conformation of each ligand–protein complex was selected for analysing the interactions between the virus NP and the inhibitor.

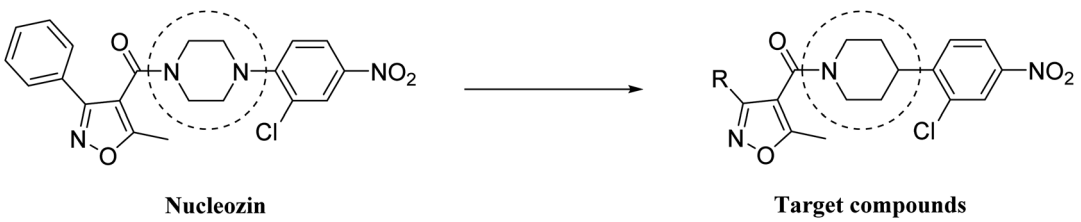
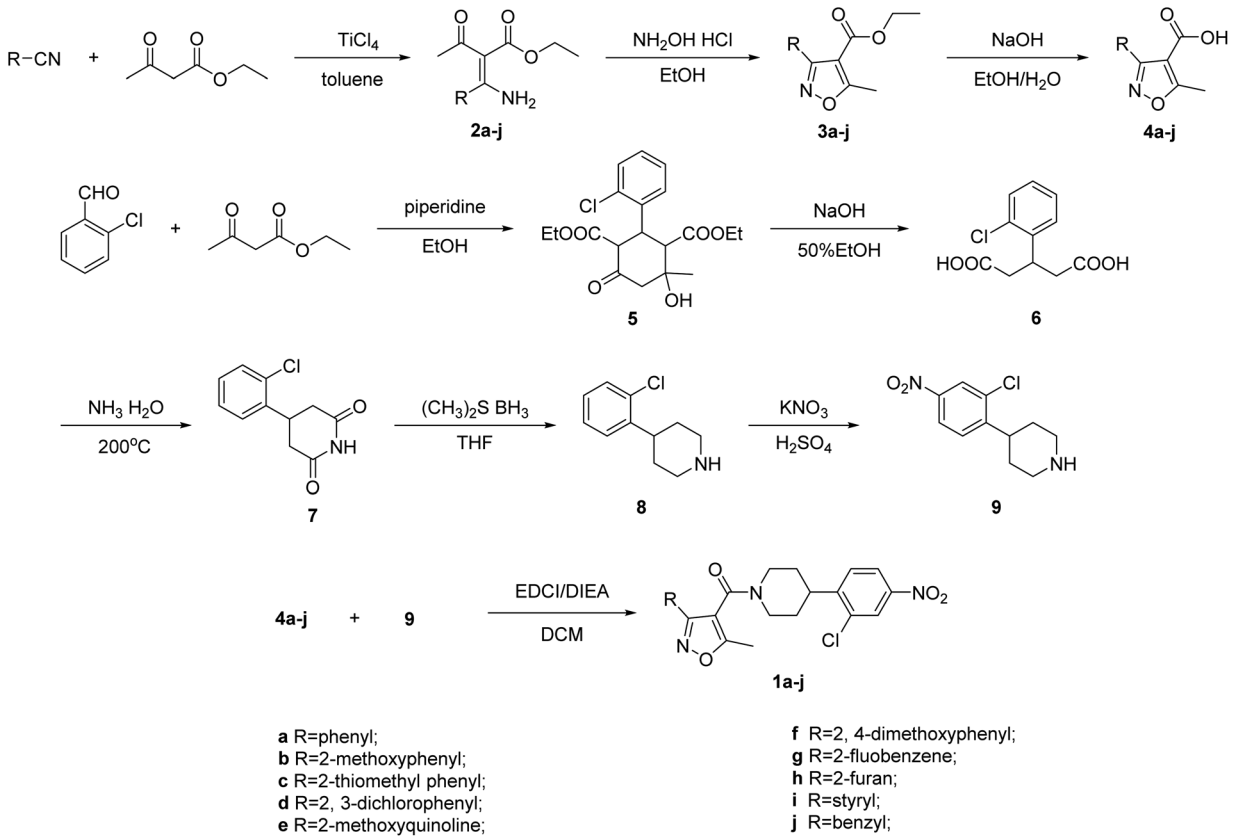


Fig. 1 Design of isoxazol-4-carboxa piperidyl derivatives.



Scheme 1 Synthesis of isoxazol-4-carboxa piperidyl derivative.

Discussion

Anti-influenza A virus activity

All the compounds prepared herein **1a–j** were screened for their anti-influenza activity against influenza virus (A/PR/8/34 H1N1 strain) and the results acquired were incorporated in Table 1. Ribavirin was used as the standard reference drug and most of the tested compound displayed good to moderate activity with respect to all cell lines. The EC_{50} values of synthesized compounds ranged from 0.235 to 34.483 μM and the standard drug was at 9.891 μM. Among them, five compounds, **1a**, **1b**, **1c**, **1f** and **1g** exhibited excellent activity than ribavirin. Further, compound **1b** (R = 2-methoxyphenyl) showed the most promising activity ($EC_{50} = 0.253 \pm 0.001$ μM, $CC_{50} = 69.05 \pm 0.11$ μM, SI = 272.93). The replacement of (R = phenyl) with (R = 2-thiomethyl phenyl) resulted in compound **1c** which exhibited lower activity ($EC_{50} = 0.502 \pm 0.002$ μM, SI = 398.41) when compared with compound **1b**. Compound **1a** (R = phenyl) displayed good activity on the cell line ($EC_{50} = 0.898 \pm 0.004$ μM, $CC_{50} = 19.78 \pm 0.07$ μM, SI = 22.02) when compared with compound **1c**. Whereas, compound **1f** with (R = 2,4-dimethoxyphenyl) groups ($EC_{50} = 2.036 \pm 0.009$ μM, $CC_{50} = 27.85 \pm 0.08$ μM, SI = 13.68) and **1g** with (R = 2-fluorobenzene) groups ($EC_5 = 1.356 \pm 0.008$ μM, $CC_{50} = 16.64 \pm 0.05$ μM, SI = 12.27) exhibited comparable activity, respectively. The rest compounds **1d**, **1e**, **1h**, **1i** and **1j** showed moderate activity on the cell line.

Cytotoxicity

Cytotoxic activities of the compounds against MDCK cells were also evaluated to monitor the potential cytotoxicity effects. Most

Table 1 Anti-influenza virus activity and cytotoxicity of target compounds[a]

Entry	Compound	Cytotoxicity CC_{50}[b]/(μM)	Anti-influenza virus activity EC_{50}[c]/(μM)	SI[d]
1	**1a**	19.78 ± 0.07	0.898 ± 0.004	22.02
2	**1b**	69.05 ± 0.11	0.253 ± 0.001	272.93
3	**1c**	>100	0.502 ± 0.002	—
4	**1d**	>100	21.572 ± 0.015	—
5	**1e**	24.96 ± 0.09	9.905 ± 0.010	2.52
6	**1f**	27.85 ± 0.08	2.036 ± 0.009	13.68
7	**1g**	16.64 ± 0.05	1.356 ± 0.008	12.27
8	**1h**	>100	16.226 ± 0.012	—
9	**1i**	>100	34.483 ± 0.014	—
10	**1j**	>100	19.410 ± 0.012	—
11	Ribavirin	63.01 ± 0.05	9.891 ± 0.06	6.37

[a] All the data were the average values from three independent assays. [b] Compound concentration that reduces cell viability by 50% relative to uninfected MDCK cells. [c] Compound concentration that reduces viral replication by 50% relative to infected MDCK cells. [d] Selectivity index: CC_{50}/EC_{50}.

of the derivatives show no obvious cellular growth inhibition against MDCK cells at concentrations below 500 μM (the highest test concentration); while ribavirin displays moderate toxicity against MDCK cells (CC_{50} = 201.80 μM). It is worth mentioning that all the compounds bear a great selectivity index.

Molecular docking study

The X-ray crystal structure of influenza A virus nucleoprotein complex with inhibitors was disclosed. It was demonstrated that six inhibitors bridged two molecules of NP (NP_A and NP_B) protein to form a stable dimer complex. A computational study was performed using Schrodinger 2013 to investigate the differences of potential interactions between compounds **1b** and nucleozin and virus nucleoprotein (PDB ID: 3RO5). The results indicate that compounds **1b** and nucleozin have similar action modes. Although the 2-chlor-4-nitrophenyl fragments of three molecules overlapped well, there is a discrepancy on the other side of the molecule, which might be the reason why there is a disparity in the anti influenza viral activity (Fig. 2).

As shown in Fig. 3, a strong π–π stacking interaction has been formed between 4-nitro-2-chloro-phenyl moiety and TYR289. The pyrazine ring of nucleozin also plays a crucial role in making the molecule into a more stable conformation so that nucleozin could link the two trimers more steadily. This gives a higher inhibitory activity. The diversity of the binding modes has aroused our great interests and encourages us to expansion additional study on this topic despite the fact that all the compounds we reported here have lower activity than nucleozin.

Experimental

Generals

All the reactions were carried out under the protection of argon (Ar) atmosphere. Most chemicals and solvents were analytical grade and used without further purification. Thin layer chromatography (TLC) was performed using precoated silica gel GF254 (0.2 mm) while column chromatography was performed using silica gel (100–200 mesh). The melting point was measured on a YRT-3 melting point apparatus (Shantou Keyi Instrument & Equipment Co., Ltd, Shantou, China) without thermometer correction. ^{1}H NMR spectra were collected on a Varian INOVA400 (Varian, Palo Alto, USA) using $CDCl_3$, DMSO-d_6 or D_2O as solvent. Chemical shifts were expressed in δ, with tetramethylsilane (TMS) as the internal reference; coupling constants (J) were expressed in Hz. The high-resolution mass (HRMS) spectra were recorded on a Bruker maxis impact Q-TOF instrument (Bruker, Billerica, USA) coupled with a Dionex Ultimate 3000 spectrometer (Dionex, Sunnyvale, USA).

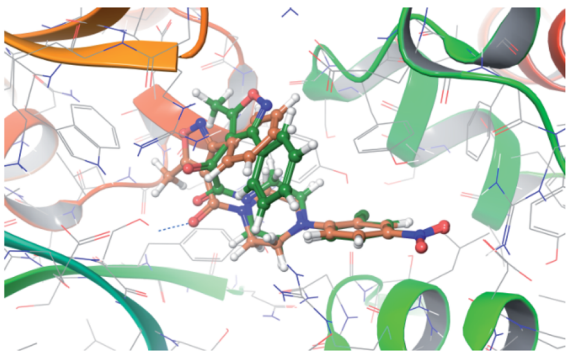


Fig. 2 Orientation of the best docked poses of compound **1b** and nucleozin in the active site of the viral nucleoprotein.

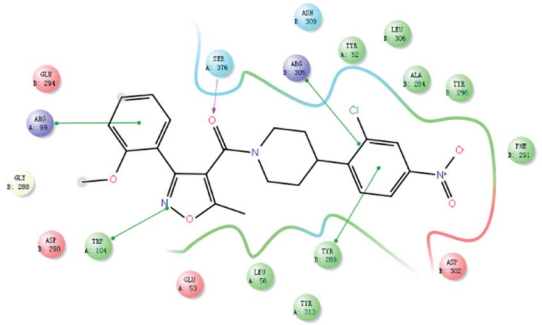


Fig. 3 Interaction mode between viral nucleoprotein and compound **1b**.

General procedure for the preparation of compounds 2a–j

To a solution of nitriles (1 eq.), $TiCl_4$ (1.2 eq.) and ethyl acetoacetate (1.2 eq.) were added at room temperature with stirring. The mixture was refluxed with stirring for 2 h. After cooling to room temperature, saturated sodium carbonate solution was added, and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel to afford pure compounds **2a–j**.

Ethyl (*Z*)-2-(amino(phenyl)methylene)-3-oxobutanoate (2a). This compound was prepared according to method described above benzonitrile (1.0 g, 0.01 mol), with ethyl acetoacetate (1.6 g, 0.012 mol) to afford compound **2a** as a yellow oil (1.9 g with 82.1% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.18 (s, 1H), 7.42–7.23 (m, 5H), 5.52 (s, 1H), 3.78 (q, 2H, J = 7.2 Hz), 2.41 (s, 3H), 0.75 (t, 3H, J = 7.2 Hz). MS (ESI): 233.1 [M^+ + H].

Ethyl (*Z*)-2-(amino(2-methoxyphenyl)methylene)-3-oxobutanoate (2b). This compound was prepared according to method described above 2-methoxybenzonitrile (1.0 g, 7.5 mmol), with ethyl acetoacetate (1.2 g, 9 mmol) to afford compound **2b** as a white solid (1.4 g with 73.2% yield). mp 98–100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.20 (s, 1H), 7.37 (t, 1H, J = 8.0 Hz), 7.18 (d, 1H, J = 7.6 Hz), 6.97 (m, 2H), 5.75 (s, 1H), 3.82 (s, 3H), 3.70 (m, 2H), 2.36 (s, 3H), 0.86 (s, 3H); MS (ESI): 264.2 [M^+ + H].

Ethyl (*Z*)-2-(amino(2-(methylthio)phenyl)methylene)-3-oxobutanoate (2c). This compound was prepared according to method described above 2-methylthiobenzonitrile (1.0 g, 6.7 mmol), with ethyl acetoacetate (1.1 g, 8 mmol) to afford compound **2c** as a yellow oil (1.3 g with 70.8% yield). ^{1}H NMR

($CDCl_3$, 400 MHz, δ ppm): 11.50 (s, 1H), 7.24 (t, 1H, $J = 8.0$ Hz), 7.07 (d, 1H, $J = 7.6$ Hz), 6.90 (m, 2H), 5.71 (s, 1H), 3.75 (s, 3H), 3.65 (m, 2H), 2.31 (s, 3H), 0.82 (s, 3H); MS (ESI): 280.6 [M^+ + H].

Ethyl (*Z*)-2-(amino(2,3-dichlorophenyl)methylene)-3-oxobutanoate (2d). This compound was prepared according to method described above 2,3-dichlorobenzonitrile (1.0 g, 5.8 mmol), with ethyl acetoacetate (0.9 g, 7 mmol) to afford compound **2d** as a colorless oil (0.7 g with 40.7% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.54 (s, 1H), 7.26 (m, 3H), 5.71 (s, 1H), 3.72 (s, 3H), 3.65 (m, 2H), 2.36 (s, 3H), 1.12 (s, 3H); MS (ESI): 302.4 [M^+ + H].

Ethyl (*Z*)-2-(amino(2-methoxyquinolin-3-yl)methylene)-3-oxobutanoate (2e). This compound was prepared according to method described above quinoline-2-carbonitrile (1.0 g, 6.5 mmol), with ethyl acetoacetate (1.0 g, 7.8 mmol) to afford compound **2e** as a colorless oil (0.69 g with 37.2% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.25 (s, 1H), 8.79 (s, 1H), 8.03 (m, 1H), 7.91 (m, 1H), 7.77 (m, 1H), 7.50 (m, 1H), 5.74 (s, 1H), 4.12 (s, 3H), 4.10 (q, 2H, $J = 6.8$ Hz), 2.37 (s, 3H), 1.30 (t, 3H, $J = 6.8$ Hz); MS (ESI): 285.3 [M^+ + H].

Ethyl (*Z*)-2-(amino(2,4-dimethoxyphenyl)methylene)-3-oxobutanoate (2f). This compound was prepared according to method described above 2,4-dimethoxybenzonitrile (1.0 g, 6.1 mmol), with ethyl acetoacetate (0.95 g, 7.3 mmol) to afford compound **2f** as a white solid (0.85 g with 58.2% yield). mp 124–127 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.15 (s, 1H), 7.46 (d, 1H, $J = 8.4$ Hz), 6.57 (d, 1H, $J = 8.4$ Hz), 6.46 (s, 1H), 5.65 (s, 1H), 4.16 (q, 2H, $J = 7.2$ Hz), 3.85 (s, 3H), 3.72 (s, 3H), 2.72 (s, 3H), 1.24 (m, 3H); MS (ESI): 294.5 [M^+ + H].

Ethyl (*Z*)-2-(amino(2-fluorophenyl)methylene)-3-oxobutanoate (2g). This compound was prepared according to method described above 2-fluorobenzonitrile (1.0 g, 8.2 mmol), with ethyl acetoacetate (1.3 g, 9.9 mmol) to afford compound **2g** as a colorless oil (0.88 g with 42.9% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.32 (s, 1H), 7.43–7.06 (m, 4H), 5.54 (s, 1H), 3.91 (q, 2H, $J = 6.8$ Hz), 2.03 (s, 3H), 1.07 (t, 3H, $J = 6.8$ Hz); MS (ESI): 252.1 [M^+ + H].

Ethyl (*Z*)-2-(amino(furan-2-yl)methylene)-3-oxobutanoate (2h). This compound was prepared according to method described above furan-2-carbonitrile (1.0 g, 10.7 mmol), with ethyl acetoacetate (1.7 g, 12.9 mmol) to afford compound **2h** as a yellow oil (1.82 g with 76.4% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 10.68 (s, 1H), 7.52 (d, 1H, $J = 0.8$ Hz), 6.77 (d, 1H, $J = 2.8$ Hz), 6.50 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 3.2$ Hz), 5.92 (s, 1H), 4.14 (q, 2H, $J = 7.2$ Hz), 2.95 (s, 3H), 1.17 (t, 3H, $J = 7.2$ Hz); MS (ESI): 224.2 [M^+ + H].

Ethyl (2*Z*,4*E*)-2-acetyl-3-amino-5-phenylpenta-2,4-dienoate (2i). This compound was prepared according to method described above cinnamonitrile (1.0 g, 7.7 mmol), with ethyl acetoacetate (1.2 g, 9.3 mmol) to afford compound **2i** as a yellow oil (1.67 g with 83.8% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.04 (s, 1H), 7.48 (d, 2H, $J = 6.8$ Hz), 7.37 (d, 3H, $J = 6.8$ Hz), 7.19 (d, 1H, $J = 1.6$ Hz), 7.05 (d, 1H, $J = 1.6$ Hz), 5.63 (s, 1H), 4.27 (q, 2H, $J = 7.2$ Hz), 2.37 (s, 3H), 1.31 (t, 3H, $J = 7.2$ Hz); MS (ESI): 260.3 [M^+ + H].

Ethyl (*Z*)-2-acetyl-3-amino-4-phenylbut-2-enoate (2j). This compound was prepared according to method described above 2-phenylacetonitrile (1.0 g, 8.5 mmol), with ethyl acetoacetate (1.3 g, 10.2 mmol) to afford compound **2j** as a yellow oil (1.71 g with 81.5% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 11.04 (s, 1H), 7.48 (d, 2H, $J = 6.8$ Hz), 7.37 (d, 3H, $J = 6.8$ Hz), 7.19 (d, 1H, $J = 1.6$ Hz), 7.05 (d, 1H, $J = 1.6$ Hz), 5.63 (s, 1H), 4.27 (q, 2H, $J = 7.2$ Hz), 2.37 (s, 3H), 1.31 (t, 3H, $J = 7.2$ Hz); MS (ESI): 248.6 [M^+ + H].

General procedure for the preparation of compounds 3a–j

To a solution of **2a–j** (1 eq.) in ethanol was added hydroxylammonium chloride (1.5 eq.). the reaction mixture was refluxed with stirring for 2 h until TLC showed no starting materials. The suspension was cooled to room temperature and evaporated to dryness in vacuum followed by the addition of H_2O and the extraction with ethyl acetate three times. The organic layer was combined, washed with brine three times and dried over Na_2SO_4. After the solvent was evaporated in vacuum to obtain a crude product, which was further purified by flash chromatography on silica gel to get **3a–j**.

Ethyl 5-methyl-3-phenylisoxazole-4-carboxylate (3a). This compound was prepared according to method described above **2a** (500 mg, 2.5 mmol) to afford compound **3a** as a colorless oil (410 mg with 82.1% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.42–7.23 (m, 5H), 3.75 (q, 2H, $J = 7.2$ Hz), 2.43 (s, 3H), 1.05 (t, 3H, $J = 7.2$ Hz). MS (ESI): 232.7 [M^+ + H].

Ethyl 3-(2-methoxyphenyl)-5-methylisoxazole-4-carboxylate (3b). This compound was prepared according to method described above **2b** (500 mg, 1.9 mmol) to afford compound **3b** as a white solid (460 mg with 92.1% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.45–7.38 (m, 2H), 7.02 (t, 1H, $J = 4$ Hz) 6.94 (d, 1H, $J = 8.4$ Hz), 4.13 (q, 2H, $J = 7.2$ Hz), 3.76 (s, 3H), 2.70 (s, 3H), 1.10 (t, 3H, $J = 7.2$ Hz). MS (ESI): 262.3 [M^+ + H].

Ethyl 5-methyl-3-(2-(methylthio)phenyl)isoxazole-4-carboxylate (3c). This compound was prepared according to method described above **2c** (500 mg, 1.8 mmol) to afford compound **3c** as a yellow oil (360 mg with 72.1% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.35–7.28 (m, 2H), 6.95 (t, 1H, $J = 4$ Hz) 6.91 (d, 1H, $J = 8.4$ Hz), 4.10 (q, 2H, $J = 7.2$ Hz), 3.72 (s, 3H), 2.66 (s, 3H), 1.02 (t, 3H, $J = 7.2$ Hz). MS (ESI): 278.4 [M^+ + H].

Ethyl 3-(2,3-dichlorophenyl)-5-methylisoxazole-4-carboxylate (3d). This compound was prepared according to method described above **2d** (500 mg, 1.6 mmol) to afford compound **3d** as a colorless oil (440 mg with 88.6% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.54–7.59 (m, 1H), 7.30–7.35 (m, 2H), 4.15 (q, 2H, $J = 7.2$ Hz), 3.76 (s, 3H), 2.74 (s, 3H), 1.14 (t, 3H, $J = 7.2$ Hz). MS (ESI): 300.9 [M^+ + H].

Ethyl 3-(2-methoxyquinolin-3-yl)-5-methylisoxazole-4-carboxylate (3e). This compound was prepared according to method described above **2e** (500 mg, 1.6 mmol) to afford compound **3e** as a colorless oil (270 mg with 53.5% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.15 (s, 1H), 8.03 (m, 1H), 7.91 (m, 1H), 7.77 (m, 1H), 7.50 (m, 1H), 4.22 (dd, 1H, $J_1 = 7.2$ Hz, $J_2 = 1.6$ Hz), 4.02 (s, 3H), 2.37 (s, 3H), 1.30 (t, 3H, $J = 7.2$ Hz). MS (ESI): 313.9 [M^+ + H].

Ethyl 3-(2,4-dimethoxyphenyl)-5-methylisoxazole-4-carboxylate (3f). This compound was prepared according to

method described above 2f (500 mg, 1.7 mmol) to afford compound **3f** as a white solid (340 mg with 68.6% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.46 (d, 1H, $J = 8.4$ Hz), 6.57 (d, 1H, $J = 8.4$ Hz), 6.45 (s, 1H), 4.16 (q, 2H, $J = 7.2$ Hz), 3.85 (s, 3H), 3.72 (s, 3H), 2.73 (s, 3H), 1.13 (t, 3H, $J = 7.2$ Hz). MS (ESI): 291.7 [M^+ + H].

Ethyl 3-(2-fluorophenyl)-5-methylisoxazole-4-carboxylate (3g). This compound was prepared according to method described above **2g** (500 mg, 2.0 mmol) to afford compound **3g** as a yellow solid (410 mg with 82.3% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.28 (m, 4H), 4.28 (q, 2H, $J = 7.6$ Hz), 2.21 (s, 3H), 1.27 (t, 3H, $J = 7.6$ Hz). MS (ESI): 250.2 [M^+ + H].

Ethyl 3-(furan-2-yl)-5-methylisoxazole-4-carboxylate (3h). This compound was prepared according to method described above **2h** (500 mg, 2.1 mmol) to afford compound **3h** as a yellow solid (315 mg with 63.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.57 (d, $J = 1.2$ Hz, 1H), 7.40 (d, $J = 3.2$ Hz, 1H), 6.53 (dd, $J_1 = 2.0$ Hz, $J_2 = 3.6$ Hz, 1H), 4.33 (q, 2H), 2.72 (s, 3H), 1.36 (t, 3H). MS (ESI): 222.4 [M^+ + H].

Ethyl (*Z*)-5-methyl-3-styrylisoxazole-4-carboxylate (3i). This compound was prepared according to method described above **2i** (500 mg, 1.9 mmol) to afford compound **3i** as a colorless solid (380 mg with 76.5% yield).^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.45 (d, 2H, $J = 6.8$ Hz), 7.33 (d, 3H, $J = 6.8$ Hz), 7.15 (d, 1H, $J = 1.6$ Hz), 7.00 (d, 1H, $J = 1.6$ Hz), 4.21 (q, 2H, $J = 7.2$ Hz), 2.32 (s, 3H), 1.21 (t, 3H, $J = 7.2$ Hz); MS (ESI): 258.1 [M^+ + H].

Ethyl 3-benzyl-5-methylisoxazole-4-carboxylate (3j). This compound was prepared according to method described above **2j** (500 mg, 2.0 mmol) to afford compound **3j** as a colorless solid (430 mg with 86.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.31 (m, 3H), 7.23 (m, 2H), 4.21 (q, 2H), 3.94 (s, 2H), 2.71 (s, 3H), 1.12 (t, 3H). MS (ESI): 258.2 [M^+ + H].

General procedure for the preparation of compounds 4a–j

To a solution of **3a–j** (1 eq.) in ethanol was added sodium hydroxide (5 eq.) in water. The reaction mixture was refluxed with stirring for 2 h until TLC showed no starting materials. The solution was evaporated in vacuum. The water layer was adjusted with 10% HCl until the pH was about 3. The mixture was filtered, the solid cake dried in a vacuum at 50 °C to give compounds **4a–j**, which was used without further purification.

Synthesis of 3-(2-chlorophenyl)pentanedioic acid (6)

To a solution of 2-chlorobenzaldehyde (20 g, 0.142 mol) in ethanol (100 ml) was added ethyl acetoacetate (36.2 ml, 0.284 mol) and piperidine (2 ml). The reaction mixture was stirred at room temperature for 4 h until TLC showed no starting materials. The reaction mixture was concentrated *in vacuo* and extracted with EtOAc (2 × 200 ml). The combined EtOAc layers were dried over Na_2SO_4 and concentrated to give the crude product **5**, which was used without further purification.

To a solution of crude product **5** in ethanol (220 ml) was added sodium hydroxide (55 g, 1.375 mol) in water (250 ml). The reaction mixture was refluxed with stirring for 3 h until TLC showed no starting materials. The reaction mixture was concentrated *in vacuo*. The pH value was adjusted to 4 with 1 mol L^{-1} HCl, and extracted with EtOAc (2 × 200 ml). The combined EtOAc layers were washed with brine and dried over Na_2SO_4. After the solvent was evaporated in vacuum, an off-white solid was obtained and further purified by recrystallization in acetone to obtain the product as a white crystal (19.5 g). Two steps yield 56.7%. mp 152–154 °C.

Synthesis of 4-(2-chlorophenyl)piperidine-2,6-dione (7)

To a solution of compound **6** (19.5 g) dissolved in ammonium hydroxide (25 ml), The reaction mixture was concentrated *in vacuo* and the residue was heated at 200 °C for 5 h. And then the residue was cooled to room temperature, and dissolved in ethyl acetate and washed with sat. $NaHCO_3$, and brine. The combined EtOAc layers were dried over Na_2SO_4. After the solvent was evaporated in vacuum, an off-white solid was obtained and further purified by recrystallization in acetone to obtain the product as a white crystal (15.4 g). Yield 85.6% mp 155–157 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.43 (dd, 1H, $J = 8.0$ Hz), 7.30 (m, 2H), 7.21 (dd, 1H, $J = 7.6$ Hz), 3.91 (m, 1H), 2.98 (d, 2H, $J = 8.0$ Hz), 2.94 (d, 1H, $J = 4.8$ Hz), 2.76 (m, 2H). MS (ESI): 224.4 [M^+ + H].

Synthesis of 4-(2-chlorophenyl)piperidine (8)

A solution of compound **7** (10 g, 44.84 mol) in THF (100 ml) was cooled to 0 °C and borane dimethyl sulfide (25 ml, 89.68 mol) was added dropwise. The mixture was refluxed for 6 h. And then the residue was cooled to room temperature, and added 6 mol L^{-1} HCl (150 ml). The mixture was refluxed for 4 h until TLC showed no starting materials. The reaction mixture was concentrated *in vacuo*. The pH value was adjusted to 10 with NaOH, and extracted with EtOAc (2 × 200 ml). The combined EtOAc layers were washed with brine and dried over Na_2SO_4. After the solvent was evaporated in vacuum to obtain a crude product, which was further purified by flash chromatography on silica gel to get white solid (5.06 g). Yield 58.2% mp 210–215 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.33 (t, 2H, $J = 6.4$ Hz), 7.23 (m, 1H), 7.14 (t, 1H, $J = 7.6$ Hz), 3.64 (t, 1H, $J = 4.8$ Hz), 3.27 (d, 1H, $J = 10.8$ Hz), 3.12 (m, 1H), 2.59 (s, 1H), 2.31 (s, 1H), 1.93 (s, 2H), 1.75 (m, 2H), 1.26 (m, 1H). MS (ESI): 196.1 [M^+ + H].

Synthesis of 4-(2-chloro-4-nitrophenyl)piperidine (9)

A solution of compound **8** (5 g, 25.64 mmol) in con. H_2SO_4 (50 ml) was cooled to 0 °C and KNO_3 (2.6 g, 25.64 mmol) was added. The mixture was stirred at room temperature overnight until TLC showed no starting materials. The solution was poured into ice. The pH value was adjusted to 10 with NaOH, and extracted with EtOAc (2 × 50 ml). The combined EtOAc layers were washed with brine and dried over Na_2SO_4. After the solvent was evaporated in vacuum to obtain a crude product, which was further purified by flash chromatography on silica gel to get yellow solid (4.94 g). Yield 80.3% ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.19 (s, 1H), 8.02 (dd, 1H, $J = 8.0$ Hz), 7.53 (d, 1H, $J = 8.8$ Hz), 3.74 (s, 1H), 3.32 (d, 1H, $J = 12.4$ Hz), 3.22 (t, 1H, $J = 12.0$ Hz), 2.86 (t, 1H, $J = 12.0$ Hz), 1.92 (d, 2H, $J = 12.4$ Hz), 1.72 (m, 2H). MS (ESI): 241.6 [M^+ + H].

General procedure for the preparation of compounds 1a–j

To a solution of **4a–j** (1 eq.) in DCM was added compound **9** (2.5 eq.), HOBT (2.5 eq.), EDCI (4 eq.) and triethylamine (2.5 eq.). The reaction mixture was stirred at room temperature overnight until TLC showed no starting materials. The reaction mixture was washed with brine and the organic layers were dried over Na_2SO_4. After the solvent was evaporated in vacuum to obtain a crude product, which was further purified by flash chromatography on silica gel to get **1a–j**.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(5-methyl-3-phenylisoxazol-4yl)methanone (1a). This compound was prepared according to method described above **4a** and **9** to afford compound **1a** as a white soild. Yield 85.6% mp 125–127 °C, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.00 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 7.6 Hz, 2H), 7.53 (m, 3H), 7.49 (d, J = 8.8 Hz, 2H), 4.91 (d, 1H), 3.55 (d, 1H), 3.13 (m, 1H), 2.91 (brs, 1H), 2.74 (m, 1H), 2.56 (s, 3H), 1.91 (m, 2H), 1.41 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 175.26, 172.56, 162.43, 146.67, 139.21, 130.42, 129.29, 129.23, 129.09, 128.76, 127.58, 124.38, 122.15, 111.67, 134.38, 45.21, 44.62, 36.62, 29.15, 29.03, 12.11. HRMS (ESI) calcd for $C_{22}H_{20}ClN_3O_4Na$ ([M + Na]$^+$) 428.1132; found: 428.1169.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-(2-methoxyphenyl)-5-methylisoxazol-4-yl)methanone (1b). This compound was prepared according to method described above **4b** and **9** to afford compound **1b** as a yellow soild. Yield 83.3% mp 164–166 °C, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 7.98 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 7.72 (s, 1H), 7.54 (m, 2H), 7.47 (d, J = 8.4 Hz, 1H), 7.07 (m, 2H), 4.73 (d, J = 10.4 Hz, 2H), 3.80 (s, 3H), 3.52–3.54 (d, 1H), 3.06–3.12 (m, 1H), 2.76–2.78 (m, 1H), 2.68–2.71 (m, 1H), 2.55 (s, 3H), 1.88 (m, 2H), 1.32 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 175.14, 172.52, 160.43, 146.67, 111.64, 118.92, 157.32, 139.23, 131.16, 130.42, 129.75, 124.34, 122.18, 121.51, 111.18, 56.10, 44.69, 44.62, 36.68, 29.08, 29.02, 12.17. HRMS (ESI) calcd for $C_{23}H_{22}ClN_3O_5Na$ ([M + Na]$^+$) 478.1252; found: 478.1292.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(5-methyl-3-(2-(methylthio)phenyl)isoxazol-4-yl)methanone (1c). This compound was prepared according to method described above **4c** and **9** to afford compound **1c** as a yellow soild. Yield 80.4% mp 182–186 °C, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 7.97 (d, J = 1.6 Hz, 1H), 7.66 (s, 1H), 7.56 (m, 1H), 7.38 (m, 2H), 7.30 (m, 1H), 7.26 (m, 1H), 4.73 (brs, 1H), 3.61 (brs, 1H), 3.06 (m, 1H), 2.85 (brs, 1H), 2.69 (brs, 1H), 2.58 (s, 3H), 2.42 (s, 3H), 1.74 (m, 2H), 1.32 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 175.23, 172.64, 160.49, 146.62, 111.60, 118.96, 157.30, 139.28, 131.12, 130.43, 129.70, 124.38, 122.12, 121.56, 111.11, 56.13, 44.68, 44.64, 36.65, 29.01, 29.04, 15.14. HRMS (ESI) calcd for $C_{23}H_{22}ClN_3O_4SNa$ ([M + Na]$^+$) 494.1042; found: 494.1071.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-(2,3-dichlorophenyl)-5-methylisoxazol-4-yl)methanone (1d). This compound was prepared according to method described above **4d** and **9** to afford compound **1d** as a yellow solid. Yield 83.6% mp 188–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.03 (m, 1H), 7.75 (m, 2H), 7.48 (m, 2H), 7.31 (m, 1H), 4.73 (brs, 1H), 3.70 (brs, 1H), 3.14 (m, 1H), 2.90 (m, 2H), 2.62 (s, 3H), 1.92 (m, 1H), 1.81 (s, 1H), 1.60 (brs, 1H), 1.30 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 175.28, 172.51, 159.13, 146.67, 139.56, 134.32, 133.92, 131.39, 131.12, 130.40, 130.21, 127.61, 127.03, 124.31, 122.18, 111.62, 44.62, 44.68, 36.60, 29.01, 29.07, 12.10. HRMS (ESI) calcd for $C_{22}H_{18}Cl_3N_3O_4Na$ ([M + Na]$^+$) 516.0452; found: 516.0447.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-(2-methoxyquinolin-3-yl)-5-methylisoxazol-4-yl)methanone (1e). This compound was prepared according to method described above **4e** and **9** to afford compound **1e** as a white solid. Yield 78.3% mp 218–220 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.37 (s, 1H), 7.96 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.0 Hz, 2H), 7.65 (m, 1H), 7.40–7.48 (m, 2H), 4.85 (brs, 1H), 4.11 (s, 3H), 3.69 (brs, 1H), 3.10 (m, 1H), 2.79 (brs, 2H), 2.62 (brs, 3H), 1.94 (brs, 1H), 1.86 (brs, 1H), 1.54 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 181.48, 175.21, 172.52, 160.49, 146.23, 144.82, 139.59, 136.86, 134.39, 131.52, 130.86, 130.42, 129.14, 127.70, 124.82, 124.38, 123.58, 122.16, 111.67, 54.42, 44.65, 44.70, 36.62, 29.02, 29.08, 12.18. HRMS (ESI) calcd for $C_{26}H_{23}ClN_4O_5Na$ ([M + Na]$^+$) 529.1435.; found: 529.1447.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-(2,4-dimethoxyphenyl)-5-methylisoxazol-4-yl)methanone (1f). This compound was prepared according to method described above **4f** and **9** to afford compound **1f** as a yellow solid. Yield 84.8% mp 114–116 °C, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.02 (dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 1H), 7.84 (s, 1H), 7.48–7.52 (m, 2H), 6.56–7.58 (dd, J_1 = 6.4 Hz, J_2 = 3.2 Hz, 1H), 4.79 (m, 1H), 3.81 (s, 6H), 3.70 (m, 1H), 3.12 (m, 1H), 2.75 (m, 2H), 2.55 (s, 3H), 1.93 (m, 2H), 1.40 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 175.28, 172.53, 161.69, 160.48, 158.23, 148.66, 139.52, 134.32, 132.53, 130.47, 124.38, 122.13, 111.23, 111.62, 107.12, 98.82, 56.19, 55.82, 44.62, 44.70, 36.67, 29.11, 29.03, 12.23. HRMS (ESI) calcd for $C_{24}H_{24}ClN_3O_6Na$ ([M + Na]$^+$) 508.1439; found: 508.1427.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-(2-fluorophenyl)-5-methylisoxazol-4-yl)methanone (1g). This compound was prepared according to method described above **4g** and **9** to afford compound **1g** as a yellow solid. Yield 83.4% mp 104–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.02–8.05 (d, J = 8.8 Hz, 1H), 7.86 (s, 1H), 7.67 (m, 1H), 7.58 (m, 1H), 7.53 (m, 1H), 7.32 (m, 2H), 4.84 (d, 1H), 3.71 (d, 1H), 3.23 (m, 1H), 3.04 (brs, 1H), 2.80 (brs, 1H), 2.58 (s, 3H), 1.94–1.99 (brs, 2H), 1.52–1.56 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 169.65, 162.26, 160.94, 159.28, 156.48, 146.84, 143.67, 140.20, 132.44, 130.77, 124.99, 124.93, 122.51, 122.16, 118.11, 116.40, 47.46, 42.32, 38.56, 29.63, 29.60, 11.97. HRMS (ESI) calcd for $C_{24}H_{24}ClN_3O_6$-Na ([M + Na]$^+$) 466.1035; found: 466.1072.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-(furan-2-yl)-5-methylisoxazol-4-yl)methanone (1h). This compound was prepared according to method described above **4h** and **9** to afford compound **1h** as a yellow solid. Yield 84.6% ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.05 (m, 2H), 7.65 (s, 1H), 7.55 (d, J = 8.8 Hz, 1H), 6.96 (s, 1H), 6.62 (s, 1H), 5.01 (m, 1H), 3.75 (m, 1H), 3.33 (m, 1H), 3.15 (m, 1H), 2.92 (m, 1H), 2.52 (s, 3H), 1.72 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 172.54, 158.92, 158.78, 154.02, 146.67, 142.95, 139.58, 134.38, 130.42, 124.36, 122.17, 112.08, 107.12, 100.54, 44.63, 44.67, 36.64, 29.36, 29.03, 9.6.

HRMS (ESI) calcd for $C_{20}H_{18}ClN_3O_5Na$ ([M + Na]$^+$) 438.0957; found: 438.0969.

(4-(2-Chloro-4-nitrophenyl)piperidin-1-yl)(3-methyl-5-styrylisoxazol-4-yl)methanone (1i). This compound was prepared according to method described above **4i** and **9** to afford compound **1i** as a yellow solid. Yield 78.9% mp 94–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.03 (m, 2H), 7.53 (m, 3H), 7.32 (m, 4H), 7.22 (d, J = 16.8 Hz, 1H), 5.02 (brs, 1H), 3.84 (brs, 1H), 3.30 (m, 1H), 3.22 (brs, 1H), 2.95 (brs, 1H), 2.51 (s, 3H), 1.71 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 172.52, 158.92, 158.76, 146.64, 139.50, 137.51, 134.97, 134.30, 130.42, 128.67, 128.64, 128.57, 128.52, 127.90, 124.39, 122.34, 122.14, 100.56, 44.67, 44.62, 36.65, 29.02, 29.08, 10.37. HRMS (ESI) calcd for $C_{24}H_{22}ClN_3O_4Na$ ([M + Na]$^+$) 474.1302; found: 474.1327.

(5-Benzyl-3-methylisoxazol-4-yl)(4-(2-chloro-4-nitrophenyl) piperidin-1-yl)methanone (1j). This compound was prepared according to method described above **4j** and **9** to afford compound **1j** as a yellow solid. Yield 78.92% mp 94–96 °C, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.06 (m, 1H), 7.94 (s, 1H), 7.55 (d, J = 8.8 Hz, 1H), 7.26 (m, 5H), 4.80 (brs, 1H), 3.17 (brs, 3H), 3.47 (brs, 1H), 3.09 (m, 1H), 2.71 (brs, 1H), 2.38 (s, 3H), 1.25–2.1 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 172.56, 158.92, 158.73, 146.68, 139.52, 136.21, 134.32, 130.47, 129.09, 129.02, 128.68, 128.63, 125.73, 124.82, 122.10, 113.58, 44.67, 44.62, 36.62, 31.18, 29.08, 29.04, 10.23. HRMS (ESI) calcd for $C_{23}H_{22}ClN_3O_4Na$ ([M + Na]$^+$) 462.1348; found: 462.1361.

Conclusions

In conclusion, we have synthesized a new series of isoxazol-4-carboxa piperidyl derivatives **1a–j** through a facile route and their anti-influenza virus activity was demonstrated. These compounds were tested for their preliminary anti-influenza virus activity against influenza virus (A/PR/8/34 H1N1) and ribavirin was used as the standard reference drug. All these molecules showed good to moderate activity. Among all the synthesized compounds, **1a**, **1b**, **1c**, **1f** and **1g** exhibited more potent activity than standard drug, and compound **1b** showed the most promising activity. Our results suggest that compound **1b** has a significant potential for further development into a new class of antiviral therapeutics targeting of the influenza nucleoprotein.

Conflicts of interest

There are no conflicts to declare.

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