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ORIGINAL RESEARCH

# Synthesis and antiviral study of novel 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamide derivatives

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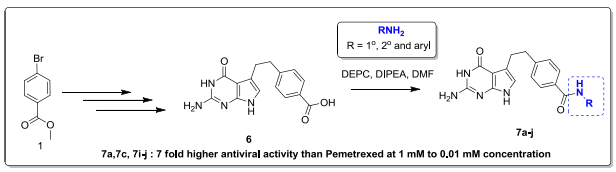
## Abstract

A series of ten new compounds (**7a–j**) has been synthesized by absolutely replacing the glutamic acid part of Pemetrexed drug, chemically known as N-{4-[2-(2-amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid, with primary, secondary, and aryl amines in high yields using diethylphosphorocyanidate (DEPC) as a peptide coupling agent. All the synthesized compounds are characterized by $^{1}H$ and $^{13}C$ NMR, LCMS, and FT-IR spectral techniques. All the synthesized novel non-glutamate 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl) ethyl)benzamide derivatives showed 4- to 7-folds higher antiviral activity than its structurally similar commercial drug Pemetrexed against Newcastle disease virus, an avian paramyxovirus. Among the lot, compounds possessing carboxamide synthesized using five-membered heteroaryl amines (**7i** and **7j**) exhibited the highest antiviral activity.

## Graphical Abstract




**Keywords** Pyrrolo[2,3-d]pyrimidine · Antiviral · Avian paramyxovirus · Diethylphosphorocyanidate

## Introduction

The folates and its derivatives, by means of structural modifications, have been extensively studied by many researchers over several decades. This is because of presence of pyrrolo[2,3-d]pyrimidine skeleton, which exhibits massive biological activities like dihydrofolate reductase (DHFR) inhibition (Berman and Werbel 1991), enzyme inhibition (Supuran et al. 1998), antiviral (Guo et al. 2011; Varaprasad et al. 2007), anti-inflammatory (Mohamed et al. 2007), anti-allgeric (Nagashima et al. 2009), antitumor (Jung et al. 2009; Asukai et al. 2010; McHardy et al. 2010), antibacterial, and antifungal (Hilmy et al. 2010) activities. These efforts have led to the discovery of Methotrexate (Suster et al. 1978) and Pemetrexed, which are the currently marketed drugs exclusively used in the treatment of cancer.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00044-018-2256-z) contains supplementary material, which is available to authorized users.

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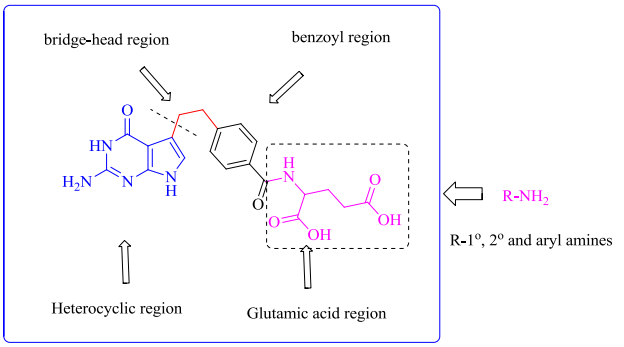



**Fig. 1** Structure of Pemetrexed

The chemical modification of Pemetrexed by either one of the four regions (heterocyclic (Suster et al. 1978; Shohreh et al. 2012), bridge (Suster et al. 1978; Smith et al. 2002; Grant et al. 1993), benzyol (Smith et al. 2002; Itoh et al.,1996) and glutamic acid (Itoh et al.,1996, 1995, 2000) regions as shown in Fig. 1) had been extensively studied by various researchers.

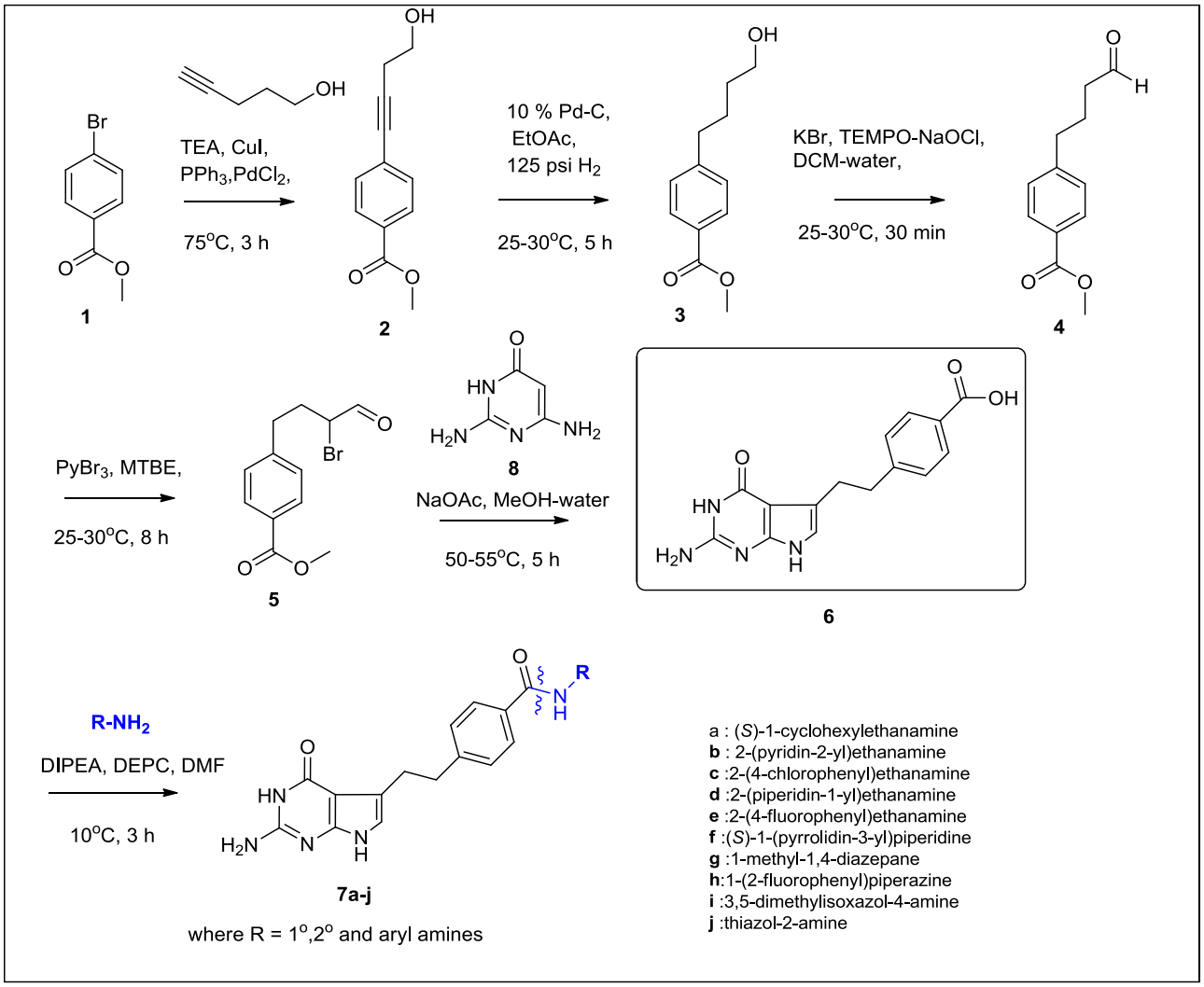
Review of literature revealed that sugar moieties of nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, and formycin were modified and evaluated for antiviral studies but showed less antiviral potency (Erik et al. 1987). Pyrrolo[2,3-d]pyrimidine compounds were screened against influenza virus and have shown promising activity against both the A and B strains (Marcos et al. 1996). Antiviral activity of some acyclic analogs of the pyrrolo[2,3-d]pyrimidine were studied against human cytomegalovirus (HCMV) and herpes simplex virus type-1 (HSV-1) where carboxamide and thioamide containing compounds were found to be active (Pranab et al. 1989). 4,5,6,7-Substituted non-nucleoside, non-phosphorylatable pyrrolo[2,3-d]pyrimidines exhibited both significantly lower levels of cytotoxicity and superior antiviral activity against human DNA viruses such as cytomegalovirus (HCMV) and herpes simplex virus type-1 (HSV-1) (Leroy and John 2000). However, literature survey revealed that only very limited studies have been carried out against paramyxovirus using 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamide template. Considering the biological significance of pyrimidine template, we have reported novel methodology to prepare 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamide derivatives and studied their antibacterial properties in our previous endeavors (Selvakumar and Elango 2017). As an extension of our research work, here in this article we have synthesized few more novel 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamide derivatives by replacing the glutamic acid part of Pemetrexed with novel 1°, 2°, and aryl amines (7**a–j**), investigated their antiviral activity against avian paramyxovirus (APMV-1) and compared with its parent drug Pemetrexed. Owing to the use of heterocyclic amines to form carboxamide, newly synthesized compounds are expected to show enhanced antiviral activity.

## Results and discussion

The overall protocol employed in the synthesis of these compounds is shown in Scheme 1. The pyrrolo[2,3-*d*]pyrimidine scaffold (**6**) was prepared from commercially available methyl-4-bromobenzoate by the reported method (Barnett et al. 1999).

Methyl-4-bromobenzaote was subjected for Sonogashira coupling, reduction, oxidation, and condensation with 2,4-diamino-6-hydroxy pyrimidine **8** to afford **6** as pale pink solid. Primary, secondary, and aryl amines were coupled with the scaffold **6** using diethylphosphorocyanidate (DEPC) as coupling agent. Several peptide coupling reagents like HOBT-EDC.HCl, TBTU, HATU, and DEPC have been tried out to prepare the novel non-glutamate pyrrolo[2,3-*d*]pyrimidine and the results showed that diethylphosphorocyanidate (DEPC) was found to be a better peptide coupling agent, which gave optimum yields. Ten new 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamides have been synthesized using primary amines with one and two carbon extensions (7**a–e**), secondary amines with five- and six-membered and hetero substitution (7**f–h**) and aryl amines with hetero substitution (7**i** and **j**). All these compounds were characterized using $^{1}H$ and $^{13}C$ Nuclear Magnetic Resonance (NMR), Liquid Chromatography-Mass Spectrometry (LCMS), and Fourier-Transform Infra-red Spectroscopy (FT-IR) techniques.

Antiviral activities of the synthesized compounds (**7a–j**) against avian paramyxovirus was screened and compared with Pemetrexed and Ribavirin, a well-known antiviral drug by plaque reduction assay. The results of the plaque reduction assay are shown in Table 1. The cytotoxicity of the compounds was evaluated by MTT assay (Denis and Nicole 1986). Vero cells were seeded in 96-well plates at an initial density of 10,000 cells per well. The cells were incubated with ten concentrations of tenfold serial dilutions of compounds (starting with 1 mM) for 24 h at 37 °C and 5% $CO_2$. MTT solution (5 mg/mL) was added to the cells, which were further incubated for 4 h. MTT was removed, and 100 µL of DMSO were added for 5 min. The optical density was measured at 570 nm. None of the compounds showed cytotoxicity over 50% at the tested concentrations and hence the same concentrations were tested for antiviral activity against Newcastle disease virus (NDV), an economically important avian paramyxovirus. A total of 100




**Scheme 1** Synthesis of 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamide derivatives **7a–j**

μL of NDV of $2^8$ HAU was incubated with same volume of different test concentrations of test compounds at 4 °C for 1 h and then added to Vero cells (90,000 cells per well) seeded in 24-well plate for 1 h. The cells were washed with phosphate buffer solution (PBS) and incubated with methyl cellulose overlay media. After 7 days, the cells were stained with 1% crystal violet and viral plaques were counted as plaque forming units (PFU). The antiviral effect was determined by reduction in number of plaques in comparison with untreated but NDV infected cells and expressed as percentage reduction of PFU. All the treatments were done in triplicates. The results obtained are depicted in Table 1 and Fig. 2.

It is evident from the results depicted in Table 1 that all the compounds, except **7b**, showed higher antiviral activity than Pemetrexed. Compounds **7c** and **7h** showed antiviral activity at relatively higher test concentrations. Compounds

**Table 1** Antiviral activity of the compounds (**7a–j**) against avian paramyxovirus (APMV-1)

| Compounds number | Minimum test concentration (mM) at which antiviral activity was observed | Plaque reduction percentage at the minimal test concentration |
|---|---|---|
| **7a** | 0.1 | 50 |
| **7b** | 1 | 13 |
| **7c** | 1 | 68 |
| **7d** | 0.01 | 31 |
| **7e** | 0.01 | 76 |
| **7f** | 0.1 | 64 |
| **7g** | 0.1 | 68 |
| **7h** | 1 | 47 |
| **7i** | 0.01 | 86 |
| **7j** | 0.01 | 78 |
| Pemetrexed | 0.001 | 12 |
| Ribavirin | 0.3 | 60 |

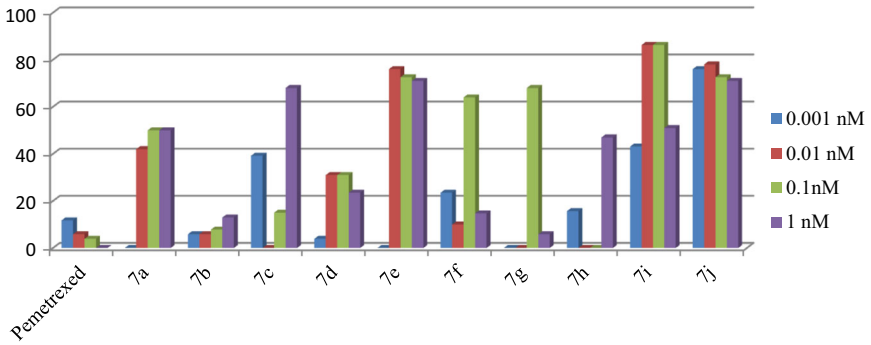



**Fig. 2** Antiviral activity of the compounds (**7a–j**) against Avian paramyxovirus (APMV-1)

**7a, 7f**, and **7g** showed antiviral activity at a concentration of 0.1 mM. While the compounds **7d, 7e, 7i**, and **7j** showed antiviral activity at lower test concentration of 0.01 mM. Overall, the compounds showed better antiviral activity against NDV at lower test concentration in comparison with Ribavirin.

It is evident from the results that the replacement of glutamic acid part of Pemetrexed with simple primary (**a–e**), secondary (**f–h**) and aryl amines (**i–j**) has significantly enhanced the antiviral activity to a larger extent. All synthesized compounds showed almost 4- to 7-fold higher antiviral activity than Pemetrexed. The compounds **7a, 7c, 7e–j** have shown almost equivalent antiviral activities against NDV as that of reference Ribavirin drug. Hence, it is presumed that enhancement of the antiviral activity of these compounds is due to carboxamide group arrived from simple amines rather than carboxamide from amino acid. It is interesting to note that, from their plaque reduction percentage values at the minimal test concentrations, we could see some behavioral similarity among these compounds with respect to their structure. Among the compounds, **7b** and **7d**, prepared using primary amines having two carbon extension with pyridyl or piperidyl attached to the second methylene carbon, have shown very less antiviral activity. Whereas, similar compounds **7c** and **7e** having two carbon extension in a primary amine wherein substituted phenyl ring attached to the second carbon showed significantly higher antiviral activity than **7b** and **7d**. The relatively higher antiviral activity shown by **7e** than **7c**, even at 100 time's lower concentration, may probably due to presence of 4-fluoro group instead of 4-chloro group in the phenyl ring. Compounds **7f, 7g**, and **7h** have exhibited similar antiviral activity wherein carboxamide was prepared using secondary amines like 5-membered pyrrolidine, 6-membered piperazine or 7-membered 1,4-diazapane. Among the compounds, the highest antiviral activity was shown by **7i** and **7j**, in which carboxamide was synthesized using five-membered heteroaryl amines.

Adjei (2004) have established the mechanism of action of the parent drug Pemetrexed. This compound inhibits thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase enzymes, which involved in folate metabolism and purine and pyrimidine synthesis. As spelt in the introduction section, Itoh et al. (Itoh et al.1996, 1995, 2000) have done various modifications on glutamic acid part of Pemetrexed and reported the significant change in the inhibitory activity. Most probably action of antiviral compounds against NDV could either involve targeting HN protein and F Protein in NDV, which binds to sialic acid receptor on the host cells or targeting the RNA depended RNA polymerase complex to inhibit the replication of virus. In the present study, from the above plaque reduction data, it is clear that parent drug Pemetrexed cannot be considered as an antiviral drug as it does not meet minimum criteria of antiviral compounds that is compounds showing 50% reduction in the plaque assay are considered as promising antiviral candidates. Only compounds **7a, 7c, 7e, 7f, 7g, 7i**, and **7j** showed >50% reduction in the plagues and hence could be considered as promising antiviral compounds. This data also revealed that the novel compounds prepared by modifying the glutamic acid portion in Pemetrexed might have impacted the viral life cycle than its parent drug Pemetrexed, hence they are showing antiviral activity. Interestingly, compounds Pemetrexed, **7d, 7f, 7g**, and **7i** did not show high antiviral activity at higher doses as one might expect. It is evident from the results that these compounds showed antiviral activity only at optimal doses.

## Conclusion

To conclude, a series of ten new non-glutamate type novel 4-(2-(6-amino-4-oxo-4,5-dihydro-1H-pyrrolo[2,3-d]pyrimidin-3-yl)ethyl)benzamide derivatives have been synthesized and characterized. The design concept involved replacement of glutamic acid group in Pemetrexed drug molecule by simple primary, secondary and aryl amines. These compounds exhibited substantial antiviral activity. Especially, compounds containing five-membered heteroaryl amines showed relatively higher antiviral activity against Newcastle disease virus almost on par with well-

known antiviral commercial drug, Ribavirin and further modification of these amines would definitely pose lead molecule towards antiviral therapeutics.

## Experimental procedure

All the chemicals used in the study are commercially available high purity grade (Aldrich or Merck, India). The solvents were of reagent grade and used as supplied commercially. Thin Lalyer Chromatography (TLC) experiments were performed on alumina-packed silica gel 40F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and $KMnO_4$. Melting points were determined using a melting point apparatus (B-540 Buchi, Germany) without corrections. All $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker 300 MHz or 400 MHz NMR. Molecular masses of unknown compounds were checked by LCMS 6200 series Agilent Technology (Agilent, Bengaluru, India). Chemical shifts are reported in ppm ($\delta$) with reference to internal standard TMS. The signals are designated as follows: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), doublet of triplet (dt), multiplet (m), and broad singlet (bs). Infrared (IR) spectra were recorded using a Bruker Alpha FT-IR spectrometer (Bruker, Germany) using a diamond attenuated total reflectance (ATR) single reflectance module (24 scans). All reactions were carried out under a nitrogen / argon atmosphere unless otherwise stated. Elemental analysis was carried out with a Thermo Scientific, model Flash 1112EA apparatus and Eagar xperience software.

The pyrrolo[2,3-*d*]pyrimidine scaffold (**6**) was prepared from commercially available methyl-4-bromobenzoate by the reported method (Barnett et al. 1999).

### 4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)benzoic acid (6)

To a solution of 2,4-diamino-6-hydroxy pyrimidine 8 (0.04 mol) in a 50:50 mixture of water and methanol (100 mL), compound 5 (0.04 mol) was added. To this, sodium acetate (0.06 mol) was added and resultant mixture was stirred for 5 h at about 50 °C. Upon completion of reaction, reaction mixture was cooled and solid obtained was collected by filtration. Wet cake was washed with water (20 mL) and dried to afford pure title compound. Yield: 75%, Pale pink solid, m.p. 250–253 °C. $^{1}H$ NMR (400 MHz, DMSO-d6) $\delta$ 2.83-2.87 (m, 2H, $CH_2$-methylene), 2.96–3.00 (m, 2H, $CH_2$-methylene), 6.03 (s, 1H, pyrimidine $NH_2$), 6.31 (s, 1H, CH-pyrrole), 7.30–7.32 (d, 2H, $J = 8.0$ Hz, *para* CH-phenyl), 7.83–7.85 (d, $J = 8.0$ Hz, 2H, *para* CH-phenyl), 10.17 (s, 2H, amine), 10.62 (s,1H, pyrimidine NH-*sec*. amide), 12.75 (bs, 1H, OH-acid) ppm. $^{13}C$ NMR (100 MHz, DMSO-d6) $\delta$ 28.3 (aliphatic methylene-C), 36.7 (aliphatic methylene-C), 99.2 (pyrrole-C), 113.9 (pyrrole-C), 118.0 (pyrrole-C), 128.6 (phenyl-C), 128.9 (phenyl-C), 129.7 (phenyl-C), 148.2 (phenyl-C), 151.6 (pyrrole-C), 152.7 (pyrimidine imine-C), 159.7 (pyrimidine cyclic amide-C), 167.8 carboxylic acid-C) ppm. FT-IR υ 841 (*para*-substituted phenyl C–H), 1099 (phenyl C–O stretching), 1390 (amine C–N bending), 1534 (NH–C=O amide bending), 1654 (aromatic C=O symmetric stretching), 2909 (NH-amine stretching), 3474 (NH-amide stretching) $cm^{-1}$. LCMS (ESI) *m/z* [M+H]+: 299.8 Da. *Anal. Calcd.* for $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78. Found: C, 60.51; H, 4.75; N, 18.85%.

### General synthetic procedure for 7a–j

To a suspension of compound 6 (0.033 mmol) in N,N-dimethylformamide (5 mL), corresponding amine ($RNH_2$) (0.033 mmol) and DIPEA (0.1 mmol) were added at about 10 °C under nitrogen atmosphere. To this, diethylphosphorocyanidate (0.04 mmol) was added slowly over a period of 15 min. The resultant solution was stirred for 3 h at about 10 °C. The reaction was complete by TLC (TLC system: 10% methanol in chloroform). Reaction was slowly quenched using saturated sodium-bi-carbonate solution and the resulted solid was stirred for 30 min. Solid was collected by filtration and the wet cake was washed with saturated sodium-bi-carbonate solution. Upon drying the wet cake for an hour under vacuum, it was further suspended in methanol and stirred for 30 min, filtered, collected and dried to get respective pure compounds.

#### (S)-4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(1-cyclohexylethyl)benzamide (7a)

Compound **7a** is a pale brown solid. Yield: 72%, m.p. 207–209 °C.$^{1}H$ NMR (300 MHz, DMSO-d6) $\delta$ 1.25–1.27 (m, 3H, $CH_3$ chiral methyl), 1.40–1.71 (m, 8H, $CH_2$ cyclohexyl methylene), 2.84–2.86 (m, 2H, $CH_2$ methylene), 2.94–2.96 (m, 2H, $CH_2$ methylene), 3.13 (m, 2H, $CH_2$ cyclohexyl methylene), 3.60 (m, 2H, $CH_2$ cyclohexyl methylene), 3.81–3.83 (m, 1H, CH methine chiral bridge), 6.04 (s, 2H, $NH_2$ pyrimidine), 6.31 (s, 1H, CH-pyrrole), 7.24–7.27 (d, 2H, $J = 8.1$ Hz, *para* CH-phenyl), 7.72–7.75 (d, 2H, $J =$ 7.5 Hz, *para* CH-phenyl), 8.00–8.03 (d,1H, $J = 8.4$ Hz, carboxamide NH), 10.18 (s, 1H, NH-pyrrole), 10.63 (s, 1H, pyrimidine NH-*sec*. amide) ppm. FT-IR υ 796 (*para*-substituted phenyl C–H), 1026 (phenyl C-O stretching, 1342 (amine C–N bending), 1532 (aryl NH-amide bending), 1624 (NH-amide bending), 2852 ($2^{o}$ amide symmetric stretching), 2923 ($2^{o}$ amide symmetric stretching), 3300 ($1^{o}$ amide stretching) $cm^{-1}$. LCMS (ESI) *m/z* [M+H]+: 408.2 Da. *Anal. Calcd.* for $C_{23}H_{29}N_5O_2$: C, 67.79; H, 7.17; N, 17.19. Found: C, 67.81; H, 7.16; N, 17.22%.

**4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(2-(pyridin-2-yl)ethyl)benzamide (7b)**

Compound **7b** is a pale pink solid. Yield: 88%, m.p. 273–275 °C.$^{1}$H NMR (300 MHz, DMSO-d6): $\delta$ 2.84–2.86 (m, 2H, $CH_2$ methylene-phenyl), 2.93–3.01(dd, 4H, $J_1 = 7.2$, $J_2 = 7.8$, two $CH_2$ methylene-phenyl and pyridyl), 3.56–3.63 (dd, 2H, $J_1 = 6.6$ Hz, $J_2 = 6.3$ Hz, $CH_2$ methylene-pyridyl), 6.02 (s, 2H, pyrimidine $NH_2$), 6.31 (s, 1H, CH-pyrrole), 7.20–7.28 (m, 4H, *para* CH-phenyl and CH-pyridyl), 7.68–7.72 (m, 3H, CH-pyridyl), 8.48–8.5 (m, 2H, *para* CH-phenyl), 10.17 (s, 1H, NH-pyrrole), 10.61 (s, 1H, pyrimidine NH-*sec*. amide) ppm. $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 166.6 (amide C=O), 159.8 (amide C=O), 159.7 (pyrimidine-C), 152.7 (pyrimidine-C), 151.8 (pyridyl-C), 149.5 (pyrrole-C), 146.2 (pyrrole-C), 136.8 (pyridyl-C), 132.4 (phenyl-C), 128.6 (phenyl-C), 127.4 (phenyl-C), 123.5 (phenyl-C), 121.9 (phenyl-C), 118.1 (pyrrole-C), 113.8 (pyrrole-C), 99.2 (pyrrole-C), 37.8 (pyridyl ethyl-C), 36.6 (phenyl methylene-C), 36.5 (pyridyl methylene-C), 28.4 ppm. FT-IR υ 775 (pyridyl C–H), 841 (*para*-substituted phenyl C–H), 1289 (phenyl ethyl-C), 1341 (amine C–N bending), 1433 (H–C–N-bending), 1534 (NH–C=O amide bending), 1632 (aromatic C=O symmetric stretching), 3235 (NH-amide stretching) cm$^{-1}$. LCMS (ESI) *m/z* [M+H]+: 403.3 Da. *Anal. Calcd.* for $C_{22}H_{22}N_6O_2$: C, 65.66; H, 5.51; N, 20.88. Found: C, 65.82; H, 5.48; N, 20.92%.

**4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(4-chlorophenethyl)benzamide (7c).**

Compound **7c** is a pale orange solid. Yield: 92%, m.p. 278–281 °C.$^{1}$H NMR (300 MHz, DMSO-d6) $\delta$ 2.83-2.85 (m, 4H, two $CH_2$ methylene-phenyl), 2.93–2.96 (m, 2H, $CH_2$ methylene -4-Cl phenyl), 3.42–3.47 (m, 2H, $CH_2$ methylene-4-Cl phenyl), 6.0 (s, 2H, pyrimidine $NH_2$), 6.31 (s, 1H, CH-pyrrole), 7.25–7.27 (m, 4H, *para* CH-4-Cl phenyl), 7.33–7.36 (d, 2H, $J = 8.4$ Hz, *para* CH-phenyl), 7.69–7.72 (d, 2H, $J = 7.8$ Hz, *para* CH-phenyl), 8.45–8.46 (m, 1H, 1$^{o}$ amide NH), 10.14 (bs, 1H, NH-pyrrole), 10.61 (s, 1H, pyrimidine NH-*sec*. amide) ppm. $^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ 28.4 (phenyl ethyl-C), 34.8 (4-Cl phenyl ethyl-C), 36.5 (phenyl ethyl-C), 36.7 (4-Cl phenyl methyl-C), 99.2 (pyrrole-C), 113.8 (pyrrole-C), 118.1 (pyrrole-C), 127.4 (phenyl-C), 128.6 (phenyl *para*-C), 128.6 (phenyl *para*-C), 131.0 (4-Cl phenyl *para*-C), 131.1 (4-Cl phenyl *para*-C), 132.4 (4-Cl phenyl-C), 139.1 (pyrrole-C), 146.2 (pyrrole-C), 151.8 (pyrimidine-C), 152.6 (pyrimidine-C), 159.7 (amide C=O), 166.6 (amide C=O) ppm. FT-IR υ 775 (*para*-substituted phenyl C–H bending), 841 (*para*-substituted phenyl C–H bending), 1286 (phenyl ethyl-C), 1362 (amine C–N bending), 1435 (H–C–N-bending), 1531 (NH–C=O amide bending), 1628 (aromatic C=O symmetric stretching), 3220 (NH-amide stretching) cm$^{-1}$. LCMS (ESI) *m/z* [M+H]+: 436.4 Da. *Anal. Calcd.* for $C_{23}H_{22}ClN_5O_2$: C, 63.37; H, 5.09; N, 16.07. Found: C, 63.53; H, 5.11; N, 16.23%.

**4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(2-(piperidin-1-yl)ethyl)benzamide (7d).**

Compound **7d** is a pale pink solid. Yield: 84%, m.p. 232–235 °C.$^{1}$H NMR (300 MHz, DMSO-d6) $\delta$ 1.37–1.38 (m, 2H, $CH_2$ piperidyl methylene), 1.47–1.48 (m, 4H, $CH_2$ piperidyl methylene), 2.37–2.43 (m, 6H, $CH_2$ piperidyl methylene and $CH_2$ aliphatic methylene), 2.82–2.86 (m, 2H, $CH_2$ methylene-phenyl), 2.94–2.96 (m, 2H, $CH_2$ methylene-phenyl), 3.3 (m, 2H, $CH_2$ aliphatic methylene), 5.99 (s, 2H, pyrimidine $NH_2$), 6.31 (s, 1H, pyrrole CH), 7.25–7.28 (d, 2H, $J = 7.5$ Hz, *para* phenyl CH), 7.71–7.73 (d, 2H, $J = 7.8$ Hz, *para* phenyl CH), 8.27 (m, 1H, 1$^{o}$ amide NH), 10.14 (s, 1H, NH-pyrrole), 10.61 (s, 1H, pyrimidine NH-*sec*. amide) ppm. $^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ 24.5 (phenyl ethyl-C), 26.0 (phenyl ethyl-C), 28.4 (piperidyl ethyl-C), 36.5 (piperidyl ethyl-C), 37.3 (piperidyl-C), 54.5 (piperidyl-C), 58.2 (piperidyl-C), 99.2 (pyrrole-C), 113.8 (pyrrole-C), 118.1 (phenyl-C), 127.4 (phenyl *para*-C), 128.6 (phenyl *para*-C), 132.5 (phenyl-C), 146.2 (pyrrole-C), 151.7 (pyrimidine-C), 152.6 (pyrimidine-C), 159.7 (amide C=O), 166.5 (amide C=O) ppm. FT-IR υ, 844 (*para*-substituted phenyl C–H bending), 1362 (amine C–N bending), 1436 (H–C–N-bending), 1532 (NH–C=O amide bending), 1633 (aromatic C=O symmetric stretching), 3221 (NH-amide stretching) cm$^{-1}$. LCMS (ESI) *m/z* [M+H]+: 409.3 Da. *Anal. Calcd*. for $C_{22}H_{28}N_6O_2$: C, 64.68; H, 6.91; N, 20.57. Found: C, 64.85; H, 6.88; N, 20.69%.

**4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(4-fluorophenethyl)benzamide (7e).**

Compound **7e** is an off-white solid. Yield: 71%, m.p. 275–278 °C. 1H NMR (300 MHz, DMSO-d6, $\delta$ ppm, 2.80–2.85 (m, 4H, two $CH_2$ methylene-phenyl), 2.93–2.96 (m, 2H, $CH_2$ methylene-4-F phenyl), 3.42–3.48 (m, 2H, $CH_2$ methylene-4-F phenyl), 6.0 (s, 2H, pyrimidine $NH_2$), 6.32 (s, 1H, CH-pyrrole), 7.08–7.14 (t, 2H, $J = 9.0$ Hz, *para* CH-4-Fluorophenyl), 7.25–7.28 (d, 4H, $J = 7.8$ Hz, *para* CH-4-Fluorophenyl and *para* phenyl CH), 7.69–7.72 (d, 2H, $J = 7.8$ Hz, *para* phenyl CH), 8.43–8.45 (m, 1H, 1$^{o}$ amide NH),10.14 (bs, 1H, NH-pyrrole), 10.62 (s,1H, pyrimidine NH-*sec*. amide), 13C NMR (300 MHz, DMSO-d6, $\delta$ ppm) 28.4 (phenyl ethyl-C), 34.6 (4-Fluorophenyl-C), 36.5 (4-Fluorophenyl-C), 99.2 (pyrrole-C), 113.8 (pyrrole-C),

115.2–115.5 (4-Fluorophenyl-C), 118.1 (phenyl-C), 127.4 (phenyl-C), 128.6 (phenyl-C), 130.8–130.9 (4-Fluorophenyl-C), 132.4, 136.1–136.2 (4-Fluorophenyl-C), 146.2 (pyrrole-C), 151.8 (pyrimidine-C), 152.6 (pyrimidine-C), 162.8 (amide C=O), 166.6 (amide C=O). IR (ATR, υ $cm^{-1}$): 829 (*para*-substituted phenyl C–H bending), 1223 (H–C–N-bending), 1504 (NH–C=O amide bending), 1631 (aromatic C=O symmetric stretching), 3221 (NH-amide stretching) $cm^{-1}$. LCMS (ESI) *m/z* $[M+H]^+$: 420.3 Da, *Anal. Calcd.* for $C_{23}H_{22}FN_5O_2$:C, 65.86; H, 5.29; N, 16.70; found: C, 65.79; H, 5.40; N, 16.77%.

### (S)-2-Amino-5-(4-(3-(piperidin-1-yl)pyrrolidine-1-carbonyl) phenethyl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (7f)

Compound **7f** is a pale pink solid. Yield: 78%, m.p. 210–212 °C.$^1$H NMR (300 MHz, DMSO-d6) $\delta$ 1.34–1.68 (m, 7H, piperazine and pyrrolidine methylene), 1.71–2.07 (m, 1H, pyrrolidine), 2.10–2.5 (m, 5H, piperazine and pyrrolidine methylene), 2.8–2.9 (m, 2H, $CH_2$ methylene-phenyl), 2.92–3.05 (m, 2H, $CH_2$ methylene-phenyl), 3.4–82 (m, 4H, piperazine and pyrrole methylene), 6.03 (s, 2H, pyrimidine $NH_2$), 6.33 (s, 1H, CH-pyrrole), 7.25–7.27 (d, 2H, $J = 6.3$ Hz, *para* CH-phenyl), 7.39–7.45 (m, 2H, *para* CH-phenyl), 10.17 (s, 1H, 1° amide NH), 10.63 (s, 1H, (pyrimidine NH-*sec*. amide) ppm. $^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ 24.24 (phenyl ethyl-C), 25.64 (phenyl ethyl-C), 28.5 (pyrrolidine-C), 36.6 (piperazine-C), 45.3 (pyrrolidine-C), 48.3 (piperazine-C), 49.8 (piperazine-C), 52.5 (pyrrolidine-C), 63.4 (piperazine-C), 64.8 (piperazine), 99.1 (pyrrole-C), 113.7 (pyrrole-C), 118.2 (*para* phenyl-C), 127.6 (*para* phenyl-C), 128.5 (*para* phenyl-C), 134.4 (pyrrole-C), 144.9 (pyrrole-C), 151.8 (pyrimidine-C), 152.8 (pyrimidine-C), 159.7 (amide C=O), 168.8 ppm (amide C=O). FT-IR υ 789 (*para*-substituted phenyl C–H bending), 1091 (phenyl ethyl-C), 1433 (H–C–N-bending), 1588 (NH–C=O amide bending), 1664 (aromatic C=O symmetric stretching), 2923 (NH-amide stretching) $cm^{-1}$. LCMS (ESI) *m/z* [M+H]+: 435.3 Da. *Anal. Calcd.* for $C_{24}H_{30}N_6O_2$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.22; H, 6.98; N, 19.25%.

### 2-Amino-5-(4-(4-methyl-1,4-diazepane-1-carbonyl) phenethyl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (7g)

Compound **7g** is a pale pink solid. Yield: 89%, m.p. 253–256 °C.$^1$H NMR (300 MHz, DMSO-d6) $\delta$ 1.72 (m, 1H, diazepane), 1.84 (m, 1H, diazepane), 2.22–2.72 (d, 3H, $J =$ 14.1 Hz, N–$CH_3$), 2.4–2.7 (m, 4H, diazepane methylene $CH_2$), 2.87–2.91 (m, 2H, methylene $CH_2$), 2.92–2.94 (m, 2H, methylene $CH_2$), 3.32 (m, 2H, diazepane methylene $CH_2$), 3.6 (m, 2H, diazepane methylene $CH_2$), 5.99 (s, 2H, pyrimidine $NH_2$), 6.33 (s, 1H, CH-pyrrole), 7.26 (s, 4H, *para* CH-phenyl), 10.13 (s, 1H, 1° amide NH), 10.62 (s,1H, (pyrimidine NH-*sec*. amide)) ppm. $^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ 26.8 (phenyl ethyl-C), 28.5 (phenyl ethyl-C), 36.5 (diazepane–C), 45.1 (diazepane–C), 45.2 (diazepane–C), 46.2–49.3 (diazepane–C), 56.4–58.4 (diazepane–C), 99.2 (pyrrole-C), 113.2 (pyrrole-C), 118.2 (*para* phenyl-C), 126.9 (*para* phenyl-C), 128.6 (*para* phenyl-C), 134.8 (*para* phenyl-C), 144.0 (pyrrole-C), 151.7 (pyrimidine-C), 159.7 (amide C=O), 170.7 (amide C=O) ppm. FT-IR υ 843 (*para*-substituted phenyl C–H bending), 1364 (amine C–N bending), 1431 (H–C–N-bending), 1540 (NH–C=O amide bending), 1661 (aromatic C=O symmetric stretching), 3186 (NH-amide stretching) $cm^{-1}$. LCMS (ESI) *m/z* [M+H]+: 393.3 Da. *Anal. Calcd.* for $C_{21}H_{26}N_6O_2$: C, 63.94; H, 6.64; N, 21.30. Found: C, 64.16; H, 6.62; N, 21.25%.

### 2-Amino-5-(4-(4-(2-fluorophenyl)piperazine-1-carbonyl) phenethyl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (7h).

Compound **7h** is a pale yellow solid. Yield: 90%, m.p. 276–278 °C. $^1$H NMR (300 MHz, DMSO-d6) $\delta$ 2.89–3.02 (m, 8H, four $CH_2$ piperazine methylene), 3.55–3.73 (m, 4H, two $CH_2$ methylene bridge head), 6.01 (s, 2H, pyrimidine $NH_2$), 6.34 (s, 1H, CH-pyrrole), 7.01–7.18 (m, 5H, CH ortho-fluorophenyl), 7.28–7.36 (dd, 4H, $J_1 = 7.5$ Hz, $J_2 = 9.9$ Hz, CH *para*-phenyl),10.15(s, 1H, NH-pyrrole), 10.64 (s, 1H, pyrimidine NH-*sec*. amide) ppm. $^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ 28.6 (phenyl ethyl-C), 31.2 (phenyl ethyl-C), 36.6 (piperazine-C), 50.7 (piperazine-C), 99.2 (pyrrole-C), 113.8 (pyrrole-C), 116.3-116.5 (phenyl-C), 118.2 (ortho-fluorophenyl C), 120.1-120.1 (ortho-fluorophenyl C), 123.2-123.3 (ortho-fluorophenyl C), 125.3 (phenyl-C), 125.3 (phenyl-C), 127.5 (phenyl-C), 128.7 (phenyl-C), 133.4 (ortho-fluorophenyl C), 139.4 (pyrrole-C), 140.0 (pyrrole-C), 144.7 (pyrimidine-C), 151.8 (pyrimidine-C), 154.2 (ortho-fluorophenyl C), 156.6 (ortho-fluorophenyl C), 159.8 (amide C=O), 169.6 (amide C=O) ppm. FT-IR υ 813 (*para*-substituted phenyl C–H bending), 1283 (H–C–N-bending), 1439 (H–C–N-bending), 1565 (NH–C=O amide bending), 1676 (aromatic C=O symmetric stretching), 3172 (NH-amide stretching) $cm^{-1}$. LCMS (ESI) *m/z* [M+H]+: 461.2 Da. *Anal. Calcd.* for $C_{25}H_{25}FN_6O_2$: C, 65.20; H, 5.47; N, 18.25. Found: C, 65.32; H, 5.45; N, 18.39%.

### 4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(3,5-dimethylisoxazol-4-yl) benzamide (7i)

Compound **7i** is a pale pink solid. Yield: 93%, m.p. 247–250 °C.$^1$H NMR (300 MHz, DMSO-d6) $\delta$ 2.12 (s, 3H, $CH_3$ isoxazol), 2.29 (s, 3H, $CH_3$ isoxazol), 2.87 (m, 2H, $CH_2$ methylene bridge head), 3.01 (m, 2H, $CH_2$ methylene bridge head), 6.01 (s, 2H, pyrimidine $NH_2$), 6.33 (s, 1H, pyrrole CH), 7.33–7.36 (d, 2H, $J = 7.2$ Hz, *para* phenyl

CH), 7.86–7.88 (d, 2H, $J = 7.2$ Hz, *para* phenyl CH), 9.7 (bs, 1H, 1° amide NH), 10.16 (s, 1H, NH-pyrrole), 10.63 (s, 1H, pyrimidine NH-*sec*. amide) ppm. $^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ 10.1 (isoxazole–aliphatic C), 10.3 (isoxazole–aliphatic C), 20.4 (methylene bridge head C), 36.5 (methylene bridge head C), 99.2 (pyrrole-C), 113.9 (pyrrole-C), 114.8 (*para*-phenyl C), 118.0 (*para*-phenyl C), 128.1 (*para*-phenyl C), 128.8 (*para*-phenyl C), 131.3 (pyrrole-C), 147.2 (pyrrole-C), 151.8 (pyrimidine-C), 152.6 (pyrimidine-C), 158.3 (isoxazole-C), 159.7 (isoxazole-C), 163.1 (amide C=O), 166.1 (amide C=O) ppm. FT-IR υ 884 (*para*-substituted phenyl C–H bending), 1239 (C–N–O stretching), 1363 (amine C–N bending), 1594 (NH–C=O amide bending), 1630 (aromatic C=O symmetric stretching), 3311 (NH-amide stretching) cm$^{-1}$. LCMS (ESI) *m/z* [M+H]+: 393.3 Da. *Anal. Calcd.* for, $C_{20}H_{20}N_6O_3$: C, 61.22; H, 5.14; N, 21.42. Found: C, 61.49; H, 5.18; N, 21.21%.

#### 4-(2-(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-5-yl)ethyl)-N-(thiazol-2-yl)benzamide (7j).

Compound **7j** is a pale brown solid. Yield: 91%, m.p. 303–306 °C. $^1$H NMR (300 MHz, DMSO-d6) $\delta$ 2.87–2.89 (m, 2H, $CH_2$ methylene bridge head), 2.98–3.01 (m, 2H, $CH_2$ methylene bridge head), 6.01 (s, 2H, pyrimidine $NH_2$), 6.33 (s, 1H, pyrrole CH), 7.25–7.26 (d, 1H, $J = 3.3$ Hz, thiazole CH), 7.35–7.37 (d, 2H, $J = 8.1$ Hz, *para* phenyl CH), 7.54–7.55 (d, 2H, $J = 3.3$ Hz, thiazole CH), 7.99–8.02 (d, 2H, $J = 8.4$ Hz, *para* phenyl CH), 10.17 (s, 1H, 1° amide NH), 10.63 (s, 1H, NH-pyrrole), 12.5 (s, 1H, pyrimidine NH-*sec*. amide) ppm. FT-IR υ 707 (*para*-substituted phenyl C–H bending), 828 (*para*-substituted phenyl C–H bending), 1434 (N–C–S stretching), 1544 (NH–C=O amide bending, 1642 (aromatic C=O symmetric stretching), 3253 (NH-amide stretching) cm$^{-1}$. LCMS (ESI) *m/z* [M+H]+: 379.1 Da. *Anal. Calcd*. for $C_{18}H_{16}N_6O_2S$: C, 56.83; H, 4.24; N, 22.09. Found: C, 57.05; H, 4.21; N, 22.23 %.

### Plaque reduction assay

The antiviral activity of the chosen compounds was carried out at National Institute of Animal Biotechnology, Hyderabad, India. To test the antiviral activity, targeted compounds were initially screened by MTT assay in African Green Monkey Kidney cell line, Vero cell line. The maximum non-cytotoxic concentration, at which no significant changes were detected in cellular morphology of Vero cells was used as the highest test dose for testing the antiviral activity of the compounds by viral plaque reduction assay using an avian paramyxovirus (APMV-1), a Newcastle disease virus. The commercially available well-known antiviral drug, Ribavirin, was used for comparing the antiviral potential of the test compounds. In a typical experiment, 100 µL of NDV of $2^8$ Hemagglutination units was incubated with same volume of different test concentrations of test compounds at 4 °C for 1 h and then added to Vero cells (90,000 cells per well) seeded in 24-well plate for 1 h. The cells were washed with PBS and incubated with methyl cellulose overlay media. After 7 days, the cells were stained with 1% crystal violet and plaques for counted. The antiviral effect was determined by reduction in plaque forming units (PFU) in comparison with untreated infected cells and expressed as percentage reduction of PFU. All the treatments were done in triplicates.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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