ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

# Multicomponent Synthesis of Diaminopurine and Guanine PNA's Analogues Active against Influenza A Virus from Prebiotic Compounds

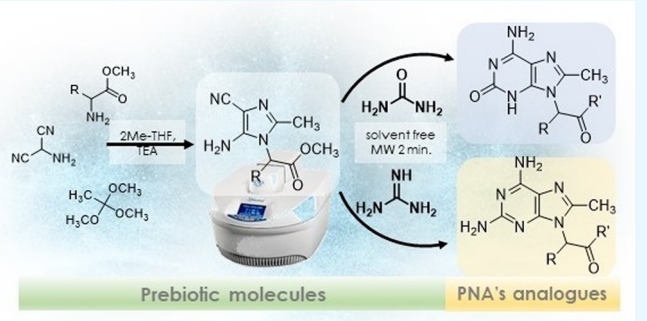
Bruno Mattia Bizzarri,* Angelica Fanelli, Stefania Ciprini, Alessandra Giorgi, Marta De Angelis, Raoul Fioravanti, Lucia Nencioni, and Raffaele Saladino

Cite This: *ACS Omega* 2022, 7, 45253−45264

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

**ABSTRACT:** Peptide nucleic acids (PNAs) play a key role in prebiotic chemistry as a chimera between RNA and proteins. We developed an alternative synthesis of bioactive PNA's diaminopurine and guanine analogues from prebiotic compounds, such as aminomalononitrile (AMN), urea, and guanidine, using a two-step multicomponent microwave-assisted and solvent-free approach in the presence of selected amino acids. The novel derivatives showed selective inhibitory activity against influenza virus A/Puerto Rico/8/34 H1N1 encompassing the range of nanomolar activity. Derivatives decorated with the tyrosine residue showed the highest inhibitory activity against the virus.



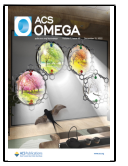

## INTRODUCTION

At the origins, RNA worked as a molecular shuttle for the translation of genetic information into the catalytic world of proteins. However, the examples of prebiotic synthesis of ribonucleosides and ribo-oligonucleotides are limited, encompassing one-pot condensation processes from simple starting compounds or multistep procedures.[1−6] As an alternative, peptide nucleic acids (PNAs) play the role of a chimera between RNA and proteins.[7] Examples of the synthesis of PNA's building blocks in prebiotic chemistry are reported, and the emergence of structural complexity associated with chemical modification of the nucleobase and sugar is discussed in detail.[8,9] PNAs showed important biological activities,[10] including the inhibitory effect against a large panel of viral diseases.[11,12] The prebiotic synthesis of PNA's building blocks includes the condensation of formamide and HCN oligomers, such as aminomalononitrile (AMN) and diaminomaleonitrile (DAMN).[5,13,14] In the latter cases, urea and guanidine[15] have been involved in multicomponent procedures with cyanoacetaldehyde,[16,17] malic acid,[18] acrylonitrile,[19] propionic acid,[20] and $\beta$-alanine,.[21−23] In addition, they favored the ring-closing annulation and aromatization in the transformation of pyrimidines and purines.[24] Recently, we reported the multicomponent synthesis of a large panel of PNA's building blocks, starting from $\alpha$-amino acids and AMN and DAMN, highlighting the role of the energy source in the chemoselectivity of the reaction.[25,26] Amino imidazole carbonitrile derivatives were recovered as key intermediates for the successive annulation step to yield purine derivatives with selective antiviral activity against influenza A virus through inhibition of the budding step in the viral replication cycle. Here, we describe an alternative synthetic pathway for the preparation of bioactive diaminopurine and guanine PNA's building blocks by microwave-assisted multicomponent synthesis of amino imidazole carbonitrile derivatives in the presence of sustainable reaction solvents. For the purpose, AMN and $\alpha$-amino acids were reacted in the presence of trimethyl orthoacetate and 2-methyltetrahydrofuran (2-MeTHF), or in alternative, ethylene glycol (EG), followed by treatment of amino imidazole carbonitrile intermediates with guanidine and urea. 2-MeTHF and EG were selected since they are a greener alternative to toxic organic solvents in tandem reactions, photocatalytic cascade, and cyclization process.[27−32] The novel purine derivatives showed high inhibitory activity against influenza A virus, encompassing the range of nanomolar activity.

## RESULTS AND DISCUSSION

As a selected case, amino imidazole carbonitrile derivative **4a** was prepared by reaction of AMN **1** (5.9 mmol) and trimethyl orthoacetate **2** (8.3 mmol; TOA) with glycine methyl ester derivative **3a** (7.1 mmol) in the appropriate reaction solvent

**Received:** September 5, 2022
**Accepted:** October 24, 2022
**Published:** November 29, 2022







https://doi.org/10.1021/acsomega.2c05754
*ACS Omega* 2022, 7, 45253−45264

**Scheme 1. Synthesis of Diaminopurine Analogues 6a−f, 7a−f (Pathway A) and Guanine Analogues 9a−g, 10a−g (Pathway B)**

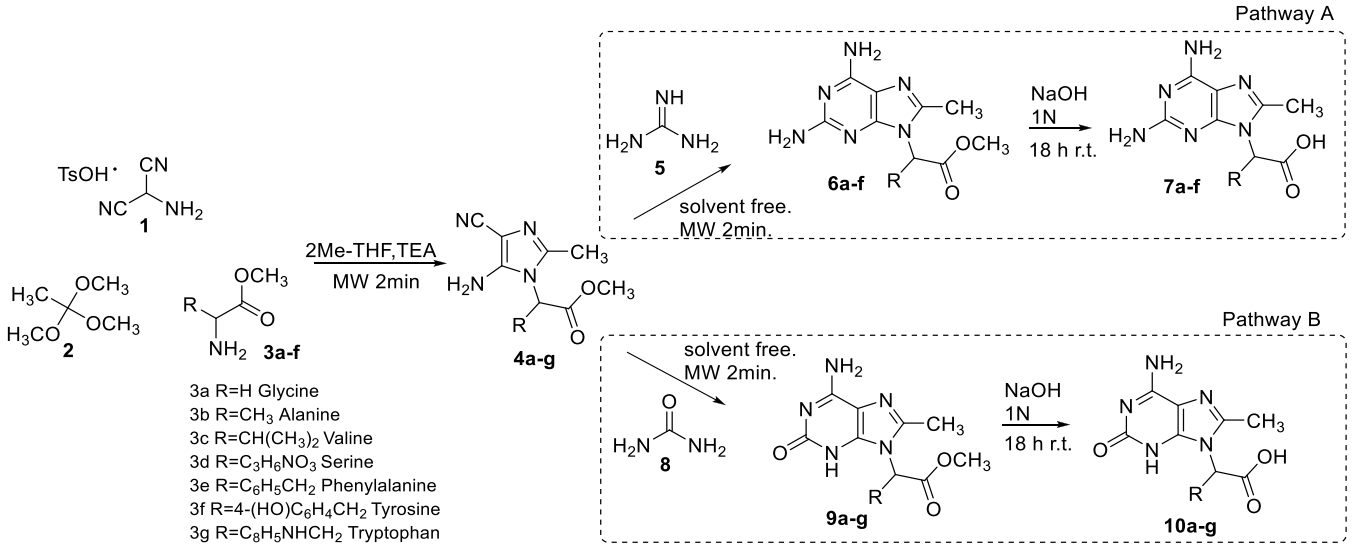
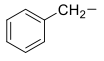
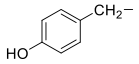
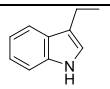



**Table 1. Synthesis of Amino Imidazole Carbonitrile Derivatives 4a−g**[a,b]

| Entry | α-amino acid | Condition | Products | R | Yield (%)[b] |
|---|---|---|---|---|---|
| 1 | Glycine | THF, 2.0 min, 250 W, 250 psi at 200 °C | 4a | H | 60 |
| 2 | | $CH_2Cl_2$, 2.0 min, 250 W, 250 psi at 200 °C | | | 20 |
| 3 | | $CH_3CN$, 2.0 min, 250 W, 250 psi at 200 °C | | | 50 |
| 4 | | $(CH_3O)_2(CH_2)_2$, 2.0 min, 250 W, 250 psi at 200 °C | | | 15 |
| 5 | | 2Me-THF, 2.0 min, 250 W, 250 psi at 200 °C | | | 66 |
| 6 | Alanine | | 4b | $CH_3$ | 37 |
| 7 | Valine | | 4c | $CH(CH_3)_2$ | 35 |
| 8 | Serine | | 4d | $CH_2OH$ | 29 |
| 9 | Phenylalanine | | 4e |  | 56 |
| 10 | Tyrosine | | 4f |  | 52 |
| 11 | Tryptophan | | 4g |  | 50 |

[a]AMN **1** (5.9 mmol), trimethyl orthoacetate **2** (8.3 mmol), amino acid (**3a−g**) (7.1 mmol), and triethylamine (7.1 mmol) under MW irradiation. [b]Yield has been calculated on the basis of the amount of the recovered product. Reactions were performed in triplicate.

(2-MeTHF, or in alternative, EG; 30 mL) and in the presence of triethylamine (7.1 mmol, 1.0 mL; TEA), working at room temperature for 30 min, followed by microwave irradiation (250 W, 250 psi) at 200 °C for 2 min (Scheme 1). The reaction with tetrahydrofuran (THF) was also performed as a reference.[25] As reported in Table 1, the yield of amino imidazole carbonitrile methyl ester derivative **4a** was higher with 2-MeTHF than with THF and EG, the latter solvent being the least efficient of the series (Table 1, entry 5 versus entries 1 and 4). This result was probably due to the high polarity of EG responsible for the undesired amino acid self-condensation.[33] Note that 2-MeTHF performed better than

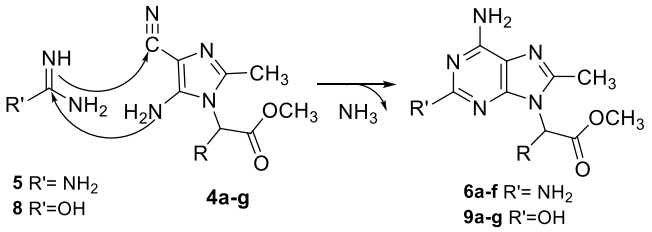


https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

traditional multicomponent reaction solvents such as methylene dichloride ($CH_2Cl_2$) and acetonitrile ($CH_3CN$) (Table 1, entry 5, versus entries 2 and 3). To generalize the procedure, the reaction was repeated using a panel of five $\alpha$-amino acids methyl ester derivatives **3b−f** (alanine **3b**, valine **3c**, serine **3d**, phenylalanine **3e**, tyrosine **3f**, and tryptophan **3g**) to yield the corresponding amino imidazole carbonitrile methyl esters **4b−g** in yield ranging from 29 to 56% (Table 1, entries 6−11).

Next, the reaction was oriented toward the preparation of PNA's diaminopurine analogues (DAPAs) 6a−f (Scheme 1, Pathway A) and guanine analogues (GAs) 9a−g (Scheme 1, Pathway B). Imidazole derivatives 4a−g reacted with guanidine 5 or, in alternative, urea 8, as one-carbon donors in the annulation process (Scheme 2).

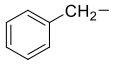
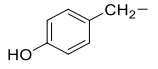
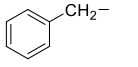
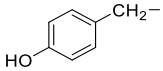
**Scheme 2. Proposed Mechanism for the Synthesis of Compounds 6a−f and 9a−g**




Briefly, imidazole derivatives 4a−g (0.80 mmol) and compounds 5 and 8 (1.60 mmol, 2 equiv) were treated under solvent-free microwave irradiation (150 W, 250 psi) for 2.0 min at 250 °C (Scheme 1, Pathways A and B) to afford 6a−f and 9a−g, respectively, in quantitative conversion of the substrate and appreciable yield of product (Table 2, entries 2−7 and Table 3, entries 2−8). The reaction of compound 4a was also carried out under simple thermal conditions (250 °C) as a reference to yield DAPA 6a and GA 7a in low yield (Tables 2 and 3; entry 1 versus 2), confirming the beneficial role of microwave in the annulation process. As a general trend, the selectivity of the reaction decreased by increasing the irradiation time, probably due to the occurrence of polycondensation side reactions with the formation of high polar derivatives not isolated under our experimental conditions.[34]

The polycondensation of urea, guanidine, and their derivatives in the presence of aromatic compound was reviewed, and the relationship between this process and the energy source and the reaction time was deeply investigated.[35] Even if a specific selectivity trend was not observed, it has not escaped our attention that the substitution pattern of imidazole intermediates **4a−g** played a significative role in the reaction. In particular, the presence of electron-donating aromatic amino acid residues generally increased the overall yield of the annulation process, with the only exception being the case of compound **9a** (Table 2 entries 6 and 7 and Table 3, entries 6, 7, and 8 vs Table 3 entry 2). Finally, to increase the solubility in water and enlarge the panel of PNA's building blocks, DAPAs **6a−f** and GAs **9a−g** (0.1 mmol) were treated with a solution of NaOH (1.0 N) at 25 °C for 18 h (Scheme 1) to

**Table 2. Synthesis of Diaminopurine Analogues 6a−f and 7a−f**[a,b,c,d]

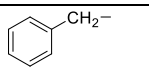
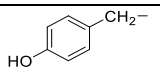
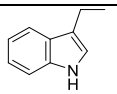
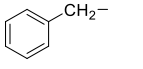
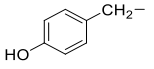
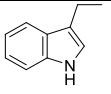
| Entry | α-amino acid | Condition | Products | R | Yield (%)[d] |
|---|---|---|---|---|---|
| 1 | Glycine | Solvent free, 120.0 min, at 250 °C[a] | **6a** | H | 21 |
| 2 | | Solvent free, MW 2.0 min, 150 W, 250 psi at 250 °C[b] | | | 38 |
| 3 | Alanine | | **6b** | $CH_3$ | 25 |
| 4 | Valine | | **6c** | $CH(CH_3)_2$ | 31 |
| 5 | Serine | | **6d** | $CH_2OH$ | 25 |
| 6 | Phenylalanine | | **6e** |  | 40 |
| 7 | Tyrosine | | **6f** |  | 42 |
| 8 | Glycine | NaOH, 18 hrs, 25 °C[c] | **7a** | H | 98 |
| 9 | Alanine | | **7b** | $CH_3$ | 95 |
| 10 | Valine | | **7c** | $CH(CH_3)_2$ | 99 |
| 11 | Serine | | **7d** | $CH_2OH$ | 96 |
| 11 | Phenylalanine | | **7e** |  | 95 |
| 12 | Tyrosine | | **7f** |  | 98 |

[a]Reaction conditions: **4a−f** (0.80 mmol), guanidine carbonate **5** (1.60 mmol, 2 equiv) under solvent-free thermal heating for 120 min at 250 °C. [b]Reaction conditions: **4a−f** (0.80 mmol), guanidine carbonate **5** (1.60 mmol, 2 equiv) under solvent-free microwave irradiation (150 W, 250 psi) for 2 min at 250 °C. [c]NaOH (1.0 N, 1.0 mL) stirring for 18 h at 25 °C. [d]Yield has been calculated on the basis of the amount of the recovered product. Reactions were performed in triplicate.



https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

Table 3. Synthesis of Guanine Analogues 9a−g and 10a−g[a,b,c,d]

| Entry | α-amino acid | Condition | Products | R | Yield (%)[d] |
|---|---|---|---|---|---|
| 1 | Glycine | Solvent free, 120.0 min, at 250 °C[a] | **9a** | H | 25 |
| 2 | | Solvent free, MW 2.0 min, 150 W, 250 psi at 250 °C[b] | | | 45 |
| 3 | Alanine | | **9b** | $CH_3$ | 32 |
| 4 | Valine | | **9c** | $CH(CH_3)_2$ | 35 |
| 5 | Serine | | **9d** | $CH_2OH$ | 27 |
| 6 | Phenylalanine | | **9e** |  | 41 |
| 7 | Tyrosine | | **9f** |  | 45 |
| 8 | Tryptophan | | **9g** |  | 47 |
| 9 | Glycine | NaOH, 18 hrs, 25 °C[c] | **10a** | H | 99 |
| 10 | Alanine | | **10b** | $CH_3$ | 94 |
| 11 | Valine | | **10c** | $CH(CH_3)_2$ | 98 |
| 12 | Serine | | **10d** | $CH_2OH$ | 97 |
| 13 | Phenylalanine | | **10e** |  | 94 |
| 14 | Tyrosine | | **10f** |  | 99 |
| 15 | Tryptophan | | **10g** |  | 98 |

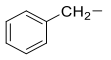
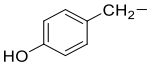
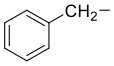
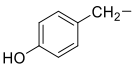
[a]Reaction conditions: **4a−g** (0.80 mmol), urea **8** (1.60 mmol, 2 equiv) under solvent-free thermal heating for 120 min at 250 °C. [b]Reaction conditions: **4a−g** (0.80 mmol), urea **8** (1.60 mmol, 2 equiv) under solvent-free microwave irradiation (150 W, 250 psi) for 2 min at 250 °C. [c]NaOH (1.0 N, 1.0 mL) stirring for 18 hrs at 25 °C. [d]Yield has been calculated on the basis of the amount of the recovered product. Reactions were performed in triplicate.

afford the corresponding carboxylic acid derivatives **7a−f** and **10a−g**, respectively. Compounds **6a−f**, **7a−f**, **9a−-g** and **10a−g** were tested against influenza virus A, one of the main respiratory pathogens responsible for seasonal epidemics or pandemic events. The use of vaccines and drugs in the therapy of influenza A virus, such as neuraminidase (NA), M2 channel, and polymerase inhibitors, is limited by the multidrug-resistant phenomenon associated with the high variability and the circulation of new influenza virus strains,[36,37] requiring a continuous effort for the search of new antiviral agents. PNA's analogues are well-recognized compounds with inhibitory activity against viral infections being able to pair with viral RNA and DNA.[38,39] For example, pyrimidine-like PNA's derivatives showed selective pair with polypurine sequences of double-helical RNA,[40] while 2-amino pyridines' counterpart[41] interferes with RNA editing.[42]

A549 cells were infected with 0.001 MOI of PR8 and treated with different concentrations (0.015−0.36 $\mu$MoL) of **6a−f**, **7a−f**, **9a−g**, and **10a−g** (Table 4) for the following 24 h. The cytotoxicity of the compounds was evaluated by standard MTT assay. The antiviral activity was evaluated by the HAU assay from supernatants of cells infected with PR8/H1N1 virus and treated for 24 h with the compounds. Control cells were treated with DMSO alone at the same concentration used for each compound. Table 4 shows the values of IC50, CC50, and relative selective index (SI). Compounds **6f**, **7f**, **9a**, **10a**, **9f**, and **10f** showed the highest SI values, and IC50 values were closely related to the most used NA inhibitor oseltamivir-carboxylate concentrations in cell cultures.[43,44] As a general trend, DAPAs and GAs showed comparable antiviral activity, and derivatives bearing a free carboxylic moiety showed an IC50 value higher than the corresponding esters (Table 4). In addition, compounds decorated with glycine, phenylalanine, and tyrosine showed the highest inhibitory activity. In both series, compounds **6f**, **7f**, **9a**, **10a**, **9f**, and **10f** showed lower toxicity (Table 4 entries 12, 13, 14, 15, 24, 25). The presence of an aromatic residue always leads to an inhibitory activity effect, the tyrosine residue producing the most active derivatives (**6f**, **7f**, **9f**, and **10f**) with IC50 values of 0.020, 0.024, 0.023, and 0.035 $\mu$M, respectively, and the highest CC50 values (0.580, 0.610, 0.582, and 0.604 $\mu$M, respectively). The major activity of compounds bearing a tyrosine residue could be explained by the high radical scavenging activity and antioxidant activity reported for this catechol derivative, which can interfere in the overall redox activity of the cell, thus modulating the viral cycle.[45−47]


https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

Table 4. Biological Activity of Compounds 6a−f, 7a−f, 9a−g, and 10a−g against Influenza A Virus[a,b,c]

| Entry | Class | Amino acid | R | Products | IC50[a] | CC50[b] | SI[c] |
|---|---|---|---|---|---|---|---|
| 1 | - | - | - | Oseltamivir | 0.180 | 0.350 | 1.95 |
| 2 | Diaminopurine analogues (DAPA) | Glycine | H | **6a** | 0.390 | 0.560 | 1.4 |
| 3 | | | | **7a** | 0.526 | 0.621 | 1.2 |
| 4 | | Alanine | $CH_3$ | **6b** | n.a | 0.790 | - |
| 5 | | | | **7b** | n.a | 0.846 | - |
| 6 | | Valine | $CH(CH_3)_2$ | **6c** | n.a | 0.718 | - |
| 7 | | | | **7c** | n.a | 0.756 | - |
| 8 | | Serine | $CH_2OH$ | **6d** | n.a | 0.318 | - |
| 9 | | | | **7d** | n.a | 0.350 | - |
| 10 | | Phenylalanine |  | **6e** | 0.039 | 0.420 | 10.5 |
| 11 | | | | **7e** | 0.210 | 0.610 | 2.9 |
| 12 | | Tyrosine |  | **6f** | 0.020 | 0.580 | 29.0 |
| 13 | | | | **7f** | 0.024 | 0.610 | 25.4 |
| 14 | Guanine analogues (GA) | Glycine | H | **9a** | 0.036 | 0.896 | 24.1 |
| 15 | | | | **10a** | 0.039 | 0.764 | 19.2 |
| 16 | | Alanine | $CH_3$ | **9b** | n.a | 0.715 | - |
| 17 | | | | **10b** | n.a | 0.821 | - |
| 18 | | Valine | $CH(CH_3)_2$ | **9c** | n.a | 0.650 | - |
| 19 | | | | **10c** | n.a | 0.420 | - |
| 20 | | Serine | $CH_2OH$ | **9d** | n.a | 0.218 | - |
| 21 | | | | **10d** | n.a | 0.323 | - |
| 22 | | Phenylalanine |  | **9e** | 0.340 | 0.710 | 2.1 |
| 23 | | | | **10e** | 0.520 | 0.654 | 1.3 |
| 24 | | Tyrosine |  | **9f** | 0.023 | 0.582 | 25.3 |
| 25 | | | | **10f** | 0.035 | 0.604 | 17.3 |
| 26 | | Tryptophan |  | **9g** | 0.310 | 0.690 | 2.2 |
| 27 | | | | **10g** | 0.510 | 0.673 | 1.3 |

[a]IC50 is the drug concentration ($\mu$moL) causing 50% inhibition of the desired activity. Each experiment was conducted in triplicate. [b]CC50 is the drug concentration ($\mu$moL) causing 50% of death of the viable cell. [c]SI is the selectivity index defined as the ratio of the CC50 to the IC50. n.a: not available.

## CONCLUSIONS

A large panel of diaminopurine and guanine PNA analogues was synthesized from aminomalononitrile multicomponent chemistry with guanidine and urea as one-carbon annulation reagents. The process was assisted by microwave irradiation to afford amino acid-decorated purine derivatives resembling the structural motif of acyclonucleosides. With respect to our previous study,[25] environmentally sustainable 2-MeTHF was selected as the best reaction solvent as an alternative to toxic THF, affording imidazole intermediates **4a−g** in yield higher than THF and other organic solvents, such as MeCN and $CH_2Cl_2$. In addition, we proved for the first time that the annulation of imidazoles **4a−g** was effective also in the presence of guanidine carbonate and urea (two widely recognized prebiotic precursors[15]), affording a large variety of novel PNA's building blocks to investigate both the chemical space and the scaffold morphing. This reflects the unexpected high IC50 and SI values in the inhibition of influenza A virus and in the range of nanomolar concentration showed by compounds **6a**, **7a**, **7e**, **9e**, and **10e**. They were decorated by glycine, phenylalanine, and tyrosine. In particular, compounds **6f**, **7f**, **9f**, and **10f**, bearing the tyrosine residue, showed IC50 values of 0.020, 0.024, 0.023, and 0.035 $\mu$M and the highest CC50 value, probably due to the high antioxidant activity reported for this amino acid. Interestingly, although a detailed investigation was not carried out about the mechanism of action of novel compounds, our attention was also attracted by the repetitive difference of activity between the derivatives bearing a free carboxylic moiety in the amino acid residue and the ester counterpart, the latter being more active. The very fact that carboxylic acid derivatives showed inhibitory activity



https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

different from that of the corresponding ester derivatives suggested that the latter were stable enough to esterase activity to interact with virus pathways. Indeed, the presence of a free carboxylic moiety may alter the microlocal pH and the hemagglutinin complex of the virus, probably inducing conformational changes by protonation of histidine residues that could favor the viral entry into the cell.[48] Therefore, we can also hypothesize that the ester compounds may inhibit specific steps of viral replication by impairing the host cell microenvironment.

## EXPERIMENTAL SECTION

**Materials.** All solvents and reagents were purchased from Aldrich Chemical Co. (purity grade >99%). Monitoring and purification of the reactions have been performed with silica gel 60 and silica 60-F254 acquired from Merck. Visualization of plates has been performed using a UV lamp at 254 nm. All products were completely dried under high vacuum ($10^{-3}$ mbar) prior to the spectroscopic characterization. All of the NMR spectra were acquired on a Bruker Advance DRX400 (400 MHz/100 MHz) spectrometer. Signals and chemical shifts of the reported $^{1}$H and $^{13}$C-NMR spectra are in parts per million and internally referenced to DMSO-$d_6$. Coupling constants ($J$) are reported in Hz. Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, dd = double doublets, m = multiplet. Microwave reactions were performed by a microwave synthesizer CEM Discover (CEM Corporation, Italy).

**General Procedure for the Synthesis of 5- Amino-1,2-Disubstituted-1H-imidazole-4-carbonitriles (4a−g).** Aminomalononitrile p-toluenesulfonate **1** (5.9 mmol) in 2-MeTHF (30 mL) and triethylamine (7.1 mmol) were stirred at room temperature. After 30 min, trimethyl orthoacetate **2** (8.3 mmol) was added, and the solution was irradiated with microwave assistance using the program in Table 5.

**Table 5. Microwave Condition Program for the Synthesis of Compounds 4a−g**

| no. of cycles | temperature | ramp time | hold time | pressure (psi) | power (W) |
|---|---|---|---|---|---|
| 1 | 200 °C | 1 min | 2 min | 250 | 250 |

Thereafter, the solution was cooled to room temperature, and triethylamine (7.1 mmol) and the corresponding amino acid (protected as methyl ester) (**3a−g**) (7.1 mmol) were added. The solution was stirred under microwave conditions as described above. Thereafter, the solvent was removed, and the precipitate was dissolved in dichloromethane (30 mL) and extracted with saturated aqueous $Na_2CO_3$ (3 × 20 mL) and saturated aqueous NaCl (1 × 20 mL). The organic layer was treated with $Na_2SO_4$ and concentrated under reduced pressure. Purification was performed by flash chromatography with ethyl acetate (AcOEt)/hexane (Hex) (2:1) to afford **4a−g** with 29 to 66% of yield.

**General Procedure for the Synthesis of Diaminopurine Analogues 6a−f and Guanine Analogues 9a−g.** Imidazole **4a−g** (0.80 mmol, 1 equiv) and guanidine carbonate **5** (for derivatives **6a−f**) or urea **8** (for derivatives **9a−g**) (1.60 mmol, 2 equiv) were irradiated under microwave conditions using the program in Table 6.

Thereafter, the solution was poured into hot water (8.0 mL), and the mixture was stirred for 10 min. After the mixture returned to room temperature, the solid residue was filtered, evaporated under reduced pressure, and purified by silica gel chromatography and eluting with 10% methanol in dichloromethane. Compounds **6a−f** and **9a−g** were obtained as brown solids in yield from 21 to 45%.

**Table 6. Microwave Condition Program for the Synthesis of Compounds 6a−f and 9a−g**

| no. of cycles | temperature | ramp Time | hold Time | pressure (psi) | power (W) |
|---|---|---|---|---|---|
| 1 | 200 °C | 1 min | 2 min | 250 | 150 |

**General Procedure for the Synthesis of Diaminopurine Analogues and Guanine Analogues Bearing Free Carboxylic Acid Moiety (7a−f and 10a−g).** Compounds **6a−f** or **9a−g** (0.10 mmol) were treated with an aqueous solution of NaOH (1.0 N, 1.0 mL) and stirred for 18 h at room temperature. The solution was acidified with HCl 1.0 N until reaching neutral pH, freeze-dried, and washed with methanol. The organic layer afforded **7a−f** or **10a−g** in quantitative yield after evaporation of the solvent.

**Spectroscopic Data.** Original $^{1}$H-NMR and $^{13}$C-NMR chromatogram of compounds **4a−g, 6a−f, 7a−f, 9a−g, and 10a−g** are in SI#1.

*Compound **4a**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)acetate. The crude residue was recovered by crystallization, as a beige solid.**$^{1}$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.13 (s, 2H, $NH_2$), 4.73 (s, 2H, $CH_2$), 3.71 (s, 3H, O-$CH_3$), 2.07 (s, 3H, $CH_3$).**$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 168.51 (C═O), 148.88 (C), 140.10 (C), 118.08 (C), 88.53 (C), 52.91 (O-$CH_3$), 43.91 ($CH_2$), 13.29 ($CH_3$).MS (ESI): $m/z$ (M + H) +195.19. Elemental analysis for $C_8H_{10}N_4O_2$ calcd C, 49.48; H, 5.19; N, 28.85; O, 16.48. Found: C, 49.45; H, 5.18; N, 28.83; O, 16.47.

*Compound **4b**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)propanoate. The crude residue was purified by silica gel chromatography eluting with ethyl acetate/petroleum ether (2:1). Compound **4b** was isolated as a beige solid.**$^{1}$H-NMR** (400 MHz, $CDCl_3$, ppm): $\delta$ 4.89−4.87 (m, 1H, CH), 4.15 (s, 2H, $NH_2$), 3.84 (s, 3H, O-$CH_3$), 2.34 (s, 3H, $CH_3$), 1.76 (d, $J$ = 7.6 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CDCl_3$, ppm): $\delta$ 170.57 (C═O), 145.71 (C), 140.66 (C), 115.58 (C), 96.24 (C), 53.37 (CH), 53.29 (O-$CH_3$), 15.96 ($CH_3$), 14.09 ($CH_3$).MS (ESI): $m/z$ (M + H) +209.22. Elemental analysis for $C_9H_{12}N_4O_2$ calcd C, 51.92; H, 5.81; N, 26.91; O, 15.37. Found: C, 51.89; H, 5.80; N, 26.89; O, 15.36.

*Compound **4c**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)-3-methylbutanoate. The crude residue was purified by silica gel chromatography eluting with ethyl acetate/hexane (2:1). Compound **4c** was isolated as an orange solid. **$^{1}$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.04 (s, 2H, $NH_2$), 4.57 (d, $J$ = 10.8 Hz, 1H, CH), 3.70 (s, 3H, O-$CH_3$), 2.59-2.55 (m, 1H, CH), 2.12 (s, 3H, $CH_3$), 1.11 (d, $J$ = 6.4 Hz, 3H, $CH_3$), 0.64 (d, $J$ = 6.4 Hz, 3H, $CH_3$).**$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.80 (C═O), 148.93 (C), 139.72 (C), 117.72 (C), 89.92 (C), 61.88 (CH), 53.19 (O-$CH_3$), 28.37 (CH), 20.67 ($CH_3$), 18.86 ($CH_3$), 14.72 ($CH_3$). MS (ESI): $m/z$ (M + H) +237.28. Elemental analysis for $C_{11}H_{16}N_4O_2$ calcd C, 55.92; H, 6.83; N, 23.71; O, 13.54. Found: C, 55.89; H, 6.82; N, 23.69; O, 13.53.

*Compound **4d**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)-3-hydroxypropanoate. The crude residue was purified by silica gel chromatography eluting with ethyl acetate.


https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

Compound **4d** was isolated as a beige solid. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.89 (s, 1H, OH), 5.87 (s, 2H, $NH_2$), 5.47−5.09 (m, 2H, $CH_2$), 3.68 (s, 3H, O-$CH_3$), 3.66−3.61 (m, 1H, CH), 2.11 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 168.27 (C═O), 148.98 (C), 140.56 (C), 117.99 (C), 90.09 (C), 62.85 ($CH_2$), 59.86 (CH), 53.09(O-$CH_3$), 14.55 ($CH_3$).MS (ESI): $m/z$ (M + H) +225.22. Elemental analysis for $C_9H_{12}N_4O_3$ calcd C, 48.21; H, 5.39; N, 24.99; O, 21.41. Found: C, 48.18; H, 5.38; N, 24.97; O, 21.39.

*Compound **4e**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)-3-phenylpropanoate. The crude residue was purified by silica gel chromatography eluting with ethyl acetate/Hexane (3:1). Compound **4e** was isolated as a yellow oil.**$^1$H-NMR** (400 MHz, $CDCl_3$, ppm): $\delta$ 7.28−7.25 (m, 3H, CH-Ar), 6.99-6.97 (m, 2H, CH-Ar), 4.84−4.79 (dd, $J$ = 4.0, 11.2 Hz, 1H, $CH_2$), 4.32 (s, 2H, $NH_2$), 3.87 (s, 3H, O-$CH_3$), 3.56 (t, $J$ = 12.8 Hz, 1H, CH), 3.38−3.34 (dd, $J$ = 4.4, 13.6 Hz, 1H, $CH_2$), 1.81 (s, 3H, $CH_3$).**$^{13}$C-NMR** (100 MHz, $CDCl_3$, ppm): $\delta$ 169.93 (C═O), 146.09 (C), 141.43 (C), 135.34 (C), 129.08 (C-Ar x2), 128.75 (C-Ar x2), 127.76 (C-Ar), 115.83 (C), 95.75 (C), 60.28 (CH), 53.49 (O-$CH_3$), 35.29 ($CH_2$), 13.45 ($CH_3$).MS (ESI): $m/z$ (M + H) +285.32. Elemental analysis for $C_{15}H_{16}N_4O_2$ calcd C, 63.37; H, 5.67; N, 19.71; O, 11.25. Found: C, 63.33; H, 5.66; N, 19.69; O, 11.24.

*Compound **4f**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)-3-(4-hydroxyphenyl)propanoate. The crude residue was purified by silica gel chromatography eluting with ethyl acetate/Petroleum ether (4:1). Compound **4f** was isolated as a yellow solid.**$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 9.25 (s, 1H, OH), 6.85 (d, $J$ = 8.4 Hz, 2H, CH-Ar),6.59 (d, $J$ = 8.4 Hz, 2H, CH-Ar), 5.70 (s, 2H, $NH_2$) 5.19−5.16 (m, 1H, CH), 3.72 (s, 3H, O-$CH_3$), 3.26−3.22 (m,2H, $CH_2$), 1.79 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.44 (C═O), 156.59 (C), 148.22 (C), 140.24 (C), 130.41 (C-Ar x2), 126.71 (C-Ar), 117.83 (C), 115.60 (C-Ar x2), 90.92 (C), 60.22 (CH), 53.26 (O-$CH_3$), 34.19 ($CH_2$), 14.56 ($CH_3$).MS (ESI): $m/z$ (M + H) + 301.32. Elemental analysis for $C_{15}H_{16}N_4O_3$ calcd C, 59.99; H, 5.37; N, 18.66; O, 15.98. Found: C, 59.96; H, 5.36; N, 18.64; O, 15.97.

*Compound **4g**.* Methyl 2-(5-amino-4-cyano-2-methyl-1H-imidazol-1-yl)-3-(1H-indol-3-yl)propanoate. The crude residue was purified by silica gel chromatography eluting with ethyl acetate/Petroleum Ether (2:1). Compound **4g** was isolated as a yellow solid.**$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 10.85 (s, 1H, NH), 7.52 (d, $J$ = 8.0 Hz, 1H, CH-Ar), 7.32 (d, $J$ = 8.0 Hz, 1H, CH-Ar), 7.08 (t, $J$ = 7.6 Hz, 1H, CH-Ar), 6.99 (t, $J$ = 7.6 Hz, 1H, CH-Ar), 6.89 (d, $J$ = 2.0 Hz, 1H, CH-Ar), 5.75 (s, 2H, $NH_2$), 5.30 (t, $J$ = 7.8 Hz, 1H, CH), 3.77 (s, 3H, O-$CH_3$), 3.54 (d, $J$ = 8 Hz, 2H, $CH_2$), 1.74 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.67 (C═O), 145.05 (C), 140.26 (C), 136.39 (C-Ar), 127.23 (C-Ar), 124.21 (C-Ar), 121.55 (C-Ar), 118.99 (C-Ar), 118.36 (C-Ar), 115.14 (C), 111.91 (C-Ar), 109.00 (C-Ar), 95.22 (C), 57.37 (CH), 53.27 (O-$CH_3$), 25.27 ($CH_2$), 14.16 ($CH_3$). MS (ESI): $m/z$ (M + H) + 324.14. Elemental analysis for $C_{17}H_{17}N_5O_2$ calcd C, 63.15; H, 5.30; N, 21.66; O, 9.90. Found: C, 63.12; H, 5.29; N, 21.64; O, 9.89.

*Compound **6a**.* Methyl 2-(2,6-diamino-8-methyl-9H-purin-9-yl) acetate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.89 (s, 2H, $NH_2$), 6.12 (s, 2H, $NH_2$), 4.71 (s, 2H, $CH_2$), 3.68 (s, 3H, O-$CH_3$), 2.24 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 171.78 (C═O), 160.09 (C), 155.33 (C), 152.94 (C), 146.03 (C), 115.53 (C), 52.46 (O-$CH_3$), 48.81 ($CH_2$), 13.15 ($CH_3$). MS (ESI): $m/z$ (M + H) + 237,24. Elemental analysis for $C_9H_{12}N_6O_2$ calcd C, 45.76; H, 5.12; N, 35.58; O, 13.54. Found: C, 45.73; H, 5.11; N, 35.56; O, 13.53.

*Compound **6b**.* Methyl 2-(2,6-diamino-8-methyl-9H-purin-9-yl) propanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.90 (s, 2H, $NH_2$), 6.27 (s, 2H, $NH_2$), 4.52−4.51 (m, 1H, CH), 3.75 (s, 3H, O-$CH_3$), 2.13 (s, 3H, $CH_3$), 1.29 (d, $J$ = 7.2 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.92 (C═O), 158.08 (C), 155.58 (C), 152.03 (C), 145.06 (C), 118.00 (C), 53.36 (CH) 52.77 (O-$CH_3$), 22.86 ($CH_3$), 13.02 ($CH_3$). MS (ESI): $m/z$ (M + H) + 251,26. Elemental analysis for $C_{10}H_{14}N_6O_2$ calcd C, 47.99; H, 5.64; N, 33.58; O, 12.79. Found: C, 47.96; H, 5.63; N, 35.56; O, 12.77.

*Compound **6c**.* Methyl 2-(2,6-diamino-8-methyl-9H-purin-9-yl)-3-methylbutanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane.**$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.76 (s, 2H, $NH_2$), 6.06 (s, 2H, $NH_2$), 5.00 (d, $J$ = 9.6 Hz, 1H, CH), 3.72 (s, 3H, O-$CH_3$), 2.71−2.67 (m, 1H, CH), 2.12 (s, 3H, $CH_3$), 1.18 (d, $J$ = 6.4 Hz, 3H, $CH_3$), 0.88 (d, $J$ = 6.4 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 168.88 (C═O), 158.13 (C), 153.93 (C), 151.48 (C), 147.00 (C), 116.82 (C), 67.32 (CH), 53.29 (O-$CH_3$), 25.02 (CH), 20.00 ($CH_3$), 16.36 ($CH_3$), 14.57 ($CH_3$). MS (ESI): $m/z$ (M + H) + 279,32. Elemental analysis for $C_{12}H_{18}N_6O_2$ calcd C, 51.79; H, 6.52; N, 30.20; O, 11.50. Found: C, 51.76; H, 6.51; N, 30.18; O, 11.48.

*Compound **6d**.* Methyl 2-(2,6-diamino-8-methyl-9H-purin-9-yl)-3-hydroxypropanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane.**$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 6.97 (s, 2H, $NH_2$), 6.46 (s, 1H, OH), 6.05 (s, 2H, $NH_2$), 4.41−4.39 (m, 2H, $CH_2$), 3.77 (s, 3H, O-$CH_3$), 3.64−3.62 (m, 1H, CH), 2.13 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 170.62 (C═O), 160.87 (C), 156.47 (C), 153.03 (C), 146.78 (C), 117.65 (C), 68.00 (CH), 61.21 ($CH_2$) 52.40 (O-$CH_3$), 14.12 ($CH_3$). MS (ESI): $m/z$ (M + H) + 267,26. Elemental analysis for $C_{10}H_{14}N_6O_3$ calcd C, 45.11; H, 5.30; N, 31.56; O, 18.03. Found: C, 45.08; H, 5.29; N, 31.54; O, 18.02.

*Compound **6e**.* Methyl 2-(2,6-diamino-8-methyl-9H-purin-9-yl)-3-phenylpropanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 7.22−7.02 (m, 5H, CH-Ar), 6.63 (s, 2H, $NH_2$), 6.21 (s, 2H, $NH_2$), 5.22−5.20 (m, 1H, CH), 3.63 (s, 3H, O-$CH_3$), 3.23-3.18 (dd, $J$ = 4.0, 14.0 Hz, 1H, $CH_2$), 2.95−2.92 (dd, $J$ = 6.8, 9.0 Hz, 1H, $CH_2$), 2.22 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 167.62 (C═O) 157.88 (C), 155.01 (C), 153.06 (C), 150.64 (C), 141.10 (C), 129.41 (C-Ar x2), 128.66 (C-Ar x2), 126.67 (C-Ar), 111.70 (C), 64.39 (CH), 53.79 (O-$CH_3$), 34.88 ($CH_2$), 13.09 ($CH_3$). MS (ESI): $m/z$ (M + H) +327.36. Elemental analysis for $C_{16}H_{18}N_6O_2$ calcd C, 58.88; H, 5.56; N, 25.75; O, 9.80. Found: C, 58.85; H, 5.55; N, 25.73; O, 9.78.

*Compound **6f**.* Methyl 2-(2,6-diamino-8-methyl-9H-purin-9-yl)-3-(4-hydroxyphenyl) propanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 9.29 (s, 1H, OH), 7.11 (s, 2H, $NH_2$), 6.77 (d, $J$ =


https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

8.8 Hz, 2H, CH-Ar), 6.61 (d, $J$ = 8.4 Hz, 2H, CH-Ar), 5.95 (s, 2H, $NH_2$), 5.19−5.17 (m, 1H, CH), 3.71 (s, 3H, O-$CH_3$), 2.89-2.85 (dd, $J$ = 6.0, 14.0 Hz, 1H, $CH_2$), 2.77−2.72 (dd, $J$ = 9.2, 14.0 Hz, 1H, $CH_2$), 2.08 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.03 (C═O), 159.38 (C), 157.04 (C), 155.86 (C), 153.71 (C), 145.29 (C), 131.20 (C-Ar), 130.36 (C-Ar x2), 120.12 (C), 115.59 (C-Ar x2), 67.81 (CH), 53.62 (O-$CH_3$), 34.73 ($CH_2$), 13.71 ($CH_3$). MS (ESI): $m/z$ (M + H) + 343,36. Elemental analysis for $C_{16}H_{18}N_6O_3$ calcd C, 56.13; H, 5.30; N, 24.55; O, 14.02. Found: C, 56.10; H, 5.29; N, 24.53; O, 14.00.

*Compound* ***7a***. 2-(2,6-Diamino-8-methyl-9H-purin-9-yl) acetic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 4.45 (s, 2H, $CH_2$), 2.17 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.03 (C═O), 160.05 (C), 155.87 (C), 152.92 (C), 147.19 (C), 115.74 (C), 49.74 ($CH_2$), 13.32 ($CH_3$). MS (ESI): $m/z$ (M + H) + 223,21. Elemental analysis for $C_8H_{10}N_6O_2$ calcd C, 43.24; H, 4.54; N, 37.82; O, 14.40. Found: C, 43.21; H, 4.53; N, 37.80; O, 14.39.

*Compound* ***7b***. 2-(2,6-Diamino-8-methyl-9H-purin-9-yl)-propanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane.**$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 4.61−4.55 (m, 1H, CH), 2.08 (s, 3H, $CH_3$), 1.77 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 168.05 (C═O), 159.15 (C), 156.27 (C), 153.19 (C), 146.24 (C), 114.96 (C), 59.32 (CH), 22.33 ($CH_3$), 13.43 ($CH_3$). MS (ESI): $m/z$ (M + H) + 237,24. Elemental analysis for $C_9H_{12}N_6O_2$ calcd C, 45.76; H, 5.12; N, 35.58; O, 13.54. Found: C, 45.73; H, 5.11; N, 35.56; O, 13.53.

*Compound* ***7c***. 2-(2,6-Diamino-8-methyl-9H-purin-9-yl)-3-methylbutanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 4.55 (d, $J$ = 3.6 Hz, 1H, CH), 2.41-2.37 (m, 1H, CH), 2.17 (s, 3H, $CH_3$), 0.99 (d, $J$ = 6.4 Hz, 3H, $CH_3$), 0.81 (d, $J$ = 6.8 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.15 (C═O), 159.19 (C), 155.96 (C), 152.24 (C), 145.22 (C), 116.46 (C), 66.23 (CH), 24.43 (CH), 21.11 ($CH_3$), 16.31 ($CH_3$), 13.55 ($CH_3$). MS (ESI): $m/z$ (M + H) + 265.29. Elemental analysis for $C_{11}H_{16}N_6O_2$ calcd C, 49.99; H, 6.10; N, 31.80; O, 12.11. Found: C, 49.96; H, 6.09; N, 31.78; O, 12.10.

*Compound* ***7d***. 2-(2,6-Diamino-8-methyl-9H-purin-9-yl)-3-hydroxypropanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane.**$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 5.45−5.41 (m, 1H, CH), 4.18−4.15 (m, 2H, $CH_2$), 2.12 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.87 (C═O), 159.75 (C), 156.11 (C), 153.30 (C), 146.42 (C), 117.18 (C), 64.82 (CH), 61.01 ($CH_2$), 13.31 ($CH_3$). MS (ESI): $m/z$ (M + H) + 253,23. Elemental analysis for $C_9H_{12}N_6O_3$ calcd C, 42.86; H, 4.80; N, 33.32; O, 19.03. Found: C, 42.83; H, 4.79; N, 33.30; O, 19.02.

*Compound* ***7e***. 2-(2,6-Diamino-8-methyl-9H-purin-9-yl)-3-phenylpropanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 7.36−7.23 (m, 5H, CH-Ar), 5.21−5.16 (m, 1H, CH), 3.15−3.11 (dd, $J$ = 4.4, 14.0 Hz, 1H, $CH_2$), 3.03-2.97 (dd, $J$ = 4.4, 14.0 Hz, 1H, $CH_2$), 2.16 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.04 (C═O), 156.95 (C), 154.78 (C), 152.15 (C), 149.98 (C), 141.92 (C), 129.58 (C-Ar x2), 127.82 (C-Ar x2), 124.88 (C-Ar), 112.64 (C), 64.45 (CH), 34.40 ($CH_2$), 14.41 ($CH_3$). MS (ESI): $m/z$ (M + H) + 313,33. Elemental analysis for $C_{15}H_{16}N_6O_2$ calcd C, 57.68; H, 5.16; N, 26.91; O, 10.24. Found: C, 57.65; H, 5.15; N, 26.89; O, 10.23.

*Compound* ***7f***. 2-(2,6-Diamino-8-methyl-9H-purin-9-yl)-3-(4-hydroxyphenyl) propanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 7.15 (d, $J$ = 8.0 Hz, 2H, CH-Ar), 6.75 (d, $J$ = 7.6 Hz, 2H, CH-Ar), 5.26−5.20 (m, 1H, CH), 3.20-3.14 (m, 2H, $CH_2$), 2.06 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.81 (C═O), 159.74 (C), 157.11 (C), 154.16 (C), 152.30 (C), 146.42 (C), 134.18 (C), 130.77 (C-Ar x2), 121.01 (C), 116.82 (C-Ar x2), 60.43 (CH), 34.71 ($CH_2$), 13.79 ($CH_3$). MS (ESI): $m/z$ (M + H) + 329,33. Elemental analysis for $C_{15}H_{16}N_6O_3$ calcd C, 54.87; H, 4.91; N, 25.60; O, 14.62. Found: C, 54.84; H, 4.90; N, 25.58; O, 14.60.

*Compound* ***9a***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl) acetate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 11.28 (s, 1H, NH), 6.13 (s, 2H, $NH_2$), 4.15 (s, 2H, $CH_2$), 3.70 (s, 3H, O-$CH_3$), 2.07 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 168.44 (C═O), 156.91 (C), 152.04 (C), 150.07 (C), 148.88 (C), 110.81 (C), 52.44 (O-$CH_3$), 45.08 ($CH_2$), 13.52 ($CH_3$). MS (ESI): $m/z$ (M + H) + 238,22. Elemental analysis for $C_9H_{11}N_5O_3$ calcd C, 45.57; H, 4.67; N, 29.52; O, 20.23. Found: C, 45.54; H, 4.66; N, 29.50; O, 20.22.

*Compound* ***9b***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)propanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 10.22 (s, 1H, NH), 6.16 (s, 2H, $NH_2$), 4.72 -4.68 (m, 1H, CH), 3.65 (s, 3H, O-$CH_3$), 2.26 (s, 3H, $CH_3$), 1.81 (d, $J$ = 7.2 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.49 (C═O), 159.83 (C), 154.84 (C), 150.51 (C), 147.34 (C), 112.21 (C), 58.27 (CH), 53.44 (O-$CH_3$), 21.48 ($CH_3$), 13.32 ($CH_3$). MS (ESI): $m/z$ (M + H) + 252,25. Elemental analysis for $C_{10}H_{13}N_5O_3$ calcd C, 47.81; H, 5.22; N, 27.88; O, 19.10. Found: C, 47.78; H, 5.21; N, 27.86; O, 19.09.

*Compound* ***9c***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-methylbutanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 11.11 (s, 1H, NH), 6.16 (s, 2H, $NH_2$), 4.69 (d, $J$ = 3.6 Hz, 1H, CH), 3.65 (s, 3H, O-$CH_3$), 2.85−2.79 (m, 1H, CH), 2.26 (s, 3H, $CH_3$), 1.15 (d, $J$ = 6.4 Hz, 3H, $CH_3$), 0.76 (d, $J$ = 6.8 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 168.99 (C═O), 157.83 (C), 152.17 (C), 149.34 (C), 147.68 (C), 110.72 (C), 64.10 (CH), 52.61 (O-$CH_3$), 26.14 (CH), 19.81($CH_3$), 17.48 ($CH_3$), 13.82 ($CH_3$). MS (ESI): $m/z$ (M + H) + 280,30. Elemental analysis for $C_{12}H_{17}N_5O_3$ calcd C, 51.60; H, 6.14; N, 25.08; O, 17.18. Found: C, 51.57; H, 6.13; N, 25.06; O, 17.16.

*Compound* ***9d***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-hydroxypropanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 10.09 (s, 1H, NH), 6.89 (s, 1H, OH), 6.17 (s, 2H, $NH_2$), 5.45−5.43 (m, 1H, CH), 5.02−4.99 (m, 1H, $CH_2$), 3.68 (s, 3H, O-$CH_3$), 3.66−3.61 (m, 1H, $CH_2$), 2.14 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.49 (C═O), 158.67 (C), 154.17 (C), 151.34 (C), 148.18 (C), 111.55 (C),


https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

62.93 (CH), 60.94 ($CH_2$), 53.11 (O-$CH_3$), 13.65 ($CH_3$). MS (ESI): $m/z$ (M + H) + 268,25. Elemental analysis for $C_{10}H_{13}N_5O_4$ calcd C, 44.94; H, 4.90; N, 26.21; O, 23.95. Found: C, 51.57; H, 6.13; N, 25.06; O, 17.16.

*Compound* ***9e***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-phenylpropanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 11.00 (s, 1H, NH), 7.19−7.12 (m, 3H, CH-Ar), 7.05−7.03 (m, 2H, CH-Ar), 6.44 (s, 2H, $NH_2$), 5.10−5.06 (m, 1H, CH), 3.69 (s, 3H, O-$CH_3$), 3.22−3.17 (dd, $J$ = 5.6, 14.0 Hz, 1H, $CH_2$), 3.13-3.07 (dd, $J$ =7.6, 14.0 Hz, 1H, $CH_2$), 2.05 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 170.49 (C), 158.88 (C), 154.19 (C), 151.15 (C), 145.13 (C), 138.15 (C-Ar), 129.17 (C-Ar x2), 128.66 (C-Ar x2), 126.89 (C-Ar), 107.61 (C), 66.22 (CH), 56.49 (O-$CH_3$), 35.01 ($CH_2$), 14.35 ($CH_3$). MS (ESI): $m/z$ (M + H) + 328,34. Elemental analysis for $C_{16}H_{17}N_5O_3$ calcd C, 58.71; H, 5.23; N, 21.39; O, 14.66. Found: C, 58.68; H, 5.22; N, 21.37; O, 14.65.

*Compound* ***9f***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-(4-hydroxyphenyl) propanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 11.11 (s, 1H, NH), 9.16 (s, 1H, OH), 6.82 (d, $J$ = 8.0 Hz, 2H, CH-Ar), 6.55 (d, $J$ = 8.0 Hz, 2H, CH-Ar), 6.33 (s, 2H, $NH_2$), 4.99-4.95 (m, 1H, CH), 3.74 (s, 3H, O-$CH_3$), 2.89-2.85 (dd, $J$ = 6.0, 14.0 Hz, 1H, $CH_2$), 2.77−2.72 (dd, $J$ = 9.2, 14.0 Hz, 1H, $CH_2$), 2.03 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 170.66 (C═O), 156.23 (C), 155.03 (C), 152.67 (C), 150.65 (C), 143.77 (C), 130.07 (C-Ar), 128.04 (C-Ar x2), 115.48 (C-Ar x2), 108.72 (C), 63.48 (CH), 54.11 (O-$CH_3$), 34.20 ($CH_2$), 15.04 ($CH_3$). MS (ESI): $m/z$ (M + H) + 344,34. Elemental analysis for $C_{16}H_{17}N_5O_4$ calcd C, 55.97; H, 4.99; N, 20.40; O, 18.64. Found: C, 55.94; H, 4.98; N, 20.38; O, 18.62.

*Compound* ***9g***. Methyl 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-(1H-indol-3-yl)propanoate. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, DMSO-$d_6$, ppm): $\delta$ 11.81 (s, 1H, NH), 10.92 (s, 1H, NH), 7.07 (t, $J$ = 7.0 Hz, 1H, CH-Ar), 6.98 (t, $J$ = 7.2 Hz, 1H, CH-Ar), 6.87 (d, $J$ = 2.0 Hz, 1H, CH-Ar), 6.78 (d, J = 2.0 Hz, 1H, CH-Ar), 6.58 (d, $J$ = 2.0 Hz, 1H, CH-Ar), 6.53 (s, 2H, $NH_2$), 5.25 (t, $J$ = 4.6 Hz, 1H, CH), 3.64 (s, 3H, O-$CH_3$), 3.45 (d, $J$ = 4.0 Hz, 2H, $CH_2$), 1.99 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$, ppm): $\delta$ 169.82 (C═O), 159.78 (C), 154.59 (C), 145.39 (C), 140.54 (C), 136.35 (C-Ar), 128.88 (C-Ar), 123.30 (C-Ar), 121.63 (C-Ar), 119.12 (C-Ar), 116.99 (C-Ar), 111.92 (C-Ar), 110.89 (C-Ar), 106.57 (C), 61.03 (CH), 52.20 (O-$CH_3$), 26.94 ($CH_2$), 13.55 ($CH_3$). MS (ESI): $m/z$ (M + H) + 367,14. Elemental analysis for $C_{18}H_{18}N_6O_3$ calcd C, 59.01; H, 4.95; N, 22.94; O, 13.10. Found: C, 58.98; H, 4.94; N, 22.92; O, 13.09.

*Compound* ***10a***. 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl) acetic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 4.55 (s, 2H, $CH_2$), 2.16 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.61 (C═O), 157.66 (C),153.33 (C), 151.25 (C), 147.96 (C), 110.88 (C), 43.83 ($CH_2$), 13.17 ($CH_3$). MS (ESI): $m/z$ (M + H) + 224,19. Elemental analysis for $C_8H_9N_5O_3$ calcd C, 43.05; H, 4.06; N, 31.38; O, 21.50. Found: C, 43.02; H, 4.05; N, 31.36; O, 21.48.

*Compound* ***10b***. 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl) propanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 4.72−4.66 (m, 1H, CH), 2.18 (s, 3H, $CH_3$), 1.88 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.65 (C═O), 158.83 (C),153.50 (C), 150.34 (C), 147.84 (C), 109.88 (C), 59.44 (CH), 20.14 ($CH_3$), 13.48 ($CH_3$). MS (ESI): $m/z$ (M + H) + 238,22. Elemental analysis for $C_9H_{11}N_5O_3$ calcd C, 45.57; H, 4.67; N, 29.52; O, 20.23. Found: C, 45.54; H, 4.66; N, 29.50; O, 20.21.

*Compound* ***10c***. 2-(6-amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-methylbutanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 4.40 (d, $J$ = 10.8 Hz, 1H, CH), 2.24-2.20 (m, 1H, CH), 2.08 (s, 3H, $CH_3$), 0.78 (d, $J$ = 6.8 Hz, 3H, $CH_3$), 0.61 (d, $J$ = 6.8 Hz, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.32 (C═O), 157.66 (C), 154.67 (C), 150.84 (C), 146.68 (C), 111.22 (C), 63.60 (CH), 26.31 (CH), 19.47 ($CH_3$), 16.82 ($CH_3$), 14.15 ($CH_3$). MS (ESI): $m/z$ (M + H) + 266,27. Elemental analysis for $C_{11}H_{15}N_5O_3$ calcd C, 49.81; H,5.70; N, 26.40; O, 18.09. Found: C, 49.78; H, 5.69; N, 26.38; O, 18.08.

*Compound* ***10d***. 2-(6-Amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-hydroxypropanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 5.42−5.40 (m, 1H, CH), 3.62-3.50 (m, 2H, $CH_2$), 2.01 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 170.65 (C═O), 159.49 (C), 154.34 (C), 152.17 (C), 149.51 (C), 113.21 (C), 64.76 (CH), 61.60 ($CH_2$), 13.48 ($CH_3$). MS (ESI): $m/z$ (M + H) + 254,22. Elemental analysis for $C_9H_{11}N_5O_4$ calcd C, 42.69; H, 4.38; N, 27.66; O, 25.27. Found: C, 42.66; H, 4.37; N, 27.64; O, 25.25.

*Compound* ***10e***. 2-(6-Amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-phenylpropanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $CD_3OD$, ppm): $\delta$ 7.18-7.12 (m, 5H, CH-Ar), 5.05−4.99 (m, 1H, CH), 3.24-3.17 (dd, $J$ =4.4, 18.8 Hz, 2H, $CH_2$), 2.12 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $CD_3OD$, ppm): $\delta$ 169.79 (C═O), 158.18 (C), 155.45 (C), 151.60 (C), 145.68 (C), 137.22 (C-Ar), 129.59 (C-Ar x2), 127.86 (C-Ar x2), 125.78 (C-Ar), 108.46 (C), 65.92 (CH), 35.52 ($CH_2$), 14.65 ($CH_3$). MS (ESI): $m/z$ (M + H) + 314,32. Elemental analysis for $C_{15}H_{15}N_5O_3$ calcd C, 57.50; H, 4.83; N, 22.35; O, 15.32. Found: C, 57.47; H, 4.82; N, 22.33; O, 15.30.

*Compound* ***10f***. 6-Amino-9-(1-(4-hydroxyphenyl)-3-oxobutan-2-yl)-8-methyl-3,9-dihydro-2*H*-purin-2-one. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $D_2O$, ppm): $\delta$ 6.82 (d, $J$ = 7.6 Hz, 2H, CH-Ar), 6.63 (d, $J$ = 6.4 Hz, 2H, CH-Ar), 5.19−5.16 (m, 1H, CH), 3.22 (d, $J$ = 14.8 Hz, 1H, $CH_2$), 2.98 (d, $J$ =14.8 Hz, 1H, $CH_2$) 2.12 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $D_2O$, ppm): $\delta$ 168.32 (C═O), 158.70 (C), 155.36 (C-Ar), 153.85 (C), 148.30 (C), 142.41 (C), 132.88 (C-Ar), 129.69 (C-Ar x2), 117.95 (C-Ar x2), 110.36 (C), 61.50 (CH), 35.86 ($CH_2$), 15.27 ($CH_3$). MS (ESI): $m/z$ (M + H) + 330.32. Elemental analysis for $C_{15}H_{15}N_5O_4$ calcd C, 54.71; H, 4.59; N, 21.27; O, 19.43. Found: C, 54.68; H, 4.58; N, 21.25; O, 19.42.



https://doi.org/10.1021/acsomega.2c05754
ACS Omega 2022, 7, 45253−45264

*Compound* ***10g***. 2-(6-Amino-8-methyl-2-oxo-2,3-dihydro-9H-purin-9-yl)-3-(1H-indol-3-yl)propanoic acid. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. **$^1$H-NMR** (400 MHz, $D_2O$, ppm): $\delta$ 7.56 (d, $J$ = 8.0 Hz, 1H, CH-Ar), 7.47 (d, $J$ = 8.0 Hz, 1H, CH-Ar), 7.24 (s, 1H, CH-Ar), 7.21 (t, $J$ = 7.2 Hz, 1H, CH-Ar), 7.13 (t, $J$ = 7.2 Hz, 1H, CH-Ar), 5.40 (t, $J$ = 6.2 Hz,1H, CH), 3.43 (t, $J$ = 6.8 Hz, 2H, $CH_2$), 1.99 (s, 3H, $CH_3$). **$^{13}$C-NMR** (100 MHz, $D_2O$, ppm): $\delta$ 169.31 (C═O), 158.44 (C), 153.98 (C), 144.22 (C), 139.03 (C), 135.52 (C-Ar), 127.49 (C-Ar), 123.81 (C-Ar), 122.13 (C-Ar), 119.62 (C-Ar), 117.92 (C-Ar), 111.76 (C-Ar), 110.17 (C-Ar), 106.91 (C), 59.56 (CH), 31.12 ($CH_2$), 13.89 ($CH_3$). MS (ESI): $m/z$ (M + H) + 353.13. Elemental analysis for $C_{17}H_{16}N_6O_3$ calcd C, 57.95; H, 4.58; N, 23.85; O, 13.62. Found: C, 57.92; H, 4.57; N, 23.83; O, 13.61.

**Cell Cultures.** A549, human lung epithelial carcinoma, (ATCC catalogue No. CCL-185) cell line was grown in a DMEM-Hi glucose medium (Sigma, Milan, Italy) supplemented with 10% fetal bovine serum (FBS) (FBS; Euroclone, Milan, Italy); glutamine 0.3 mg/mL; penicillin 100 U/mL; and streptomycin 100 mg/mL (Euroclone, Milan, Italy).

**Cell Toxicity Assay.** The cytotoxicities of **6a−f**, **7a−f**, **9a−g**, and **10a−g** were evaluated by the inhibition of MTT test and the trypan blue staining assay. In the MTT test, A549 cells were seeded in 96-well plates at a density of $2 \times 10^4$ cells/well in 100 $\mu$L of complete DMEM without phenol red for 24 h at 37 °C. Thereafter, cell monolayers were treated, when required, in a concentration range of 0.015−0.36 $\mu$MoL with the selected compound for 24 h at 37 °C. After 24 h, 10 $\mu$L of MTT solution (5 mg/ml) was added to each well for 3−4 h at 37 °C. Each sample was then treated with a solution of isopropanol and HCl (0.1 N, 100 $\mu$L/well) for 30 min under mild stirring. Results were recorded at 570 nm using an automatic plate reader (Multiskan EX, Ascent Software, Thermo Fisher Scientific). Untreated cells were used as control. CC50 was defined as the compound concentration required to reduce cell viability by 50% and obtained by the regression analysis considering untreated cells as control (100%).

**Antiviral Activity Assay.** Monolayers of A549 epithelial cells were treated for 1 h at 37 °C with Influenza virus A/Puerto Rico/8/34 H1N1 (PR8) at a multiplicity of infection (m.o.i.) of 0.001 (TCID50%/cell) by incubation for 1 h at 37 °C, washed with buffer sodium phosphate, and again incubated with medium supplemented with 2% of fetal bovine serum. Mock infection was conducted with the same dilution of allantoic fluid from uninfected eggs. Activity of **6a−f**, **7a−f**, **9a−g**, and **10a−g** have been evaluated in the culture medium until 24 h post-infection. The highest DMSO concentration present in the culture medium was 0.2%. Control cells were treated with DMSO alone at the same concentration present in the test substance being evaluated, and it was used as negative control of the antiviral assay. Viral titration was performed by hemagglutination assay (HAU) in human type 0 Rh+ erythrocytes, as already reported.[49]

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05754.

Original $^1$H-NMR and $^{13}$C-NMR spectra of compounds **4a−g, 6a−f, 7a−f, 9a−g and 10a−g** (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

**Bruno Mattia Bizzarri** − *Department of Biological and Ecological Sciences, University of Tuscia, Viterbo 01100, Italy;* orcid.org/0000-0001-7085-5432; Email: bm.bizzarri@unitus.it

### Authors

**Angelica Fanelli** − *Department of Biological and Ecological Sciences, University of Tuscia, Viterbo 01100, Italy*

**Stefania Ciprini** − *Department of Biological and Ecological Sciences, University of Tuscia, Viterbo 01100, Italy*

**Alessandra Giorgi** − *Department of Biological and Ecological Sciences, University of Tuscia, Viterbo 01100, Italy*

**Marta De Angelis** − *Department of Public Health and Infectious Diseases, Laboratory Affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome 00185, Italy*

**Raoul Fioravanti** − *Department of Public Health and Infectious Diseases, Laboratory Affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome 00185, Italy;* orcid.org/0000-0002-0151-0331

**Lucia Nencioni** − *Department of Public Health and Infectious Diseases, Laboratory Affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome 00185, Italy*

**Raffaele Saladino** − *Department of Biological and Ecological Sciences, University of Tuscia, Viterbo 01100, Italy;* orcid.org/0000-0002-4420-9063

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c05754


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported by the Italian Space Agency (ASI) DCVUM-2017-034 contratto ASI N. 2019-3-U.0, CUP F86C16000000006 "Vita nello spazio − Origine, presenza, persistenza della vita nello spazio, dalle molecole agli estremofili"and MIUR Ministero dell'Istruzione, dell'Università e della Ricerca Italiano, project PRIN 2017, ORIGINALE CHEMIAE in Antiviral Strategy−Origin and Modernization of Multi-Component Chemistry as a Source of Innovative Broad Spectrum Antiviral Strategy, cod. 2017BMK8JR.

## ■ REFERENCES

(1) Bizzarri, B. M.; Fanelli, A.; Kapralov, M.; Krasavin, E.; Saladino, R. Meteorite-Catalyzed Intermolecular Trans-Glycosylation Produces Nucleosides under Proton Beam Irradiation. *RSC Adv.* **2021**, *11*, 19258−19264.

(2) Saladino, R.; Bizzarri, B. M.; Botta, L.; Šponer, J.; Šponer, J. E.; Georgelin, T.; Jaber, M.; Rigaud, B.; Kapralov, M.; Timoshenko, G. N.; Rozanov, A.; Krasavin, E.; Timperio, A. M.; Mauro, E. Di. Proton Irradiation: A Key to the Challenge of N-Glycosidic Bond Formation in a Prebiotic Context. *Sci. Rep.* **2017**, *7*, No. 14709.

(3) Botta, L.; Bizzarri, B. M.; Piccinino, D.; Fornaro, T.; Robert Brucato, J.; Saladino, R. Prebiotic Synthesis of Carboxylic Acids,

Amino Acids and Nucleic Acid Bases from Formamide under Photochemical Conditions. *Eur. Phys. J. Plus* **2017**, *132*, 317−324.
(4) Saladino, R.; Botta, G.; Bizzarri, B. M.; Di Mauro, E.; Garcia Ruiz, J. M. A Global Scale Scenario for Prebiotic Chemistry: Silica-Based Self-Assembled Mineral Structures and Formamide. *Biochemistry* **2016**, *55*, 2806−2811.
(5) Mattia Bizzarri, B.; Botta, L.; Pérez-Valverde, M. I.; Saladino, R.; Di Mauro, E.; García-Ruiz, J. M. Silica Metal Oxide Vesicles Catalyze Comprehensive Prebiotic Chemistry. *Chem. − A Eur. J.* **2018**, *24*, 8126−8132.
(6) Botta, L.; Saladino, R.; Bizzarri, B. M.; Cobucci-Ponzano, B.; Iacono, R.; Avino, R.; Caliro, S.; Carandente, A.; Lorenzini, F.; Tortora, A.; Di Mauro, E.; Moracci, M. Formamide-Based Prebiotic Chemistry in the Phlegrean Fields. *Adv. Space Res.* **2018**, *62*, 2372−2379.
(7) Moggio, L.; Romanelli, A.; Gambari, R.; Bianchi, N.; Borgatti, M.; Fabbri, E.; Mancini, I.; Di Blasio, B.; Pedone, C.; Messere, A. Alternate PNA-DNA chimeras $(PNA\text{-}DNA)_n$: Synthesis, binding properties and biological activity. *Biopolymers* **2007**, *88*, 815−822.
(8) Kitadai, N.; Maruyama, S. Origins of building blocks of life: A review. *Geosci. Front.* **2018**, *9*, 1117−1153.
(9) Saladino, R.; Šponer, J. E.; Šponer, J.; Costanzo, G.; Pino, S.; Di Mauro, E. Chemomimesis and Molecular Darwinism in Action: From Abiotic Generation of Nucleobases to Nucleosides and RNA. *Life* **2018**, *8*, 24−39.
(10) Nielsen, P. E. Peptide Nucleic Acids and the Origin of Life. *Chem. Biodivers.* **2007**, *4*, 1996−2002.
(11) Pandey, V. N.; Upadhyay, A.; Chaubey, B. Prospects for antisense peptide nucleic acid (PNA) therapies for HIV. *Expert Opin. Biol. Ther.* **2009**, *9*, 975−989.
(12) Ghaffari, E.; Rezatofighi, S. E.; Ardakani, M. R.; Rastegarzadeh, S. Delivery of antisense peptide nucleic acid by gold nanoparticles for the inhibition of virus replication. *Nanomedicine* **2019**, *14*, 1827−1840.
(13) Robertson, P. S.; Vaughan, J. Derivatives of the Hydrogen Cyanide Tetramer: Structure and Chemistry. *J. Am. Chem. Soc.* **1958**, *80*, 2691−2693.
(14) Di Mauro, E.; Bizzarri, B. M.; Raffaele, S. *Comprehensive Series in Photochemical and Photobiological Sciences*; Saija, F.; Cassone, G., Eds.; Royal Society of Chemistry, 2021; Vol. *20*, Ch. 6, pp 107−123.
(15) Lohrmann, R. Formation of urea and guanidine by irradiation of ammonium cyanide. *J. Mol. Evol.* **1972**, *1*, 263−269.
(16) Ferris, J. P.; Zamek, O. S.; Altbuch, A. M.; Freiman, H. Chemial evolution XVIII. Synthesis of Pyrimidines from Guanidine and Cyanoacetaldehyde. *J. Mol. Evol.* **1974**, *3*, 301−309.
(17) Robertson, M. P.; Miller, S. L. An efficient prebiotic synthesis of cytosine and uracil. *Nature* **1995**, *375*, 772−774.
(18) Fox, S. W.; Harada, K. Synthesis of uracil under conditions of a thermal model of prebiological chemistry. *Science* **1961**, *133*, 1923−1924.
(19) Oro, J. In *The Origin of Prebiotic Systems*; Fox, S. W., Ed.; Academic Press: New York, 1965; pp 137−171.
(20) Harada, K.; Suzuki, S. The new synthesis of uracil and 1,3-dimethyluracil. *Tetrahedron Lett.* **1976**, *17*, 2321−2322.
(21) Chittenden, G. J. F.; Schwartz, A. W. Possible pathway for prebiotic uracil synthesis by photodehydrogenation. *Nature* **1976**, *263*, 350−351.
(22) Schwartz, A. W.; Chittenden, G. J. Synthesis of uracil and thymine under simulated prebiotic conditions. *Biosystems* **1977**, *9*, 87−92.
(23) Sarma, R.; Prajapati, D. Microwave-promoted efficient synthesis of dihydroquinazolines. *Green Chem.* **2011**, *13*, 718−722.
(24) Kruse, F. M.; Teichert, J. S.; Trapp, O. Prebiotic Nucleoside Synthesis: The Selectivity of Simplicity. *Chem. − Eur. J.* **2020**, *26*, 14776−14790.
(25) Bizzarri, B. M.; Fanelli, A.; Botta, L.; De Angelis, M.; Palamara, A. T.; Nencioni, L.; Saladino, R. Aminomalononitrile inspired prebiotic chemistry as a novel multicomponent tool for the synthesis of imidazole and purine derivatives with anti-influenza A virus activity. *RSC Adv.* **2021**, *11*, 30020−30029.
(26) Bizzarri, B. M.; Fanelli, A.; Cesarini, S.; Saladino, R. A Three-Way Regioselective Synthesis of Amino Acid Decorated Imidazole, Purine and Pyrimidine Derivatives by Multicomponent Chemistry Starting from Prebiotic Diaminomaleonitrile. *Eur. J. Org.Chem.* **2022**, *2022*, No. e202200598.
(27) Monticelli, S.; Castoldi, L.; Murgia, I.; Senatore, R.; Mazzeo, E.; Wackerlig, J.; Urban, E.; Langer, T.; Pace, V. Recent advancements on the use of 2-methyltetrahydrofuran in organometallic chemistry. *Monatsh Chem.* **2017**, *148*, 37−48.
(28) Ismael, A.; Gevorgyan, A.; Skrydstrup, T.; Bayer, A. Renewable Solvents for Palladium-Catalyzed Carbonylation Reactions. *Org. Process Res. Dev.* **2020**, *24*, 2665−2675.
(29) Zippilli, C.; Bizzarri, B. M.; Gabellone, S.; Botta, L.; Saladino, R. Oxidative Coupling of Coumarins by Blue-LED-Driven in situ Activation of Horseradish Peroxidase in a Two-Liquid-Phase System. *ChemCatChem* **2021**, *13*, 4151−4158.
(30) Huber, G. W.; Iborra, S.; Corma, A. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. *Chem. Rev.* **2006**, *106*, 4044−4098.
(31) Yue, H.; Zhao, Y.; Ma, X.; Gong, J. Ethylene glycol: properties, synthesis, and applications. *Chem. Soc. Rev.* **2012**, *41*, 4218−4244.
(32) Sun, K.; Zhang, Y. L.; Chen, X. L.; Su, H. F.; Peng, Q. C.; Yu, B.; Qu, L. B.; Li, K. A Type of Atypical AIEgen Used for One-Photon/Two-Photon Targeted Imaging in Live Cells. *ACS Appl. Bio Mater.* **2020**, *3*, 505−511.
(33) Lyman, D. J. *J. Polym. Sci., Part A: Gen. Pap.* **1960**, *45*, 49−59.
(34) Chemat, F.; Poux, M. Microwave assisted pyrolysis of urea supported on graphite under solvent-free conditions. *Tetrahedron Lett.* **2001**, *42*, 3693−3695.
(35) Banihashemi, A.; Hazarkhani, H.; Abdolmaleki, A. Efficient and rapid synthesis of polyureas and polythioureas from the reaction of urea and thiourea with diamines under microwave irradiation. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 2106−2111.
(36) Zhang, W.; Xu, H.; Guan, S.; Wang, C.; Dong, G. Frequency and distribution of H1N1 influenza A viruses with oseltamivir-resistant mutations worldwide before and after the 2009 pandemic. *J. Med. Virol.* **2022**, *94*, 4406−4416.
(37) Lampejo, T. Influenza and antiviral resistance: an overview. *Eur. J. Clin. Microbiol. Infect. Dis.* **2020**, *39*, 1201−1208.
(38) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* **1991**, *254*, 1497−1500.
(39) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. *Nature* **1993**, *365*, 566−568.
(40) Devi, G.; Yuan, Z.; Lu, Y.; Zhao, Y.; Chen, G. Incorporation of thio-pseudoisocytosine into triplex-forming peptide nucleic acids for enhanced recognition of RNA duplexes. *Nucleic Acids Res.* **2014**, *42*, 4008−4018.
(41) Annoni, C.; Endoh, T.; Hnedzko, D.; Rozners, E.; Sugimoto, N. Triplex-forming peptide nucleic acid modified with 2-aminopyridine as a new tool for detection of A-to-I editing. *Chem. Commun.* **2016**, *52*, 13417−13418.
(42) Wojciechowski, F.; Hudson, R. E. H. Peptide nucleic acid containing a meta-substituted phenylpyrrolocytosine exhibits a fluorescence response and increased binding affinity toward RNA. *Org. Lett.* **2009**, *11*, 4878−4881.
(43) Nguyen, J. T.; Hoopes, J. D.; Le, M. H.; Smee, D. F.; Patick, A. K.; Faix, D. J.; Blair, P. J.; De Jong, M. D.; Prichard, M. N.; Went, G. T. Triple combination of amantadine, ribavirin, and oseltamivir is highly active and synergistic against drug resistant influenza virus strains in vitro. *PLoS One* **2010**, *5*, No. e9332.
(44) Smee, D. F.; Hurst, B. L.; Wong, M. H.; Tarbet, E. B.; Babu, Y. S.; Klumpp, K.; Morrey, J. D. Combinations of oseltamivir and peramivir for the treatment of influenza A (H1N1) virus infections in cell culture and in mice. *Antiviral Res.* **2010**, *88*, 38−44.


https://doi.org/10.1021/acsomega.2c05754
*ACS Omega* 2022, 7, 45253−45264

(45) Saladino, R.; Barontini, M.; Crucianelli, M.; Nencioni, L.; Sgarbanti, R.; Palamara, A. T. Current advances in anti-influenza therapy. *Curr. Med. Chem.* **2010**, *17*, 2101−2140.
(46) Sgarbanti, R.; Amatore, D.; Celestino, I.; Marcocci, M. E.; Fraternale, A.; Ciriolo, M. R.; Magnani, M.; Saladino, R.; Garaci, E.; Palamara, A. T.; Nencioni, L. Intracellular redox state as target for anti-influenza therapy: are antioxidants always effective? *Curr. Top. Med. Chem.* **2014**, *14*, 2529−2541.
(47) Checconi, P.; De Angelis, M.; Marcocci, M. E.; Fraternale, A.; Magnani, M.; Palamara, A. T.; Nencioni, L. Redox-Modulating Agents in the Treatment of Viral Infections. *Int. J. Mol. Sci.* **2020**, *21*, 4084−4105.
(48) Caffrey, M.; Lavie, A. pH-Dependent Mechanisms of Influenza Infection Mediated by Hemagglutinin. *Front. Mol. Biosci.* **2021**, *8*, No. 777095.
(49) De Angelis, M.; Della-Morte, D.; Buttinelli, G.; Di Martino, A.; Pacifici, F.; Checconi, P.; Ambrosio, L.; Stefanelli, P.; Palamara, A. T.; Garaci, E.; Ricordi, C.; Nencioni, L. Protective Role of Combined Polyphenols and Micronutrients against Influenza A Virus and SARS-CoV-2 Infection In Vitro. *Biomedicines.* **2021**, *9*, 1721.



https://doi.org/10.1021/acsomega.2c05754
*ACS Omega* 2022, 7, 45253−45264