

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

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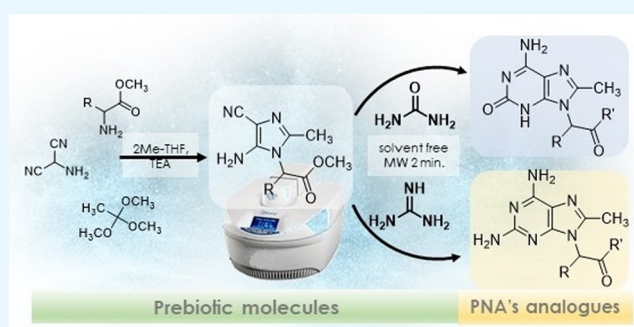
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**ABSTRACT:** Peptide nucleic acids (PNAs) play a key role in prebiotic chemistry as a chimera between RNA and proteins. We developed an alternative synthesis of bioactive PNA's diaminopurine and guanine analogues from prebiotic compounds, such as aminomalonnitrile (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.<sup>1–6</sup> As an alternative, peptide nucleic acids (PNAs) play the role of a chimera between RNA and proteins.<sup>7</sup> 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.<sup>8,9</sup> PNAs showed important biological activities,<sup>10</sup> including the inhibitory effect against a large panel of viral diseases.<sup>11,12</sup> The prebiotic synthesis of PNA's building blocks includes the condensation of formamide and HCN oligomers, such as aminomalonnitrile (AMN) and diaminomaleonitrile (DAMN).<sup>5,13,14</sup> In the latter cases, urea and guanidine<sup>15</sup> have been involved in multicomponent procedures with cyanoacetaldehyde,<sup>16,17</sup> malic acid,<sup>18</sup> acrylonitrile,<sup>19</sup> propionic acid,<sup>20</sup> and  $\beta$ -alanine.<sup>21–23</sup> In addition, they favored the ring-closing annulation and aromatization in the transformation of pyrimidines and purines.<sup>24</sup> 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.<sup>25,26</sup> 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.<sup>27–32</sup> The novel purine derivatives showed high inhibitory activity against influenza A virus, encompassing the range of nanomolar activity.

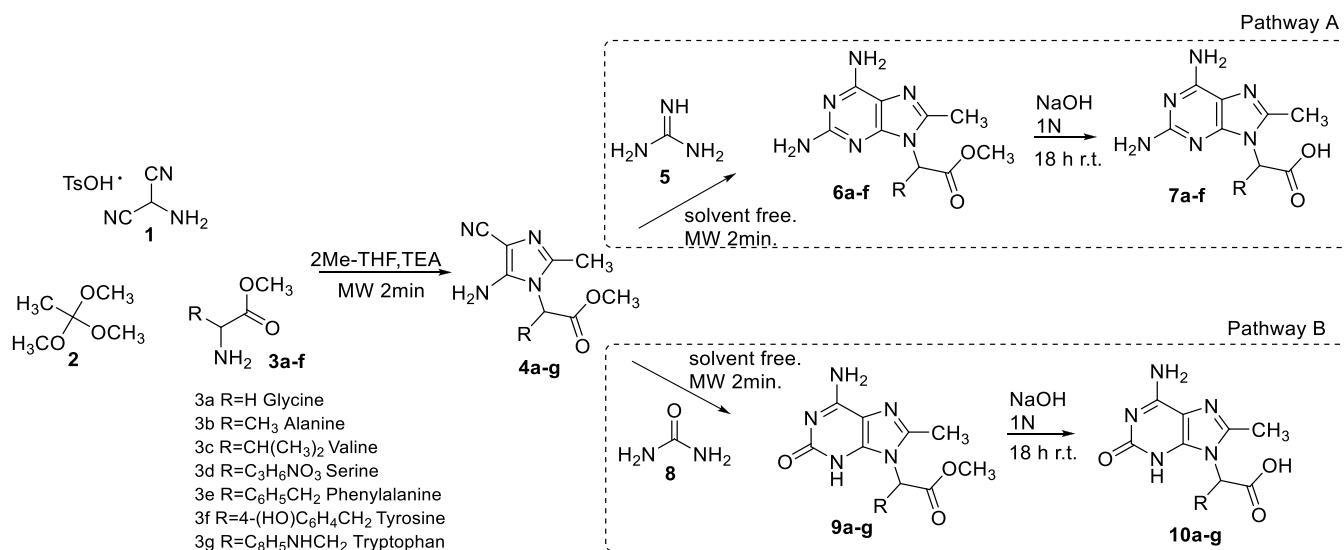
## RESULTS AND DISCUSSION

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

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## 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<sup>a,b</sup>

Entry	$\alpha$ -amino acid	Condition	Products	R	Yield (%) <sup>[b]</sup>
1	Glycine	THF, 2.0 min, 250 W, 250 psi at 200 °C	4a	H	60
2		CH <sub>2</sub> Cl <sub>2</sub> , 2.0 min, 250 W, 250 psi at 200 °C			20
3		CH <sub>3</sub> CN, 2.0 min, 250 W, 250 psi at 200 °C			50
4		(CH <sub>3</sub> O) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> , 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	2Me-THF, 2.0 min, 250 W, 250 psi at 200 °C	4b	CH <sub>3</sub>	37
7	Valine		4c	CH(CH <sub>3</sub> ) <sub>2</sub>	35
8	Serine		4d	CH <sub>2</sub> OH	29
9	Phenylalanine		4e		56
10	Tyrosine		4f		52
11	Tryptophan		4g		50

<sup>a</sup>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.

<sup>b</sup>Yield has been calculated on the basis of the amount of the recovered product. Reactions were performed in triplicate.

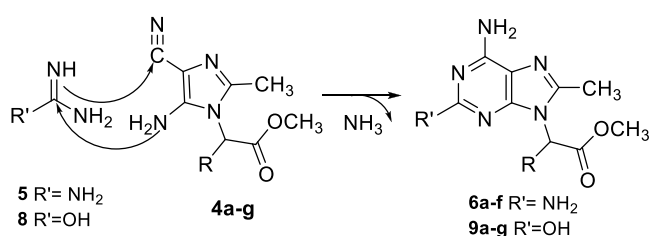
(2-MeTHF, or in alternative, EG; 30 mL) and in the presence of triethylamine (7.1 mmol, 1.0 mL; TEA), working at room temperature for 30 min, followed by microwave irradiation (250 W, 250 psi) at 200 °C for 2 min (Scheme 1). The reaction with tetrahydrofuran (THF) was also performed as a reference.<sup>25</sup> 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.<sup>33</sup> Note that 2-MeTHF performed better than

traditional multicomponent reaction solvents such as methylene dichloride (CH<sub>2</sub>Cl<sub>2</sub>) and acetonitrile (CH<sub>3</sub>CN) (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.<sup>34</sup>

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.<sup>35</sup> 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 significant 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

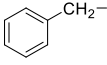
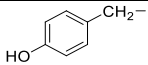
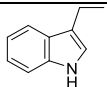
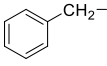
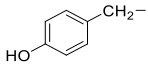
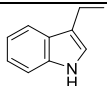
Table 2. Synthesis of Diaminopurine Analogues **6a–f** and **7a–f**<sup>a,b,c,d</sup>

Entry	$\alpha$ -amino acid	Condition	Products	R	Yield (%) <sup>d1</sup>
1	Glycine	Solvent free, 120.0 min, at 250 °C <sup>a1</sup>	<b>6a</b>	H	21
2					38
3	Alanine	Solvent free, MW 2.0 min, 150 W, 250 psi at 250 °C <sup>b1</sup>	<b>6b</b>	CH <sub>3</sub>	25
4	Valine		<b>6c</b>	CH(CH <sub>3</sub> ) <sub>2</sub>	31
5	Serine		<b>6d</b>	CH <sub>2</sub> OH	25
6	Phenylalanine		<b>6e</b>		40
7	Tyrosine		<b>6f</b>		42
8	Glycine	NaOH, 18 hrs, 25 °C <sup>c1</sup>	<b>7a</b>	H	98
9	Alanine		<b>7b</b>	CH <sub>3</sub>	95
10	Valine		<b>7c</b>	CH(CH <sub>3</sub> ) <sub>2</sub>	99
11	Serine		<b>7d</b>	CH <sub>2</sub> OH	96
11	Phenylalanine		<b>7e</b>		95
12	Tyrosine		<b>7f</b>		98

<sup>a1</sup>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.

<sup>b1</sup>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. <sup>c1</sup>NaOH (1.0 N, 1.0 mL) stirring for 18 h at 25 °C. <sup>d1</sup>Yield has been calculated on the basis of the amount of the recovered product. Reactions were performed in triplicate.

Table 3. Synthesis of Guanine Analogues 9a–g and 10a–g<sup>a,b,c,d</sup>

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

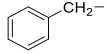
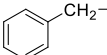
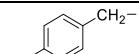

<sup>a</sup>Reaction conditions: **4a–g** (0.80 mmol), urea **8** (1.60 mmol, 2 equiv) under solvent-free thermal heating for 120 min at 250 °C. <sup>b</sup>Reaction conditions: **4a–g** (0.80 mmol), urea **8** (1.60 mmol, 2 equiv) under solvent-free microwave irradiation (150 W, 250 psi) for 2 min at 250 °C. <sup>c</sup>NaOH (1.0 N, 1.0 mL) stirring for 18 hrs at 25 °C. <sup>d</sup>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,<sup>36,37</sup> 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.<sup>38,39</sup> For example, pyrimidine-like PNA's derivatives showed selective pair with polypurine sequences of double-helical RNA,<sup>40</sup> while 2-amino pyridines' counterpart<sup>41</sup> interferes with RNA editing.<sup>42</sup>

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 IC<sub>50</sub>, CC<sub>50</sub>, and relative selective index (SI). Compounds **6f**, **7f**, **9a**, **10a**, **9f**, and **10f** showed the highest SI values, and IC<sub>50</sub> values were closely related to the most used NA inhibitor oseltamivir-carboxylate concentrations in cell cultures.<sup>43,44</sup> As a general trend, DAPAs and GAs showed comparable antiviral activity, and derivatives bearing a free carboxylic moiety showed an IC<sub>50</sub> 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 IC<sub>50</sub> values of 0.020, 0.024, 0.023, and 0.035  $\mu$ M, respectively, and the highest CC<sub>50</sub> 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.<sup>45–47</sup>

Table 4. Biological Activity of Compounds 6a–f, 7a–f, 9a–g, and 10a–g against Influenza A Virus<sup>a,b,c</sup>

Entry	Class	Amino acid	R	Products	IC50 <sup>[a]</sup>	CC50 <sup>[b]</sup>	SI <sup>[c]</sup>
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				6b	n.a	0.790	-
5		Alanine	CH <sub>3</sub>	7b	n.a	0.846	-
6				6c	n.a	0.718	-
7				7c	n.a	0.756	-
8		Valine	CH(CH <sub>3</sub> ) <sub>2</sub>	6d	n.a	0.318	-
9				7d	n.a	0.350	-
10				6e	0.039	0.420	10.5
11		Phenylalanine		7e	0.210	0.610	2.9
12				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
15	10a				0.039	0.764	19.2
16	9b				n.a	0.715	-
17	Alanine		CH <sub>3</sub>	10b	n.a	0.821	-
18				9c	n.a	0.650	-
19				10c	n.a	0.420	-
20	Valine		CH(CH <sub>3</sub> ) <sub>2</sub>	9d	n.a	0.218	-
21				10d	n.a	0.323	-
22				9e	0.340	0.710	2.1
23	Phenylalanine			10e	0.520	0.654	1.3
24				9f	0.023	0.582	25.3
25				10f	0.035	0.604	17.3
26	Tyrosine			9g	0.310	0.690	2.2
27		10g		0.510	0.673	1.3	
	Tryptophan						

<sup>a</sup>IC50 is the drug concentration ( $\mu\text{mol}$ ) causing 50% inhibition of the desired activity. Each experiment was conducted in triplicate. <sup>b</sup>CC50 is the drug concentration ( $\mu\text{mol}$ ) causing 50% of death of the viable cell. <sup>c</sup>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,<sup>25</sup> 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<sub>2</sub>Cl<sub>2</sub>. 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<sup>15</sup>), 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  $\mu\text{M}$  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



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.<sup>48</sup> 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 <sup>1</sup>H and <sup>13</sup>C-NMR spectra are in parts per million and internally referenced to DMSO-*d*<sub>6</sub>. 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<sub>2</sub>CO<sub>3</sub> (3 × 20 mL) and saturated aqueous NaCl (1 × 20 mL). The organic layer was treated with Na<sub>2</sub>SO<sub>4</sub> 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

**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

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%.

**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 <sup>1</sup>H-NMR and <sup>13</sup>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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 6.13 (s, 2H, NH<sub>2</sub>), 4.73 (s, 2H, CH<sub>2</sub>), 3.71 (s, 3H, O-CH<sub>3</sub>), 2.07 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 168.51 (C=O), 148.88 (C), 140.10 (C), 118.08 (C), 88.53 (C), 52.91 (O-CH<sub>3</sub>), 43.91 (CH<sub>2</sub>), 13.29 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) +195.19. Elemental analysis for C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub> 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. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, ppm): δ 4.89–4.87 (m, 1H, CH), 4.15 (s, 2H, NH<sub>2</sub>), 3.84 (s, 3H, O-CH<sub>3</sub>), 2.34 (s, 3H, CH<sub>3</sub>), 1.76 (d, *J* = 7.6 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>, ppm): δ 170.57 (C=O), 145.71 (C), 140.66 (C), 115.58 (C), 96.24 (C), 53.37 (CH), 53.29 (O-CH<sub>3</sub>), 15.96 (CH<sub>3</sub>), 14.09 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) +209.22. Elemental analysis for C<sub>9</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 6.04 (s, 2H, NH<sub>2</sub>), 4.57 (d, *J* = 10.8 Hz, 1H, CH), 3.70 (s, 3H, O-CH<sub>3</sub>), 2.59–2.55 (m, 1H, CH), 2.12 (s, 3H, CH<sub>3</sub>), 1.11 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub>), 0.64 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 169.80 (C=O), 148.93 (C), 139.72 (C), 117.72 (C), 89.92 (C), 61.88 (CH), 53.19 (O-CH<sub>3</sub>), 28.37 (CH), 20.67 (CH<sub>3</sub>), 18.86 (CH<sub>3</sub>), 14.72 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) +237.28. Elemental analysis for C<sub>11</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub> 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.

Compound **4d** was isolated as a beige solid.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  6.89 (s, 1H, OH), 5.87 (s, 2H, NH<sub>2</sub>), 5.47–5.09 (m, 2H, CH<sub>2</sub>), 3.68 (s, 3H, O-CH<sub>3</sub>), 3.66–3.61 (m, 1H, CH), 2.11 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>2</sub>), 59.86 (CH), 53.09 (O-CH<sub>3</sub>), 14.55 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 225.22. Elemental analysis for C<sub>9</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub> 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.  $^1\text{H-NMR}$  (400 MHz, CDCl<sub>3</sub>, 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<sub>2</sub>), 4.32 (s, 2H, NH<sub>2</sub>), 3.87 (s, 3H, O-CH<sub>3</sub>), 3.56 (t,  $J$  = 12.8 Hz, 1H, CH), 3.38–3.34 (dd,  $J$  = 4.4, 13.6 Hz, 1H, CH<sub>2</sub>), 1.81 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (100 MHz, CDCl<sub>3</sub>, 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<sub>3</sub>), 35.29 (CH<sub>2</sub>), 13.45 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 285.32. Elemental analysis for C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub> 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.  $^1\text{H-NMR}$  (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<sub>2</sub>), 5.19–5.16 (m, 1H, CH), 3.72 (s, 3H, O-CH<sub>3</sub>), 3.26–3.22 (m, 2H, CH<sub>2</sub>), 1.79 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>3</sub>), 34.19 (CH<sub>2</sub>), 14.56 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 301.32. Elemental analysis for C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub> 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.  $^1\text{H-NMR}$  (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<sub>2</sub>), 5.30 (t,  $J$  = 7.8 Hz, 1H, CH), 3.77 (s, 3H, O-CH<sub>3</sub>), 3.54 (d,  $J$  = 8 Hz, 2H, CH<sub>2</sub>), 1.74 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>3</sub>), 25.27 (CH<sub>2</sub>), 14.16 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 324.14. Elemental analysis for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub> 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.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  6.89 (s, 2H, NH<sub>2</sub>), 6.12 (s, 2H, NH<sub>2</sub>), 4.71 (s, 2H, CH<sub>2</sub>), 3.68 (s, 3H, O-CH<sub>3</sub>), 2.24 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>3</sub>), 48.81 (CH<sub>2</sub>), 13.15 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 237.24. Elemental analysis for C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>2</sub> 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.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  6.90 (s, 2H, NH<sub>2</sub>), 6.27 (s, 2H, NH<sub>2</sub>), 4.52–4.51 (m, 1H, CH), 3.75 (s, 3H, O-CH<sub>3</sub>), 2.13 (s, 3H, CH<sub>3</sub>), 1.29 (d,  $J$  = 7.2 Hz, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>3</sub>), 22.86 (CH<sub>3</sub>), 13.02 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 251.26. Elemental analysis for C<sub>10</sub>H<sub>14</sub>N<sub>6</sub>O<sub>2</sub> 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.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  6.76 (s, 2H, NH<sub>2</sub>), 6.06 (s, 2H, NH<sub>2</sub>), 5.00 (d,  $J$  = 9.6 Hz, 1H, CH), 3.72 (s, 3H, O-CH<sub>3</sub>), 2.71–2.67 (m, 1H, CH), 2.12 (s, 3H, CH<sub>3</sub>), 1.18 (d,  $J$  = 6.4 Hz, 3H, CH<sub>3</sub>), 0.88 (d,  $J$  = 6.4 Hz, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>3</sub>), 25.02 (CH), 20.00 (CH<sub>3</sub>), 16.36 (CH<sub>3</sub>), 14.57 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 279.32. Elemental analysis for C<sub>12</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub> 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.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  6.97 (s, 2H, NH<sub>2</sub>), 6.46 (s, 1H, OH), 6.05 (s, 2H, NH<sub>2</sub>), 4.41–4.39 (m, 2H, CH<sub>2</sub>), 3.77 (s, 3H, O-CH<sub>3</sub>), 3.64–3.62 (m, 1H, CH), 2.13 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>2</sub>), 52.40 (O-CH<sub>3</sub>), 14.12 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 267.26. Elemental analysis for C<sub>10</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub> 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.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  7.22–7.02 (m, 5H, CH-Ar), 6.63 (s, 2H, NH<sub>2</sub>), 6.21 (s, 2H, NH<sub>2</sub>), 5.22–5.20 (m, 1H, CH), 3.63 (s, 3H, O-CH<sub>3</sub>), 3.23–3.18 (dd,  $J$  = 4.0, 14.0 Hz, 1H, CH<sub>2</sub>), 2.95–2.92 (dd,  $J$  = 6.8, 9.0 Hz, 1H, CH<sub>2</sub>), 2.22 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  (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<sub>3</sub>), 34.88 (CH<sub>2</sub>), 13.09 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 327.36. Elemental analysis for C<sub>16</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub> 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.  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ , ppm):  $\delta$  9.29 (s, 1H, OH), 7.11 (s, 2H, NH<sub>2</sub>), 6.77 (d,  $J$  =

8.8 Hz, 2H, CH-Ar), 6.61 (d,  $J = 8.4$  Hz, 2H, CH-Ar), 5.95 (s, 2H, NH<sub>2</sub>), 5.19–5.17 (m, 1H, CH), 3.71 (s, 3H, O-CH<sub>3</sub>), 2.89–2.85 (dd,  $J = 6.0, 14.0$  Hz, 1H, CH<sub>2</sub>), 2.77–2.72 (dd,  $J = 9.2, 14.0$  Hz, 1H, CH<sub>2</sub>), 2.08 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, 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<sub>3</sub>), 34.73 (CH<sub>2</sub>), 13.71 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 343,36. Elemental analysis for C<sub>16</sub>H<sub>18</sub>N<sub>6</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm):  $\delta$  4.45 (s, 2H, CH<sub>2</sub>), 2.17 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, ppm):  $\delta$  169.03 (C=O), 160.05 (C), 155.87 (C), 152.92 (C), 147.19 (C), 115.74 (C), 49.74 (CH<sub>2</sub>), 13.32 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 223,21. Elemental analysis for C<sub>8</sub>H<sub>10</sub>N<sub>6</sub>O<sub>2</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm):  $\delta$  4.61–4.55 (m, 1H, CH), 2.08 (s, 3H, CH<sub>3</sub>), 1.77 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, 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<sub>3</sub>), 13.43 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 237,24. Elemental analysis for C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>2</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm):  $\delta$  4.55 (d,  $J = 3.6$  Hz, 1H, CH), 2.41–2.37 (m, 1H, CH), 2.17 (s, 3H, CH<sub>3</sub>), 0.99 (d,  $J = 6.4$  Hz, 3H, CH<sub>3</sub>), 0.81 (d,  $J = 6.8$  Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, 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<sub>3</sub>), 16.31 (CH<sub>3</sub>), 13.55 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 265,29. Elemental analysis for C<sub>11</sub>H<sub>16</sub>N<sub>6</sub>O<sub>2</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm):  $\delta$  5.45–5.41 (m, 1H, CH), 4.18–4.15 (m, 2H, CH<sub>2</sub>), 2.12 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, 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<sub>2</sub>), 13.31 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 253,23. Elemental analysis for C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, 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<sub>2</sub>), 3.03–2.97 (dd,  $J = 4.4, 14.0$  Hz, 1H, CH<sub>2</sub>), 2.16 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, 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<sub>2</sub>), 14.41 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 313,33. Elemental analysis for C<sub>15</sub>H<sub>16</sub>N<sub>6</sub>O<sub>2</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, 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<sub>2</sub>), 2.06 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, 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<sub>2</sub>), 13.79 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 329,33. Elemental analysis for C<sub>15</sub>H<sub>16</sub>N<sub>6</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm):  $\delta$  11.28 (s, 1H, NH), 6.13 (s, 2H, NH<sub>2</sub>), 4.15 (s, 2H, CH<sub>2</sub>), 3.70 (s, 3H, O-CH<sub>3</sub>), 2.07 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, 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<sub>3</sub>), 45.08 (CH<sub>2</sub>), 13.52 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 238,22. Elemental analysis for C<sub>9</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm):  $\delta$  10.22 (s, 1H, NH), 6.16 (s, 2H, NH<sub>2</sub>), 4.72–4.68 (m, 1H, CH), 3.65 (s, 3H, O-CH<sub>3</sub>), 2.26 (s, 3H, CH<sub>3</sub>), 1.81 (d,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, 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<sub>3</sub>), 21.48 (CH<sub>3</sub>), 13.32 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 252,25. Elemental analysis for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm):  $\delta$  11.11 (s, 1H, NH), 6.16 (s, 2H, NH<sub>2</sub>), 4.69 (d,  $J = 3.6$  Hz, 1H, CH), 3.65 (s, 3H, O-CH<sub>3</sub>), 2.85–2.79 (m, 1H, CH), 2.26 (s, 3H, CH<sub>3</sub>), 1.15 (d,  $J = 6.4$  Hz, 3H, CH<sub>3</sub>), 0.76 (d,  $J = 6.8$  Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, 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<sub>3</sub>), 26.14 (CH), 19.81 (CH<sub>3</sub>), 17.48 (CH<sub>3</sub>), 13.82 (CH<sub>3</sub>). MS (ESI):  $m/z$  (M + H) + 280,30. Elemental analysis for C<sub>12</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm):  $\delta$  10.09 (s, 1H, NH), 6.89 (s, 1H, OH), 6.17 (s, 2H, NH<sub>2</sub>), 5.45–5.43 (m, 1H, CH), 5.02–4.99 (m, 1H, CH<sub>2</sub>), 3.68 (s, 3H, O-CH<sub>3</sub>), 3.66–3.61 (m, 1H, CH<sub>2</sub>), 2.14 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm):  $\delta$  169.49 (C=O), 158.67 (C), 154.17 (C), 151.34 (C), 148.18 (C), 111.55 (C),



62.93 (CH), 60.94 (CH<sub>2</sub>), 53.11 (O-CH<sub>3</sub>), 13.65 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 268,25. Elemental analysis for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 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<sub>2</sub>), 5.10–5.06 (m, 1H, CH), 3.69 (s, 3H, O-CH<sub>3</sub>), 3.22–3.17 (dd, *J* = 5.6, 14.0 Hz, 1H, CH<sub>2</sub>), 3.13–3.07 (dd, *J* = 7.6, 14.0 Hz, 1H, CH<sub>2</sub>), 2.05 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 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<sub>3</sub>), 35.01 (CH<sub>2</sub>), 14.35 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 328,34. Elemental analysis for C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 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<sub>2</sub>), 4.99–4.95 (m, 1H, CH), 3.74 (s, 3H, O-CH<sub>3</sub>), 2.89–2.85 (dd, *J* = 6.0, 14.0 Hz, 1H, CH<sub>2</sub>), 2.77–2.72 (dd, *J* = 9.2, 14.0 Hz, 1H, CH<sub>2</sub>), 2.03 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 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<sub>3</sub>), 34.20 (CH<sub>2</sub>), 15.04 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 344,34. Elemental analysis for C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub> 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. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 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<sub>2</sub>), 5.25 (t, *J* = 4.6 Hz, 1H, CH), 3.64 (s, 3H, O-CH<sub>3</sub>), 3.45 (d, *J* = 4.0 Hz, 2H, CH<sub>2</sub>), 1.99 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm): δ 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<sub>3</sub>), 26.94 (CH<sub>2</sub>), 13.55 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 367,14. Elemental analysis for C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm): δ 4.55 (s, 2H, CH<sub>2</sub>), 2.16 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, ppm): δ 169.61 (C=O), 157.66 (C), 153.33 (C), 151.25 (C), 147.96 (C), 110.88 (C), 43.83 (CH<sub>2</sub>), 13.17 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 224,19. Elemental analysis for C<sub>8</sub>H<sub>9</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm): δ 4.72–4.66 (m, 1H, CH), 2.18 (s, 3H, CH<sub>3</sub>), 1.88 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, ppm): δ 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<sub>3</sub>), 13.48 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 238,22. Elemental analysis for C<sub>9</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm): δ 4.40 (d, *J* = 10.8 Hz, 1H, CH), 2.24–2.20 (m, 1H, CH), 2.08 (s, 3H, CH<sub>3</sub>), 0.78 (d, *J* = 6.8 Hz, 3H, CH<sub>3</sub>), 0.61 (d, *J* = 6.8 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, ppm): δ 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<sub>3</sub>), 16.82 (CH<sub>3</sub>), 14.15 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 266,27. Elemental analysis for C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm): δ 5.42–5.40 (m, 1H, CH), 3.62–3.50 (m, 2H, CH<sub>2</sub>), 2.01 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, ppm): δ 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<sub>2</sub>), 13.48 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 254,22. Elemental analysis for C<sub>9</sub>H<sub>11</sub>N<sub>5</sub>O<sub>4</sub> 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. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD, ppm): δ 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<sub>2</sub>), 2.12 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD, ppm): δ 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<sub>2</sub>), 14.65 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 314,32. Elemental analysis for C<sub>15</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub> 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-2H-purin-2-one. The crude residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane. <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O, ppm): δ 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<sub>2</sub>), 2.98 (d, *J* = 14.8 Hz, 1H, CH<sub>2</sub>), 2.12 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, D<sub>2</sub>O, ppm): δ 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<sub>2</sub>), 15.27 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 330,32. Elemental analysis for C<sub>15</sub>H<sub>15</sub>N<sub>5</sub>O<sub>4</sub> 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.

**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. <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O, ppm): δ 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<sub>2</sub>), 1.99 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, D<sub>2</sub>O, ppm): δ 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<sub>2</sub>), 13.89 (CH<sub>3</sub>). MS (ESI): *m/z* (M + H) + 353.13. Elemental analysis for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>O<sub>3</sub> 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 μL 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 μMol with the selected compound for 24 h at 37 °C. After 24 h, 10 μL 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 μL/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.<sup>49</sup>

## ■ ASSOCIATED CONTENT

### SI Supporting Information

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

Original <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of compounds **4a–g**, **6a–f**, **7a–f**, **9a–g** and **10a–g** (PDF)

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### Notes

The authors declare no competing financial interest.

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