

Microwave Assisted Synthesis of 6-Substituted Aminopurine Analogs in Water

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Aminação de derivados de 6-cloropurina em água, assistida por microondas, resultou na preparação de análogos de aminopurina 6-substituídas, em bons rendimentos. Usando um forno de microondas simples, modificado com aparelhagem para refluxo, a aaminação do 6-cloro na estrutura da purina ocorreu em condições brandas. Foram preparados 19 análogos conhecidos e 16 desconhecidos de aminopurinas substituídas, através de substituição aromática nucleofílica com filtração simples ou coluna de cromatografia.

Microwave assisted amination of 6-chloropurine derivatives with various amines in water resulted in a "green chemistry" protocol for the preparation of 6-substituted aminopurine analogs in very good yields. Using a simply modified microwave oven with the refluxing apparatus, the amination of the 6-chloro in the purine structure occurred smoothly. 19 known and 16 unknown 6-substituted aminopurine analogs were prepared through nucleophilic aromatic substitution with simple filtration or column chromatography.

Keywords: amination, 6-substituted aminopurine analogs, nucleophilic substitution, microwave irradiation, amines

Introduction

In modern antiviral and antitumor therapy, an important role is played by modified nucleosides and their analogs, in which modified purine structures are frequently found.^{1,2} 6-Substituted aminopurine analogs, the aminated products of the 6-functional groups in purine structures, continued focusing attention due to their wide range of biological activities (inhibitors of *Clostridium fesi* growth,³ cytokinin activity,⁴ CIV-CDK (CIV1) and *Candida albicans* as antifungal medicines,⁵ selective kinase,⁶ agonists of the A₁ adenosine receptor,⁷ the cysteine protease cathepsin K,⁸ and platelet aggregation⁹). Prominent examples of synthetic 6-substituted aminopurine analogs are *N*-cyclopentyl adenosine (CPA) and *N*-cyclohexyl adenosine (CHA) (two agonists for the adenosine A₁ receptors).¹⁰

The traditional method for the synthesis of 6-substituted aminopurine analogs is the amination of halo,¹¹ oxo,¹² mercapto or methylmercapto¹³ groups with various amines, which can be performed smoothly in organic solvents (BuOH,¹¹ CH₃CN,¹³ dioxane,¹⁴ DMF,¹⁵ or

DMSO¹⁶) in the presence of tertiary amines (Et₃N, *N,N*-dimethyl cyclohexylamine or diisopropylethylamine) and the catalysts (P₂O₅,¹⁴ K₂CO₃ and Cu/K₃PO₄¹⁶). However, the complete conversion usually needs 2-24 h and the use of toxic organic solvents or relatively expensive reagents could not be avoided, which dose not conform to the requirement of modern pharmaceutical industry and environmental friendliness.

In any case, the development of efficient protocols for the synthesis of aminopurine analogs is still an important goal. Mild reaction conditions, short reaction times and high selectivity and yields are preferable, which makes us pay attention to microwave irradiation. Microwave assisted organic synthesis and functional group conversions have been an increasingly popular field as indicated by numerous publications in the past few years.¹⁷ It often leads to rate enhancement, higher yields, easier work-up and better selectivity as well as shortening reaction times compared with the conventional heating methods. In spite of the body of literature about microwave accelerated organic synthesis, the references on the microwave-assisted synthesis of nucleoside compounds are only a few examples. In our previous research, we have prepared a series of modified nucleosides under microwave irradiation.¹⁸

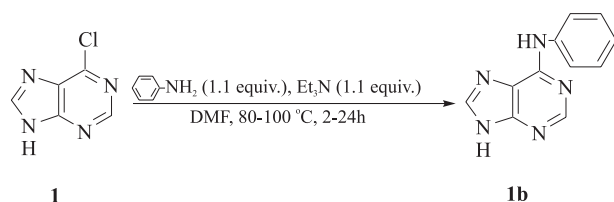
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To avoid the contamination of organic solvents, we turn to water. Water as a kind of environmentally benign solvent for organic reactions has attracted more and more attention in recent years.¹⁹ Synthesis using water as the solvent has several advantages, such as low toxicity, low cost, high yields and ease of manipulation.^{20,21}

In this paper, we described a facile, efficient and eco-friendly protocol for the preparation of 6-substituted aminopurine analogs based on the nucleophilic substitution of 6-chloropurine derivatives with various amines in water under microwave irradiation. Using this green method, we obtained 19 known and 16 unknown 6-substituted aminopurine analog compounds in moderate to high yields.

Results and Discussion

In view of the limitations of the existing methods, a study of microwave assisted synthesis of 6-substituted aminopurine analogs in water using a simply modified microwave oven with the refluxing apparatus was undertaken. First of all the amination of model 6-chloropurine **1** with aniline producing **1b** by conventional heating methods was reexamined (Scheme 1).



Scheme 1. Amination of **1** with aniline in the original condition.

As shown in Scheme 1, the original conditions involved the use of Et₃N (1.1 equiv.) and the keeping of the reaction mixture in DMF at 80-100 °C for 2-24 h. Based on these conditions, different solvents and different equivalents of

aniline and Et₃N to the precursor 6-chloropurine **1** were tested, widening the set of parameters (Table 1).

Table 1 summarized the studies carried out into variation of these conditions. In the original condition, the maximum conversion was reached at approximately 18h and 80 °C (compare entries 1, 2, 3 and 4 in Table 1), so we fixed 80 °C and 18h as the reaction temperature and reaction time to test the other conditions. It was quickly proved that the presence of Et₃N was unnecessary. We found **1b** could be also obtained in good yields when the equivalent of aniline was increased to 5 (compare entries 6, 9, 11, 12 in Table 1) but in lower yields when it was remained to 1.1 (entries 5 and 8) in the absence of Et₃N. The results showed that the presence of Et₃N strengthened the base of the reaction mixture and the nucleophilicity of aniline, which could be complemented by the enhanced amount of aniline.

The solvent was examined subsequently. As shown in Table 1 (entries 6, 9, 11, 12), the solvent could be replaced by H₂O and the product **1b** was still formed in good yield. The possible explanation is that the polarity of water is lower than DMF and higher than BuOH and EtOH, which makes water an appropriate medium to allow **1b** to crystallize from the reaction system. The experimental fact proved it that 6-chloropurine **1** solved completely after the addition of colorless aniline to its suspension in water and **1b** precipitated out from the aqueous solution because of lower polarity and solubility of **1b** in water than that of **1**.

Finally, the amount of aniline was examined. Although **1b** formed in good yields as the equivalent of aniline to **1** was 5, there was unreacted aniline left and need to wash many times, which made some loss of **1b**. The excellent results were obtained when the equivalent was 3 (compare entries 9 and 10 in Table 1).

Table 1. Influence of variation of conditions on the yields **1b**

Entry	Aniline equiv.	Et ₃ N equiv.	Solvent	Reaction T/°C	time/h	Yield/ % ^a
1	1.1	1.1	DMF	80	12	65
2	1.1	1.1	DMF	80	18	71
3	1.1	1.1	DMF	80	24	68
4	1.1	1.1	DMF	100	18	63
5	1.1	0	DMF	80	18	52
6	5	0	DMF	80	18	73
7	1.1	1.1	H ₂ O	80	18	79
8	1.1	0	H ₂ O	80	18	56
9	5	0	H ₂ O	80	18	80
10	3	0	H ₂ O	80	18	83
11	5	0	BuOH	80	18	70
12	5	0	EtOH	80	18	71
13 ^b	3	0	H ₂ O	72	10 min	87
14 ^b	3	0	H ₂ O	72	8 min	85

^a Isolated yield. ^b Microwave Irradiation (200 W, 72 °C). The temperature was measured immediately after the irradiation.

Running the reaction with 3 equiv. aniline in water under microwave irradiation appeared to proceed more efficiently (entries 13 and 14 in Table 1). At 200W for 2 min, the reaction mixture began to boil and could be condensed by the refluxing apparatus. The attempt to increase microwave power in order to reduce reaction time is unsuccessful because the reaction vapor could not be condensed as soon as possible with the elevated temperature quickly, which would increase the possibility of the release of aniline to the air.

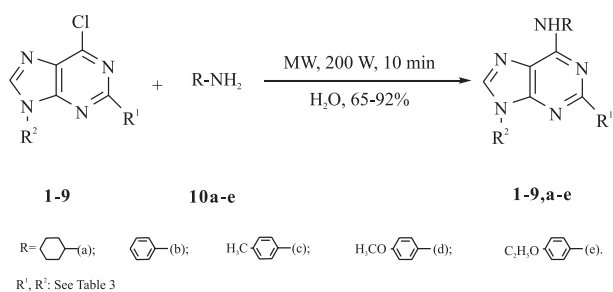
After some experiments, the best procedure for the irradiation is described as follows. The reaction mixtures were irradiated successively for 2.5 min periods followed by a 3 min cooling interval between irradiations. This method was designed to avoid overheating of reactants since the simply modified microwave oven lacks the attributes of stirring and temperature control.

After fixed the procedure, irradiation time was tested (Table 2). Inspection of data showed the conversion increased with the prolonging of reaction time and the best result was achieved between 8 and 10 min. Compared Table 1 and 2, the conclusion can be easily reached that microwave assisted synthesis of 6-phenylaminopurine **1b** can reduce the long reaction time of conventional thermal methods to 10 min.

Table 2. Influence of different irradiation time on the yields **1b** in water

Entry	Aniline equiv.	Irradiation time/min	Isolated yield/%
1	3	5	72
2	3	8	85
3	3	10	87
4	3	13	84

Having established a simplified reaction protocol (Scheme 2), we applied the modified conditions to a series of 6-chloropurine derivatives (**1-9**) and various amines (**10a-e**) (Table 3). For comparison most reactions were run for the same irradiation power (200W) and time (10min). The by-product of these reactions, hydrogen



Scheme 2. The synthesis of 6-substituted aminopurine analogs **1-9,a-e** by the modified amination protocol of 6-chloropurine derivatives **1-9** with various amines **10a-e**.

chloride, was quenched by amines, used as nucleophiles and bases, forming ammonium salts, thus avoiding its release into the environment. The existence of ammonium salts was not an obstacle to the purification of 6-substituted aminopurine analogs because the latter are the crystalline solids, which are poorly soluble in water and can be separated easily from the former by direct filtration.

We found this protocol allowed 6-substituted aminopurine **1-3,a-e** to be isolated with moderate to high yields (entries

Table 3. Amination of 6-chloropurine derivatives **1-9** with various primary amines **10a-e** in water under microwave irradiation

Entry	6-chloropurine derivatives	6-substituted aminopurine analogs	Yield/% ^a
1		1a	79
2		1b	87
3		1c	85
4		1d	82
5		1e	81
6		2a	90
7		2b	87
8		2c	82
9		2d	88
10		3a	78
11		3b	72
12		3c	65
13		3d	68
14		3e	70
15		4a	86
16		4b	92
17		4c	82
18		4d	83
19		5a	80
20		5b	89
21		5c	91
22		5d	83
23		5e	85
24		6a	82
25		6b	80
26		6c	81
27		6d	81
28		6e	79
29		7a	87
30		7b	91
31		7c	88
32		7d	85
33		7e	82
34		8a	34 ^b
		8a'	26
35		9a	42 ^b
		9	
		9	

^a Isolated yield. ^b Deacetylated products.

1-14 in Table 2). For 6-chloropurine **1** and 2,6-dichloropurine **2**, on adding the amines to their suspension a clean solution and a heterogeneous oil phase at the bottom of the flask could be obtained because the amines helped **1** and **2** solve in water. When irradiated, the reaction mixture boiled within a few seconds and the two phases merged well and reacted quickly. The refluxing apparatus made the reaction vapor and the amines with low boiling points return to the reaction systems. Then the mixtures were cooled to room temperature and some solids crystallized slowly. After filtering and washing, the crystals obtained showed short melting ranges and comprehensive attributes of amine and purine structure in NMR and IR, which indicated the desired products **1a-e** and **2a-d** were prepared. After the mixture of **2** and *p*-ethoxyphenyl amine **10e** was irradiated, a white solid emerged which polarity was so high that it could not solve even in DMF and DMSO, so it was given up. For 2-amino-6-chloropurine **3**, after the addition of amines and during the irradiation, no clean solutions were obtained except the reaction with cyclohexylamine **10a**. **3a** crystallized slowly from the solution after cooled to room temperature about 30 min, but **3b-e** were formed during the reaction, which could be proved by NMR that the solids filtered completely were expected products.

We prepared 6-chloro substituted purine acyclic nucleosides **4**, **5**, **6** and **9** according to the reported methods,²² and applied **4-6** to our aminating protocol, then obtained the desired acyclic 6-substituted aminopurine nucleosides **4-6,a-e** in high yields after column chromatography purification (entries 15-28 in Table 3). The products **4-6,a-e** are new compounds and their structures, proven by NMR, IR and MS, are described in the experimental section and Supplementary Information. Under the same conditions, 6-chloropurine nucleoside **7** obtained **7a-e** in 82-91% after simply filtration (entries 29-33 in Table 3). As 2', 3', 5'-triacetyl-2,6-dichloropurine nucleoside **8** and 2,6-dichloro-9-[(2-acetoxyethoxy) methyl]purine **9** reacted with **10a**, the deacetylated products **8a** (entry 34) and **9a** (entry 35 in Table 3) were prepared and no satisfactory results were given: (i) No obvious reaction was monitored by TLC immediately after the irradiation; (ii) On a long time standing (2 days), there is no any solid precipitated out. TLC showed the disappearance of **8** and the appearance of deacetylated product **8a** and acetylated product **8a'**. And for **9**, there was about half conversion to the desired product **9a**. After chromatography, **8a**, **8a'** and **9a** were isolated in rather low yields (26-42%). NMR proved the replacement of -OAc to -OH and 6-chloro to 6-cyclohexylamino, which made **8a** and **9a** easily solve in water because of

their increased polarity compared to **8** and **9** and was the possible reason to the above experimental phenomena. It indicated that the differences of solubility in water between the starting materials and the products played an important role when water acted as the reaction medium.

Conclusions

In conclusion, we reported an environmentally benign microwave-assisted protocol for the rapid and direct amination of 6-chloropurine derivatives to prepare 6-substituted aminopurine analogs **1-7,a-e** in 10 min via nucleophilic substitution, making full use of the soluble differences in water between the starting materials and the products. Products with moderate to high purity were isolated by filtration directly and column chromatography. The by-product, hydrogen chloride, is quenched as an ammonium salt in the course of the reaction, avoiding its release to the air, which presents an additional environmentally friendly synthetic advantage.

Experimental

¹H and ¹³C NMR spectra were recorded in DMSO-*d*₆ solutions on a Bruker DPX-400 spectrometer (at 400 MHz and 100 MHz, respectively) using TMS as internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) are given in Hz. IR spectra were recorded on a Bruker Vector 22 spectrometer, using KBr tablets, and the frequency being expressed in cm⁻¹. Mass spectra (ESI) were recorded on an Agilent 1100 (LC-MSD-Tarp-SL) mass spectrometer. Elemental analyses were performed on an EA-1110 (CE Instruments) instrument. Melting points were determined with an XRC-1 micro melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel plates (Merck, silica gel 60F₂₅₄) and column chromatography was performed on silica gel (Merck, 200-300 mesh). All the reactions were carried out in a simply modified microwave oven (SANYO EM-202MSI, 2450MHz) attaching the refluxing apparatus. A hole is designed in the microwave oven through which a round-bottomed flask is fixed and can be refluxed directly. Irradiation time can be prefixed by the time apparatus and output power can be shown in the electric current bell according to the working curve of the oven. For example, 90 mA in electric current bell stands for output power 200W. The reaction temperatures are measured immediately after the stop of irradiation by putting the thermometer into the reaction mixtures.

Procedure for the optimization of the amination conditions

6-chloropurine **1** (0.3 g, 2 mmol) is dissolved in 10 mL of different solvents (see Table 1) in a 50 mL round-bottomed flask and aniline is added to the suspension, which equivalents are 1.1 (0.22 mL, 0.22 mmol), 3 (0.6 mL, 0.6 mmol), 5 (1.0 mL, 10 mmol), respectively. Different amount of Et₃N is added as the design in Table 1, and then keep the oil bath temperature to 80 °C (or 100 °C) for 12h, 18h or 24h. After the reaction, the mixtures (DMF, BuOH, EtOH as the solvents) are concentrated in vacuum. As a result, they become thicker and their colors become deeper (red for DMF and yellow for BuOH, EtOH). Add appropriate amount silica gel (Merck, 200-300 mesh) to the concentrated reaction mixtures and make them disperse well, and then purify by column chromatography with CHCl₃/CH₃OH (9/1, v/v). Solid can be filtered from the mixtures and the filtrate is concentrated to 1/3 volume, some products crystallized again and collected. The yields are listed in Table 1.

Procedure for the optimization of irradiation time and power

On adding 0.6 mL aniline (3 equiv.) to the suspension of 0.3 g **1** in 10 mL water, a clean solution obtained as well as a heterogeneous oil phase at the bottom of the flask. The flask is fixed to the microwave oven and irradiated successively for 2.5 min periods followed by a 3 min cooling interval between irradiation at 200 W for 5 min, 8 min, 10 min and 13 min (that is the irradiation is stopped at the second, third, fourth and fifth interval). Different amounts of product **1b** are obtained and the yields are listed in Table 2.

When irradiated at the power of above 200 W, the reaction mixture boiled violently within 40 seconds and cannot be condensed to return to the flask as soon as possible, which made it spill to the air. To avoid that, we choose 200 W as the irradiation power.

General procedure for the synthesis of 6-substituted aminopurine analogs **1-7,a-e** and **8a, 8a', 9a**

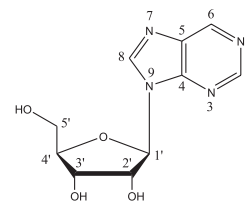
Amine (6 mmol) is added to a stirred suspension of 6-chloropurine derivatives **1-9** (2mmol) in water (10 mL) in a 50 mL round-bottomed flask. After vibration, the flask is moved into microwave oven and irradiated at 200W for 10 minutes. At each interval, TLC monitors the reaction progress. When the reaction completed, the mixture is cooled to room temperature and desired 6-substituted aminopurine analogs **1-3,a-e** and **7a-e** precipitate out. Then filter the solid directly followed by washing with

cold water (3×5 mL). The filtrate is concentrated to 1/3 volume, and collects the products crystallized again from the mixture. The products **4-6,a-e** and **8a, 8a', 9a** are purified by column chromatography with CHCl₃/CH₃OH (9/1, v/v) for **4-6,a-e** and CHCl₃/CH₃OH (97/3, v/v) for **8a, 8a', 9a** after the concentration of the reaction mixtures.

Physical data of the known compounds had been partly reported in the literatures^{4,23-26} and melting points of **2a** and **3a** are not identified with the literatures^{4,23} although the NMR spectra have proved their structures.

Spectroscopic data of new compounds

Note: Assignments of H and C are given according to purine nucleoside numbering:



9-β-cyanoethyl-6-cyclohexylamino purine (4a). White needle crystal; mp 137-139 °C. ¹H NMR (DMSO-*d*₆) δ 1.112-1.881 (m, 10H, H cyclohexyl), 3.168 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.101 (br, 1H, H cyclohexyl), 4.447 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 7.554 (d, 1H, *J* 8.0Hz, NH), 8.187 (s, 1H, H-2), 8.219 (s, 1H, H-8). ¹³C NMR (DMSO-*d*₆) δ 18.56 (CH₂CN), 39.26 (NCH₂CH₂), 24.80, 25.56, 26.25, 32.78, 33.35, 49.11 (C cyclohexyl), 118.72 (CN), 119.33 (5-C), 140.59 (8-C), 149.18 (6-C), 153.03 (4-C), 154.30 (2-C). MS (ESI) *m/z* [M⁺Na⁺-1, 292.8], 270.8, 254.8, 228.8, 203.7, 150.8. IR (KBr) ν_{max}/cm⁻¹: 3398, 3385, 3084, 3036, 2933, 2854, 2250, 1607, 1586, 1475, 1366, 1299, 772. Anal. Calc. for C₁₄H₁₈N₆: C, 62.22; H, 6.67; N, 31.11. Found: C, 61.97; H, 6.73; N, 31.34 %.

9-β-cyanoethyl-6-phenylamino purine (4b). Lustrous flakes; mp 168-170 °C. ¹H NMR (DMSO-*d*₆) δ 3.229 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.538 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 7.049 (t, 1H, *J* 6.4Hz, H_{Ar}), 7.340 (t, 2H, *J* 6.0Hz, H_{Ar}), 7.972 (d, 2H, *J* 8.8Hz, H_{Ar}), 8.390 (s, 1H, H-2), 8.445 (s, 1H, H-8), 9.917 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 18.63 (CH₂CN), 39.46 (NCH₂CH₂), 118.71 (CN), 120.20 (C-5), 121.33, 123.10, 128.82, 140.06 (C_{Ar}), 141.94 (C-8), 149.75 (C-6), 150.04 (C-4), 152.53 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 286.7], 233.8. IR (KBr) ν_{max}/cm⁻¹: 3352, 3087, 3050, 2967, 2261, 1622, 1580, 1476, 1300, 1239, 1148, 1018, 753. Anal. Calc. for C₁₄H₁₂N₆: C, 63.64; H, 4.54; N, 31.82. Found: C, 63.51; H, 4.59; N, 31.93 %.

9-β-cyanoethyl-6-(p-tolylamino) purine (4c). Broken-white powder; mp 173-174 °C. ¹H NMR (DMSO-*d*₆) δ 2.284 (s, 3H, CH₃), 3.218 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.521 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 7.141 (d, 2H, *J* 8.4Hz, H_{Ar}), 7.816 (d, 2H, *J* 8.4Hz, H_{Ar}), 8.364 (s, 1H, H-2), 8.403 (s, 1H, H-8), 9.811 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 18.60 (CH₂CN), 20.91 (CH₃), 39.42 (NCH₂CH₂), 118.71 (CN), 120.07 (C-5), 121.42, 129.23, 132.06, 137.46 (C_{Ar}), 141.78 (C-8), 149.93 (C-6), 151.23 (C-4), 152.56 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 300.7], 145.9. IR (KBr) ν_{\max} /cm⁻¹: 3386, 3089, 2919, 2860, 2249, 1616, 1585, 1476, 1367, 821. Anal. Calc. for C₁₅H₁₄N₆: C, 64.75; H, 5.04; N, 30.22. Found: C, 64.49; H, 5.21; N, 30.35%.

9-β-cyanoethyl-6-(p-methoxyphenylamino) purine (4d). Colorless column crystal; mp 80-82 °C. ¹H NMR (DMSO-*d*₆) δ 3.216 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 3.750 (s, 3H, OCH₃), 4.517 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 6.924 (d, 2H, *J* 8.8Hz, H_{Ar}), 7.794 (d, 2H, *J* 9.2Hz, H_{Ar}), 8.344 (s, 1H, H-2), 8.370 (s, 1H, H-8), 9.765 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 18.62 (CH₂CN), 39.41 (NCH₂CH₂), 55.64 (OCH₃), 118.72 (CN), 119.91 (C-5), 114.06, 123.23, 132.95, 152.63 (C_{Ar}), 141.61 (C-8), 149.82 (C-6), 152.67 (C-4), 155.61 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 316.7], 263.7. IR (KBr) ν_{\max} /cm⁻¹: 3466, 3301, 3204, 3101, 2978, 2881, 2253, 1620, 1587, 1512, 1471, 1294, 1035, 797. Anal. Calc. for C₁₅H₁₄N₆O: C, 61.22; H, 4.76; N, 28.57. Found: C, 61.09; H, 4.84; N, 28.64%.

9-β-cyanoethyl-6-(p-ethoxyphenylamino) purine (4e). Gray flakes; mp 146-148 °C. ¹H NMR (DMSO-*d*₆) δ 1.328 (t, 3H, *J* 7.2Hz, CH₂CH₃), 3.215 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.009 (q, 2H, *J* 7.2Hz, OCH₂CH₃), 4.513 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 6.907 (d, 2H, *J* 9.2Hz, H_{Ar}), 7.783 (d, 2H, *J* 8.8Hz, H_{Ar}), 8.343 (s, 1H, H-2), 8.365 (s, 1H, H-8), 9.758 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 15.16 (OCH₂CH₃), 18.60 (CH₂CN), 39.42 (NCH₂CH₂), 118.72 (CN), 119.90 (C-5), 114.60, 123.15, 132.88, 152.62 (C_{Ar}), 141.61 (C-8), 149.81 (C-6), 154.84 (C-4), 158.64 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 330.8], 308.8. IR (KBr) ν_{\max} /cm⁻¹: 3309, 3231, 3107, 2979, 1930, 2881, 2252, 1618, 1587, 1512, 1479, 1299, 1048, 840. Anal. Calc. for C₁₆H₁₆N₆O: C, 62.34; H, 5.19; N, 27.27. Found: C, 62.11; H, 5.31; N, 27.46%.

2-chloro-9-β-cyanoethyl-6-cyclohexylamino purine (5a). White powder; mp 179-181 °C. ¹H NMR (DMSO-*d*₆) δ 1.107-1.923 (m, 10H, H cyclohexyl), 3.135 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.005 (br, 1H, H cyclohexyl), 4.411 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 8.163 (d, 1H, *J* 8.0Hz, NH), 8.199 (s, 1H, H-8). ¹³C NMR (DMSO-*d*₆) δ 18.62 (CH₂CN), 25.13, 25.36, 25.56, 32.47, 33.48, 49.45 (C cyclohexyl), 38.76

(NCH₂CH₂), 118.38 (C-5), 118.65 (CN), 141.15 (C-8), 150.13 (C-6), 153.79 (C-4), 154.66 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 326.7], 233.8. Anal. Calc. for C₁₄H₁₇ClN₆: C, 55.17; H, 5.62; N, 27.57. Found: C, 54.97; H, 5.69; N, 27.73%.

2-chloro-9-β-cyanoethyl-6-phenylamino purine (5b). White powder; mp 266-268 °C. ¹H NMR (DMSO-*d*₆) δ 3.187 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.492 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 7.113 (t, 1H, *J* 7.2Hz, H_{Ar}), 7.374 (t, 2H, *J* 7.6Hz, H_{Ar}), 7.845 (d, 2H, *J* 8.0Hz, H_{Ar}), 8.388 (s, 1H, H-8), 10.348 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 18.64 (CH₂CN), 39.94 (NCH₂CH₂), 118.65 (CN), 119.20 (C-5), 121.81, 124.03, 128.97, 139.14 (C_{Ar}), 142.49 (C-8), 149.32 (C-6), 151.15 (C-4), 152.91 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 320.7], 298.8, 256.9, 101.9, 88.0. IR (KBr) ν_{\max} /cm⁻¹: 3346, 3087, 3062, 2973, 2935, 2258, 1622, 1578, 1500, 1452, 1318, 1284, 756. Anal. Calc. for C₁₄H₁₁ClN₆: C, 56.28; H, 3.69; N, 28.14. Found: C, 55.97; H, 3.81; N, 28.36%.

2-chloro-9-β-cyanoethyl-6-(p-tolylamino) purine (5c). Lustrous needle crystal; mp 254-256 °C. ¹H NMR (DMSO-*d*₆) δ 2.297 (s, 3H, CH₃), 3.180 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 4.480 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 7.174 (d, 2H, *J* 8.0Hz, H_{Ar}), 7.690 (d, 2H, *J* 8.4Hz, H_{Ar}), 8.363 (s, 1H, H-8), 10.256 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 18.63 (CH₂CN), 20.94 (CH₃), 39.99 (NCH₂CH₂), 118.65 (CN), 119.08 (C-5), 121.99, 129.39, 133.18, 136.51 (C_{Ar}), 142.32 (C-8), 149.54 (C-6), 151.04 (C-4), 152.98 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 334.7], 271.9, 227.9, 145.8, 96.9. IR (KBr) ν_{\max} /cm⁻¹: 3339, 3055, 2930, 2264, 1623, 1579, 1514, 1456, 1252, 818. Anal. Calc. for C₁₅H₁₃ClN₆: C, 57.60; H, 4.16; N, 26.88. Found: C, 57.51; H, 4.22; N, 26.93%.

2-chloro-9-β-cyanoethyl-6-(p-methoxyphenylamino) purine (5d). Lustrous flakes; mp 239-240 °C. ¹H NMR (DMSO-*d*₆) δ 3.178 (t, 2H, *J* 6.4Hz, CH₂CH₂CN), 3.764 (s, 3H, OCH₃), 4.476 (t, 2H, *J* 6.4Hz, NCH₂CH₂), 6.954 (d, 2H, *J* 8.8Hz, H_{Ar}), 7.691 (d, 2H, *J* 8.8Hz, H_{Ar}), 8.343 (s, 1H, H-8), 10.205 (s, 1H, NH). ¹³C NMR (DMSO-*d*₆) δ 18.65 (CH₂CN), 39.49 (NCH₂CH₂), 55.68 (OCH₃), 118.65 (CN), 118.94 (C-5), 114.20, 123.71, 131.96, 150.91 (C_{Ar}), 142.15 (C-8), 153.06 (C-6), 153.12 (C-4), 156.23 (C-2). MS (ESI) *m/z* [M⁺Na⁺-1, 350.7], 328.8, 292.8, 239.8, 224.7, 196.7. IR (KBr) ν_{\max} /cm⁻¹: 3337, 3063, 2962, 2935, 2835, 2258, 1624, 1589, 1512, 1478, 1309, 1248, 1048, 827. Anal. Calc. for C₁₅H₁₃ClN₆O: C, 54.79; H, 3.96; N, 25.57. Found: C, 54.66; H, 4.03; N, 25.64%.

2-amino-9-β-cyanoethyl-6-cyclohexylamino purine (6a). White powder; mp 135-136 °C. ¹H NMR (DMSO-*d*₆) δ

1.085-1.861 (m, 10H, H cyclohexyl), 3.088 (t, 2H, J 6.4Hz, $\text{CH}_2\text{CH}_2\text{CN}$), 4.060 (br, 1H, H cyclohexyl), 4.243 (t, 2H, J 6.4Hz, NCH_2CH_2), 5.846 (s, 2H, NH_2), 6.914 (s, 1H, NH), 7.742 (s, 1H, H-8). ^{13}C NMR ($\text{DMSO}-d_6$) δ 18.34 (CH_2CN), 25.51, 25.69, 33.13, 48.54 (C cyclohexyl), 38.76 (NCH_2CH_2), 113.48 (C-5), 118.85 (CN), 137.04 (C-8), 152.31 (C-6), 154.63 (C-4), 160.76 (C-2). MS (ESI) m/z [M^+Na^+-1 , 307.8], 285.9, 254.8, 203.7, 150.8. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3331, 3218, 3103, 2929, 2854, 2246, 1637, 1602, 1486, 1399, 791. Anal. Calc. for $\text{C}_{14}\text{H}_{19}\text{N}_7$: C, 58.95; H, 6.67; N, 34.39. Found: C, 58.82; H, 6.79; N, 34.45 %.

2-amino-9- β -cyanoethyl-6-phenylamino purine (6b). Colorless flake crystal; mp 246-247 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 3.141 (t, 2H, J 6.4Hz, $\text{CH}_2\text{CH}_2\text{CN}$), 4.313 (t, 2H, J 6.4Hz, NCH_2CH_2), 6.204 (s, 2H, NH_2), 6.981 (t, 1H, J 7.2Hz, H_{Ar}), 7.280 (t, 2H, J 8.0Hz, H_{Ar}), 7.910 (s, 1H, H-8), 8.017 (d, 2H, J 8.0Hz, H_{Ar}), 9.380 (s, 1H, NH). ^{13}C NMR ($\text{DMSO}-d_6$) δ 18.36 (CH_2CN), 38.87 (NCH_2CH_2), 118.83 (CN), 120.65 (C-5), 114.17, 122.25, 128.71, 138.13 (C_{Ar}), 140.77 (C-8), 152.18 (C-6), 152.82 (C-4), 160.44 (C-2). MS (ESI) m/z [M^+Na^+-1 , 301.6], 248.7. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3445, 3391, 3329, 3202, 3099, 2984, 2935, 2252, 1645, 1617, 1579, 1498, 1439, 787. Anal. Calc. for $\text{C}_{14}\text{H}_{13}\text{N}_7$: C, 60.22; H, 4.66; N, 35.13. Found: C, 60.13; H, 4.73; N, 35.19 %.

2-amino-9- β -cyanoethyl-6-(p-tolylamino) purine (6c). White needle crystal; mp 208-209 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 2.269 (s, 3H, CH_3), 3.134 (t, 2H, J 6.4Hz, $\text{CH}_2\text{CH}_2\text{CN}$), 4.305 (t, 2H, J 6.4Hz, NCH_2CH_2), 6.153 (s, 2H, NH_2), 7.083 (d, 2H, J 8.4Hz, H_{Ar}), 7.864 (d, 2H, J 8.4Hz, H_{Ar}), 7.889 (s, 1H, H-8), 9.271 (s, 1H, NH). ^{13}C NMR ($\text{DMSO}-d_6$) δ 18.36 (CH_2CN), 20.89 (CH_3), 38.86 (NCH_2CH_2), 118.83 (CN), 120.82 (C-5), 114.10, 129.14, 131.13, 137.98 (C_{Ar}), 138.17 (C-8), 152.06 (C-6), 152.87 (C-4), 160.46 (C-2). MS (ESI) m/z [M^+Na^+-1 , 315.8], 293.8, 245.9, 228.8, 198.7, 156.8, 144.7, 117.9, 82.0, 65.1. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3457, 3337, 3104, 2944, 2855, 2252, 1627, 1597, 1513, 1482, 1417, 825, 788. Anal. Calc. for $\text{C}_{15}\text{H}_{15}\text{N}_7$: C, 61.43; H, 5.12; N, 33.45. Found: C, 61.36; H, 5.17; N, 33.48%.

2-amino-9- β -cyanoethyl-6-(p-methoxyphenylamino) purine (6d). Colorless needle crystal; mp 174-176 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 3.133 (t, 2H, J 6.4Hz, $\text{CH}_2\text{CH}_2\text{CN}$), 3.738 (s, 3H, OCH_3), 4.303 (t, 2H, J 6.4Hz, NCH_2CH_2), 6.116 (s, 2H, NH_2), 6.863 (d, 2H, J 8.8Hz, H_{Ar}), 7.856 (d, 2H, J 8.8Hz, H_{Ar}), 7.877 (s, 1H, H-8), 9.249 (s, 1H, NH). ^{13}C NMR ($\text{DMSO}-d_6$) δ 18.37 (CH_2CN), 38.86 (NCH_2CH_2), 55.61 (OCH_3), 118.84 (CN), 120.15 (C-5), 113.95, 122.46, 133.82, 137.86, 152.92 (C_{Ar}), 141.59 (C-8), 151.96 (C-6),

154.98 (C-4), 160.50 (C-2). MS (ESI) m/z [M^+Na^+-1 , 331.7], 309.8, 292.8, 267.8, 252.7, 224.7, 214.7, 199.7, 160.8, 92.4. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3483, 3327, 3203, 3008, 2946, 2848, 2250, 1596, 1514, 1486, 1243, 1031, 786. Anal. Calc. for $\text{C}_{15}\text{H}_{15}\text{N}_7\text{O}$: C, 58.25; H, 4.85; N, 31.72. Found: C, 58.16; H, 4.78; N, 31.91 %.

2-amino-9- β -cyanoethyl-6-(p-ethoxyphenylamino) purine (6e). White needle crystal; mp 177-178 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.324 (t, 3H, J 6.8Hz, OCH_2CH_3), 3.131 (t, 2H, J 6.4Hz, $\text{CH}_2\text{CH}_2\text{CN}$), 3.998 (q, 2H, J 6.8Hz, OCH_2CH_3), 4.301 (t, 2H, J 6.4Hz, NCH_2CH_2), 6.114 (s, 2H, NH_2), 6.846 (d, 2H, J 8.8Hz, H_{Ar}), 7.851 (d, 2H, J 9.2Hz, H_{Ar}), 7.876 (s, 1H, H-8), 9.243 (s, 1H, NH). ^{13}C NMR ($\text{DMSO}-d_6$) δ 15.20 (OCH_2CH_3), 18.60 (CH_2CN), 38.86 (NCH_2CH_2), 63.52 (OCH_2CH_3), 118.84 (CN), 120.15 (C-5), 113.97, 114.52, 122.41, 133.73, 137.85, 152.89 (C_{Ar}), 141.59 (C-8), 151.93 (C-6), 154.23 (C-4), 160.47 (C-2). MS (ESI) m/z [M^+Na^+-1 , 323.8], 281.8, 253.7, 224.7, 200.7, 146.8, 119.8, 80.9. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3487, 3322, 3195, 2975, 2930, 2884, 2248, 1599, 1512, 1456, 1418, 1235, 1053, 832, 786. Anal. Calc. for $\text{C}_{16}\text{H}_{17}\text{N}_7\text{O}$: C, 59.44; H, 5.26; N, 30.34. Found: C, 59.35; H, 5.29; N, 30.42 %.

6-(p-ethoxyphenylamino)-9-(β -D-ribofuranosyl) purine (7e). White powder; mp 191-192 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.330 (t, 3H, J 7.2Hz, CH_2CH_3), 3.650 (dd, 2H, J 12Hz, H-5'), 3.898 (m, 1H, H-4'), 4.012 (q, 2H, J 7.2Hz, OCH_2CH_3), 4.183 (m, 1H, H-3'), 4.648 (m, 1H, H-2'), 5.953 (d, 1H, J 7.0Hz, H-1'), 6.908 (d, 2H, J 8.8Hz, H_{Ar}), 7.777 (d, 2H, J 8.8Hz, H_{Ar}), 8.341 (s, 1H, H-2), 8.504 (s, 1H, H-8), 9.792 (s, 1H, NH). ^{13}C NMR ($\text{DMSO}-d_6$) δ 15.16 (CH_2CH_3), 62.05 (5'-C), 63.57 (CH_2CH_3), 71.03 (3'-C), 74.03 (2'-C), 86.32 (4'-C), 88.35 (1'-C), 117.41 (5-C), 114.61, 115.77, 120.56, 123.21, 132.78, 149.53 (C_{Ar}), 140.85 (8-C), 152.45 (6-C), 152.75 (4-C), 154.89 (2-C). MS (ESI) m/z [M^+Na^+-1 , 409.7], 387.8, 362.0, 318.0, 274.0, 255.8, 227.7, 199.8, 171.7, 134.8, 119.9, 108.9. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3338, 3223, 3151, 2983, 2930, 2871, 1645, 1596, 1512, 1478, 1240, 1058, 826, 791. Anal. Calc. for $\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_5$: C, 55.81; H, 5.43; N, 18.09. Found: C, 55.72; H, 5.51; N, 18.13 %.

2-chloro-6-cyclohexylamino-9-[(2-hydroxyethoxy) methyl]purine (9a). White powder; mp 165-167 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.107-1.922 (m, 10H, H cyclohexyl), 3.493 (m, 4H, $\text{HOCH}_2\text{CH}_2\text{O}$), 4.004 (br, 1H, H cyclohexyl), 5.518 (s, 2H, NCH_2O), 8.154 (d, 1H, J 7.2Hz, NH), 8.283 (s, 1H, H-8). ^{13}C NMR ($\text{DMSO}-d_6$) δ 33.48, 32.47, 25.56, 25.35 (C cyclohexyl), 49.47 (NCH), 60.34 ($\text{HOCH}_2\text{CH}_2\text{O}$), 71.23 ($\text{HOCH}_2\text{CH}_2\text{O}$), 72.92 (NCH_2O), 118.33 (5-C), 141.77 (8-C), 150.41 (6-C), 154.08 (4-C), 154.70 (2-C).

MS (ESI) m/z [M^+Na^+-1 347.7], 325.8, 311.6, 284.0, 228.8, 101.9. IR (KBr) ν_{max}/cm^{-1} : 3441, 3236, 2937, 2923, 2857, 1621, 1310, 1216. Anal. Calc. for $C_{14}H_{20}ClN_5O_2$: C, 51.61; H, 6.14; N, 21.51. Found: C, 51.47; H, 6.18; N, 21.63 %.

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Supplementary Information

General procedure, characterization of all compounds and 1H NMR, ^{13}C NMR and IR of selected compounds. Supplementary data are available free of charge as PDF file at <http://jbcbs.sbq.org.br>

References

1. Dalpozzo, R.; De Nino, A.; Maiuolo, L.; Procopio, A.; De Munno, G.; Sindona, G.; *Tetrahedron* **2001**, *57*, 4035.
2. Bisacchi, G. S.; Singh, J.; Godfrey, J. D.; Kissick, J. T. P.; Mitt, T.; Malley, M. F.; Marco, J. D. D.; Gougoutas, J. Z.; Mueller, R. H.; Zahler, R.; *J. Org. Chem.* **1995**, *60*, 2902.
3. Cappuccino, J. G.; George, M.; Merker, P. C.; Tarnowski, G. S.; *Cancer Res.* **1964**, *24*, 1243.
4. Tret'yakova, G. S.; Nedel'kina, N. N.; Cherkasov, V. M.; *Ukrainskii Khimicheskii Zhurnal* **1972**, *38*, 602.
5. Florence, B.-P.; Haesslein; Jean-Luc.; *PCT Int. Appl. WO 2002051843 A1 20020704*, **2002**.
6. Merckle GmbH.; *Eur. Pat. Appl. EP 1444982 A1 20040811*, **2004**.
7. Kwatra, M. M.; Leung, E.; Hosey, M. M.; Green, R. D.; *J. Med. Chem.* **1987**, *30*, 954; Vittori, S.; Lorenzen, A.; Stannek, C.; Costanzi, S.; Volpini, R.; IJerman, A. P.; Kunzel, J. K. V. F. D.; Cristalli, G.; *J. Med. Chem.* **2000**, *43*, 250; Moos, W. H.; Szotek, D. S.; Bruns, R. F.; *J. Med. Chem.* **1985**, *28*, 1383.
8. Altmann, E.; Cowan-Jacob, S. W.; Missbach, M.; *J. Med. Chem.* **2004**, *47*, 5833.
9. Kikugawa, K.; Iizuka, K.; Ichino, M.; *J. Med. Chem.* **1973**, *16*, 358.
10. Knutsen, L. J. S.; Lau, J.; Petersen, H.; Thomsen, C.; Weis, J. U.; Shalmi, M.; Judge, M. E.; Hansen, A. J.; Sheardown, M. J.; *J. Med. Chem.* **1999**, *42*, 3463.
11. Lanver, A.; Schmalz, H.-G.; *Molecules* **2005**, *10*, 508.
12. Elion, G. B.; Burgi, E.; Hitchings, G. H.; *J. Am. Chem. Soc.* **1952**, *74*, 411.
13. Girgis, N. S.; Pedersen, E. B.; *Synthesis* **1982**, *6*, 480; Fu, R.; Xu, X.; Dang, Q.; Bai, X.; *J. Org. Chem.* **2005**, *70*, 10810.
14. Wu, T. Y. H.; Schultz, P. G.; Ding, S.; *Org. Lett.* **2003**, *5*, 3587.
15. Fiorini, M. T.; Abell, C.; *Tetrahedron Lett.* **1998**, *39*, 1827.
16. Ran, C.; Dai, Q.; Harvey, R. G.; *J. Org. Chem.* **2005**, *70*, 3724.
17. Khalafi-Nezhad, A.; Rad, M. N. S.; Hakimelahi, G. H.; *Helv. Chim. Acta.* **2003**, *86*, 2396; Varma, R. S.; *Green Chem.* **1999**, *1*, 43; Kappe, C. O.; *Angew. Chem., Int. Ed.* **2004**, *43*, 6250; Perreux, L.; Loupy, A.; *Tetrahedron* **2001**, *57*, 9199; Lidström, P.; Tierney, J.; Wathey, B.; Westman, J.; *Tetrahedron* **2001**, *57*, 9225.
18. Qu, G.-R.; Li, Y.; Han, H.-S.; *J. Chem. Res.* **2005**, 167; Qu, G.-R.; Liu, Q.-B.; *Indian. J. Chem. B* **2005**, *44*, 196.
19. Bai, L.; Wang, J.-X.; Zhang, Y.-M.; *Green Chem.* **2003**, *5*, 615.
20. Tundo, P.; Anastas, P. T. In *Green Chemistry: Challenging Perspectives*, Oxford University Press: Oxford, 1999; Fringuelli, F.; Piermatti, O.; Pizzo, F.; Vaccaro, L.; *Eur. J. Org. Chem.* **2001**, 439.
21. Fringuelli, F.; Pizzo, F.; Tortoioli, S.; Vaccaro, L.; *Green Chem.* **2003**, *5*, 436.
22. Lira, E. P.; Huffman, C. W.; *J. Org. Chem.* **1966**, *31*, 2188; Baker, B. R.; Tanna, P. M.; *J. Org. Chem.* **1965**, *30*, 2857; Morris, J. R.; Peter, W. H.; *Can. J. Chem.* **1982**, *60*, 547.
23. *Chem. Abstr.* **1966**, *65*, 16970.
24. Okono, T.; Goya, S.; Takadate, A.; Eto, Y.; *J. Pharm. Soc. Jpn.* **1966**, *86*, 694.
25. Hitchings, G. H.; Elion, G. B.; *US 2691654*, **1954**.
26. Fleysner, M. H.; Bloch, A.; Hakala M. T.; Nichol, C. A.; *J. Med. Chem.* **1969**, *12*, 1056.

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