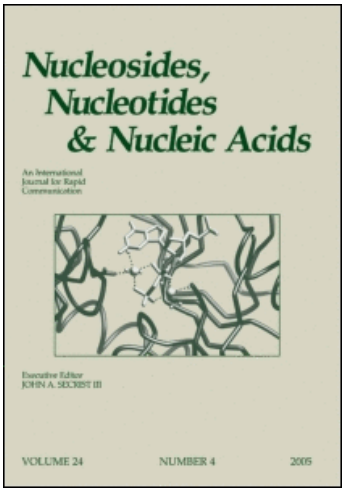

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Synthesis of 6-Alkyl- and Arylamino-9-(tetrahydro-2-pyranyl)purines via 6-Methylsulfonylpurine

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SYNTHESIS OF 6-ALKYL- AND ARYLAMINO-9-(TETRAHYDRO-2-PYRANYL)PURINES *VIA* 6-METHYLSULFONYLPURINE

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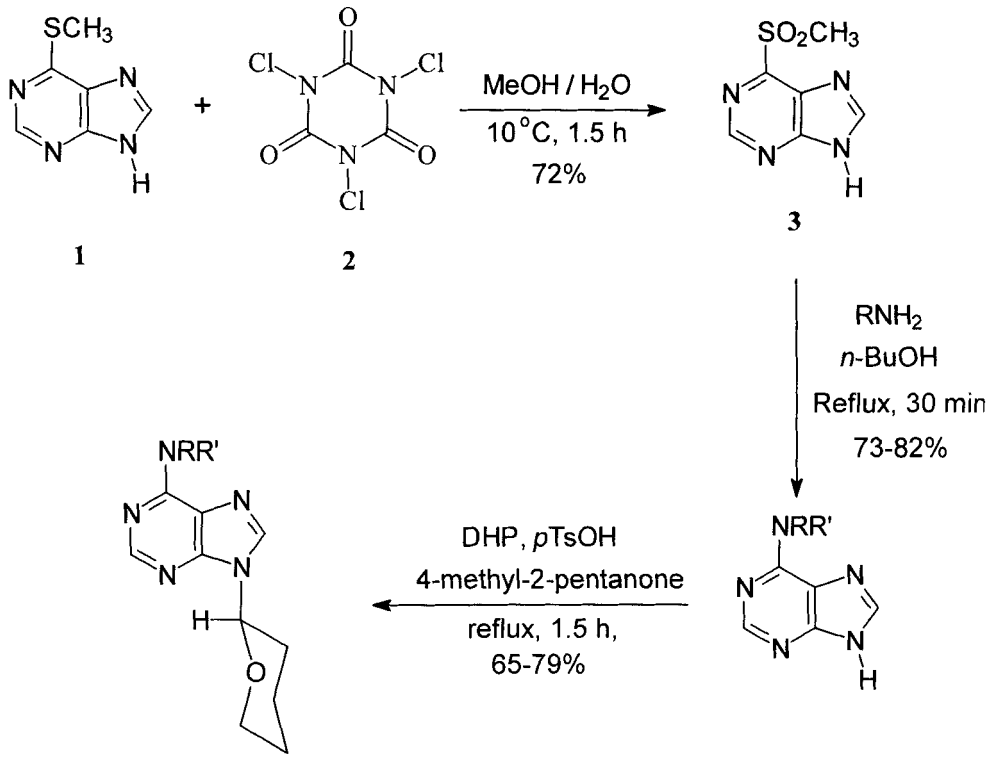
Abstract: A series of six potential plant hormones, 6-alky- and arylamino-9-(tetrahydro-2-pyranyl)purines were prepared in three steps in 35 to 45% overall yield from 6-methylthiopurine *via* 6-methylsulfonylpurine.

The chemistry of 6-aminopurines is of great interest as this family of compounds displays a wide variety of interesting biological properties, including antiallergenic,[1] acceleration of cellular multiplication,[2] plant hormones,[3-5] antibacterial,[6] and hair growth stimulant.[7] Interestingly, traces this kind of compounds, such as kinetin, have been found in human cells.[8] The biological activity of 6-aminopurines is usually enhanced when there is substitution at N-9, becoming more alike the natural nucleosides.[9,10] For example, the zeatins, a recently reviewed family of 6-alkylaminopurines and their 9-β-D-ribofuranosyl derivatives display very important cytokinin activity.[11] The substitution at N-9 by groups that resemble ribose or deoxyribose leads to enhanced activity, for example 6-benzylamino-9-(tetrahydro-2-pyranyl)purine is a potent plant hormone.[12] The synthesis of 6-aminopurines is usually carried out using 6-methylthiopurine (**1**)[13-16] or 6-chloropurine[17,18] as substrates and amines[19-20] as nucleophiles. An alternative route to 6-alkylaminopurines have been the reaction of 6-aminopurines with alcohols.[21-23] The disadvantage of such methodologies is the long reaction times (up to 24 hours) and high reaction temperatures (>130 °C), principally when **1** is the starting material. Some 6-aminopurines have also been prepared using 4-amino-6-chloro-5-nitropyrimidine as

starting material.[24] In this work, we have used 6-methylsulfonylpurine (**3**) as substrate for the preparation of a series of six novel 6-aminopurines and their respective 9-tetrahydro-2-pyranyl derivatives, which are potential plant hormones.

The nucleophilic substitution of the SCH_3 group of **1** with primary amines occurs only under relatively high temperatures (>130 °C) and long reaction times (12 to 24 h). Shorter reaction times can be used if the leaving group were Cl (1.5 to 2.0 h) at 124 °C. We decided to convert the SCH_3 group into a better leaving group, SO_2CH_3, to allow for shorter reaction times and lower reaction temperatures. The methylsulfonyl group have been previously used with success in nucleophilic substitutions on similar heterocyclic systems,[25-30] with special notice of the work of Wetzel and Eckstein,[30] that used various oxygen, nitrogen and sulfur nucleophiles to substitute the methylsulfonyl group in 6-methylsulfonyl-9-β-D-ribofuranosylpurine. Compound **1** was converted to the sulfone **3** by oxidation with trichloroisocyanuric acid (**2**) in methanol-water at 10 °C in 72% yield, as shown in Scheme 1. This oxidation reaction could also be carried out using chlorine or calcium hypochlorite as oxidants under similar conditions and with similar yields but with longer reaction times. Attempts to prepare the sulfone **3** by oxidation of **1** with peroxides led to the sulfoxide **16**. For example, as shown in Scheme 2, treatment of **1** with excess of MCPBA afforded **16** in 58% yield. Our oxidation methodology is inferior to the procedure reported by Yamane, Matsuda and Ueda, in which 6-methylsulfonyl-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine was obtained in 96% yield by oxidation of the methylthio derivative with $KmnO_4$ in 90% HOAc .[25]

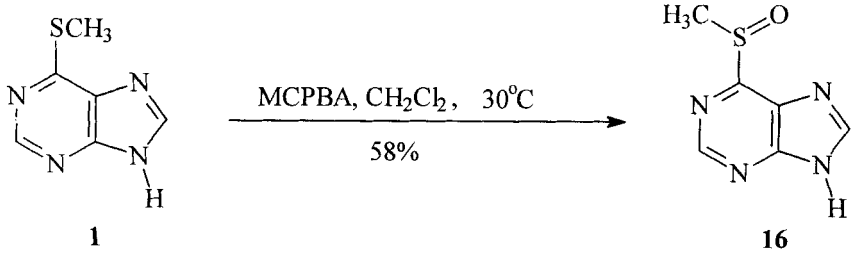
Reaction of the sulfone **3** with primary or secondary amines in refluxing 1-butanol led to the desired 6-aminopurines (**4** to **9**) in good yields in 20-30 minutes. This reaction is temperature dependent, as it does not occur at room temperature and takes 2 h to complete under reflux in ethanol. The nucleophilicity of the amine is also important, as poor nucleophiles, such as 2-aminopyridine, do not react, even under reflux in DMSO for 6 h. The lowest yields in the series of tested amines occurred with isobutylamine (73%) and pyrrolidine (74%), possibly due to the greater steric hindrance of these amines. Conversion of the 6-aminopurines to the 9-(tetrahydro-2-pyranyl) derivatives was easily accomplished using the literature procedure;[13] by treatment with DHP and *p*-toluenesulfonic acid in an appropriate solvent.



10: R' = H, R = benzyl
11: R' = H, R = furfuryl
12: R' = H, R = *n*-butyl
13: R' = H, R = isobutyl
14: R' = H, R = phenyl
15: R' = R = pyrrolidino

4: R' = H, R = benzyl
5: R' = H, R = furfuryl
6: R' = H, R = *n*-butyl
7: R' = H, R = isobutyl
8: R' = H, R = phenyl
9: R' = R = pyrrolidino

SCHEME 1



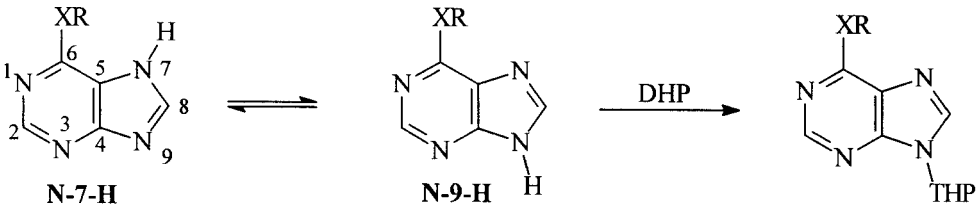
1 **16**

SCHEME 2

The lack of NOE between the hydrogens of the THP group and the hydrogens of the amine side chains showed that DHP reacts selectively at N-9 affording the thermodynamic product. Interestingly, the ^{13}C NMR spectra of the sulfone (**3**) and all the 6-aminopurines did not show all the expected signals. When the sample temperature was increased from 20 to 60 °C for the determination of the ^{13}C NMR spectra, some of the previously undetected signals appeared as broad low intensity peaks, which were assigned to the quaternary carbons of the purine ring system. We believe that this behavior of some of the carbon peaks is caused by two factors. First, some signal broadening may be due to the quadrupolar effect of the ^{14}N atoms. Second, the existence of a slow equilibrium between the two possible protonated forms, N-7-H and N-9-H, in purines which are not substituted at neither nitrogen (see Scheme 3), would lead to broadening of the signals of C-4, C-5 and C-6. This means that both N-9 and N-7 would, in principle, be available for reaction with DHP. Since, as for all the 6-aminopurines, 6-methylthiopurine and 6-methylsulfonylpurine are converted to the 9-DHP derivatives, it seems that the selectivity of this reaction is explained by the steric hindrance caused by the groups at C-6 on N-7. This observation is in agreement with previous observations reported in the literature.[31] Furthermore, the equilibrium between N-7-H and N-9-H in Scheme 3 is supported by the observation that introduction of the DHP at N-9, besides increasing the solubility of the products, leads to the easy observation of all the expected ^{13}C NMR signals in the spectra.

Even though the oxidation reaction have not been optimized, this new methodology for the preparation of 6-amino-9-(tetrahydro-2-pyranyl)purines is very efficient, as it requires milder conditions and leads to good overall yields of the products (35 to 45%).

Preliminary tests of hormonal activity were carried out only with compounds **12** (*n*-butyl) and **14** (phenyl). The effects of these compounds on plant growth and fruit production were tested on kidney beans (*Phaseolus vulgaris*). It was observed that, after 20 days of growth, the plants cultivated on soil containing 0.001 % w/w of **14** had 20% more leaves than the plants grown in untreated soil. Compound **12** afforded a more discrete result, with an increase of less than 10% of the treated plants foliage. Also, after 45 days of growth, it was observed that the plants treated with **14** produced, in the average, 5% more pods than the untreated plants. Compound **12** did not have any observable effect on pod production under the conditions that the tests were conducted.



XR = SCH_3 , $SOCH_3$, SO_2CH_3, NHR'

SCHEME 3

These are only preliminary results which are in the process of being confirmed and expanded to all the compounds and different concentrations.

Experimental:

All reagents used in this work were purified by recrystallization or distillation according to approved procedures. The NMR spectra were determined in a Varian UNITY-300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C) using TMS as internal standard. The UV spectra were recorded in a Varian DMS-80 UV-visible spectrophotometer using spectrometric grade methanol as solvent. The IR spectra were determined in a Perkin Elmer 1420 spectrometer using KBr pellets. The low resolution mass spectra were recorded in a Kratos MS-50 mass spectrometer with electron impact ionization (70 eV). Melting points were measured in a Fisher-Johns melting point apparatus and are not corrected. The thin layer chromatographic analyses were carried out using silica gel 60 HR from Merck.

6-Methylsulfonylpurine (3): A suspension of 1.0 g (6.0 mmol) of 6-methylthiopurine (**1**) in 20 ml of a 30-70% methanol-water solution was cooled below 10 °C. Slowly and with constant stirring, 0.93 g (4.0 mmol) of trichloroisocyanuric acid (**2**) was added maintaining the temperature below 10 °C. The reaction mixture was magnetically stirred for 1.5 h at this temperature. The precipitate formed after this time was filtered, washed with water and cold ethanol and then recrystallized from hot ethanol to afford pure **3** in 60 to 72 % yield; m.p. 234 °C (dec.); UV (MeOH) λ_{max} nm (ε): 288 (28800) and 218 (17200); IR (KBr) ν_{max}: 3400, 3020, 3000, 1600, 1560, 1320, 1130, 750 and 630 cm^{-1}; ^1H

NMR (DMSO-d_6) δ: 14.0 (1H, s), 9.1 (1H, s), 8.9 (1H, s), 3.5 (3H, s); ^{13}C NMR (DMSO-d_6) δ: 151.2, 149.9, 40.7; LREIMS m/z (intensity %): 198 (30, M^+, $C_6H_6N_4O_2$), 119 (100). Compound **3** was also prepared using the above procedure but changing the oxidizing agent to chlorine gas (2.6 h) or calcium hypochloride (7 h) with similar results.

Oxidation of 6-methylmercaptopurine with MCPBA: A portion of 1. 0 g (6.0 mmol) of 6-methylthiopurine was treated with 2.08 g (12 mmol) of MCPBA in methanol : water 3:7 at 10 °C for 1.5 h. Filtration of the reaction mixture afforded white crystals which were recrystallized from methanol to give 6-methylsulfinylpurine (**16**) in 58% yield; m.p. 240 °C (dec.); UV (MeOH) λ_{max} nm (ε): 276 (27,778) and 205 (15,185). IR (KBr) ν_{max}: 3400, 3050, 1590, 1570, 1060 and 650 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 13.0 (1H, s), 9.0 (1H, s), 8.7 (1H, s) and 3.0 (3H, s). ^{13}C NMR (DMSO-d_6): δ 158.9, 148.2 and 11.2. LREIMS m/z (int.): 182 (22, M^+, $C_6H_6N_3SO$), 166 (20), 137 (57), 119 (100).

General Procedure for the Synthesis of 6-Aminopurines: All the 6-aminopurines were prepared by refluxing a solution of 0.2 g (1.0 mmol) of **3** and 2.0 mmol of the respective amine in 10.0 mL of *n*-butanol in a nitrogen atmosphere. The solvent was then removed under reduced pressure and the crude product recrystallized from an appropriate solvent.

6-Benzylaminopurine (**4**): Compound **4** was prepared according to the general procedure using 45 min. of reflux. The crude product was recrystallized from ethanol to afford pure **4** in 78.2% yield; m.p. 245-247 °C; UV (MeOH) λ_{max} nm (ε): 265 (47727) and 207 (56250); IR (KBr) ν_{max}: 3260, 3200, 1610, 1590, 1330, 1290, 690 and 640, cm^{-1}; ^{1}H NMR (DMSO-d_6) 13.0 (1H, s), 8.3 (1H, s), 8.2 (2H, s), 7.3 (5H, m) and 5.8 (2H, s); ^{13}C NMR (DMSO-d_6) δ: 153.8, 152.8, 152.1, 150.6, 141.1, 138.8, 110.8, 106.4 and 36.8.

6-Furfurylaminopurine (**5**): Compound **5** was prepared in exactly the same manner as **4**. The crude product was recrystallized from ethanol to afford pure **5** in 81.8% yield; m.p. 292-295 °C; UV (MeOH) λ_{max} nm (ε): 265 (17391) and 210 (26521); IR (KBr) ν_{max}: 3200, 3050, 1610, 1580, 1300, 1250, 690 and 640, cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.9 (1H, s), 8.2 (1H, s), 8.1 (2H, s), 7.5 (1H, bs), 6.3 (1H, bs), 6.2 (1H, bs) and 4.7 (2H, bs); ^{13}C NMR (DMSO-d_6) δ: 152.8, 151.9, 141.1, 138.8, 110.8, 106.4 and 36.8.

6-Butylaminopurine (**6**): The reaction occurred in 15 minutes of reflux. The crude product was recrystallized from hot ethanol to afford pure **6** in 77.7% yield; m.p. 233-

234 °C; UV (MeOH) λ_{max} nm (ε): 266 (28077) and 205 (23461); IR (KBr) ν_{max}: 3210, 2960, 2880, 1630, 1560, 1330, 660 and 610 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 12.9 (1H, s), 8.2 (1H, s), 8.1 (1H, s), 7.5 (1H, s), 3.4 (2H, bs), 1.6 (2H, quintet), 1.4 (2H, sextet) and 0.9 (3H, t); ^{13}C NMR (DMSO-d_6) δ: 154.5, 152.4, 149.5, 138.5, 118.7, 31.3, 19.6 and 13.7.

6-Isobutylaminopurine (**7**): Compound **7** was prepared in the same way as **6**, but was recrystallized from ethyl acetate to afford pure **7** in 73% yield; m.p. 234-236 °C; UV (MeOH) λ_{max} nm (ε): 268 (15769) and 205 (20000); IR (KBr) ν_{max}: 3280, 1610, 1600, 1340, 1330, 1290, 1240, 1230 and 640, cm^{-1}; ^1H NMR (DMSO-d_6) 12.9 (1H, s), 8.2 (1H, s), 8.1 (1H, s), 7.6 (1H, s), 3.2 (2H, bs), 2.0 (1H, m) and 0.9 (6H, d); ^{13}C NMR (DMSO-d_6) δ: 154.1, 152.1, 150.6, 138.7, 117.3, 47.4, 27.8 and 19.8.

6-Anilinopurine (**8**): The time of reflux for this product was 3 hours, and the crude product was recrystallized from ethanol to afford pure **8** in 76.2% yield; m.p. 271-273 °C; UV (MeOH) λ_{max} nm (ε): 296 (33511) and 204 (27.128); IR (KBr) ν_{max}: 1630, 1560, 1470, 1350, 13200, 750 and 600 cm^{-1}; ^1H NMR (DMSO-d_6) 10.0 (1H, s), 8.5 (1H, s), 8.4 (1H, s), 7.9 (2H, d), 7.4 (2H, t) and 7.3 (1H, t); ^{13}C NMR (DMSO-d_6) δ: 151.1, 150.9, 140.4, 139.3, 128, 122.6 and 120.

6-Pyrrolidinopurine (**9**): This compound was prepared in the same way as **6**, but the crude reaction product was recrystallized from DMSO to afford pure **9** in 74% yield; m.p. 309-310 °C; UV (MeOH) λ_{max} nm (ε): 273 (29327) and 210 (28846); IR (KBr) ν_{max}: 3400, 2950, 1610, 1330, 1590, 790 and 640, cm^{-1}; ^1H NMR (DMSO-d_6) 13.0 (1H, s), 8.2 (1H, s), 8.1 (1H, s), 4.0 (2H, bs), 3.6 (2H, bs), and 1.9 (4H, bs), ^{13}C NMR (DMSO-d_6) δ: 152.4, 151.9, 137.8, 117.3, 47.4 and 24.6.

6-Benzylamino-9-(tetrahydro-2-pyranyl)purine (**10**): This compound was prepared by reaction of 0.61 g (2.60 mmol) of **4**, 0.66 ml (7.28 mmol) of 2,3 dihydropyran and 0.05 g (0.02 mmol) of *p*-toluenesulfonic acid in 7.0 ml of dry 4-methyl-2-pentanone. The stirring reaction mixture was heated under reflux for 2.0 h. After completion of the reaction (tlc), the solution was washed with a dilute sodium hydroxide solution and water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give a solid which was recrystallized from ethanol affording pure **10** in 71.4% yield; m.p. 116-118 °C; UV (MeOH) λ_{max} nm (ε): 265 (48406), 203 (141406); IR (KBr) ν

$_{max}$: 2940, 1610, 1590, 1330, 1290, 1080, 690, 640 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.5 (1H, s), 7.9 (1H, s), 7.3 (5H, m), 6.6 (1H, s), 5.7 (1H, dd), 4.9 (2H, s), 4.1 (1H, dd), 3.9 (1H, td), 1.6-2.0 (6H, m); C^{13} NMR (DMSO-d_6) δ: 154.3, 152.7, 151.8, 148.9, 141.5, 137.2, 110.0, 107.3, 81.9, 81.7, 68.6, 37.9, 31.8, 24.9, 22.7; LREIMS m/z (intensity %): 309 (50, M^+, $C_{17}H_{19}N_5O$), 225 (98), 120 (50), 91 (98).

6-Furfurylamino-9-(tetrahydro-2-pyranyl)purine (11): Compound **11** was prepared in the same way as **10**, but using **5** as substrate, in 78.9% yield; m.p. 149-152 °C; UV (MeOH) λ_{max} nm (ε): 265 (39395), 210 (53030); IR (KBr) ν_{max}: 3300, 2940, 2930, 2920, 1610, 1580, 1340, 1230, 1080, 750, 640 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.3 (1H, s), 7.9 (1H, s), 7.4 (1H, bs), 6.5 (1H, bs), 6.2 (2H, bs), 5.9 (1H, dd), 4.9 (2H, bs), 4.1 (1H, dd), 3.9 (1H, td), 1.6-2.0 (6H, m); C^{13} NMR (DMSO-d_6) δ: 154.3, 152.7, 151.8, 148.9, 141.5, 137.2, 110.0, 107.3, 81.9, 81.7, 68.6, 37.9, 31.8, 24.9, 22.7; LREIMS m/z (intensity %): 299 (50, M^+, $C_{14}H_{17}N_5O_2$), 215 (96), 186 (100), 120 (35).

6-Butylamino-9-(tetrahydro-2-pyrany)purine (12): This compound was prepared as **10**, but using **6** as substrate and 1.5 h of reflux. After completion of the reaction (tlc), the solution was washed with a dilute sodium hydroxide solution and water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give a viscous oil. This oil was purified by elution from an aluminum oxide chromatographic column with dichloromethane and ethyl acetate giving 64.4% yield of **12** as a crystalline product; m.p. 56-59 °C; UV (MeOH) λ_{max} nm (ε): 263 (56944), 210 (54861); IR (KBr) ν $_{max}$: 3280, 2960, 2880, 1620, 1560, 1330, 1080, 650 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.3 (1H, s), 7.9 (1H, s), 6.2 (1H, s), 5.6 (1H, dd), 4.1(1H, dd), 3.7 (1H, td), 3.2 (2H, bs), 2.0-1.6 (8H, m), 1.3 (2H, sextet), 0.9 (3H, t); C^{13} NMR (DMSO-d_6) δ: 154.7, 153.0, 137.1, 119.3, 81.6, 81.5, 68.6, 40.3, 31.7, 24.8, 22.7, 13.7; LREIMS m/z (intensity %): 275 (35, M^+, $C_{14}H_{21}N_5O$), 191 (95),162 (95), 148 (100), 119 (35).

6-Isobutylamino-9-(tetrahydro-2-pyranyl)purine (13): This compound was prepared in the same way as **12**, but using **7** as substrate and in 67.8% yield; m.p. 117-119 °C; UV (MeOH) λ_{max} nm (ε): 264 (56.944), 212 (62944); IR (KBr) ν_{max}: 3290, 1610, 1560, 1330, 1290, 1240, 1230, 1080, 650 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.2 (1H, s), 8.0 (1H, s), 6.6 (1H, s), 5.7 (1H, dd), 4.1(1H, dt), 3.9 (1H, td), 3.5 (2H, bs), 1.6-2.0 (7H, m), 1.0 (6H, d); C^{13} NMR (DMSO-d_6) δ: 154.7, 153.0, 137.1, 119.3, 81.6, 81.5, 68.6, 40.3,

31.7, 24.8, 22.7, 13.7; LREIMS m/z (intensity %): 275 (35, M^+, $C_{14}H_{21}N_5O$), 191 (80), 176 (80), 161 (90), 119 (55).

6-Anilino-9-(tetrahydro-2-pyranyl)purine (14): This product was prepared in the same way as **12** but using **8** as substrate and a reaction time of 2.5 hours. Compound **14** was obtained in 69.7% yield; m.p. 76-79 °C; UV (MeOH) λ_{max} nm (ε): 295 (45454), 203 (61364); IR (KBr) ν_{max}: 3050, 2960, 1620, 1580, 1320, 1090, 750, 640 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.5 (1H, s), 8.2 (1H, s), 8.1 (1H, s), 7.8 (2H, d), 7.3 (2H, t), 7.1 (1H, t), 5.8 (1H, dd), 4.1 (1H, dd), 3.9 (1H, td), 1.6-2.0 (6H, m); C^{13} NMR (DMSO-d_6) δ: 152.89, 148.7, 141.5, 138.5, 138.1, 128.9, 123.5, 120.3, 119.8, 81.9, 81.8, 68.8, 31.8, 24.8, 22.7; LREIMS m/z (intensity %): 295 (30, M^+, $C_{16}H_{17}N_5O$), 210 (100), 186 (30), 119 (10).

6-Pyrrolidino-9-(tetrahydro-2-pyranyl)purine (15): Compound **15** was prepared in the same way as **12** but using **9** as substrate and in 71.1% yield; m.p. 152-159 °C; UV (MeOH) λ_{max} nm (ε): 280 (49305), 218 (42361); IR (KBr) ν_{max}: 3100, 2940, 2930, 1640, 1590, 1320, 1080, 790 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.4 (1H, s), 7.9 (1H, s), 5.7 (1H, dd), 4.1(1H, dt), 4.0 (4H, bs), 3.9 (1H, td), 2.1-1.9 (6H, m), 1.5-1.8 (4H,m); C^{13} NMR (DMSO-d_6) δ: 152.9, 152.6, 149.0, 136.3, 120.1, 81.7, 81.6, 68.6, 50.6, 48.2, 31.9, 24.9, 22.8; LREIMS m/z (intensity %): 273 (70, M^+, $C_{14}H_{19}N_5O$), 189 (95), 161 (100), 120 (55).

Bioassays: Gardening soil (5 kg) was esterilized in an oven at 120 °C for 2 hours. After cooling, the soil was carefully sprayed with a 1% solution of the desired compound (**12** or **14**) in 1:5 DMSO: H_2O, dryed and homogeneized in a mortar in such a way as to leave a 0.001% w/w final concentration of the compounds in the soil. The soil was set in 200 mL plastic pots with three selected seeds of kidney beans (*Phaseolus vulgaris*) in each pot. The pots were wet with 50 mL of water and treated with 9 hours of light, from a 250 Watt lamp, daily for 45 days. Foliage and bean production control were carried out by counting the number of leaves and pods in every plant after 20 and 45 days of growth, respectively. All experiments were carried out in duplicate and the blank experiments were conducted with soil treated exactly in the same manner but spraying with pure solvent.

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