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Efficient synthesis and biological evaluation of 1,2,9-trisubstituted 1,9-dihydro-6*H*-purin-6-ones

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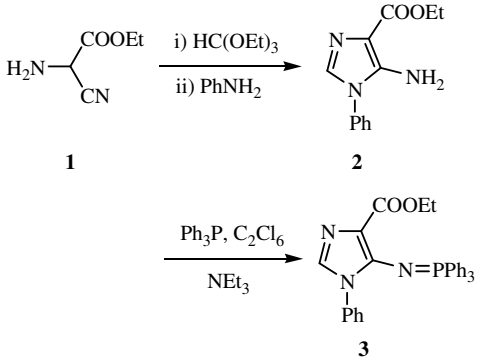
ABSTRACT

The carbodiimides **4**, obtained from aza-Wittig reactions of iminophosphorane **3** with aromatic isocyanates, reacted with amines in the presence of a catalytic amount of RO^-Na^+ to give the 1,2,9-trisubstituted 1,9-dihydro-6*H*-purin-6-ones **6** in good yields. Compound **6** exhibited cytotoxicity against various cancer cells. For example, compounds **6b** showed the best inhibition activities against KB, HepG2 and OVCAR3 with IC_{50} 9.5, 20.4 and 10.0 μM.

1,9-Dihydro-6*H*-purin-6-ones, which are also named as guanines, are of great importances because of their fundamental role in nucleic acid chemistry and cellular biochemistry. Some derivatives of them have shown remarkable biological properties such as antitumor and antiviral activities,[1–5] whereas others were evaluated as corticotropin releasing factor-1 (CRF1) receptor antagonists or as DPP-IV inhibitors.[6,7] The methods described for the preparation of this ring system either involves reaction of properly substituted diaminopyrimidines with aldehyde or acid anhydride, or reaction of aminoimidazolecarbohydrazides with orthoesters, or cyclization of 4-acetamido-5-ethoxycarbonylimidazoles with amine in the presence of phosphorus pentoxide.[8–13] However, these methods often require relatively harsh acid, dehydrating conditions or heating at high temperature, and there is no report of a generally useful synthesis of 2-amino substituted 1,9-dihydro-6*H*-purin-6-ones starting from easily accessible 5-amino-4-ethoxycarbonylimidazoles.

Recently, we have been interested in the synthesis of quinazolinones, thienopyrimidinones and imidazolinones via aza-Wittig reaction, with the aim of evaluating their biological activities.[14–19] Here, we wish to reported a new efficient synthesis and antitumor activities of 1,2,9-trisubstituted-1,9-dihydro-6*H*-purin-6-ones **6** from easily accessible iminophosphorane **3**.

The 5-aminoimidazole **2**, obtained by reaction of ethyl 2-amino-2-cyanoacetate **1** with triethyl orthoformate and phenylamine,[20] was converted easily to iminophosphorane **3** via reaction with triphenylphosphine, hexachloroethane and triethylamine in good yield.[21]



Iminophosphorane **3** reacted with aromatic isocyanates to give carbodiimides **4**, which were allowed to react with secondary amines to provide guanidine intermediates **5**. In the presence of catalytic amount of sodium ethoxide, **5** were converted easily to 2-dialkylamino-1,9-dihydro-6*H*-purin-6-ones **6** in satisfactory yields at room temperature.[22] It is noteworthy that the isolated yield of **6** was good

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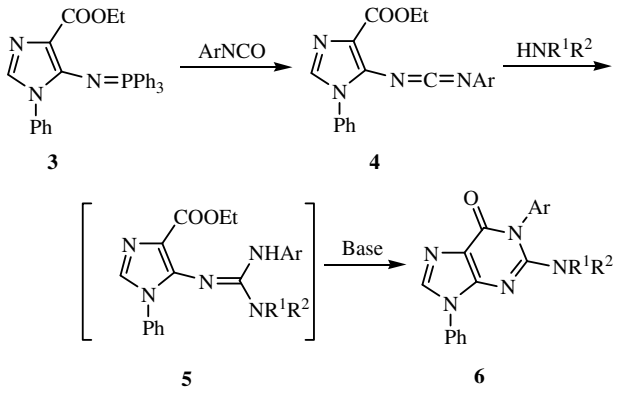
even when NR^1R^2 is bulky di-*iso*-propylamino group. The results are listed in Table 1.



The reaction of carbodiimides **4** with primary amine R^1R^2NH (R^1 = H) in the presence of EtONa provided only 2-alkylamino-1,9-dihydro-6*H*-purin-6-ones **6**, one of the possible regioisomers.[22] We obtained only **6** from the reaction mixture after recrystallization; the other isomer was not found by ^1H NMR analysis of the reaction mixture. Whenever the primary amine used is small or bulky, the cyclization was achieved all in good yields with similar selectivity. The results are also listed in Table 1. The solitary formation of **6** can be rationalized in terms of a base catalytic cyclization of the guanidine intermediate **5** to give **6** across the arylamino group rather than the alkylamino one. This may probably be due to the preferential generation of $-N^-Ar$ from more acidic –NHAr. The same selectivity is also observed in similar cases.[17,23]

The structure of 1,9-dihydro-6*H*-purin-6-ones **6** was confirmed by their spectral data. For example, the IR spectra of **6g** revealed N–H and C=O absorption bands at 3445 and 1703 cm^{-1}, respectively. The ^1H NMR spectrum of **6g** shows the signals of NH at 4.14 ppm as a broad absorption and NCH_2 at 3.40–3.33 ppm as multiple absorption, which strongly suggest the existence of $NHCH_2CH_3$ group in **6g**. The signals attributable to the imidazole-H and other Ar–Hs are found at 7.82 and 7.74–7.26 ppm as singlet and multiplet. The MS spectrum of **6g** shows strong molecular ion peak at *m*/*z* 331 with 100% abundance.

The biological activities of **6** were investigated, and the results showed that some of them exhibited cytotoxicity against various cancer cells.[24] As indicated in Table 2, most of the compounds showed good to moderate cytotoxicity against KB. Compounds **6b** showed the best inhibition activities against KB, HepG2 and OVCAR3 with IC_{50} 9.5, 20.4 and 10.0 μM (Table 2).

In conclusion, we have developed a new efficient synthesis of 2-substituted 1,9-dihydro-6*H*-purin-6-ones via base-catalyzed reaction of functionalized carbodiimides with various amines. The preliminary investigation on the biological activities of **6** shows that some of them exhibited cytotoxicity against various cancer cells.

Table 1
Preparation of 2-substituted 1,9-dihydro-6*H*-purin-6-ones **6**

Compound	Ar	NR^1R^2	Conditions	Yield[a] (%)
6a	Ph	$-NMe_2$	rt/4 h	81
6b	Ph	$-N(n\text{-}Bu)_2$	rt/6 h	72
6c	Ph	$-N(i\text{-}Pr)_2$	rt/10 h	71
6d	4-Cl-C_6H_5	$-N(n\text{-}C_5H_{11})_2$	rt/6 h	70
6e	4-Cl-C_6H_5	Piperidin-1-yl	rt/4 h	85
6f	Ph	MeNH	rt/4 h	85
6g	Ph	EtNH	rt/5 h	84
6h	4-Cl-C_6H_5	MeNH	rt/6 h	89
6i	4-Cl-C_6H_5	*n*-BuNH	rt/6 h	82
6j	4-Cl-C_6H_5	$PhCH_2NH$	rt/6 h	88

[a] Isolated yields based on iminophosphorane **3**.

Table 2
In vitro cytotoxicity (IC_{50}[a], μM) of 1,9-dihydro-6*H*-purin-6-ones **6**

Compound	KB	HepG2	OVCAR3
6a	83.8	>100	>100
6b	9.5	20.4	10.0
6c	53.5	>100	>100
6d	10.6	>100	>100
6e	72.8	>100	>100
6f	>100	>100	>100
6g	>100	>100	>100
6h	>100	>100	>100
6i	37.7	77.6	94.8
6j	34.3	62.1	46.7

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.

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21. *Preparation of iminophosphorane (**3**):* To a mixture of aminoimidazole (**2**) (1.85 g, 8 mmol), PPh_3 (3.14 g, 12 mmol) and C_2Cl_6 (2.84 g, 12 mmol) in dry CH_2Cl_2 (40 mL), was added dropwise NEt_3 (2.42 g, 24 mmol) at room temperature. The color of the reaction mixture quickly turned yellow. After stirred for 4–6 h, the solvent was removed under reduced pressure and the residue was recrystallized from ethanol–petroleum ether (1:3) to give iminophosphorane **3** as pale yellow crystals (3.4 g, yield 85%), mp 123–125 °C. IR (KBr): 1689 (C=O), 1542, 1502, 1438, 1417, 1137. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.69–7.34 (m, 21H, Ar–H), 3.86 (q, *J* = 7.0 Hz, 2H, OCH_2), 1.05 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.0 (C=O), 145.4, 145.3, 136.0, 132.4, 132.0, 131.9, 131.7, 131.6, 131.5, 130.9, 130.8, 128.4, 128.1, 128.0, 127.7, 127.6, 127.0, 126.1, 114.6, 58.2, 14.2. MS *m*/*z* (%): 491 (M^+, 100), 446 (12), 418 (16), 262 (66), 185 (11), 103 (21), 77 (29). Anal. Calcd for $C_{30}H_{26}N_3O_2P$: C, 73.31; H, 5.33; N, 8.55. Found: C, 73.42; H, 5.19; N, 8.61.
22. *Preparation of 1,9-dihydro-6H-purin-6-ones (**6**):* To a solution of iminophosphorane (**3**) (1.5 g, 3 mmol) in dry methylene dichloride (15 mL) was added phenyl isocyanate (3 mmol) under nitrogen at room temperature.

After the reaction mixture was left unstirred for 8–12 h at 0–5 °C, the solvent was removed off under reduced pressure and Et_2O/petroleum ether (1:2, 12 mL) was added to precipitate triphenylphosphine oxide. Removal of the solvent gave carbodiimides **4**, which were used directly without further purification. To the solution of carbodiimide (**4**) prepared above in methylene dichloride (15 mL) was added amines (3 mmol). After the reaction mixture was stirred for 6 h, the solvent was removed and anhydrous ethanol (10 mL) containing several drops of EtONa in EtOH was added. The reaction mixture was stirred for 6–12 h at room temperature. The solution was condensed and the residual was recrystallized from ethanol to give 1,9-dihydro-6*H*-purin-6-ones **6**.

2-Dimethylamino-1,9-diphenyl-1,9-dihydro-6H-purin-6-one (***6a***)*:* White crystals. Mp 243–245 °C. IR (KBr): 1693 (C=O), 1525, 1382, 1189 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.90 (s, 1H, Ar–H), 7.74–7.34 (m, 10H, Ar–H), 2.64 (s, 6H, $2CH_3$). MS *m*/*z* (%): 331 (M^+, 100), 316 (18), 287 (49), 274 (25), 259 (32), 232 (18), 184 (15), 120 (21), 104 (36). Anal. Calcd for $C_{19}H_{17}N_5O$: C, 68.87; H, 5.17; N, 21.13. Found: C, 68.62; H, 5.25; N, 21.01.

2-Di(n-butyl)amino-1,9-diphenyl-1,9-dihydro-6H-purin-6-one (***6b***)*:* White crystals. Mp 122–123 °C. IR (KBr): 1718 (C=O), 1606, 1535, 1494, 1464, 1372, 1118 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.96 (s, 1H, Ar–H), 7.73–7.26 (m, 10H, Ar–H), 2.97 (t, *J* = 7.4 Hz, 4H, $2NCH_2$), 1.21–1.08 (m, 8H, $4CH_2$), 0.82 (t, *J* = 7.2 Hz, 6H, $2CH_3$). MS *m*/*z* (%): 415 (M^+, 100), 372 (54), 358 (60), 316 (27), 287 (88), 240 (51), 128 (17), 104 (16). Anal. Calcd for $C_{25}H_{29}N_5O$: C, 72.26; H, 7.03; N, 16.85. Found: C, 72.19; H, 6.95; N, 16.92.

1,9-Diphenyl-2-di(i-propyl)amino-1,9-dihydro-6H-purin-6-one (***6c***)*:* White crystals. Mp 182–183 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.87 (s, 1H, Ar–H), 7.69–7.28 (m, 10H, Ar–H), 3.56–3.49 (m, 2H, 2NCH), 1.04 (d, *J* = 6.8 Hz, 12H, $4CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 158.9, 155.7, 147.6, 138.9, 137.1, 134.8, 129.4129.3, 128.7, 127.8, 127.7, 123.4, 119.9, 50.1, 21.3. MS *m*/*z* (%): 387 (M^+, 31), 344 (100), 302 (13), 287 (67), 104 (19), 77 (33). Anal. Calcd for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 71.47; H, 6.32; N, 18.23.

1-(4-Chlorophenyl)-2-di(n-pentyl)amino-9-phenyl-1,9-dihydro-6H-purin-6-one (***6d***)*:* White crystals. Mp 177–178 °C. IR (KBr): 1698 (C=O), 1525, 1494, 1377, 1347, 1092 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.94 (s, 1H, Ar–H), 7.70–7.25 (m, 9H, Ar–H), 2.96 (t, *J* = 7.8 Hz, 4H, $2NCH_2$), 1.26–1.05 (m, 12H, $6CH_2$), 0.84 (t, *J* = 7.4 Hz, 6H, $2CH_3$). MS *m*/*z* (%): 477 (M^+, 98), 406 (58), 350 (28), 321 (100), 296 (27), 240 (28), 183 (7). Anal. Calcd for $C_{27}H_{32}ClN_5O$: C, 67.84; H, 6.75; N, 14.65. Found: C, 67.91; H, 6.82; N, 14.52.

1-(4-Chlorophenyl)-2-(piperidin-1-yl)-9-phenyl-1,9-dihydro-6H-purin-6-one (***6e***)*:* White crystals. Mp 266–268 °C. IR (KBr): 1708 (C=O), 1550, 1515, 1245, 1092 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.94 (s, 1H, Ar–H), 7.72–7.26 (m, 9H, Ar–H), 3.05 (t, *J* = 5.4 Hz, 4H, $2NCH_2$), 1.45–1.28 (m, 6H, $3CH_2$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 157.8, 157.2, 147.4, 137.3, 136.2, 134.8, 133.7, 130.2, 129.5, 129.0, 127.8, 123.0, 120.2, 50.2, 24.7, 23.9. MS *m*/*z* (%): 405 (M^+, 100), 376 (15), 321 (28), 266 (15), 183 (7), 103 (11). Anal. Calcd for $C_{22}H_{20}ClN_5O$: C, 65.10; H, 4.97; N, 17.25. Found: C, 65.01; H, 4.88; N, 17.28.

1,9-Diphenyl-2-methylamino-1,9-dihydro-6H-purin-6-one (***6f***)*:* White crystals. Mp >300 °C. IR (KBr): 3445 (N–H), 1703 (C=O), 1571, 1545, 1515, 1443, 1382, 1077 cm^{-1}. ^1H NMR (DMSO-d^6, 400 MHz): δ = 8.20 (s, 1H, Ar–H), 7.91–7.30 (m, 10H, Ar–H), 5.92 (d, *J* = 4.0 Hz, 1H, NH), 2.72 (d, *J* = 4.0 Hz, 3H, NCH_3). MS *m*/*z* (%): 317 (M^+, 100), 287 (15), 183 (12), 125 (19), 104 (17), 77 (65). Anal. Calcd for $C_{18}H_{15}N_5O$: C, 68.13; H, 4.76; N, 22.07. Found: C, 68.24; H, 4.90; N, 21.98.

1,9-Diphenyl-2-ethylamino-1,9-dihydro-6H-purin-6-one (***6g***)*:* White crystals. Mp >300 °C. IR (KBr): 3445 (N–H), 1703 (C=O), 1556, 1535, 1505 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.82 (s, 1H, Ar–H), 7.74–7.26 (m, 10H, Ar–H), 4.14 (br, 1H, NH), 3.40–3.33 (m, 2H, NCH_2), 1.10 (t, *J* = 7.2 Hz, 3H, CH_3). MS *m*/*z* (%): 331 (M^+, 100), 302 (21), 287 (27), 227 (18), 184 (11), 103 (30). Anal. Calcd for $C_{19}H_{17}N_5O$: C, 68.87; H, 5.17; N, 21.13. Found: C, 68.91; H, 5.31; N, 21.02.

1-(4-Chlorophenyl)-2-methylamino-9-phenyl-1,9-dihydro-6H-purin-6-one (***6h***)*:* White crystals. Mp >300 °C. IR (KBr): 3442 (N–H), 1703 (C=O), 1555, 1489, 1433, 1306 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.82 (s, 1H, Ar–H), 7.74–7.25 (m, 9H, Ar–H), 4.23 (br, 1H, NH), 2.90 (d, *J* = 4.0 Hz, 3H, CH_3). MS *m*/*z* (%): 351 (100, M^+), 321 (11), 185 (9), 184 (9), 159 (7), 77 (8). Anal. Calcd for $C_{18}H_{14}ClN_5O$: C, 61.46; H, 4.01; N, 19.91. Found: C, 61.61; H, 4.20; N, 20.03.

2-(n-Butylamino)-1-(4-chlorophenyl)-9-phenyl-1,9-dihydro-6H-purin-6-one (***6i***)*:* White crystals. Mp 185–187 °C. IR (KBr): 3440 (N–H), 1708 (C=O), 1540, 1433 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.83 (s, 1H, Ar–H), 7.73–7.24 (m, 9H, Ar–H), 4.17 (br, 1H, NH), 3.32 (m, 2H, NCH_2), 1.51–1.23 (m, 4H, $2CH_2$), 0.87 (t, *J* = 7.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 157.2, 151.9, 149.1, 136.2, 135.8, 135.1, 133.2, 130.8, 130.5, 129.4, 127.5, 123.0, 117.8, 41.8, 31.0, 19.8, 13.6. MS *m*/*z* (%): 393 (M^+, 100), 352 (11), 337 (26), 335 (79), 322 (11), 321 (34), 77 (20). Anal. Calcd for $C_{21}H_{20}ClN_5O$: C, 64.04; H, 5.12; N, 17.78. Found: C, 64.15; H, 5.25; N, 17.90.

1-(4-Chlorophenyl)-9-phenyl-2-phenylmethylamino-1,9-dihydro-6H-purin-6-one (***6j***)*:* White crystals. Mp 251–253 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.80 (s, 1H, Ar–H), 7.58–7.19 (m, 14H, Ar–H), 4.70 (br, 1H, NH), 4.50 (d, *J* = 5.6 Hz, 2H, NCH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 157.2, 151.7, 148.8, 138.1, 136.4, 136.0, 134.9, 133.1, 130.9, 130.5, 129.4, 128.5, 127.6, 127.4, 127.3, 123.2, 118.1, 46.0. MS *m*/*z* (%): 427 (M^+, 100), 336 (6), 201 (2), 103 (17), 91 (65), 77 (27). Anal. Calcd for $C_{24}H_{18}ClN_5O$: C, 67.37; H, 4.24; N, 16.37. Found: C, 67.46; H, 4.40; N, 16.19.

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24. Cytotoxic activities were evaluated by using standard MTT assay after exposure of cells to the tested compounds for 72 h. Results are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%.