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The first total synthesis of heteromine B, and an improved synthesis of heteromine A; natural products with antitumor activities

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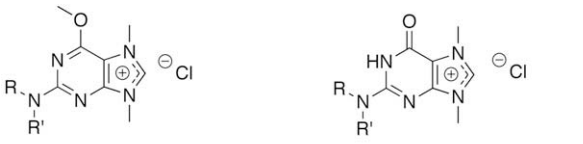
ABSTRACT

Efficient total syntheses of heteromines A and B (antitumor compounds previously isolated from the plant *Heterostemma brownii* Hayata) from commercially available 2-amino-6-chloropurine have been developed. The synthesis of heteromine A is considerably shorter than the previously reported synthesis, only requiring four steps, whereas the iodide salt of heteromine B was prepared in five steps. Heteromines A and B showed no significant antibacterial activity and therefore appear to be selective toward cancer cell lines.

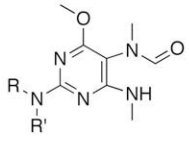
1. Introduction

Heteromines A–H (**1a**–**h**, Fig. 1) have been isolated from *Heterostemma brownii* Hayata (Asclepiadaceae),[1,2] a plant used for the treatment of tumors in Taipei folk medicine. Heteromines A and B are shown to be cytotoxic to several cancer cell lines.[2]



Heteromine A (**1a**) R=R'=Me
Heteromine B (**1b**) R=Me, R'=H
Heteromine C (**1c**) R=R'=H

Heteromine D (**1d**) R=R'=Me
Heteromine E (**1e**) R=Me, R'=H



Heteromine F (**1f**) R=R'=Me
Heteromine G (**1g**) R=Me, R'=H
Heteromine H (**1h**) R=R'=H

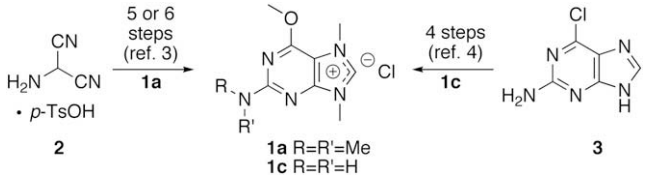
Figure 1. Structures of heteromines A–H.

The previously published synthesis of heteromine A (**1a**) involves five or six steps, the five-step synthesis involves the use of highly toxic methyl chloride, from the malononitrile **2** (Scheme 1).[3] We have synthesized heteromine C (**1c**) in four steps from the commercially available purine **3**.[4] No total synthesis of heteromine B has been described until now. Heteromines F, G, and H have been prepared from heteromines A, B, and C, respectively, isolated from the plant.[2]

As a continuation of our synthetic studies directed toward bioactive purine natural products,[4–11] we herein report the first total synthesis of heteromine B, an improved synthesis of heteromine A as well as an evaluation of antimicrobial activities of heteromines A–C.

2. Results and discussion

We have previously developed an efficient synthesis of heteromine C from 2-amino-6-chloropurine (**3**) (Scheme 1),[4] and we now wanted to explore the possibility of synthesizing heteromines A



Scheme 1. Previously reported total syntheses of heteromines A and C.

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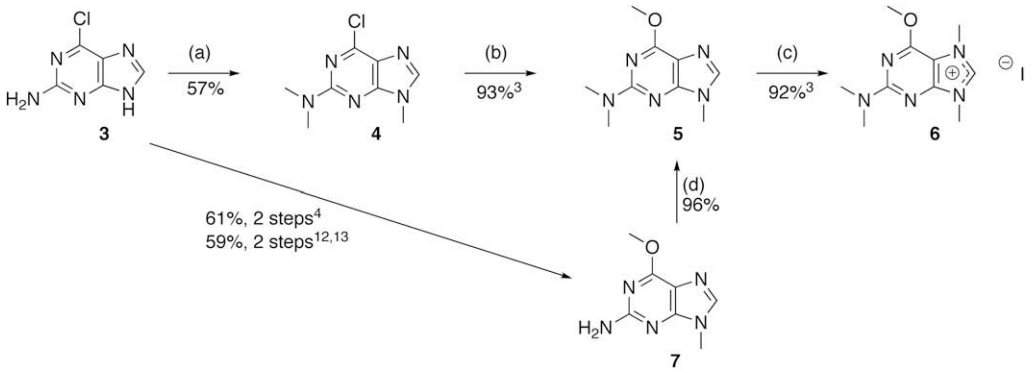
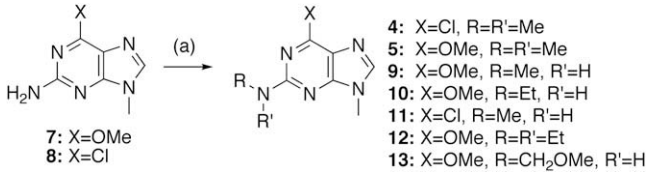


Scheme 2. (a) MeI, NaH, TBAB, THF; (b) NaOMe, MeOH, Δ, see Ref. 3; (c) MeI, acetone, see Ref. 3; (d) HCHO, $NaCNBH_3$, AcOH, MeOH, H_2O.

and B from the same starting material using a similar strategy. The most efficient route to heteromine A (as iodide, **6**) was found to be a one-step trimethylation of the 2-aminopurine **3** with methyl iodide in the presence of tributylammonium bromide (TBAB) and NaH (Scheme 2) to give 57% of **4**, followed by further synthetic transformations according to the previously reported heteromine A synthesis.[3] The *N*-7-methylated isomer of **4** was isolated in 17% yield as a by-product in the synthesis of **4**. Alternatively, compound **5** was available by reductive amination of formaldehyde employing 2-aminopurine **7** (see also Table 1 below). The latter can be prepared by *N*-9 methylation of purine **3** followed by nucleophilic displacement of the chlorine,[4] or the substituents may be introduced in reverse order.[12,13] Attempts to trimethylate 2-amino-6-methoxypurine[12] with methyl iodide in the presence of TBAB resulted only in complex mixtures.

Our first strategy for the synthesis of heteromine B involved attempts to monomethylate the amino group in compound **8**[4] employing the same set of reaction conditions used in trimethylation of compound **3** (Scheme 3) only with a reduced amount of all reagents [MeI (1.1 equiv), NaH (1.2 equiv), TBAB (1.2 equiv)], but compound **4** was the only isolated product, in 24% yield. Reduction in temperature or reaction time, or a combination of the two, gave either the same product or no conversion of starting material. Alkylation of **8** by the Mitsunobu reaction, using triphenylphosphine, diethyl azodicarboxylate (DEAD), and methanol was also unsuccessful.

Next, we turned to reductive amination using purines **7** and **8** (Scheme 3 and Table 1). Guanosine derivatives have been monoalkylated on N^2 when reacted with the desired aldehyde and $NaCNBH_3$.[14] However, dimethylation has been achieved when the aldehyde employed was formaldehyde.[15] Several attempts were



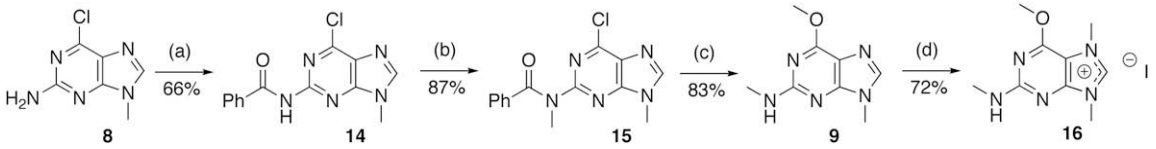
Scheme 3. (a) RCHO, $NaCNBH_3$, AcOH, 50 °C, for further details see Table 1.

made to adjust the reaction conditions in the reductive alkylation of **7** to obtain the monomethylated product **9**, instead of the dimethylated compound **5**. We found the aminopurines to be essentially unreactive toward formaldehyde unless acetic acid (AcOH) was present, and that using only a small excess of AcOH (1.3 equiv), resulted in a rather complex mixture. In addition to **5**, compound **13** was the only compound isolated from the mixture. The latter compound is probably formed by nucleophilic attack by methanol on an iminium ion intermediate in the reductive alkylation. Similar reactions are known in the literature.[16] Interestingly, acetaldehyde gave a mixture of the monoethylated and diethylated purines **10** and **12**, with the former as the major product and without complete conversion, under the same set of reaction conditions. Removal of methanol as a solvent in the reaction with formaldehyde, resulted in the formation of **5** and a range of by-products. No reaction was observed when the hydride reagent was changed to $NaBH_4$. The highest isolated yield of **5** was achieved using 35 equiv of formaldehyde. The amount could be reduced to 12 equiv, but still only the dimethylated product was observed. Lowering the reaction time to only 1.25 or 3 h showed full conversion by ^1H NMR spectroscopy of the crude, but only 62 and 81% yield of **5** was isolated. Employing the latter reaction

Table 1
Reductive alkylation of 2-aminopurines **7** and **8**

X	R (equiv HCHO or MeCHO)	Equiv AcOH	Equiv $NaCNBH_3$	Time (h)	Solvent[a]	Yield (%) **9**–**11**	Yield (%) **4**, **5** or **12**	Yield (%) **13**
Cl	Me (35)	1.6	6.0	71	$MeOH/H_2O$	—[b]	—[b]	—[b]
OMe	Me (35)	1.3	6.3	69	$MeOH/H_2O$	—[c]	—[c]	37 (**13**)
OMe	Me (35)	1.3	6.0	70	H_2O	—[c]	—[c]	—[c]
OMe	Me (12)	2.4	6.3	70	$MeOH/H_2O$	<12 (**9**)	45 (**5**)	4 (**13**)
OMe	Me (35)	23	6.3	70	$MeOH/H_2O$	—[c]	97[d] (**5**)	—[c]
OMe	Me (12)	23	6.3	70	$MeOH/H_2O$	—[c]	92 (**5**)	—[c]
OMe	Me (12)	23	6.3[f]	70	$MeOH/H_2O$	—[c]	—[c]	—[c]
OMe	Me (12)	23	6.3	1.25	$MeOH/H_2O$	—[c]	62[d] (**5**)	—[c]
OMe	Me (12)	23	6.3	3	$MeOH/H_2O$	—[c]	81 (**5**)	—[c]
OMe	Et (35)	1.3	6.3	70	$MeOH/H_2O$	ca. 55 (**10**)	<10 (**12**)	—[c]
OMe	Et (12)	23	6.3	3	$MeOH/H_2O$	49[d,e] (**10**)	51[d,e] (**12**)	—[c]

[a] $MeOH/H_2O$ was used in a ratio of 3:2.
[b] A complex mixture was formed, no products were identified.
[c] Not observed.
[d] From ^1H NMR.
[e] Isolated yields were 11% of **10** and 19% of **12**.
[f] $NaBH_4$ used instead of $NaCNBH_3$.



Scheme 4. (a) PhCOCl, pyridine; (b) K_2CO_3, MeI, acetone; (c) NaOMe, MeOH; (d) MeI, acetone.

conditions only using acetaldehyde, resulted in equivalent amounts of monoalkylated and dialkylated products. Attempts to employ 2-amino-6-methoxypurine[6] or the 2-aminopurine **3** in reductive aminations, resulted only in complex mixtures.

Since none of the 2-methylaminopurines **9** or **11** were available by direct monomethylation, the N^2 amino function was protected by a benzoyl group before methylation to give compound **15** (Scheme 4). Introduction of the methoxy group and removal of the protecting group was achieved in one step, by treatment with sodium methoxide. Compound **9** was finally reacted with methyl iodide to give the iodide salt of heteromine B (**16**). Since heteromines F (**1f**) and G (**1g**) earlier have been synthesized from heteromines A and B isolated from plant material,[2] the work described herein may also be regarded as a formal synthesis of heteromines F and G.

Heteromines A and B are reported to show cytotoxicity toward cancer cell lines; esophageal carcinoma, hepatoma, lymphoma, and leukemia cells.[2] Agelasines (cationic purinium salts isolated from marine sponges) and derivatives often display cytotoxicity both toward cancer cells and microorganisms.[6,7,9,11,17–19] Hence we also evaluated iodide salts of heteromines A–B, as well as the previously synthesized heteromine C,[4] as antimicrobial compounds. As also argued in a study of heteromine analogs, the identity of the counter ion is not expected to influence bioactivity since chloride anions will be present in a large excess in the bioassays used.[20] However the cytotoxicity displayed by these compounds appears to be selective toward cancer cell lines, with none of the heteromines examined exhibiting any significant inhibitory activity against a variety of microorganisms [MIC *Staphylococcus aureus* (Gram positive bacterium) and *Escherichia coli* (Gram negative bacterium) >32 μg/mL, MIC *Candida albicans* (yeast) >16 μg/mL, 0% inhibition of *Mycobacterium tuberculosis* (mycobacterium) at 6.25 μg/mL].

3. Conclusion

We have shown the first total synthesis of the iodide salt of heteromine B, in five steps from commercially available 2-amino-6-chloropurine (**3**). All steps were completed in good to very good yields. Direct trimethylation of 2-amino-6-chloropurine (**3**) greatly improved the synthesis of heteromine A. Reductive alkylation of 2-amino-6-methoxy-9-methyl-9*H*-purine (**7**) resulted in dimethylation of the amino group and thus opens up an alternative pathway to heteromine A. Heteromines A and B are previously reported to be active against cancer cell lines and were now evaluated as antimicrobials. Neither of the two displayed any significant growth inhibition of the different microorganisms examined, and therefore appear to display a selective activity against (certain) cancer cell lines. The narrow spectrum of toxicity and the efficient syntheses reported herein, makes heteromines A and B more interesting as potential anticancer drugs or drug leads.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded at 500 MHz with a Bruker Avance DRX 500 instrument or at 300 MHz with a Bruker Avance DPX 300 instrument. Decoupled ^{13}C NMR spectra were recorded at 125 or 75 MHz using the instruments mentioned above. Assignments of ^{1}H and ^{13}C resonances are inferred from 1D ^{1}H NMR, 1D ^{13}C NMR, as well as relevant 2D NMR spectral data. Mass spectra under electron impact conditions were recorded with a VG Prospec instrument at 70 eV ionizing voltage, and are presented as *m*/*z* (% rel int.). Electrospray MS spectra were recorded with a Micromass Q-Tof-2 mass spectrometer. Elemental analyses were carried out at the Centre for Chemical and Biochemical Analysis, School of Chemistry, University of Birmingham, UK. Melting points were determined with a Büchi Melting Point B-545 apparatus and are uncorrected. THF was purified by a solvent purification system, MB SPS-800 from MBraun. Pyridine was distilled from CaH_2 and stored over molecular sieves (4 Å). Acetone was distilled from potassium carbonate and stored over molecular sieves (4 Å). Methanol used as solvent in reactions was distilled from Mg and stored over molecular sieves (4 Å). Silica gel for flash chromatography was purchased from Merck, Darmstadt, Germany (Merck No. 09385) and flash chromatography were performed manually or with an Isco Inc. CombiFlash Companion instrument. Compounds available by literature methods: **5**,[3] **6**,[3] **7**,[4,21] and **8**.[21] All other reagents were commercially available and used as received. Antimicrobial activities were determined as described before.[7]

4.1.1. 6-Chloro-N,N,9-trimethyl-9H-purin-2-amine (*4*)

2-Amino-6-chloropurine **3** (0.848 g, 5.00 mmol) in dry THF (50 mL) was cooled to 0 °C under an argon atmosphere and NaH (0.86 g, 36 mmol) was added. The cooling bath was removed, TBAB (5.80 g, 18.0 mmol) followed by methyl iodide (1.1 mL, 18 mmol) was added at ambient temperature, and the resulting reaction mixture stirred for 21 h and filtered. The filtrate was evaporated in vacuo and the residue was purified twice by flash chromatography (CombiFlash, 0–5% MeOH in CH_2Cl_2) to give **4** (0.604 g, 2.85 mmol) in 57% yield and 6-chloro-*N*,*N*,7-trimethyl-7*H*-purin-2-amine (0.179 g, 0.846 mmol) in 17% yield, both as colorless solids.

Compound **4**. Mp 151.1–151.4 °C (from MeOH–CH_2Cl_2), (lit. 152–153 °C).[3] ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.11 [s, 6H, $N(CH_3)_2$], 3.63 (s, 3H, N^9CH_3), 8.07 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.3 (N^9CH_3), 37.1 [$N(CH_3)_2$], 122.5 (C-5), 144.0 (C-8), 148.8 (C-6), 154.2 (C-4), 158.3 (C-2); MS EI *m*/*z* (rel %) 213/211 (M^+, 33/100), 198/196 (18/50), 184/182 (15/45), 170/168 (7/20), 133 (31); HRMS (EI) calcd for $C_8H_{10}ClN_5$ 211.0625, found 211.0632. More spectral data can be found in Ref. 3.

4.1.2. 6-Chloro-N,N,7-trimethyl-7H-purin-2-amine

Mp 172–173 °C (from MeOH–CH_2Cl_2, decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.09 [s, 6H, $N(CH_3)_2$], 3.90 (s, 3H, NCH_3), 8.31 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 33.5 (NCH_3), 37.2 [$N(CH_3)_2$], 115.1 (C-5), 142.3 (C-6), 150.0 (C-8), 158.7 (C-2), 163.9 (C-4); MS EI *m*/*z* (rel %) 213/211 (M^+, 32/100), 198/196 (20/62), 184/182 (23/70), 170/168 (10/28), 133 (25); HRMS (EI) calcd for $C_8H_{10}ClN_5$ 211.0625, found 211.0621.

4.1.3. 6-Methoxy-N,9-dimethyl-9H-purin-2-amine (*9*)

Sodium methoxide (0.95 M, 4.0 mL, 3.8 mmol) in methanol was added to *N*-(6-chloro-9-methyl-9*H*-purin-2-yl)-*N*-methylbenzamide **14** (0.227 g, 0.752 mmol) and the resulting mixture was stirred at ambient temperature under an argon atmosphere for 22 h. The

reaction was quenched by addition of ammonium chloride (0.041 g, 0.77 mmol) and water (ca. 2 mL) and evaporated in vacuo. The residue was purified by flash chromatography (CombiFlash, 40 g column, 0–5% MeOH in CH_2Cl_2) to give **9** (0.134 g, 0.695 mmol) in 92% yield as a colorless solid. Mp 173.6–175.6 °C (from MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, CD_2Cl_2) δ 3.01 (d, J=5.1 Hz, 3H, N^2CH_3), 3.66 (s, 3H, N^9CH_3), 4.05 (s, 3H, OCH_3), 5.03 (br s, 1H, NH), 7.50 (s, 1H, H-8); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 28.9 (N^2CH_3), 29.6 (N^9CH_3), 53.8 (OCH_3), 115.0 (C-5), 139.7 (C-8), 155.0 (C-4), 160.3 (C-2), 161.5 (C-6); MS EI m/z (rel %) 193 (M^+, 100), 164 (28), 149 (28); HRMS (EI) calcd for $C_8H_{11}N_5O$ 193.0964, found 193.0958. Anal. Calcd C, 49.73; H, 5.74; N, 36.25. Found C, 49.65; H, 5.65; N, 36.18%.

4.1.4. *N-Ethyl-6-methoxy-9-methyl-9H-purin-2-amine (**10**) and N,N-diethyl-6-methoxy-9-methyl-9H-purin-2-amine (**12**)*

Methanol (3 mL), water (2 mL), formaldehyde (0.34 mL, 6.0 mmol), and $NaBH_3CN$ (0.20 g, 3.2 mmol) were added to 6-methoxy-9-methyl-9*H*-purin-2-amine **7** (0.091 g, 0.51 mmol) and the resulting reaction mixture was stirred at ambient temperature for ca. 3 min. Glacial acetic acid (0.67 mL, 12 mmol) was added and the reaction mixture was stirred at 50 °C in a sealed container for 3 h. The solvents were evaporated in vacuo and the crude product was purified twice by flash chromatography (CombiFlash, 0–6% MeOH in CH_2Cl_2) to give **10** (0.012 g, 0.058 mmol) in 11% yield and **12** (0.023 g, 0.098 mmol) in 19% yield, both as colorless solids.

Compound **10**. Mp 129.9–131.2 °C (MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.13 (t, J=7.1 Hz, 3H, CH_2CH_3), 3.31 (m, 2H, CH_2), 3.59 (s, 3H, NCH_3), 3.95 (s, 3H, OCH_3), 6.86 (t, J=5.4 Hz, 1H, NH), 7.79 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.7 (CH_2CH_3), 29.1 (NCH_3), 35.8 (CH_2), 52.9 (OCH_3), 113.4 (C-5), 140.1 (C-8), ca. 152 (br, C-4), 158.7 (C-2), 160.4 (C-6); MS EI m/z (rel %) 207 (M^+, 89), 192 (100), 164 (25), 163 (27), 149 (25); HRMS (EI) calcd for $C_9H_{13}N_5O$ 207.1120, found 207.1115.

Compound **12**. Mp 89.8–91.0 °C (from MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (t, J=7.0 Hz, 6H, 2×CH_2CH_3), 3.59 (s, 3H, NCH_3), 3.61 (q, J=7.0 Hz, 4H, 2×CH_2), 3.97 (s, 3H, OCH_3), 7.81 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.1 (2×CH_2CH_3), 29.0 (NCH_3), 41.6 (2×CH_2), 52.8 (OCH_3), 112.9 (C-5), 140.3 (C-8), 154.6 (C-4), 157.2 (C-2), 160.0 (C-6); MS EI m/z (rel %) 235 (M^+, 78), 220 (100), 206 (66), 192 (150); HRMS (EI) calcd for $C_{11}H_{17}N_5O$ 235.1433, found 235.1433. Anal. Calcd C, 56.15; H, 7.28; N, 29.77. Found C, 56.33; H, 7.29; N, 29.78%.

4.1.5. *6-Methoxy-N-(methoxymethyl)-9-methyl-9H-purin-2-amine (**13**)*

$NaBH_3CN$ (0.105 g, 1.67 mmol), aq formaldehyde (36%, 0.75 mL, 9.7 mmol), methanol (1.5 mL), water (1.0 mL), and finally glacial acetic acid (0.02 mL, 0.4 mmol) were added to 2-amino-6-methoxy-9-methyl-9*H*-purine **7** (0.050 g, 0.28 mmol) and the resulting mixture was stirred at 50 °C in a sealed container for three days. The reaction mixture was allowed to cool to ambient temperature and the solvents were evaporated in vacuo. The residue was purified by manual flash chromatography (3×12 cm column, 2–10% MeOH in CH_2Cl_2) to give **13** (0.029 g, ca. 80% purity) as an off-white wax. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.22 (s, 3H, CH_3, CH_2OCH_3), 3.61 (s, 3H, NCH_3), 3.98 (s, 3H, O^6CH_3), 4.76 (d, J=7.0 Hz, 2H, CH_2), 7.79 (t, J=7.0 Hz, 1H, NH), 7.87 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.0 (NCH_3), 53.2 (O^6CH_3), 54.7 (CH_2OCH_3), 73.5 (CH_2), 114.4 (C-5), 141.0 (C-8), 154.2 (C-4), 158.2 (C-2), 160.5 (C-6); MS EI m/z (rel %) 223 (M^+, 54), 208 (100), 192 (100), 191 (77), 180 (48), 164 (82), 163 (53), 149 (75); HRMS (EI) calcd for $C_9H_{13}N_5O_2$ 223.1069, found 223.1072.

4.1.6. *N-(6-Chloro-9-methyl-9H-purin-2-yl)benzamide (**14**)*

Distilled benzoyl chloride (0.39 mL, 3.3 mmol) was added to a suspension of 2-amino-6-chloro-9-methyl-9*H*-purine **8** (0.305 g, 1.66 mmol) in pyridine (13.4 mL) at 0 °C, and the resulting mixture was stirred at ambient temperature under an argon atmosphere for 2.5 h. The solution was evaporated in vacuo and the residue was purified by column flash chromatography (3×12 cm column, 3% MeOH in CH_2Cl_2) to give **14** (0.314 g, 1.09 mmol) in 66% yield as a colorless solid. Mp 213.7–215.1 °C (from MeOH–CH_2Cl_2, decomp.). ^{1}H NMR (300 MHz, CD_2Cl_2) δ 3.85 (s, 3H, CH_3), 7.49–7.54 (m, 2H, Ph), 7.57–7.60 (m, 1H, Ph), 7.93 (m, 2H, Ph), 8.01 (s, 1H, H-8), 8.88 (s, 1H, NH); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 30.5 (CH_3), 127.8 (2×CH in Ph), 128.7 (C-5), 129.1 (2×CH in Ph), 132.7 (Ph), 134.6 (Ph), 146.0 (C-8), 150.9 (C-2 or C-6), 152.5 (C-2 or C-6), 153.7 (C-4), 164.8 (C=O); MS EI m/z (rel %) 289/287 (M^+, 8/23), 260/258 (12/29), 105 (100), 77 (49); HRMS (EI) calcd for $C_{13}H_{10}ClN_5O$ 287.0574, found 287.0577. Anal. Calcd C, 54.27; H, 3.50; N, 24.34. Found C, 54.08; H, 3.30; N, 24.01%.

4.1.7. *N-(6-Chloro-9-methyl-9H-purin-2-yl)-N-methyl benzamide (**15**)*

K_2CO_3 (0.282 g, 2.04 mmol) and methyl iodide (0.60 mL, 9.6 mmol) were added to *N*-(6-chloro-9-methyl-9*H*-purin-2-yl)benzamide **14** (0.279 g, 0.970 mmol) in acetone (24 mL), and the resulting mixture was stirred at ambient temperature under an argon atmosphere for 21 h. The reaction mixture was evaporated in vacuo and the resulting residue was purified by flash chromatography to give **15** (0.254 g, 0.842 mmol) in 87% yield as a yellow solid. Mp 119.3–120.8 °C (from MeOH–CH_2Cl_2, decomp.). ^{1}H NMR (300 MHz, CD_2Cl_2) δ 3.46 (s, 3H, N^9CH_3), 3.67 (s, 3H, N^2CH_3), 7.19–7.34 (m, 5H, Ph), 7.92 (s, 1H, H-8); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 29.9 (N^9CH_3), 35.1 (N^2CH_3), 128.0 (2×CH in Ph), 128.2 (2×CH in Ph), 128.4 (C-5), 130.2 (Ph), 138.0 (Ph), 146.2 (C-8), 150.2 (C-6), 152.9 (C-4), 156.7 (C-2), 172.5 (C=O); MS EI m/z (rel %) 303/301 (M^+, 14/40), 274/272 (11/29), 105 (100), 77 (57); HRMS (EI) calcd for $C_{14}H_{12}ClN_5O$ 301.0730, found 301.0735. Anal. Calcd C, 57.73; H, 4.01; N, 23.21. Found C, 57.34; H, 3.65; N, 23.26%.

4.1.8. *6-Methoxy-7,9-dimethyl-2-(methylamino)-9H-purin-7-ium iodide (**16**)*

Methyl iodide (0.64 mL, 10 mmol) was added to 6-methoxy-*N*,9-dimethyl-9*H*-purin-2-amine **9** (0.142 g, 0.735 mmol) in acetone (5.5 mL) and the resulting mixture was stirred at ambient temperature under an argon atmosphere for four days and filtered. The solid was washed with acetone (5 mL) and dried in vacuo to give **1b** (0.178 g, 0.531 mmol) in 78% as a pinkish solid. Mp 293–294 °C (decomp.) ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) δ 2.87 (d, J=4.8 Hz, 3H, $NHCH_3$), 3.78 (s, 3H, N^9CH_3), 4.00 (s, 3H, N^7CH_3), 4.08 (s, 3H, OCH_3), 7.65 (br d, J=4.8 Hz, 1H, NH), 9.32 (s, 1H, H-8); ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) δ 27.8 ($NHCH_3$), 30.7 (N^9CH_3), 35.7 (N^7CH_3), 54.3 (OCH_3), 104.5 (C-5), 139.9 (C-8), 151.7 (C-4), 157.8 (C-6), 160.6 (C-2); MS ESI m/z (rel %) 208 (M^+, 100%); HRMS (ESI) calcd for $C_9H_{14}N_5O$ 208.1198, found 208.1199. Anal. Calcd C, 32.25; H, 4.21; N, 20.90. Found C, 32.66; H, 3.97; N, 20.57%. More spectral data (of the chloride salt) can be found in Ref. 1.

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