TRICYCLIC PURINE ANALOGS DERIVED FROM 2-AMINO-6-CHLOROPURINE AND 2,6-DIAMINOPURINE AND THEIR METHYLATED QUATERNARY SALTS

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A novel series of tricyclic, etheno-bridged purine analogs was sythesized from 2-amino-6-(substituted amino)-9-methylpurines by cyclization with chloroacetaldehyde, with particular focus on the regioselectivity of the cyclization reaction and fluorescence properties. The analogs as well as the starting purines were alkylated with iodomethane, affording a new class of quaternary salts with potential biological activity. Neither significant fluorescence nor cytostatic effect was found.

Keywords: Purines; Alkylation; Chloroacetaldehyde; Fluorescence; Fused heterocycles; Quaternization; Nucleobases.

In pharmacology, fluorescence labeling is widely used for specific and sensitive analytical determination of bioactive compounds in body fluids or other complex biological systems as well as for visualization of such compounds inside the cells or in their compartments. The latter purpose could gain a high importance particularly in those cases where the compound in question cannot bear radionuclide label, e.g. in order to determine influx into the cell (conditio sine qua non for biological activity)[1]. The analytical application is very important for preclinical phase of drug development – the practical use of fluorescent labeling depends (except for rare situations where the drug or its metabolite fluoresce per se) on the completeness of transformation, which must proceed in water and must be able to stand validation. In nucleic acid chemistry such a situation was first handled by Leonard et al.[2] who realized the potential of tricyclic heteroaromatic compounds specifically formed from adenine derivatives by reaction with chloroacetaldehyde in weakly acidic aqueous solution. The method was practically applied for diverse purposes including the determination of our FDA- and EMEA-approved anti-HBV drug adefovir, the parent active com-

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pound that is used in an oral prodrug formulation as adefovir dipivoxil (Hepsera™)[3].

Linear and angular etheno-bridged tricyclic bases are formed from purine nucleosides in the organisms exposed to vinyl chloride which is in liver converted to chloroacetaldehyde; the DNA of the exposed animals contains N^2,3-ethenoguanine – a possible reason for mutagenesis[4,5]. Such a considerable sensitivity of guanine to the transformation attracted an increased attention to this process and numerous synthetic materials were prepared and their characteristics investigated[6–10].

Leonard also realized the potential of extended adenine base in linear benzoadenine for investigation of the topology of active centre of an enzyme that binds adenine derivatives[11]. He also showed that the enlargement of the base does not hinder some reactions taking place at this base or its nucleosides[12].

As some of 2,6-diaminopurine (2-aminoadenine) derivatives in the acyclic nucleoside phosphonate (ANP) series display anticancer activity and many are potent antivirals[13], we are interested in development of a methodology of fluorescent visualization of these compounds. In first approximation we decided to prepare potentially fluorescent 9-methyl derivatives of etheno 2,6-diaminopurine and 2-amino-6-(substituted amino) purines for that purpose. This study deals with their synthesis by cyclization of appropriate 2,6-diaminopurine derivatives with chloroacetaldehyde. Additionally, the influence of substitution on 6-amino function on the regiospecificity of cyclization was investigated, as both linear and angular products of cyclization are possible.

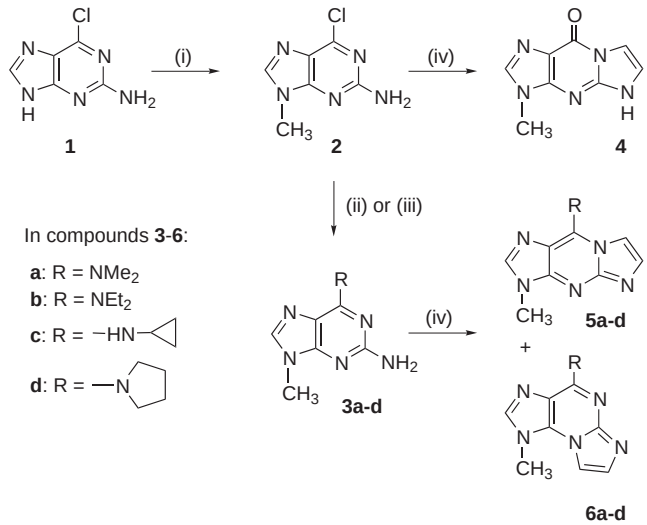
Furthermore, some of naturally occurring highly methylated quaternary purinium salts exhibit a significant cytostatic activity[14]. Therefore, we decided to perform a quaternization study on our tricyclic etheno-bridged derivatives and on their direct precursors (2-amino-6-(substituted amino) purines). We were particularly interested in the biological activity of the latter, as they are close analogs of heteromines[15].

RESULTS AND DISCUSSION

The 2-amino-6-(substituted amino) purines were prepared from commercial 2-amino-6-chloropurine. It was methylated with methyl iodide[16], the 7- and 9-regioisomers were separated and the desired 6-chloro-9-methylpurin-2-amine (**2**) was obtained in 65% yield (Scheme 1). Subsequent nucleophilic substitution of the chloro atom with different amines[17,18] led to a se-

ries of 2-amino-9-methyl-6-(substituted amino) purines **3a**–**3d**. The reaction proceeded smoothly, with high yields (70–85%).

Our initial cyclization experiments employing compound **2** and excess of 2-chloroacetaldehyde or 2-chloroacetaldehyde diethylacetal were carried out in aqueous solution at pH 6 and ambient temperature as published before in the case of ethenoguanine derivatives[2,6]. Cyclization took place together with hydrolysis of chlorine and only linear product of cyclization was isolated. However, yields were only about 10% without complete conversion even after several days which corresponded to previously reported reactions of guanine derivatives[19,20]. As there are no sensitive nucleoside linkages either in our model methyl derivative, or in the future applications to ANP, we decided to increase reaction temperature to 70 °C. The reaction of compound **2** with excess of 2-chloroacetaldehyde performed under heating in water/dioxane mixture (1:1, dioxane was added in order to enhance the solubility of reactant) led to disappearance of the starting material within 5 h and afforded only linear product **4** in 17% yield (Scheme 1).



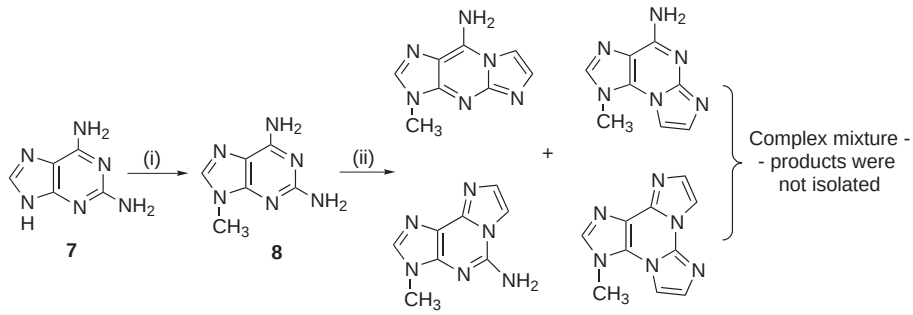
SCHEME 1

(i) 1.5 eq. MeI, 1.5 eq. NaH, DMF, 70 °C, 3 h, 65%; (ii) 3 eq. $(CH_3)_2NCOONH_2(CH_3)_2$, acetonitrile, reflux, 2 h, 80%; (iii) 4 eq. amine RH, abs. EtOH, reflux, 2 h, 70–85%; (iv) 1 M aq. $ClCH_2CHO$, H_2O/dioxane, 70 °C, 5–8 h, for yields see Table I

TABLE I
Yields of linear and angular isomers

Reactant	Linear product (yield in %)	Angular product (yield in %)
3a	**5a** (0)	**6a** (19)
3b	**5b** (17)	**6b** (56)
3c	**5c** (30)	**6c** (0)
3d	**5d** (7)	**6d** (15)

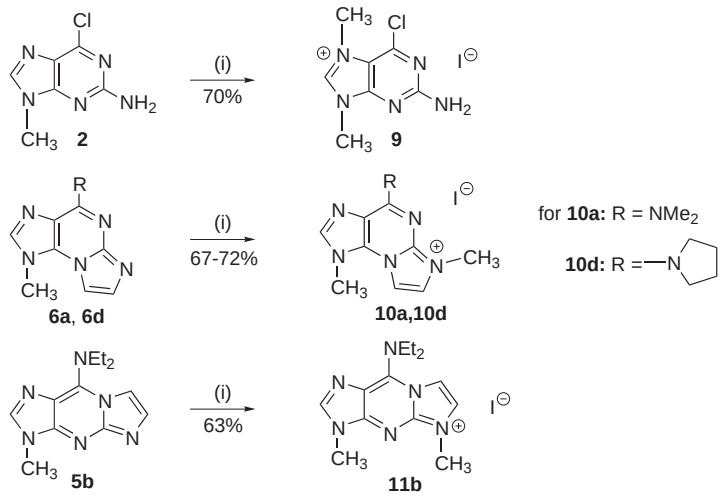
Cyclization of 2,6-diamino-9-methylpurine **8** with 2-chloroacetaldehyde was carried out under the same conditions and resulted in formation of a complex reaction mixture (three tricyclic and one tetracyclic products are theoretically possible, Scheme 2), however, we did not succeed in product isolation. Reactions of 2-amino-6-(substituted amino) purines led to two types of cyclized structures – linear **5** and angular **6** (Scheme 1, Table I). The influence of the different alkylamino substituents in position six of the purine scaffold was notable. The cyclopropylamino-substituted derivative **3c** gave the linear isomer **5c** as the only isolable product in 30% yield (Table I), while in the case of the derivatives **3a**, **3b** and **3d** the angular product predominates (Table I). The 6-dimethylamino analog **3a** provided the angular product **6a** in yield of 19% as the only identifiable product. The pyrrolidino **3d** and diethylamino derivative **3b** gave a mixture of angular (**6d** and **6b**) and linear (**5d** and **5b**) isomers, respectively, with an excess of the first one in both cases (Table I). These results could be attributed probably to the sterical hindrance of the alkylamino substituents. Another important ele-



SCHEME 2
(i) methyl 4-toluenesulfonate, Cs_2CO_3, DMF, 70 °C, 5 h, 50%; (ii) 1 M aq. $ClCH_2CHO$, water/dioxane, 70 °C, 5 h

ment is the possibility of stabilization as the 5 H tautomeric form (**3c** and **4**) which leads to strong preference of the linear isomer (Table I, Scheme 1).

Quaternization experiments with compounds **2**, **3a**–**3d**, **5b**, **6a**, **6d** were performed analogously to the procedures reported in the literature[21] by treatment with iodomethane in acetone at room temperature. The reactions are slow, taking several days to reach complete disappearance of the starting material. In the 2-amino-9-methyl-6-(substituted amino) purine series, compound **2** afforded quaternary salt **9** that crystallized directly from the reaction mixture in 70% yield (Scheme 3). On the other hand, no crystallization from reaction mixture occurred in the case of compounds **3a**–**3d** that afforded inseparable mixtures of products. In the angular tricyclic series, derivatives **6a** and **6d** were selected as model compounds, leading to **10a** and **10d**, while quaternization of **5b**, a representative of the linear tricyclic series, led to **11b** (Scheme 3). Similarly to the examples reported in the literature[21] and our product **9**, salt **10a** crystallized directly from the reaction mixture. In the case of the remaining tricyclic compounds, the quaternized products **10d** and **11b** did not crystallize from the reaction mixture, but chromatography on silica gel afforded pure products in good yields. (Scheme 3).



SCHEME 3
(i) MeI, acetone, r.t., 7 days

NMR Spectroscopy

Structures of new compounds were confirmed by NMR spectroscopy. Complete assignment of all ^{1}H and ^{13}C resonances is based on combination ^{1}H, ^{13}C APT, H,C-HSQC and H,C-HMBC experiments. DPFGSE-NOE was found to be the ultimate tool in structure distinguishing of the linear and the angular derivatives. There is only NOE contact between NCH_3 and H-2 in the case of the linear isomer, while the NOE enhancements of H-2 and H-8 could be observed upon selective irradiation of the NCH_3 protons of the angular isomer (Fig. 1).

Comparison of the NMR spectra of both isomers serves as an additional support for the structure elucidation. For example, chemical shift of NCH_3 group differs for the linear isomer (3.64–3.73 ppm in ^{1}H; 29.23–29.68 ppm in ^{13}C) and the angular one (4.07–4.13 ppm in ^{1}H; 32.71–33.06 ppm in ^{13}C) reflecting deshielding of NCH_3 group in the angular derivative by the formed heteroaromatic ring. Also, slow motion of the R group in the angular derivative leads to broadening of its NMR signals, which is not observable in the case of the linear isomer (Tables II and III).

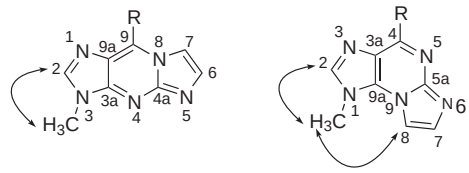
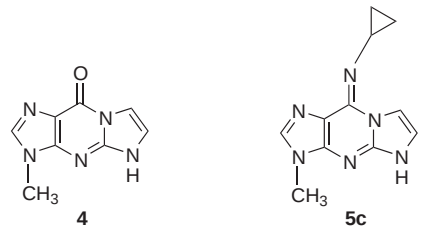


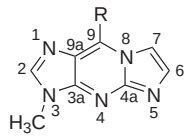
FIG. 1
NOE contacts of CH_3N group in the linear and the angular isomers



4 **5c**

FIG. 2
5 H tautomer of **4** and **5c**

TABLE II
^{1}H and ^{13}C NMR chemical shifts of the linear isomers



a) ^{1}H NMR (DMSO-d_6)

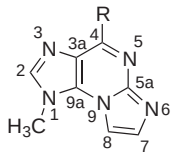
Compd	R	CH_3	H-2	H-6	H-7
4	OH	3.65	7.87	7.42 (2.7)	7.60 (2.7)
5b	Et_2N	3.70	8.26	7.55 (1.7)	7.57 (1.7)
5c	cyclopropyl-NH	3.73	8.24	7.71 (2.4)	8.50 (2.4)
5d	pyrrolidin-1-yl	3.64	8.02	7.36 (1.9)	7.95 (1.9)

b) ^{13}C NMR (DMSO-d_6)

Compd	R	CH_3	CH-2	C-3a	C-4a	CH-6	CH-7	C-9	C-9a
4	OH	29.57	139.80	151.00 or 151.50	146.19	116.47	106.94	151.00 or 151.50	115.01
5b	Et_2N	29.41	146.16	151.29	149.40	132.72	107.47	142.04	117.70
5c	cyclopropyl-NH	29.68	143.79	151.44	145.53	123.19	108.05	142.45	111.65
5d	pyrrolidin-1-yl	29.23	142.66	151.09	150.57	131.62	108.32	142.19	112.84

The linear isomer containing R substituent capable of forming tautomeric forms (R = OH or cyclopropyl-NH) exists exclusively as the 5 H tautomer as shown in Fig. 2. This observation is supported by the presence of NOE contact between 5-NH and H-6. Also differences of ^{13}C NMR chemical shift for C-4a (~146 ppm for 5-NH and ~150 ppm for R-NH or OH tautomers, respectively) and CH-6 (116.47–123.19 ppm for 5-NH and ~132 ppm for R-NH or OH tautomers, respectively) indicate shielding and electron density changes of the particular tautomer.

TABLE III
^{1}H and ^{13}C NMR chemical shifts of the angular isomers



a) ^{1}H NMR (DMSO-d_6)

Compd	R	CH_3	H-2	H-7	H-8
6a	Me_2N	4.07	7.92	7.27 (1.7)	7.85 (1.7)
6b	Et_2N	4.13	8.19	7.59 (2.3)	8.09 (2.3)
6d	pyrrolidin-1-yl	4.08	7.97	7.31 (1.7)	7.88 (1.7)

b) ^{13}C NMR (DMSO-d_6)

Compd	R	CH_3	CH-2	C-3a	C-4	C-5a	CH-7	CH-8	C-9a
6a	OH	32.71	137.29	116.30	150.79	148.08	129.92	105.98	134.72
6b	Et_2N	33.06	139.13	116.57	150.68	144.79	122.20	108.04	134.73
6d	pyrrolidin-1-yl	32.73	138.04	116.55	149.32	147.88	128.41	106.26	134.43

Fluorescence and Cytostatic Properties

Generally, the fluorescence properties of etheno-bridged triheterocyclic bases are ambivalent and, particularly in the case of the linear structures, strongly dependent on substitution[2,22,23]. Naturally occurring rare nucleoside, wyosine[22] and its analog acyclowyosine[23] are strongly fluorescent and so is the angular N^2,3-ethenoguanine[2]. On the other side, the linear N^2,1-ethenoguanine does not fluoresce[2]. Obviously, aromatic substitution leads to fluorescent derivatives[10]. As our compounds represent a so far undescribed class of tricyclic derivatives, we wanted to investigate whether they possess fluorescence properties despite the absence of aromatic substituent or fluorescence labeling.

However, none of our cyclized compounds **5a**–**5d** and **6a**–**6d** showed any significant fluorescence.

In vitro cytostatic activity tests (cell growth inhibition) were performed with cultures of murine leukemia L1210 cells (ATCC CCL 219), human promyelocytic leukemia HL60 cells (ATCC CCL 240), human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) and the human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119). None of the tested compounds **3a**–**3d**, **4**, **5a**–**5d**, **6a**–**6d**, **9**, **10a**, **10d** and **11b** showed any significant cytostatic effect.

Conclusions

We have synthesized a novel type of etheno-bridged tricyclic purine analogs and related methylated quaternary salts. In cyclization of 2-amino-6-(substituted amino) purines with chloroacetaldehyde, significant control of regioselectivity by substituent in the 6-amino function was observed. As none of the prepared coumpounds is fluorescent, our original proposal to use this type of compounds as fluorescent probes is not viable.

EXPERIMENTAL

General

Melting points were determined on a Kofler block and are uncorrected. Analytical TLC was performed on silica gel pre-coated aluminium plates with fluorescent indicator (Merck 5554, 60 F_{254}). Spots were visualized with UV light (254 nm). Column chromatography was carried out on silica gel (Sigma S-0507, 40–63 μm). Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using EI (electron energy 70 eV) or FAB (ionisation with Xe, accelerating voltage 8 kV, thioglycerol/glycerol 3:1 mixture or bis(2-hydroxyethyl) disulfide were used as matrix). ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.7 MHz on a Varian Unity 500 instrument in DMSO-d_6 (referenced to the solvent signal δ 2.50 and 39.70 ppm, respectively). Chemical shifts are given in ppm and coupling constants (*J*) in Hz. UV spectra were taken on a Beckman DU-65 spectrophotometer in methanol solution. IR spectra were obtained on an FT IR Bruker Equinox IFS 55 spectrometer in KBr pellets. Elemental analyses were carried out on a Perkin Elmer CHN Analyser 2400, Series II Sys (Perkin Elmer, Norwolk, CT, U.S.A.). Fluorescence excitation and emission spectra were measured on an Aminco Bowman Series 2 luminescence spectrometer.

6-Chloro-9-methyl-9*H*-purin-2-amine (**2**)

5.5 g of 6-chloropurine-2-amine (33 mmol) was dissolved in dry DMF. 1.5 g of NaH (60 wt.%, 38 mmol) was added, followed by 1.5 g of iodomethane (38 mmol). The reaction mixture was stirred at room temperature for 12 h, then evaporated in vacuo. The residue was codistilled with ethanol and then dissolved in ethyl acetate. The solution was washed

with water, dried over anhydrous $MgSO_4$ and evaporated. Chromatography over silica gel in MeOH/$CHCl_3$ 1:99 and recrystallization from ethyl acetate/petroleum ether afforded 3.7 g (65%) of (**2**) and 0.5 g of the corresponding 7-methyl derivative (9%). The structure was determined from MS and NMR spectra by comparison with the literature[16].

6-(Dimethylamino)-9-methyl-9*H*-purin-2-amine (**3a**)

90 mg of (**2**) was dissolved in dry acetonitrile. 0.2 ml (1.5 mmol, 3 eq.) of dimethylammonium *N*,*N*-dimethylcarbamate was added and the reaction mixture heated to reflux for 2 h. Then the solvent was evaporated in vacuo. Chromatography in MeOH/$CHCl_3$ 4:96 afforded 82 mg (86%) of slightly colored crystals, m.p. 209–212 °C. MS (EI), *m/z* (rel. intensity): 192 (85, M), 177 (86, M – CH_3), 163 (100, M – NCH_3), 149 (41, N – $(CH_3)_2$ + H), 148 (74, M – $N(CH_3)_2$). UV (MeOH): 285 (12370), 261 (8150), 232 (16950). FT IR (KBr): 3367, 3338, 3211, 1648, 1588, 1494, 1464, 1421, 1401, 1339, 1295, 1249, 1211, 1048, 1012, 787, 639. ^{1}H NMR (500 MHz, $CDCl_3$): 3.45 (bs, 6 H, CH_3-N); 3.65 (s, 3 H, CH_3-9); 4.72 (bs, 2 H, NH_2); 7.44 (s, 1 H, H-8). ^{13}C NMR (125.8 MHz, $CDCl_3$): 29.33 (CH_3-9); 38.16 (CH_3-N); 115.01 (C-5); 136.49 (CH-8); 152.99 (C-4); 155.29 (C-6); 159.27 (C-2). For $C_8H_{12}N_6$ (192.2) calculated: 49.99% C, 6.29% H, 43.72% N; found: 49.83% C, 6.45% H, 43.40% N.

N^6-Substituted 9-Methyl-9*H*-purine-2,6-diamines. General Procedure

0.5 mmol of compound (**2**) was dissolved in dry EtOH. The corresponding primary or secondary amine (2 mmol, 4 eq.) was added and the reaction mixture was heated to reflux for 3–5 h. Then it was evaporated in vacuo, codistilled with ethanol and chromatographed over silica gel. This procedure was used for the following compounds.

6-(Diethylamino)-9-methyl-9H-purin-2-amine (**3b**). Chromatographed in MeOH/$CHCl_3$ 3:97, yield 100 mg (92%) of white crystals, m.p. 133–135 °C. MS (EI), *m/z* (rel. intensity): 220 (100, M), 205 (58, M – CH_3), 191 (91, M – Et), 177 (75, M – NEt), 149 (19, M – NEt_2 + H), 148 (29, M – NEt_2). UV (MeOH): 284.5 (12840), 262 (7680), 230 (18180). FT IR (KBr): 3394, 3328, 3202, 3097, 2967, 2928, 2868, 1648, 1591, 1494, 1460, 1444, 1432, 1425, 1399, 1375, 1361, 1319, 1311, 1274, 1262, 1255, 1213, 1084, 1065, 1046, 1014, 788, 720, 639. ^{1}H NMR (400 MHz, DMSO-d_6): 1.14 (t, 6 H, J_{vic} = 7.0, CH_3); 3.53 (s, 3 H, CH_3-9); 3.85 (br, 4 H, CH_2); 5.80 (bs, 2 H, NH_2); 7.66 (s, 1 H, H-8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 13.81 (CH_3); 29.16 (CH_3-9); 41.77 (CH_2); 113.26 (C-5); 137.35 (CH-8); 153.23 (C-4); 153.65 (C-6); 159.90 (C-2). For $C_{10}H_{16}N_6$ (220.3) calculated: 54.53% C, 7.32% H, 38.15% N; found: 54.24% C, 7.32% H, 37.87% N.

6-(Cyclopropylamino)-9-methyl-9H-purin-2-amine (**3c**). Chromatographed in MeOH/$CHCl_3$ 1:99, yield 82 mg (80%) of slightly colored crystals, m.p. 173–176 °C. MS (EI), *m/z* (rel. intensity): 204 (46, M), 189 (100, M – CH_3), 176 (19, M – CH_2CH_2), 148 (29, M – cyclopropylamine + H). UV (MeOH): 285 (13940), 259 (8230), 229 (17710). FT IR (KBr): 3489, 3465, 3300, 3191, 1674, 1624, 1596, 1491, 1477, 1446, 1420, 1392, 1358, 1333, 1277, 1216, 1021, 790. ^{1}H NMR (400 MHz, $CDCl_3$): 0.60 and 0.84 (2 × m, 2 × 2 H, CH_2-cyclopropyl); 3.01 (bm, 1 H, CH-NH); 3.66 (s, 3 H, CH_3-9); 4.94 (bs, 2 H, NH_2); 5.82 (bs, 1 H, NH); 7.45 (s, 1 H, H-8). ^{13}C NMR (100.6 MHz, $CDCl_3$): 7.36 (CH_2-cyclopropyl); 23.68 (CH-NH); 29.31 (CH_3-9); 114.61 (C-5); 137.88 (CH-8); 151.62 (C-4); 156.30 (C-6); 160.14 (C-2).

9-Methyl-6-pyrrolidin-1-yl-9H-purin-2-amine (**3d**). Chromatographed in MeOH/$CHCl_3$ 1:99, yield 90 mg (83%) of slightly colored crystals, m.p. 196–199 °C. MS (EI), *m/z* (rel. intensity):

218 (100, M), 189 (74), 176 (23), 163 (25), 149 (35, M – pyrrolidine + 2 H). UV (MeOH): 286 (16140), 263 (9160), 236 (19280). FT IR (KBr): 3354, 3330, 3204, 3098, 3056, 2975, 2952, 2880, 1647, 1584, 1529, 1478, 1454, 1426, 1403, 1350, 1210, 1096, 1013, 789, 639. ^{1}H NMR (400 MHz, $CDCl_3$): 1.98 (bm, 4 H, pyrrolidine CH_2); 3.66 (s, 3 H, CH_3-9); 3.70 and 4.10 (2 × bm, 2 × 2 H, CH_2-N); 4.69 (bs, 2 H, NH_2); 7.44 (s, 1 H, H-8). ^{13}C NMR (100.6 MHz, $CDCl_3$): 24.39 and 26.12 (pyrrolidine CH_2); 29.30 (CH_3-9); 46.74 (CH_2-N); 115.19 (C-5); 137.03 (CH-8); 152.60 (C-4); 153.53 (C-6); 159.65 (C-2). For $C_{10}H_{14}N_6$ (218.3) calculated: 55.03% C, 6.47% H, 38.50% N; found: 54.91% C, 6.60% H, 38.14% N.

Cyclization Reactions with 2-Chloroacetaldehyde. General Procedure

2 mmol of compounds **2** and **3a**–**3d** was dissolved in water/dioxane. 1 M aqueous solution of 2-chloroacetaldehyde (20 ml) was added (resulting pH of reaction mixture 6). The reaction mixture was heated to 60 °C for 5–15 h until the starting compound disappeared. The solvent was evaporated in vacuo, the residue codistilled with ethanol and chromatographed over silica gel. This procedure was used for the following compounds.

3-Methyl-3,9-dihydro-5H-imidazo[1,2-a]purin-9-one (**4**). Prepared from **2**. Chromatographed in MeOH/$CHCl_3$ 3:97, yield 64 mg (17%) of slightly colored crystals, m.p. > 300°C. MS (EI), *m/z* (rel. intensity): 189 (100, M). HRMS (EI): for $C_8H_7N_5O$ calculated 189.06506, found 189.06597. UV (MeOH): 284 (8020), 225.5 (24200). FT IR (KBr): 3424, 3123, 1690, 1604, 1553, 1534, 1457, 1424, 1385, 1278, 1196, 1050, 769, 697. ^{1}H NMR (400 MHz, DMSO-d_6): 3.65 (s, 3 H, CH_3-3); 7.42 (d, 1 H, J = 2.7, H-6); 7.60 (d, 1 H, J = 2.7, H-7); 7.87 (s, 1 H, H-2); 12.41 (bs, 1 H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 29.57 (CH_3-3); 106.94 (CH-7); 115.01 (C-9a); 116.47 (CH-6); 139.80 (CH-2); 146.19 (C-4a); 151.00, 151.50 (C-3a and C-9). For $C_8H_7N_5O$ (189.2) calculated: 50.79% C, 3.73% H, 37.02% N; found: 51.03% C, 3.95% H, 37.06% N.

N,N-Diethyl-3-methyl-3H-imidazo[1,2-a]purin-9-amine (**5b**). Prepared from **3b**. Chromatographed in MeOH/$CHCl_3$ 5:95, yield 84 mg (17%) of yellow syrupy product. MS (EI), *m/z* (rel. intensity): 244 (85, M), 229 (14, M – CH_3), 215 (100, M – Et), 201 (60, M – NEt), 188 (25), 172 (74, M – NEt_2). HRMS (EI): for $C_{12}H_{16}N_6$ calculated 244.1436, found 244.1437. UV (MeOH): 299 (3840), 243 (16500). FT IR (KBr): 3430, 3099, 2970, 2927, 2873, 2855, 1630, 1581, 1539, 1515, 1467, 1456, 1395, 1270, 1172, 1159, 1043, 775, 686, 667, 611. ^{1}H NMR (400 MHz, DMSO-d_6): 1.15 (t, 6 H, J_{vic} = 7.0, CH_3); 3.70 (s, 3 H, CH_3-3); 3.74 (q, 4 H, J_{vic} = 7.0, CH_2); 7.55 (d, 1 H, J = 1.7, H-6); 7.57 (d, 1 H, J = 1.7, H-7); 8.26 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 13.39 (CH_3); 29.41 (CH_3-3); 44.37 (CH_2); 107.47 (CH-7); 117.70 (C-9a); 132.72 (CH-6); 142.04 (C-9); 146.16 (CH-2); 149.40 (C-4a); 151.29 (C-3a).

N-Cyclopropyl-3-methyl-3H-imidazo[1,2-a]purin-9-amine (**5c**). Prepared from **3c**. Chromatographed in MeOH/$CHCl_3$ 5:95, yield 80 mg (30%) of yellow syrupy product. MS (EI), *m/z* (rel. intensity): 228 (94, M), 213 (26, M – CH_3), 201 (100, M – CH_2CH_2 + H), 187 (18, M – cyclopropyl), 173 (18). HRMS (EI): for $C_{11}H_{12}N_6$ calculated 228.1123, found 228.1125. UV (MeOH): 290 (3000), 238 (17860). FT IR (KBr): 3419, 3065, 3022, 2962, 2904, 1668, 1588, 1533, 1516, 1451, 1401, 1337, 1200, 1178, 1047, 701. ^{1}H NMR (400 MHz, DMSO-d_6): 0.88–1.00 (m, 4 H, CH_2-cyclopropyl); 3.73 (s, 3 H, CH_3-3); 3.81 (tt, 1 H, J_{vic} = 7.0, 4.1, CH-cyclopropyl); 7.71 (d, 1 H, J = 2.4, H-6); 8.24 (s, 1 H, H-2); 8.50 (d, 1 H, J = 2.4, H-7). ^{13}C NMR (100.6 MHz, DMSO-d_6): 7.66 (CH_2-cyclopropyl); 27.80 (CH-cyclopropyl); 29.68 (CH_3-3); 108.05 (CH-7); 111.65 (C-9a); 123.19 (CH-6); 142.45 (C-9); 143.79 (CH-2); 145.53 (C-4a); 151.44 (C-3a).

3-Methyl-9-pyrrolidin-1-yl-3H-imidazo[1,2-a]purine (**5d**). Prepared from **3d**. Chromatographed in MeOH/$CHCl_3$ 6:94, yield 35 mg (7%) of brown crystals, m.p. 120–125 °C. MS (EI), *m/z* (rel. intensity): 242 (100, M), 214 (48), 199 (13), 187 (30), 173 (53). HRMS (EI): for $C_{12}H_{14}N_6$ calculated 242.1279, found 242.1265. UV (MeOH): 293 (2060), 285 (2340), 246 (22400). FT IR (KBr): 3417, 3093, 3029, 2955, 2924, 2853, 1620, 1585, 1542, 1518, 1457, 1396, 1351, 1244, 1143, 1048, 1031, 854, 758, 676, 603. ^{1}H NMR (400 MHz, DMSO-d_6): 1.98 (m, 4 H, pyrrolidine CH_2); 3.64 (s, 3 H, CH_3-3); 4.20 (m, 4 H, pyrrolidine CH_2N); 7.36 (d, 1 H, J = 1.9, H-6); 7.95 (d, 1 H, J = 1.9, H-7); 8.02 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 25.60 (pyrrolidine CH_2); 29.23 (CH_3-3); 52.26 (pyrrolidine CH_2N); 108.32 (CH-7); 112.84 (C-9a); 131.62 (CH-6); 142.19 (C-9); 142.66 (CH-2); 150.57 (C-4a); 151.09 (C-3a). For $C_{12}H_{14}N_6$ (242.3) calculated: 59.49% C, 5.82% H, 34.69% N; found: 59.23% C, 6.06% H, 34.30% N.

N,N,1-Trimethyl-1H-imidazo[2,1-b]purin-4-amine (**6a**). Prepared from **3a**. Chromatographed in MeOH/$CHCl_3$ 2:98, yield 83 mg (19%) of yellow syrupy product. MS (EI), *m/z* (rel. intensity): 216 (100, M), 201 (39, M – CH_3), 187 (56, M – NCH_3), 172 (28, M – $N(CH_3)_2$. HRMS (EI): for $C_{10}H_{12}N_6$ calculated 216.1123, found 216.1111. UV (MeOH): 287 (7720), 238 (15040). FT IR (KBr): 3454, 3109, 3080, 2929, 1651, 1592, 1567, 1456, 1401, 1271, 1229, 1170, 1023, 876, 757, 649. ^{1}H NMR (400 MHz, DMSO-d_6): 3.42 (bs, 6 H, CH_3); 4.07 (s, 3 H, CH_3-1); 7.27 (d, 1 H, J = 1.7, H-7); 7.85 (d, 1 H, J = 1.7, H-8); 7.92 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 32.71 (CH_3-1); 38.61 (CH_3); 105.98 (CH-8); 116.30 (C-3a); 129.92 (CH-7); 134.72 (C-9a); 137.29 (CH-2); 148.08 (C-5a); 150.79 (C-4).

N,N-Diethyl-1-methyl-1H-imidazo[2,1-b]purin-4-amine (**6b**). Prepared from **3b**. Chromatographed in MeOH/$CHCl_3$ 5:95, yield 275 mg (56%) of slightly colored crystals, m.p. 110–115 °C. MS (EI), *m/z* (rel. intensity): 244 (44, M), 229 (9, M – CH_3), 215 (10, M – Et), 201 (29, M – NEt), 188 (10), 172 (43, M – NEt_2). HRMS (EI): for $C_{12}H_{16}N_6$ calculated 244.1436, found 244.1437. UV (MeOH): 295 (6800), 238 (13020). FT IR (KBr): 3430, 3073, 2974, 2933, 1653, 1576, 1508, 1459, 1435, 1379, 1362, 1313, 1254, 1097, 1080, 1035, 900, 864, 760, 655. ^{1}H NMR (400 MHz, DMSO-d_6): 1.23 (t, 6 H, J_{vic} = 7.0, CH_3); 3.99 (br, 4 H, CH_2); 4.13 (s, 3 H, CH_3-1); 7.59 (d, 1 H, J = 2.3, H-7); 8.09 (d, 1 H, J = 2.3, H-8); 8.19 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 13.60 (CH_3); 33.06 (CH_3-1); 43.37 (CH_2); 108.04 (CH-8); 116.57 (C-3a); 122.20 (CH-7); 134.73 (C-9a); 139.13 (CH-2); 144.79 (C-5a); 150.68 (C-4). For $C_{12}H_{16}N_6$ (244.3) calculated: 59.00% C, 6.60% H, 34.40% N; found: 58.72% C, 6.38% H, 34.63% N.

1-Methyl-9-pyrrolidin-1-yl-1H-imidazo[2,1-b]purine (**6d**). Prepared from **3d**. Chromatographed in MeOH/$CHCl_3$ 6:94, yield 72 mg (15%) of slightly colored crystals, m.p. 215–218 °C. MS (EI), *m/z* (rel. intensity): 242 (52, M), 213 (53), 178 (100). HRMS (EI): for $C_{12}H_{14}N_6$ calculated 242.1279, found 242.1292. UV (MeOH): 288 (7400), 239 (15440). FT IR (KBr): 3409, 3103, 2955, 2921, 2875, 2853, 1648, 1581, 1507, 1451, 1348, 1267, 1228, 1058, 1025, 847, 759, 650. ^{1}H NMR (400 MHz, DMSO-d_6): 1.94 (bm, 4 H, pyrrolidine CH_2); 3.50–4.00 (bm, 4 H, pyrrolidine CH_2N); 4.08 (s, 3 H, CH_3-1); 7.31 (d, 1 H, J = 1.7, H-7); 7.88 (d, 1 H, J = 1.7, H-8); 7.97 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 24.15 and 25.30 (pyrrolidine CH_2); 32.73 (CH_3-1); 47.93 (pyrrolidine CH_2N); 106.26 (CH-8); 116.55 (C-3a); 128.41 (CH-7); 134.43 (C-9a); 138.04 (CH-2); 147.88 (C-5a); 149.32 (C-4). For $C_{12}H_{14}N_6$ (242.3) calculated: 59.49% C, 5.82% H, 34.69% N; found: 59.17% C, 5.54% H, 34.82% N.

9-Methyl-9*H*-purine-2,6-diamine (**8**)

750 mg (5 mmol) of commercial 2,6-diaminopurine was dissolved in dry DMF, 2.5 g (7.5 mmol, 1.5 eq.) of Cs_2CO_3 was added, followed by 1.8 g (10 mmol, 2 eq.) of methyl 4-toluenesulfonate. The reaction mixture was heated to 70 °C for 5 h, then allowed to cool and the solvent was removed in vacuo. The residue was dissolved in ethyl acetate, washed with water, dried with anhydrous $MgSO_4$ and evaporated. Chromatography on silica gel in MeOH/$CHCl_3$ 2:98 afforded 410 mg (50%) of white crystals. The structure was determined by MS and NMR, by comparison with the literature[24].

Quaternization Reactions with Iodomethane. General Procedure

0.5 mmol of compound **2**, **3a**–**3d**, **5b**, **6a** or **6d** was dissolved in acetone. 1 ml (16 mmol) of iodomethane was added dropwise and the reaction mixture was stirred at room temperature for 7 days. This procedure was used for the following compounds.

2-Amino-6-chloro-7,9-dimethylpurinium iodide (**9**). Prepared from **2**. The product crystallized from reaction mixture was filtered off, washed with diethyl ether and dried. Yield 112 mg (70%) of white crystals, m.p. > 300 °C. MS (FAB), *m/z* (rel. intensity): 200 (37), 198 (100, M^+), 164 (50, M – Cl). UV (MeOH): 323 (4100), 219 (28400). FT IR (KBr): 3384, 3293, 3197, 3056, 2997, 1631, 1586, 1506, 1467, 1399, 1388, 1312, 1033. ^{1}H NMR (400 MHz, DMSO-d_6): 3.79 (s, 3 H, CH_3-9); 4.09 (s, 3 H, CH_3-7); 7.80 (bs, 2 H, NH_2); 9.53 (s, 1 H, H-8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 31.58 (CH_3-9); 36.19 (CH_3-9); 113.52 (C-5); 143.98 (CH-8); 146.40 (C-6); 152.39 (C-4); 161.34 (C-2).

4-(Dimethylamino)-1,6-dimethyl-1H-imidazo[2,1-b]purin-6-ium iodide (**10a**). Prepared from **6a**. The product crystallized from reaction mixture, was filtered off, washed with diethyl ether and dried. Yield 120 mg (67%) of yellowish crystals, m.p. 230 °C (decomp.). MS (FAB), *m/z* (rel. intensity): 231 (86, M^+). UV (MeOH): 285 (7000), 242 (7250) sh, 227 (10380). FT IR (KBr): 3423, 3075, 3050, 2926, 2816, 1651, 1590, 1513, 1458, 1419, 1403, 1309, 1284, 1228, 1181, 1062, 1024, 993. ^{1}H NMR (400 MHz, DMSO-d_6): 3.36 (s, 3 H, C**H**$_3$NCH$_3$); 3.77 (s, 3 H, CH_3-6); 3.85 (s, 3 H, C**H**$_3$NCH$_3$); 4.13 (s, 3 H, CH_3-1); 7.87 (d, 1 H, *J* = 2.6, H-7); 8.22 (d, 1 H, *J* = 1.7, H-8); 8.23 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 32.35 (CH_3-6); 33.18 (CH_3-1); 38.42 and 39.58 ($(CH_3)_2$N); 108.10 (CH-8); 117.43 (C-3a); 121.83 (CH-7); 134.77 (C-9a); 139.66 (CH-2); 141.84 (C-5a); 152.00 (C-4)

1,6-Dimethyl-4-pyrrolidin-1-yl-1-H-imidazo[2,1-b]purin-6-ium iodide (**10d**). Prepared from **6d**. The solvent was removed in vacuo and the reaction mixture was chromatographed over silica gel in MeOH/$CHCl_3$ 10:90. Yield 137 mg (72%) of crimson crystals, m.p. > 300 °C. MS (FAB), *m/z* (rel. intensity): 257 (100, M^+). UV (MeOH): 287 (8300), 243 (8100) sh, 222 (16600). FT IR (KBr): 3360, 3048, 2923, 2854, 1648, 1588, 1508, 1475, 1456, 1413, 1278, 1234, 1061, 1030. ^{1}H NMR (500 MHz, DMSO-d_6): 1.97 and 2.07 (2 × p, 4 H, *J* = 6.9, pyrrolidine CH_2); 3.76 (s, 3 H, CH_3-6); 3.79 (t, 2 H, *J* = 6.9, pyrrolidine CH_2N); 4.13 (s, 3 H, CH_3-1); 4.19 (t, 2 H, *J* = 6.9, pyrrolidine CH_2N); 7.85 (d, 1 H, *J* = 2.7, H-7); 8.20 (s, 1 H, H-2); 8.21 (d, 1 H, *J* = 2.7, H-8). ^{13}C NMR (125.8 MHz, DMSO-d_6): 24.53 and 26.99 (pyrrolidine CH_2); 33.32 (CH_3-6); 34.09 (CH_3-1); 49.40 and 50.51 (pyrrolidine CH_2N); 109.06 (CH-8); 118.65 (C-3a); 122.69 (CH-7); 135.45 (C-9a); 141.13 (CH-2); 143.18 (C-5a); 151.27 (C-4).

9-(Diethylamino)-3,5-dimethyl-3H-imidazo[1,2-a]purin-5-ium iodide (**11b**). Prepared from **5b**. The solvent was removed in vacuo. Chromatography over silica gel in MeOH/$CHCl_3$ 10:90 yielded 120 mg (63%) of slightly colored crystals, m.p. 147 °C. MS (FAB), *m/z* (rel. intensity): 259 (100, M^+). UV (MeOH): 336 (5800), 244 (12200). FT IR (KBr): 3431, 3059, 3020, 2970,

2925, 2870, 2854, 1646, 1554, 1457, 1399, 1060, 1047. ^{1}H NMR (400 MHz, DMSO-d_6): 1.28 (t, 6 H, J_{vic} = 7.1, C**H**$_3$CH$_2$); 3.81 (s, 3 H, CH$_3$-3); 3.85 (s, 3 H, CH$_3$-5); 3.95 (q, 4 H, J_{vic} = 7.1, C**H**$_2$CH$_3$); 7.90 (d, 1 H, J = 2.8, H-7); 8.06 (d, 1 H, J = 2.8, H-6); 8.49 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 13.50 (CH$_3$CH$_2$); 30.06 (CH$_3$-3); 32.55 (CH$_3$-5); 45.73 (CH$_2$CH$_3$); 109.72 (CH-7); 118.07 (C-9a); 123.70 (CH-6); 144.25 (C-4a); 144.63 (C-9); 146.10 (CH-2); 151.57 (C-3a).

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