Bull. Chem. Soc. Ethiop. **2010**, 24(3), 425-432. ISSN 1011-3924
Printed in Ethiopia

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF O^6-ALKYLGUANINE DERIVATIVES

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(Received October 10, 2009; revised March 1, 2010)

ABSTRACT. The synthesis of some biologically active O^6-alkylguanine derivatives was achieved by alkoxylation of 2-amino-6-chloropurine with sodium alkoxides in polar aprotic solvent (DMSO) conditions. The starting material 2-amino-6-chloropurine was prepared by chlorination of 2,9-diacetylguanine (obtained from acetylation of commercially available guanine) by PEG-2000 phase transfer catalysis. The structures of the products were deduced from elemental analysis and spectral data (IR, ^{1}H NMR, and mass spectra). All the title compounds were screened for their antifungal activities, and some of the compounds showed promising activities.

KEY WORDS: O^6-Alkylguanine derivatives, 2-Amino-6-chloropurine, Synthesis, Fungicidal activity

INTRODUCTION

The purine derivatives are of great importance to chemists as well as to biologists as they are found in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities [1-3]. Moreover, they are known to possess antitubercular [1], antiulcer [2], antimicrobial [2], antineoplastic [1], antitumor [4], antiviral and cardiotonic properties [5]. O^6-Alkylguanine derivatives are considered to be of major importance for the induction of cancer, mutation, and cell death [6]. These adducts direct the incorporation of either thymine or cytosine without blocking DNA replication, resulting in GC to AT transition mutations, which provide a mean to effectively inactivate the O^6-alkylguanine-DNA alkyltransferase (AGT) protein and increase the chemotherapeutic effectiveness of alkylating agents *in vitro* and in human tumorxen ograft models [6-10].

Encouraged by the above reports and as a part of a research program on the synthesis of some biologically active heterocyclic compounds containing nitrogen, it was planned to synthesize some new substituted purines carrying guanine, aiming at an investigation of the new heterocycles of enhanced biological activities. In 2006, we have reported the synthetic method to prepare the four 2-amino-6-alkoxy substituted purines [11]. Based on the preliminary experimental results obtained from the above procedure and the task to broaden the synthesis of a variety of substituted purines, herein we report the synthesis of a series of O^6-alkylguanine derivatives including some unreported compounds (Scheme 1) and an evaluation of their antifungal activities.

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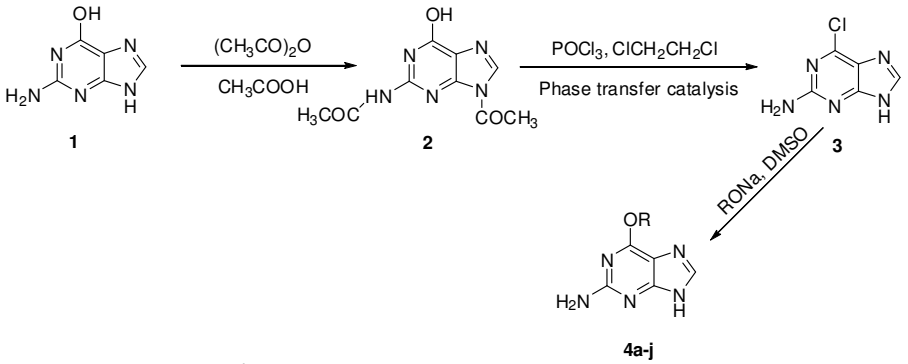


Scheme 1. Synthesis of O^6-alkylguanine derivatives.

RESULTS AND DISCUSSION

The key intermediate 2-amino-6-chloropurine **3** was synthesized by a two-step procedure from commercially available guanine **1**. The acetylation of **1** with acetic anhydride in the presence acetic acid at 135 °C yielded 2,9-diacetylguanine **2**. Further treatment of **2** with $POCl_3$ by PEG-2000 phase transfer catalysis furnished the desired compound **3**. In this process of chlorination, the reaction was initially carried out by vigorously stirring the two phase system (solvent and chlorination agent) in the absence of PEG-2000 at 80 °C, the chlorination reaction proceeded not well, and the yield was only 52% after 12 h. When the reaction was performed with PEG-2000, it proceeded very rapidly and the yield reached 84% in a shorter time (6 h), which displays distinctly the advantage of high efficiency of the phase transfer catalysis (Figure 1). Besides polyethylene glycol 2000 (PEG-2000), we also tried to use another types of phase transfer catalysts such as benzyltriethylammonium chloride (BTEAC), trioctylmethylammonium chloride (TOMAC) and triethylmethylammonium chloride (TEMAC) as catalysts in the reaction (Figure 2), and it was observed that PEG-2000 demonstrated the best performance, providing a 84% high yield in a shorter time (6 h), which presented a striking contrast to other reported procedures whose yields for **3** were usually 60-75% [12-15].

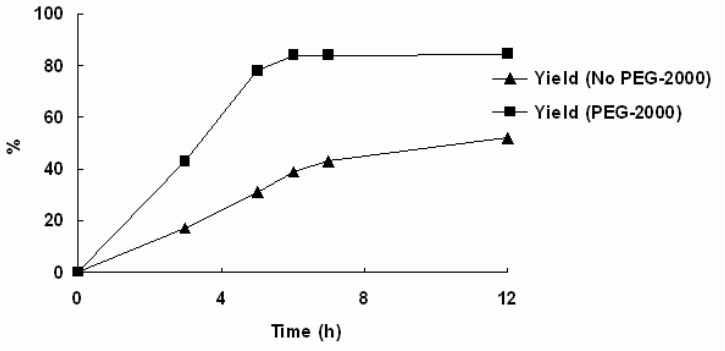


Figure 1. Relationship between yield and reaction time in the presence and absence of PEG-2000. Reaction conditions: 2,9-diacetylguanine (10 mmol), $ClCH_2CH_2Cl$ (10 mL), $POCl_3$ (35 mmol), PEG-2000 (2 mmol), 80 °C.

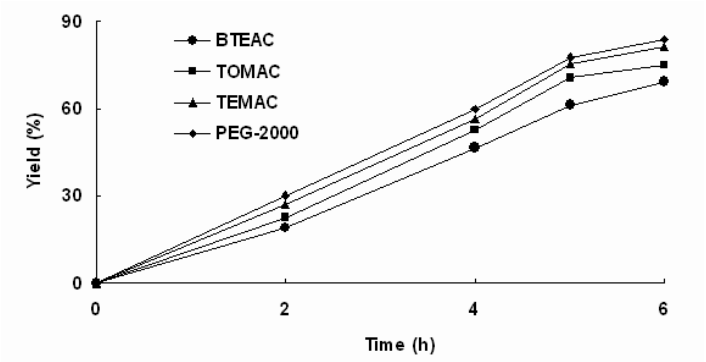
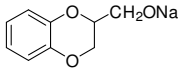
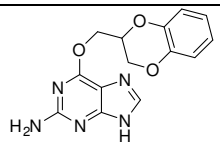
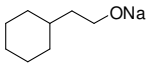
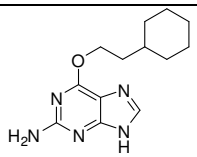
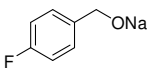
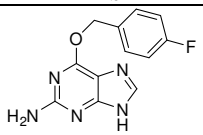
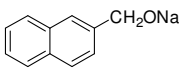
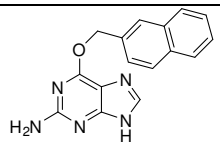
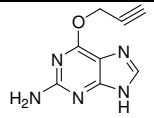
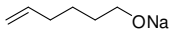
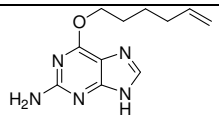
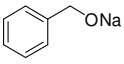
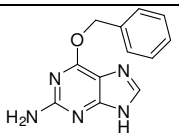
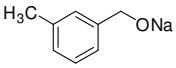
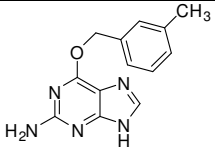
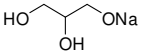
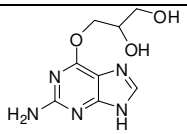
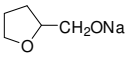
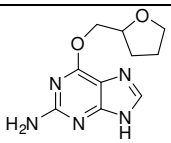


Figure 2. Influences of different types of phase transfer catalysts on the chlorination. Reaction conditions: 2,9-diacetylguanine (10 mmol), $ClCH_2CH_2Cl$ (10 mL), $POCl_3$ (35 mmol), phase transfer catalyst (2 mmol), 80 °C, 6 h.

O^6-Alkylguanine derivatives (**4a**-**j**) were then prepared according to the well established procedures [11, 16-18], by treating **3** with sodium alkoxides under DMSO solvent conditions to afford the desired alkylguanines in good to excellent isolated yield, and seven new compounds **4a**, **4b**, **4c**, **4d**, **4h**, **4i** and **4j** were also synthesized (Table 1). The structures of the synthesized compounds were established on the basis of spectral data and elemental analyses.

Table 1. Synthesis of O^6-alkylguanine derivatives via an alkoxylation[a].

Entry	Sodium alkoxide	Temp. (°C)	Time (h)	Product	Yield (%)[b]
1		90	10	 **4a**	87
2		90	12	 **4b**	74
3		85	10	 **4c**	83
4		85	10	 **4d**	94

5		90	10	 **4e**	87
6		120	16	 **4f**	75
7		90	10	 **4g**	86
8		90	12	 **4h**	81
9		110	14	 **4i**	73
10		95	10	 **4j**	79

[a] Reaction conditions: 2-amino-6-chloropurine (10 mmol), RONa (50 mmol), DMSO (15 mL). [b] Isolated yield.

Antifungal activity

The synthesized O^6-alkylguanine derivatives were screened for their antifungal activities against three species of fungi, namely *Bacillus subtillis*, *Aspergillus niger* and *Cardida tropicals* using the disc diffusion method [19, 20]. The tested compounds were dissolved in 1% NaOH solution (which has no inhibitory activity) to get concentrations of 1 mg/mL solution. The fluconazole was used as standard antifungal reference. The inhibition zones of microbial growth surrounding the filter paper disc (2.5 mm) were measured in millimeters at the end of an incubation period at 30 °C for 3 days. Inhibition of the organisms was evidenced by a clear zone surrounding each disk (Table 2).

All the tested compounds showed variable activities toward the three species of fungi, some of them comparable to standard fluconazole. The results of the antifungal screening showed that compounds **4a**-**d** and **4h** displayed good activity against *Bacillus subtillis*, the compounds **4a**, **4c**, **4d**, **4h** and **4j** displayed good activity against *Aspergillus niger*, and compounds **4a-d**, **4h** and **4j** showed good activity against *Cardida tropicals*, the compounds **4a**, **4c**, **4d** and **4h**

showed fairly good activity against the three fungal strains, while the remaining compounds exhibited moderate activity. The lowest activity was observed for the compounds **3**, **4f, 4g** and **4i** (Table 2).

Table 2. Fungicidal activity of the title compounds **4a**-**j**.

Compound	Diameter of inhibition zone		
	Bacillus subtillis	*Aspergillus niger*	*Cardida tropicals*
3	1.4	2.5	1.5
4a	0.2	0.4	0.1
4b	0.4	1.0	0.3
4c	0.1	0.3	0
4d	0.3	0.2	0.1
4e	0.8	1.1	0.9
4f	1.5	1.2	1.8
4g	1.4	2.3	2.0
4h	0.1	0.2	0.1
4i	1.3	2.4	1.3
4j	0.8	0.5	0.4
fluconazole	0	0.1	0

CONCLUSIONS

The successful synthesis of a series of some biologically active O^6-alkylguanine derivatives **4a-j** from commercially available guanine and an evaluation of the fungicidal activities of the title compounds were reported. One of the noticeable points in our paper was that the PEG-2000 catalyzed chlorination to prepare 2-amino-6-chloropurine **3** was carried out successfully, the yield under phase transfer catalysis could be remarkably improved to 84% which presented a striking contrast to other reported procedures whose yields for **3** were usually 60-75% [12-15]. Another important point was that ten derivatives were prepared from **3** via an alkoxylation with sodium alkoxide in polar aprotic solvent DMSO conditions, and seven new ones were obtained. From the results of the fungicidal screening, it can be concluded that the four new compounds (**4a**, **4c**, **4d** and **4h**) having the good properties of biological activities have been synthesized. Therefore they may be used as lead compounds for further development.

EXPERIMENTAL

Materials and apparatus

All the chemicals and reagents were of analytical grade and used as obtained. ^{1}H NMR spectra were recorded on a Bruker 400-MHz spectrometer (Bruker Daltonic, Germany) using DMSO-d_6 as the solvent with tetramethylsilane (TMS) as an internal standard. High performance liquid chromatography (HPLC) experiments were performed on a liquid chromatograph (Dionex Softron GmbH, USA), consisting of a pump (P680) and ultraviolet-visible light detector (UVD) system (170U). The experiments were performed on Diacovery C18 column, ø 4.6 × 250 mm. Melting points were recorded on Digital Melting Point Apparatus WRS-1B (All-Time Commerical Co. Ltd, China) and are uncorrected. Mass spectra were measured on an

HP1100LC (Agilent Technologies, USA). Infrared spectra were recorded on FT-IR Bruker Vector 22 spectrophotometer (Bruker Optics Inc. USA) using KBr wafer technique. Elemental analysis was performed on a Vario EL III instrument (Elmentar Anlalysensy Teme GmbH, Germany).

*Preparation of 2,9-diacetylguanine (**2**)*

A mixture of guanine (**1**, 2.5 g, 16.5 mmol), acetic anhydride (30 mL) and acetic acid (60 mL) was stirred in a 250 mL round flask for 7.5 h at 135 °C. After completion of the reaction, as indicated by HPLC, the solvent and the superfluous acetic anhydride were evaporated under vacuum, the crude product was recrystallized from distilled water to give **2** as white powders (3.7 g, yield 95.2%). M.p. 251-256 °C. IR (KBr) ν_{max}/cm^{-1}: 3200 (NH), 1680 (C=O), 1530 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.16-2.50 (m, 6H, CH_3), 8.11 (s, 1H, CH), 8.15 (s, 1H, NH), 11.56 (s, 1H, OH). MS (EI, 70 eV): *m/z* (%) = 234 (M^+, 20), 192 (100), 150 (23). Anal. calcd. for $C_9H_9N_5O_3$: C, 45.96%; H, 3.86%; N, 29.78%; O, 20.41%. Found: C, 45.93%; H, 3.87%; N, 29.76%; O, 20.37%.

*Preparation of 2-amino-6-chloropurine (**3**)*

A mixture of 2,9-diacetylguanine (**2**, 2.35 g, 10 mmol), PEG-2000 (4 g, 2 mmol) and $ClCH_2CH_2Cl$ (10 mL) was stirred in a 100 mL round flask at 80 °C, then $POCl_3$ (5.4 g, 35 mmol) was added dropwise within 20 min. The reaction progress was monitored by HPLC. The reaction was completed in 6 h, and then cooled to room temperature, the precipitate was filtered off. The solvent was removed and the residue was recrystallized from DMSO to give **3** as white powders (1.42 g, yield 84%). M.p. 298-302 °C. IR (KBr) ν_{max}/cm^{-1}: 1636, 1292, 822 (Cl), 630 (NH_2). ^{1}H NMR (400 MHz, DMSO-d_6): 6.75-6.79 (m, 3H, NH_2 and NH), 8.01 (m, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 169 (M^+, 47), 151 (100), 135 (32), 117 (6). Anal. calcd. for $C_5H_4ClN_5$: C, 35.41%; H, 2.38%; Cl, 20.91%; N, 41.30%. Found: C, 35.37%; H, 2.34%; Cl, 20.93%; N, 41.29%.

*General procedure for the preparation of O^6-alkylguanine derivatives (**4a-j**)*

The three-necked flask was loaded with 2-amino-6-chloropurine (1.7 g, 10 mmol), DMSO (15 mL), and RONa (50 mmol). The reaction mixture was vigorously stirred at an appropriate temperature for an appropriate time (Table 1), the reaction progress was monitored by HPLC. After the reaction, the solvent was removed under vacuum, the residue was dissolved in water (30 mL) and toluene (20 mL), the water layer was neutralized with concentrated HCl, the solid obtained was filtered, and then washed with water (3 × 20 mL) to give the desired products **4a-j**.

*O^6-(1,4-Benzodioxan-2-yl)methoxylguanine (**4a**)*. 2.6 g, yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6): 4.18 (d, 2H, CH_2), 4.47 (t, 1H, CH), 4.94 (d, 2H, OCH_2), 6.82 (s, 3H, NH_2 and NH), 7.05-7.16 (m, 4H, ArH), 7.96 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 299 (M^+, 54), 258 (100), 167 (32). Anal. calcd. for $C_{14}H_{13}N_5O_3$: C, 56.18%; H, 4.38%; N, 23.40%; O, 16.04%. Found: C, 56.14%; H, 4.39%; N, 23.38%; O, 16.05%.

*O^6-(2-Cyclohexyl)ethoxylguanine (**4b**)*. 1.93 g, yield 74%. ^{1}H NMR (400 MHz, DMSO-d_6): 1.04 (m, 2H, CH_2), 1.28-1.46 (m, 10H, CH_2), 1.97 (m, 1H, CH), 4.49 (t, 2H, OCH_2), 6.57 (s, 3H, NH_2 and NH), 7.88 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 261 (M^+, 100), 236 (72), 189 (90), 117 (48). Anal. calcd. for $C_{13}H_{19}N_5O$: C, 59.75%; H, 7.33%; N, 26.80%; O, 6.12%. Found: C, 59.73%; H, 7.33%; N, 26.81%; O, 6.10%.

*O^6-4-Fluorobenzylguanine (**4c**)*. 2.15 g, yield 83%. ^{1}H NMR (400 MHz, DMSO-d_6): 5.43 (s, 2H, OCH_2), 6.69 (s, 3H, NH_2 and NH), 7.23-7.52 (m, 4H, ArH), 7.86 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 259 (M^+, 38), 238 (100), 162 (56). Anal. calcd. for $C_{12}H_{10}FN_5O$: C, 55.60%; H, 3.89%; F, 7.33%; N, 27.02%; O, 6.17%. Found: C, 55.57%; H, 3.88%; F, 7.32%; N, 27.05%; O, 6.16%.

*O^6-(2-Naphthyl)methoxylguanine (**4d**)*. 2.73 g, yield 94%. ^{1}H NMR (400 MHz, DMSO-d_6): 5.73 (s, 2H, OCH_2), 6.53 (s, 3H, NH_2 and NH), 7.67 (m, 3H, $C_{10}H_7$), 7.96-8.02 (m, 5H, CH and $C_{10}H_7$). MS (EI, 70 eV): *m/z* (%) = 291 (M^+, 100), 141 (46), 95 (72), 81 (54). Anal. calcd. for $C_{16}H_{13}N_5O$: C, 65.97%; H, 4.50%; N, 24.04%; O, 5.49%. Found: C, 65.95%; H, 4.48%; N, 24.05%; O, 5.49%.

*O^6-Propargylguanine (**4e**)*. 1.64 g, yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6): 2.41 (s, 1H, CH), 5.02 (s, 2H, CH_2), 7.99 (s, 1H, CH), 8.10 (s, 3H, NH_2 and NH). MS (EI, 70 eV): *m/z* (%) = 190 (M^+, 76), 174 (100), 151 (38). Anal. calcd. for $C_8H_7N_5O$: C, 50.79%; H, 3.73%; N, 37.02%; O, 8.46%. Found: C, 50.73%; H, 3.71%; N, 37.04%; O, 8.47%.

*O^6-(1-Hexenyl)guanine (**4f**)*. 1.75 g, yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6): 1.6-2.3 (m, 6H, CH_2), 4.51-4.57 (t, 2H, CH_2), 5.13 (m, 2H, CH_2), 8.01 (s, 1H, CH), 8.12 (s, 3H, NH_2 and NH). MS (EI, 70 eV): *m/z* (%) = 234 (M^+, 55), 218 (100), 152 (44). Anal. calcd. for $C_{11}H_{15}N_5O$: C, 56.64%; H, 6.48%; N, 30.02%; O, 6.86%. Found: C, 56.58%; H, 6.47%; N, 30.04%; O, 6.83%.

*O^6-Benzylguanine (**4g**)*. 2.1 g, yield 86%. ^{1}H NMR (400 MHz, DMSO-d_6): 5.61 (s, 2H, CH_2), 6.63 (s, 3H, NH_2 and NH), 7.31-7.44 (m, 5H, ArH), 7.99 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 242 (M^+, 48), 226 (100), 151 (68). Anal. calcd. for $C_{12}H_{11}N_5O$: C, 59.74%; H, 4.60%; N, 29.03%; O, 6.63%. Found: C, 59.69%; H, 4.58%; N, 29.01%; O, 6.61%.

*O^6-(m-Methylbenzyl)guanine (**4h**)*. 2.07 g, yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6): 2.43 (s, 3H, CH_3), 5.52 (s, 2H, CH_2), 6.41 (s, 3H, NH_2 and NH), 7.41-7.48 (m, 4H, ArH), 7.97 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 256 (M^+, 43), 240 (100), 151 (76). Anal. calcd. for $C_{13}H_{13}N_5O$: C, 61.17%; H, 5.13%; N, 27.43%; O, 6.27%. Found: C, 61.13%; H, 5.13%; N, 27.39%; O, 6.28%.

*O^6-(2,3-Dihydroxypropyl)guanine (**4i**)*. 1.65 g, yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6): 3.61 (m, 2H, CH_2), 3.94 (s, 1H, CH), 4.43 (m, 2H, CH_2), 4.87 (t, 1H, OH), 5.15 (d, 1H, OH), 6.37 (s, 3H, NH_2 and NH), 7.99 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 226 (M^+, 18), 209 (100), 151 (49). Anal. calcd. for $C_8H_{11}N_5O_3$: C, 42.67%; H, 4.92%; N, 31.10%; O, 21.31%. Found: C, 42.64%; H, 4.91%; N, 31.09%; O, 21.27%.

*O^6-(2-Tetrahydrofuranyl)methoxylguanine (**4j**)*. 1.85 g, yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6): 1.87 (m, 4H, CH_2CH_2), 3.78 (m, 2H, CH_2), 4.27 (m, 1H, CH), 4.53 (d, 2H, OCH_2), 4.87 (t, 1H, OH), 5.15 (d, 1H, OH), 6.41 (s, 3H, NH_2 and NH), 7.96 (s, 1H, CH). MS (EI, 70 eV): *m/z* (%) = 235 (M^+, 32), 165 (100), 151 (58), 134 (34), 78 (62). Anal. calcd. for $C_{10}H_{13}N_5O_2$: C, 51.06%; H, 5.57%; N, 29.77%; O, 13.60%. Found: C, 51.03%; H, 5.56%; N, 29.79%; O, 13.58%.

AKNOWLEDGEMENTS

We thank the National Basic Research Program (973 Program) of China and Ministry of Education of the Republic of China for support of this research.

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