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생물 활성이 있는 Halogenopurines의 합성

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Synthesis of Some Biologically Active Halogenopurines

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요약. Guanine (**1**)으로부터 생물활성이 있는 halogenopurines계 화합물을 합성하였다. Guanine을 acetic anhydride와 반응시켜서 2,9-diacetylguanine (**2-1**)을 합성하여 얻어진 화합물을 $POCl_3$와 반응시켜서 화합물 **3a**를 합성하고, 다음 단계에서 2-amino-6-halogenopurines (**3b-d**)를 합성하였다. 2-Halogenopurines (**2-2a-d**, **4-2a-d**, **5a-d**)을 2-amino-6-substituted purines (**1**, **3a**, **4-1**)로부터 효율적으로 합성한 후에, 새로운 화합물인 **2-2a**, **2-2c**, **2-2d**, **4-2c**, **4-2d**, **5b**, **5c** 및 **5d**를 합성하였다.
합성한 화합물의 구조를 원소분석, ^{1}H NMR, mass spectral data로 확인하였으며, 합성한 화합물에 대한 항균 활성을 시험하였다.

주제어: Halogenopurines, 2-Amino-6-chloropurine, Phase transfer catalyst, Chlorine-exchange halogenation, Diazotization, Fungicidal activity

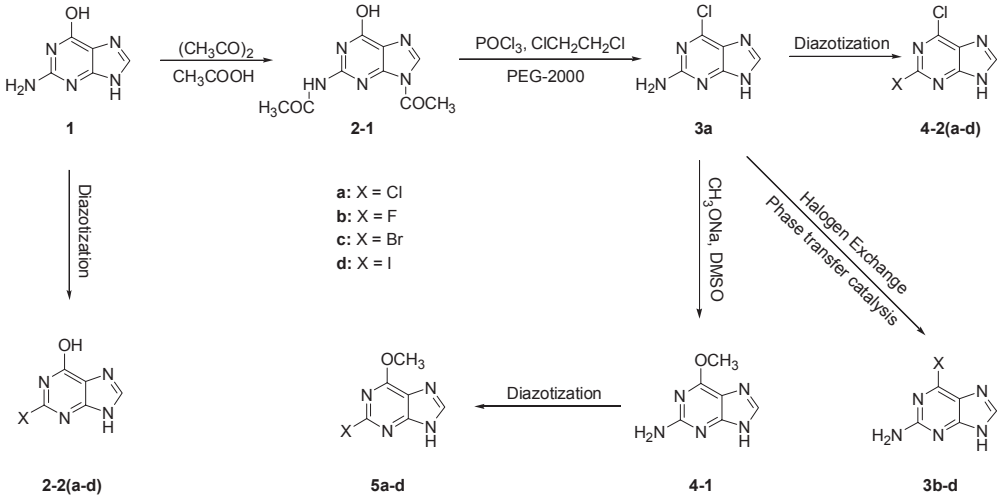
ABSTRACT. A series of some biologically active halogenopurines were synthesized from commercially available guanine (**1**). The reaction of guanine with acetic anhydride yielded 2,9-diacetylguanine (**2-1**) by acetylation reaction. Further treatment of **2-1** with $POCl_3$ by PEG-2000 phase transfer catalysis furnished the important compound **3a**, then 2-amino-6-halogenopurines (**3b-d**) were obtained through chlorine-exchange halogenations between KX and **3a** by TPPB phase transfer catalyst. Further, 2-halogenopurines (**2-2a-d**, **4-2a-d**, **5a-d**) were efficiently prepared from 2-amino-6-substituted purines (**1**, **3a**, **4-1**) *via* a diazotization catalyzed by their corresponding CuX, and some new compounds **2-2a**, **2-2c**, **2-2d**, **4-2c**, **4-2d**, **5b**, **5c** and **5d** have been discovered. The structures of synthesized compounds were mainly established on the basis of their elemental analysis, ^{1}H NMR, as well as their mass spectral data. All the title compounds were screened for their antifungal activities, and some of the compounds showed promising activity.

Keywords: Halogenopurines, 2-Amino-6-chloropurine, Phase transfer catalyst, Chlorine-exchange halogenation, Diazotization, Fungicidal activity

INTRODUCTION

The purine derivatives are of great importance to chemists as well as to biologists as they have been found in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities.[1-3] Halogenopurines are such an important purine derivatives that have been widespread applications of in the synthesis of a variety of biologically active purine derivatives, purine nucleoside derivetives, *etc*.[4-6] Moreover, some halogenopurines displayed a wide range of biological activities themselves such as fungicide, antitumour, immunosuppressant and anticonvulsant, *etc*.[7,8]

2-Amino-6-halogenopurines are usually prepared by chlorine/halogen exchange reactions between potassium halide (KX) and 2-amino-6-chloropurine, and a variety of catalysts have been developed for such halogen exchanges, such as ammonium salts,[9-11] metal carbonyls,[12-14] chromium oxide on alumina,[15-17] *etc*.[18-21] Unfortunately, many of these procedures are associated with one or more disadvantages such as high cost, low selectivity, use of stoichiometric and even excess amounts of catalysts, complicate the separation of the products, and drastic reaction conditions. The classical method for synthesizing 2-halogenopurines constitutes the diazotization of 2-amino-6-substituted purines, although only a few publications in the open literature have been



***Scheme* 1.** Synthesis of halogenopurines

found in synthesis of them.[22,23] The diazotization is generally carried out using accessible sodium nitrite in strongly acidic medium at reduced temperature.[22-27] Alternative procedures involve more expensive alkyl nitrites as diazotizing agents in the presence of dihalogenomethane or other sources of halogeno.[28,29] Other notable procedures for diazotization include the use of sodium nitrite in weakly acidic media created by *p*-toluenesulfonic acid,[30,31] sulfonated cation exchangers,[32,33] *etc*.[34-37] However, many of these procedures require high reaction temperature, expensive and special apparatus, troublesome work-up procedures, harsh reaction conditions, *etc*.

Phase transfer catalysis (PTC) is a versatile synthetic technique that has been widely applied to intensify otherwise slow heterogeneous reactions involving an organic substrate and an ionic reactant, either dissolved in water (liquid-liquid) or present in solid state (liquid-solid).[38,39] In addition, phase transfer catalysis can make reaction condition gentle, can effectively avoid side reaction to occur, can reduce consumption of organic solvent and raw materials and enhance the efficiency of organic synthesis. At present, phase transfer catalysis has been applied in various organic syntheses, such as electrophilic substitution reaction, nucleophilic substitution reaction, hydrolysis and so on.[40-43]

As a part of a research program on the synthesis of various heterocyclic compounds containing nitrogen and on the basis of these observations, the present study describes an improved and economic procedure for synthesis of a variety of halogenopurines of interest as imaging agent precursors for radiolabeling and unlabeled standard samples (*Scheme* 1) and an evaluation of their in vitro antifungal activities.

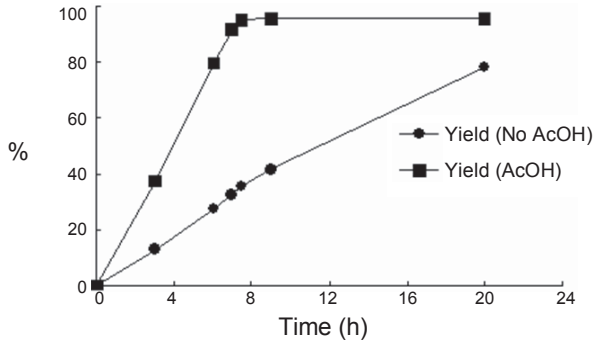


***Fig.* 1.** Influences of the presence and absence of acetic acid on the acetylation degree of guanine. Reaction conditions: **1** (16.5 mmol), acetic anhydride (30 mL), 135 °C.

RESULTS AND DISCUSSION

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

In a preliminary study, the acetylation reaction of guanine (**1**) was carried out in the presence and absence of the solvent acetic acid at 135 °C. As shown in *Figure* 1, in the absence of acetic acid, the acetylation reaction proceeded slowly, the yield was only 78% after 20 h. Reaction performed with acetic acid at a amount of 63 equiv (60 mL) proceeded very rapidly and the yield increased to 95.2% in a shorter time (7.5 h). However, under the same conditions, the yield was not enhanced significantly with further increase in the reaction time.

In the process of chlorination, 2,9-diacetylguanine (**2-1**) was initially carried out by vigorously stirring the two phase system (solvent and chlorination agent) in absence of PEG-2000 at 80 °C. As shown in *Figure* 2, the chlorination reaction proceeded not well, and the yield was only 47% after 8 h.

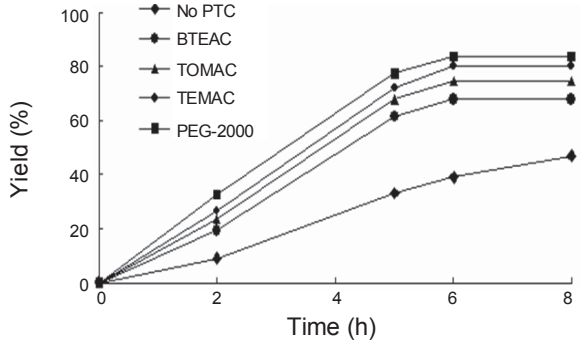


***Fig.* 2.** Relationship between yield and reaction time in the presence and absence of phase transfer catalysts. Reaction conditions: **2-1** (10 mmol), $ClCH_2CH_2Cl$ (10 mL), $POCl_3$ (35 mmol), phase transfer catalyst (2 mmol), 80 °C.

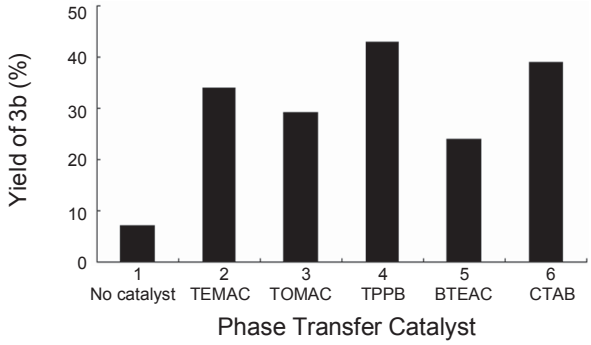


***Fig.* 3.** Influences of phase transfer catalysts on the chlorine-exchange fluorination. Reaction conditions: **3a** (10 mmol), sulfolane (30 mL), KF (50 mmol), phase transfer catalyst (0.5 mmol), 190 °C, 15 h.

***Table* 1.** The chlorination of 2,9-diacetylguanine with different type of solvents and chlorination agents by phase transfer catalysis[a]

Entry	Solvent	Chlorination agent	Time (h)	Temp. (°C)	Yield (%)[b]
1	$ClCH_2CH_2Cl$	$POCl_3$	6	80	84
2	CH_2Cl_2	$POCl_3$	10	40	51
3	$CH_3C_6H_5$	$POCl_3$	6	100	82
4	$CHCl_3$	$POCl_3$	8	60	64
5	CH_3CN	$POCl_3$	6	80	81
6	-	$POCl_3$	12	90	43
7	$ClCH_2CH_2Cl$	PCl_5	6	80	68
8	$ClCH_2CH_2Cl$	PCl_3	12	80	37
9	$ClCH_2CH_2Cl$	$SOCl_2$	6	80	80

[a]Reaction conditions: **2-1** (10 mmol), solvent (10 mL), chlorination agent (35 mmol), PEG-2000 (2 mmol). [b]Isolated yield.

However, when chlorination with PEG-2000 using a catalytic amount of 0.2 equiv 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. 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) in the reaction, it was observed that the highest yield obtained was the reaction condition when using PEG-2000, which showed that PEG-2000 demonstrated the best performance. The different catalytic abilities of phase transfer catalysts may be attributed to their different solubilization abilities in the reaction.

Table 1 shows the influences of type of solvents and chlorination agents on the reaction. The chlorination of 2,9-diacetylguanine (**2-1**) was examined with different type of solvents at first. Typical results are shown in *Table* 1, entries 1-5. The solvents such as $ClCH_2CH_2Cl$, $CH_3C_6H_5$ and CH_3CN are all the excellent solvents for the chlorination (*Table* 1, entries 1, 3 and 5), and the yields were more than 80% under almost the same conditions, although their yields were decreased respectively in the order: $ClCH_2CH_2Cl$ > $CH_3C_6H_5$ > CH_3CN. The solvent was essential for the chlorination because the yield decreased to 43% in the absence of solvent (*Table* 1, entry 6) but increased to 84% in the presence of $ClCH_2CH_2Cl$, which should be attributed to the solid state of the substrate (**2**) and its poor solubilization abilities with $POCl_3$ in the reaction. When in the presence of $ClCH_2CH_2Cl$, the solvent fulfills several functions during a chemical reaction.[44] One is that it solvates the reactans and reagents so that they dissolve, which facilitates collisions between the reactant(s) and reagents that must occur in order to transform the reactant(s) to product(s); the other is that it also provides a means of temperature control, either to increase the energy of the colliding particles so that they will react more quickly, or to absorb heat that is generated during an exothermic reaction. As a result, a high yield was obtained ccordingly. Besides the influences of different type of solvents, we also found the chlorination agents have great influences on the reaction, we tried to use chlorination agents such as $POCl_3$, PCl_5, PCl_3 and $SOCl_2$ in the reaction, however, the yield were 84%, 68%, 37% and 80%, respectively (*Table* 1, entries 1 and 7-9). Thus, the best chlorination agent is $POCl_3$.

Preparation of 2-amino-6-halogenopurines (3b-d)

The chlorine-exchange fluorination taking place between KF and 2-amino-6-chloropurine is a solid (KF)-liquid (solution of substrates) two-phase procedure in absence of whatsoever phase transfer catalyst, the fluorination proceeded very slowly, and the yield was less than 10% after 15 h. So, an appropriate phase transfer catalyst for this reaction is essen-

***Table* 2.** The halogeno exchange reaction of 2-amino-6-chloropurine with different type of solvents by phase transfer catalysis[a]

Entry	Solvent	Temp. (°C)			Time (h)			Yield (%)[b]		
		3b	3c	3d	3b	3c	3d	3b	3c	3d
1	-	200	80	80	24	10	10	0	7	9
2	DMF	190	40	35	15	2	2	12	62	74
3	DMSO	190	40	35	15	2	2	20	71	80
4	Nitrobenzene	190	40	35	15	2	2	40	83	88
5	Ketopyrrolidine	190	40	35	15	2	2	39	80	86
6	Sulfolane	190	40	35	15	2	2	43	88	92

[a]Reaction conditions: **3a** (10 mmol), solvent (30 mL), KX (50 mmol), TPPB (0.5 mmol). [b]Isolated yield.

***Table* 3.** Fluoro-, bromo- and iodo-dediazoniations of 2-amino-6-substituted purines[a]

Entry	Compound	Aqueous acid	Temp. (°C)	Yield (%)[b]
1	**2-2b**	40%HBF_4	-10	77
2	**4-2b**	40%HBF_4	-10	91
3	**5b**	40%HBF_4	-10	64
4	**2-2c**	40%HBr	-5	67
5	**4-2c**	40%HBr	-5	54
6	**5c**	40%HBr	-5	71
7	**2-2d**	50%HI	5	57
8	**4-2d**	50%HI	5	68
9	**5d**	50%HI	5	49

[a]Reaction conditions: **1** or **3a** or **4-1** (10 mmol), aqueous acid (40 mL), CuX (11 mmol), 7 h. [b]Isolated yield.

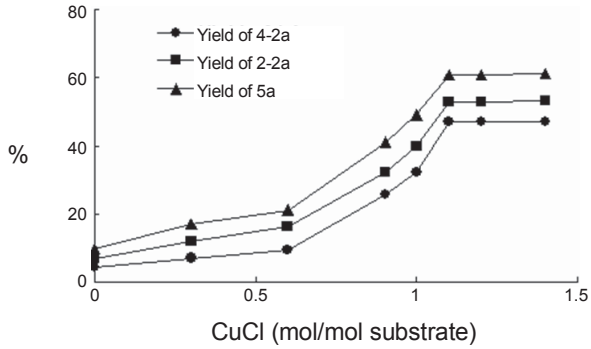


***Fig.* 4.** Influences of amount of CuCl on the diazotization. Reaction conditions: **1** or **3a** or **4-1** (10 mmol), conc. HCl (40 mL), CuCl (11 mmol), –5 °C, 7 h.

tial. The phase transfer catalysts such as TEMAC, TOMAC, BTEAC, cetyltrimethylammonium bromide (CTAB), tetraphenylphosphonium bromide (TPPB) were screened in the reaction, we found that the best phase transfer catalyst is TPPB (*Figure* 3). It is noteworthy that the exchange reaction taking place between KBr or KI and 2-amino-6-chloropurine in the presence of TPPB is very rapid, being usually completed within 2 h in a high yield 88% or 92% under relatively milder conditions (*Table* 2, entry 6), which means that the TPPB phase transfer catalysis is also adapt to the chlorine-exchange bromination or iodination. Besides the phase transfer catalysis, we also found that appropriate solvents are essential for the reaction because of the solid state of the substrate (**3a**), which has been evidenced by our experiments. No reaction occurred in the absence of whatsoever solvent for chlorine-exchange fluorination even under more drastic conditions, and the yields of **3c** and **3d** were only 7% and 9%, respectively (*Table* 2, entry 1). The influences of different types of solvents on the chlorine-exchange halogenations are shown in *Table* 2. From the different solvents screened (DMF, DMSO, nitrobenzene, ketopyrrolidine, and Sulfolane) we found the best solvents to be sulfolane for the reactions (*Table* 2, entries 2-6), and their yields (**3b**, **3c** and **3d**) were decreased respectively in the order: Sulfolane > Nitrobenzene > Ketopyrrolidine > DMSO > DMF.

Preparation of 2-halogenopurines (2-2a-d, 4-2a-d, 5a-d)

In the process of diazotization, we found that CuX has great influences on the reaction, and in this reaciton, the role of CuX is as an catalyst to convert diazonium ion to ArX. To optimize the reaction conditions, we studied the condensation of 2-amino-6-substituted purines (**1**, **3a**, **4-1**) with hydrochloric acid and CuCl at first, and the influences of amount of CuCl on the diazotization are shown in *Figure* 4. It is obvious that the reaction proceeded very slowly, and all the yields were less than 10% in absence of CuCl, and we also found that the increase in the amount of CuCl (i.e. the mole ratio of CuCl to the substrate (**1** or **3a** or **4-1**) increased) could enhance the reaction and the yields reached maximum at 1.1 of the ratio. It was found that the yields were not significantly affected by further adding amount of CuCl, and 1.1 equiv. of CuCl was sufficient. With these results in hand, we subjected guanine (**1**), 2-amino-6-methoxylpurine

***Table* 4.** Fungicidal activity of the title compounds

Compound	Diameter of inhibition zone		
	Bacillus subtillis	Aspergillus niger	Cardida tropicals
1	1.5	3.5	2.3
2-2a	0.3	2.5	2.0
2-2b	1.0	3.2	2.2
2-2c	1.2	3.0	2.3
2-2d	0.7	2.8	1.7
3a	1.4	2.5	1.5
3b	0.3	2.4	1.1
3c	0.6	2.0	1.3
3d	0.7	1.7	1.0
4-2a	1.0	0	1.6
4-2b	1.2	3.5	2.0
4-2c	1.5	3.0	2.0
4-2d	0.7	3.0	2.0
5a	0.6	0	2.0
5b	1.2	3.5	2.0
5c	1.3	3.0	1.1
5d	1.8	2.8	1.5

(**4-1**), and 2-amino-6-chloropurine (**3a**) to the optimized conditions, they were efficiently converted to the corresponding 2-halogenopurines (**2-2b-d**, **4-2b-d**, **5b-d**), and the results were summarized in *Table* 3.

Fungicidal activity

Halogenopurines synthesized were screened for their antifungal activities against three species of fungi, namely bacillus subtillis, aspergillus niger and cardida tropicals using the disc diffusion method.[45,46] The tested compounds were dissolved in 1% NaOH solution (which has no inhibitory activity) to get concentrations of 1 mg/mL solution. The guanine (**1**) 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 ^{o}C for 3 days. Inhibition of the organisms was evidenced by a clear zone surrounding each disk (*Table* 4).

All the tested compounds showed variable activities toward the three species of fungi, some of them comparable to standard guanine. The results of the antifungal screening showed that compounds **2-2a**, **2-2d**, **3b**, **3c**, **3d**, **4-2d** and **5a** displayed good activity against bacillus subtillis, the compounds **3d**, **4-2a** and **5a** displayed good activity against aspergillus niger, and compounds **3b**, **3d** and **5c** showed good activity against cardida tropicals, the compound **3d** showed fairly good activity against the three fungal strains, while the remaining compounds exhibited moderate activity. The lowest activity were observed by compounds **2-2b**, **2-2c**, **4-2c** and **4-2b**, which displayed almost the same activity as **1** against the three fungal strains (*Table* 4).

CONCLUSION

In conclusion, we have developed an efficient synthetic pathway for the preparation of halogenopurines starting from commercially available guanine. The desired products have been obtained in satisfactory yields under mild reaction conditions after simple work-up. One of the important aspects in our paper was that the PEG-2000 catalyzed chlorination of 2,9-diacetylguanine was carried out successfully, the yield for **3a** under PEG-2000 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%.[47-49] Another noticeable point was that 2-amino-6-halogenopurines (**3b-d**) have been efficiently obtained through chlorine/halogen exchange reactions between KX and **3a** by TPPB phase transfer catalysis, the yields under such conditions could be remarkably improved by 10 ~ 20% contrast to the reported procedures.[9-21] Further, we have described a facile and convenient procedure for the preparation of 2-halogenopurines (**2-2a-d**, **4-2a-d**, **5a-d**) from 2-amino-6-substituted purines (**1**, **3a**, **4-1**) *via* a diazotization catalyzed by their corresponding CuX, and some biologically new compounds **2-2a**, **2-2c**, **2-2d**, **4-2c**, **4-2d**, **5b**, **5c** and **5d** have been discovered.

EXPERIMENTAL

General

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

Preparation of 2,9-diacetylguanine (2-1): A mixture of guanine (**1**, 2.5 g, 16.5 mmol) and acetic anhydride (30 mL) in acetic acid (60 mL) was stirred in 250 mL round flask at 135 °C for 7.5 h. After completion of the reaction, as indicated by HPLC, the solvent and the superfluous acetic anhydride were evaporated under vacuum and the crude product was recrystallized from distilled water afforded a white powder (**2-1**, 3.7 g, yield 95%). m.p. 251 - 256 °C. IR (KBr) 3200, 1680, 1530 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.16-2.50 (m, 6H, CH_3), 8.11 (s, 1H, CH), 8.15 (s, 1H, NH), 11.56 (s, 1H, OH). MS (70 eV) *m*/*z* 234.09 (M^-); MS *m*/*z* (%) 234.09 (M^-, 20), 192.12 (100), 150.08 (23). Analysis Calcd for $C_9H_9N_5O_3$: C, 45.93; H, 3.87; N, 29.76; O, 20.37. Found: C, 45.96; H, 3.86; N, 29.78; O, 20.41.

Preparation of 2-amino-6-chloropurine (3a): 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 100 mL round flask. Then $POCl_3$ (5.4 g, 35 mmol) was added dropwise while the mixture was heated at 80 °C. 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 afforded a white powder (**3a**, 1.42 g, yield 84%). m.p. 298-302 °C. IR (KBr) 1636, 1292, 822, 630 cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.75-6.79 (m, 3H, NH_2 and NH), 8.01 (m, 1H, CH). MS (70 eV) *m*/*z* 169.13 (M^-); MS *m*/*z* (%) 169.13 (M^-), 151.20, 135.13, 116.84. Analysis Calcd for $C_5H_4ClN_5$: C, 35.37; H, 2.34; Cl, 20.93; N, 41.29. Found: C, 35.41; H, 2.38; Cl, 20.91; N, 41.30.

Preparation of 2-amino-6-methoxylpurine (4-1): A mixture of 2-amino-6-chloropurine (**3a**, 1.7 g, 10 mmol), DMSO (15 mL), and CH_3ONa (50 mmol) was stirred in 250 mL round flask at 70 °C for 6.5 h. After the reaction, the solvent was removed under vacuum, the residue was dissolved in water (30 mL), then added toluene (20 mL) to the mixture and filtered, the water layer was neutralized with conc. HCl. The solid obtained was filtered, washed several times with water to give a white solid (**4-1**, 1.5 g, yield 91%). ^1H NMR (DMSO-d_6) δ 3.98 (s, 3H, OCH_3), 6.81 (s, 1H, CH), 8.13 (s, 3H, NH_2 and NH). MS (70 eV) *m*/*z* 166.02 (M^+); MS *m*/*z* (%) 166.02 (M^+, 35), 149.01 (100), 134.02 (17). Analysis Calcd for $C_6H_7N_5O$: C, 43.64; H, 4.27; N, 42.39; O, 9.67. Found: C, 43.63; H, 4.27; N, 42.41; O, 9.69.

General procedure for synthesis of compounds 3b-d: All the three-necked flasks were loaded with 2-amino-6-chloropurine (**3a**, 1.7 g, 10 mmol), KX (50 mmol), TPPB (0.21 g, 0.5 mmol), and sulfolane (30 mL). The reaction mixture was stirred at an appropriate temperature for an appropriate time, and then cooled to room temperature, the precipitate was filtered off, the solvent was removed and the residue was washed several times with water to give a white powder. The yield and spectral data of the compounds are given below.

2-amino-6-fluoropurine (3b): The product was obtained with 43% yield. ^1H NMR (DMSO-d_6) δ 7.76 (s, 3H, NH_2 and NH), 8.15 (s, 1H, CH). MS (70 eV) *m*/*z* 153.06 (M); MS *m*/*z* (%) 153.06 (M), 137.07, 133.12. Analysis Calcd for $C_5H_4FN_5$: C, 39.19; H, 2.62; F, 12.43; N, 45.76. Found: C, 39.22; H, 2.63; F, 12.41; N, 45.74.

2-amino-6-bromopurine (3c): The product was obtained with 88% yield. ^1H NMR (DMSO-d_6) δ 5.75 (m, 3H, NH_2 and NH), 8.03 (m, 1H, CH). MS (70 eV) *m*/*z* 213.15 (M^-); MS *m*/*z* (%) 213.15 (M^-), 134.14. Analysis Calcd for $C_5H_4BrN_5$: C, 28.03; H, 1.86; Br, 37.35; N, 32.71. Found: C, 28.06; H, 1.88; Br, 37.33; N, 32.72.

2-amino-6-iodopurine (3d): The product was obtained with 92% yield. ^1H NMR (DMSO-d_6) δ 3.81 (s, 3H, NH_2 and NH), 8.33 (s, 1H, CH). MS (70 eV) *m*/*z* 261.15 (M); MS *m*/*z* (%) 261.15 (M), 217.32, 171.61. Analysis Calcd for $C_5H_4IN_5$: C, 22.97; H, 1.54; I, 48.63; N, 26.87. Found: C, 23.01; H, 1.54; I, 48.62; N, 26.83.

General procedure for synthesis of compounds 2-2a-d, 4-2a-d and 5a-d: All the three-necked flasks were loaded with 2-amino-6-substituted purine (**1** or **3a** or **4-1**; 10 mmol), aqueous acid (40 mL), and CuX (11 mmol). The mixture was stirred at room temperature for 30 min. After cooling down to -5 °C, the solution of sodium nitrite (0.76 g, 11 mmol) in H_2O (2 mL) was added dropwise slowly, and an evolution of nitrogen gas was observed immediately, then the reaction mixture was stirred at -10 ~ 5 °C for 2 h, urea (0.3 g, 5 mmol) was then added to decompose the excess sodium nitrite. After which, the reaction mixture was stirred at -10 ~ 5°C for another 5 h. Then 50%NaOH solution was added to regulate pH value of 3 ~ 4, the solid so formed was filtered, and then was dissolved completely with NaOH solution (15%, 20 mL) and separated by silica-gel column chromatography using dichloromethane-methanol (9/1) as eluent. The solution obtained was neutralized with conc. HCl. The solid was separated out and filtered to give the corresponding product. The yield and spectral data of each compound is given below.

2-chloro-6-hydroxylpurine (2-2a): The product was obtained with 53% yield. m.p. 182 - 184 °C. ^1H NMR (DMSO-d_6) δ 7.99 (s, 1H, CH), 10.90 (s, 1H, NH), 8.15 (s, 1H, OH). MS (70 eV) *m*/*z* 171.30 (M^+); MS *m*/*z* (%) 171.30 (M^+, 100), 169.33 (22), 157.39 (36), 141.58 (40). Analysis Calcd for $C_5H_3ClN_4O$: C, 35.18; H, 1.77; Cl, 20.79; N, 32.83; O, 9.37. Found: C, 35.21; H, 1.77; Cl, 20.79; N, 32.85; O, 9.38.

2-fluoro-6-hydroxylpurine (2-2b): The product was ob-

tained with 77% yield. m.p. 175 - 176 °C. ^{1}H NMR (DMSO-d_6) δ 7.99 (s, 1H, CH), 10.11 (s, 1H, NH), 10.25 (s, 1H, OH). MS (70 eV) *m/z* 154.99 (M^+); MS *m/z* (%) 154.99 (M^+, 50), 134.98 (100). Analysis Calcd for $C_5H_3FN_4O$: C, 38.98; H, 1.96; F, 12.35; N, 36.34; O, 10.36. Found: C, 38.97; H, 1.96; F, 12.33; N, 36.36; O, 10.38.

2-bromo-6-hydroxylpurine (2-2c): The product was obtained with 67% yield. m.p. 214 - 216 °C. ^{1}H NMR (DMSO-d_6) δ 7.23 (s, 1H, CH), 10.91 (s, 1H, NH), 11.60 (s, 1H, OH). MS (70 eV) *m/z* 217.01 (M^+); MS *m/z* (%) 217.01 (M^+, 39), 139.66 (100). Analysis Calcd For $C_5H_3BrN_4O$: C, 27.89; H, 1.41; Br, 37.17; N, 26.09; O, 7.41. Found: C, 27.93; H, 1.41; Br, 37.16; N, 26.06; O, 7.44.

2-iodo-6-hydroxylpurine (2-2d): The product was obtained with 57% yield. m.p. 224 - 227 °C. ^{1}H NMR (DMSO-d_6) δ 6.98 (s, 1H, CH), 10.63 (s, 1H, NH), 11.37 (s, 1H, OH). MS (70 eV) *m/z* 260.89 (M^-); MS *m/z* (%) 260.89 (M^-, 100), 234.44 (21), 157.21 (28), 146.92 (13). Analysis Calcd for $C_5H_3IN_4O$: C, 22.89; H, 1.17; I, 48.46; N, 21.37; O, 6.09. Found: C, 22.92; H, 1.15; I, 48.44; N, 21.38; O, 6.11.

2-chloro-6-chloropurine (4-2a): The product was obtained with 47% yield. m.p. 182 - 183 °C. ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H, CH), 11.99 (s, 1H, NH). MS (70 eV) *m/z* 187.11 (M^-); MS *m/z* (%) 187.11 (M^-, 72), 151.55 (100), 149.59 (83), 114.93 (34), 89.29 (79). Analysis Calcd for $C_5H_2Cl_2N_4$: C, 31.74; H, 1.08; Cl, 37.53; N, 29.62. Found: C, 31.77; H, 1.07; Cl, 37.52; N, 29.64.

2-fluoro-6-chloropurine (4-2b): The product was obtained with 91% yield. m.p. 162 - 163 °C. ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H, CH), 11.97 (s, 1H, NH). MS (70 eV) *m/z* 171.03 (M^-); MS *m/z* (%) 171.03 (M^-, 88), 134.95 (100), 90.08 (80). Analysis Calcd for $C_5H_2ClFN_4$: C, 34.76; H, 1.17; Cl, 20.53; F, 11.02; N, 32.48. Found: C, 34.80; H, 1.17; Cl, 20.55; F, 11.01; N, 32.47.

2-bromo-6-chloropurine (4-2c): The product was obtained with 54% yield. m.p. 175 - 177 °C. ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H, CH), 11.99 (s, 1H, NH). MS (70 eV) *m/z* 232.86 (M^-); MS *m/z* (%) 232.86 (M^-, 25), 214.94 (20), 194.16 (100), 169.29 (30), 166.56 (38). Analysis Calcd for $C_5H_2ClBrN_4$: C, 25.69; H, 0.84; Br, 34.25; Cl, 15.18; N, 23.97. Found: C, 25.72; H, 0.86; Br, 34.23; Cl, 15.19; N, 24.00.

2-iodo-6-chloropurine (4-2d): The product was obtained with 68% yield. m.p. 218 - 220 °C. ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H, CH), 11.98 (s, 1H, NH). MS (70 eV) *m/z* 278.92 (M^-); MS *m/z* (%) 278.92 (M^-, 100), 243.21 (56), 152.11 (28). Analysis Calcd for $C_5H_2ClIN_4$: C, 21.37; H, 0.71; Cl, 12.63; I, 45.27; N, 19.98. Found: C, 21.41; H, 0.72; Cl, 12.64; I, 45.25; N, 19.98.

2-chloro-6-methoxylpurine (5a): The product was obtained with 61% yield. m.p. 197 - 199 °C. ^{1}H NMR (DMSO-d_6) δ 3.97 (s, 3H, OCH_3), 7.55 (s, 1H, CH), 11.97 (s, 1H, NH). MS (70 eV) *m/z* 206.95 (M^+); MS *m/z* (%) 206.95 (M^+, 38), 193.08 (30), 175.24 (100). Analysis Calcd for $C_6H_5ClN_4O$: C, 39.02; H, 2.71; Cl, 19.23; N, 30.34; O, 8.68. Found: C, 39.04; H, 2.73; Cl, 19.21; N, 30.35; O, 8.67.

2-fluoro-6-methoxylpurine (5b): The product was obtained with 64% yield. m.p. 180 - 182 °C. ^{1}H NMR (DMSO-d_6) δ 3.96 (s, 3H, OCH_3), 7.23 (s, 1H, CH), 12.00 (s, 1H, NH). MS (70 eV) *m/z* 169.01 (M^+); MS *m/z* (%) 169.01 (M^+, 58), 149.05 (100), 121.04 (16). Analysis Calcd for $C_6H_5FN_4O$: C, 42.81; H, 2.97; F, 11.34; N, 33.30; O, 9.53. Found: C, 42.86; H, 3.00; F, 11.30; N, 33.32; O, 9.52.

2-bromo-6-methoxylpurine (5c): The product was obtained with 71% yield. m.p. 195 - 199 °C. ^{1}H NMR (DMSO-d_6) δ 3.98 (s, 3H, OCH_3), 7.41 (s, 1H, CH), 11.99 (s, 1H, NH). MS (70 eV) *m/z* 230.03 (M^+); MS *m/z* (%) 230.03 (M^+, 45), 198.14 (100). Analysis Calcd for $C_6H_5BrN_4O$: C, 31.43; H, 2.19; Br, 34.91; N, 24.43; O, 6.97. Found: C, 31.46; H, 2.20; Br, 34.89; N, 24.46; O, 6.99.

2-iodo-6-methoxylpurine (5d): The product was obtained with 49% yield. m.p. 233 - 238 °C. ^{1}H NMR (DMSO-d_6) δ 3.97 (s, 3H, OCH_3), 7.11 (s, 1H, CH), 12.51 (s, 1H, NH). MS (70 eV) *m/z* (M^-); MS *m/z* (%) 274.96 (M^-, 67), 243.02 (100), 147.87 (45). Analysis Calcd for $C_6H_5IN_4O$: C, 26.07; H, 1.83; I, 45.99; N, 20.31; O, 5.79. Found: C, 26.11; H, 1.83; I, 45.97; N, 20.30; O, 5.80.

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