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High Yielding, Base Catalyzed C^6 Regioselective Amination and N^9 Alkylation in Purine Nucleotide

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2,6-Dichloropurine is an interesting new nucleoside which gave regioselectively various 2-derivatized or 6-derivatized purines by using a secondary amines. An efficient, simple and regioselective synthesis of C^6 morpholine, N^9 alkylated purine nucleoside derivatives were attained *via* chloro-amine coupling reaction between 2,6-dichloropurine with morpholine followed by commercial alkylation method using DMF and K_2CO_3. Over the traditionally used protocols and procedure, it have been exhibited advance benefits such as admirable yield, simple reaction conditions and modest influence.

Keywords: 2,6-Dichloropurine, Regioselective, Alkylation, Amination.

INTRODUCTION

Organic chemist is performing one of the most significant C-N bond formations in organic synthesis [1]. The variety of amines and its derivatives are utilized as solvents, drug intermediates, agrochemicals, medicines and chemical agent for various chemical reactions. The SN reaction between different alkyl halides and primary/secondary amines is convenient for the research of tertiary amines but the downside of this pathway is to longer reaction time and generate binary product of secondary and tertiary amines. On the other hand, thermal irradiated reaction using base catalyst needs more reaction time and low yield of target molecules. To minimize the above drawbacks, cooper and palladium catalyzed coupling reactions have been performed for the successful amination [2].

For such transformation two concepts are utilizing by researchers *i.e*. microwave and traditional method [3]. Out of these two, microwave irradiation has concerned substantial attention for quick synthesis of a library of organic amalgams but due to initiation of side reaction and other byproduct formation, it is not used in diverse range of reaction [4]. Traditional heating method and combinatorial synthesis for the lead molecule identification is well developed and used [5].

Recent literature shows that modified nucleosides are becoming sizzling area of research due to their captivating biological and pharmacological properties [6,7]. The well-studied nucleoside *i.e*. purines nucleosides and their various novel substituted adducts are found biologically efficient and strongly dispersed heterocycles having N-atoms in the core ring structure. Various types of biologically diverse study have been exhibited by purine motif like anticancer, anti-HIV, anti-hypertensive, *etc*. [8]. It has been identified that purine with substitution at 9th position in imidazole ring side shows unbeatable medicinal properties [6,7,9,10] and many of its derivatives are commercial available as active drug molecules like acyclovir and famciclovir (antiviral) [11,12], 6-mercaptopurine and thioguanine (anticancer), neuropsychiatric disorders, neurodegenerative diseases, inflammatory diseases (theophylline, azathioprine,6′-mercaptopurine), tuberculosis or impotence [13].

Traditionally, the protection using halogenated substance, mesylate or tosylate is an imperative synthetic route for the modification on purine to generate its derivatives but it typically suffers from deprived regioselectivity, resultant in a mixture of N^9 and N^7 isomers [8,14]. Literature shows that the desired N^9 compound is normally the major product, but formation of significant amounts of the N^7 isomer as well as other alkylation products is often observed [15]. Addition of acyclic/heterocycles analogues in to the nucleosides have remained major biomedical research targets in current drug discovery process and it has been becomes fast after the discovery of potent anti-

viral activity of 9-[(2-hydroxyethoxy)methyl]guanine, the standard drug for the treatment of herpes viral infections [2]. On the basis of above modifications, researchers turn their attention to insert saturated heterocycles to the nucleoside to generate active lead molecules. It has been also noted that the morpholine/aromatic amines analogous to the nucleosides build up their supreme space in the current medicinal chemistry [16]. In continuation of our interest on the modification of nucleosides [17] and obtain these diverse scaffolds in higher yields with shorter reaction times and under milder reaction conditions, we turned our attention on insertion of acyclic part (morpholine and alkyl group) in to the purine motif. In this article, we report the modified purine nucleosides of 2-chloro-9-alkylated-6-morpholino-9*H*-purine *via* highly regioselective C^6 acylation of purines with morpholine catalyzed by Hunig's base (DIPEA-N,N-diisopropylethylamine). Alkylation of morpholine-purine was oriented to N^9 using DMF/K_2CO_3 with 89 % regioselectivity [18].

EXPERIMENTAL

All the chemicals and reagents were received from Sigma-Aldrich and Merck. Silica gel plate G60 F_{254} (Merck) was used in thin layer chromatography to monitor the completion of the reaction. Visualization was made under UV light (254 and 365 nm). Infrared spectra of the compounds were recorded on IR Affinity-1S spectrophotometer (Shimadzu). ^{1}H (400 MHz) and ^{13}C (101.1 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in DMSO-d_6. Mass spectrometer GCMS-QP 2010 (Shimadzu) was used to resolute the mass spectra of compounds and rotary evaporator was used for drying the compounds. Melting points were measured by open capillary method and are uncorrected.

Synthesis of 2-chloro-6-morpholino-9H-purine (3) (step I): To a *n*-butanol containing round bottom flask, 2,6-dichloropurine (**1**) (50 mmol), N,N-diisopropylethylamine (DIPEA) (10 mmol) and morpholine (**2**) (50 mmol) were added and was refluxed at 80 ºC for 2 h to generate a reactive intermediate (**3**). Thin layer chromatography [hexane(7):ethyl acetate (3)] was utilized to identify the complete formation of product. After 12 h stirring at room temperature, it was poured into ice-cold water and stirred for 1 h to isolate free material in powder form. The separated product was filtered and washed with cold water. The isolated product was dried for next 12 h at room temperature.

For the purification purpose, hexane stripping was performed using roteva evaporator at 60 ºC and 500 mm pressure through 100 rpm speed. The step-I crude product was characterized by ^{1}H NMR and IR techniques to carry out further reactions.

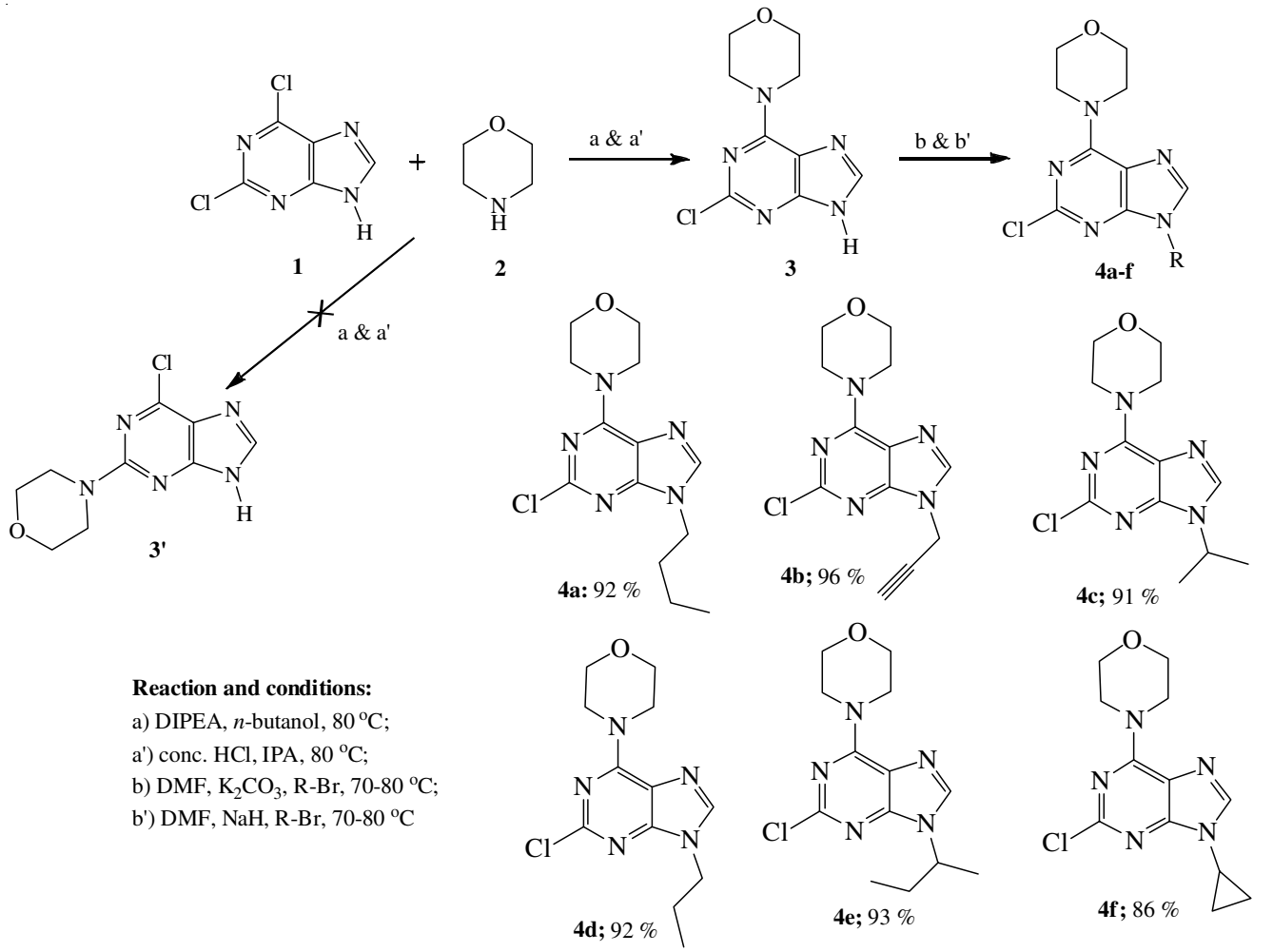
Synthesis of 2-chloro-9-alkylated-6-morpholino-9*H*-purine (4a-f) (step II): Into a vaccum dried round bottom flask, product (**3**) (3 mmol), powdered form of potassium carbonate (K_2CO_3) (10 mmol) and DMF (1 mL) were heated at 80 ºC for 2 h to generate nucleophile at N^9 position of purine. After 2 h heating, various alkyl halides (5.5 mmol) were charged at heating condition using micropipette to the reaction mixture and continue to heating for 3 h for the completion of reaction. It was monitored by TLC using two different mobile phase systems [hexane (7): ethyl acetate (3) & ethyl acetate (100 %)]. After completion of the reaction, it was poured into crushed ice water, dried over vaccum buchner funnel and purified by column chromatography using silica 60-120 mesh size as stationary phase [mobile phase: hexane (60 %):ethyl acetate (40 %)] (**Scheme-I**). The isolated purine derivatives were characterized and identified by various spectroscopic techniques like MS, IR, ^{1}H and ^{13}C NMR.

9-Butyl-2-chloro-6-morpholino-9*H*-purine (4a): Dark yellow, yield: 92 %; m.p.: 152 ºC; IR (KBr, cm^{-1}, ν_{max}): 3024.84 (arom. ring C-H *str*.), 2966.76 (aliph. C-H *str*. asym.), 2935.25 (aliph. C-H *str*. sym.), 1584.21, 1514.38, 1487.24 (arom. ring skeleton), 1258.11 (C-N *str*.), 1163.33 (C-O *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.23 (s, 1H, CH of imidazole ring), 4.52-4.33 (broad-m, 4H, morpholine -CH_2), 4.13-3.95 (m, 2H, morpholine -CH_2), 3.73-3.51 (m, 2H, morpholine -CH_2), 1.79-1.71 (m, 2H, -CH_2 of alkyl chain), 1.29-1.18 (m, 2H, -CH_2 of alkyl chain), 1.19-1.17 (t, 3H, -CH_3 of alkyl chain), 0.91-0.85 (m, 2H, N-CH_2 of alkyl chain); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 153.32, 152.40, 151.73, 140.69, 117.98, 66.01, 31.17, 22.07, 19.15; MS (*m/z*): 295.76 (M+); Anal. calcd. (found) % for $C_{13}H_{18}N_5OCl$: C, 52.79 (52.80); H, 6.13 (6.15); N, 23.68 (23.67); O, 5.41 (5.40); Cl, 11.99 (11.98).

2-Chloro-6-morpholino-9-(prop-2-ynyl)-9*H*-purine (4b): Bright yellow, yield: 96 %; m.p.: 140 ºC; IR (KBr, cm^{-1}, ν_{max}): 3101.51 (arom. ring C-H *str*.), 2979.73 (aliph. C-H *str*. asym.), 2967.96 (aliph. C-H *str*. sym.), 2128.56 (C≡C *str*.), 1584.09, 1514.40, 1460.71 (arom. ring skeleton), 1261.00 (C-N *str*.), 1140.71 (C-O *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.27 (s, 1H, CH of imidazole ring), 5.04-5.03 (m, 2H, propargyl -CH_2), 4.43 (broad, 4H, morpholine-CH_2), 3.91-3.84 (m, 4H, morpholine -CH_2), 3.53 (s, 1H, ≡CH of propargyl); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 152.72, 151.26, 139.94, 118.22, 77.77, 76.28, 65.98, 32.63; MS (*m/z*): 277.70 (M^+); Anal. calcd. (found) % for $C_{12}H_{12}N_5OCl$: C, 51.90 (51.93); H, 4.36 (4.38); Cl, 12.77(12.75); N, 25.22 (25.20); O, 5.76 (5.75).

2-Chloro-9-isopropyl-6-morpholino-9*H*-purine (4c): Yellow, yield: 91 %; m.p.: 152 ºC; IR (KBr, cm^{-1}, ν_{max}): 3031.26 (arom. ring C-H *str*.), 2965.22 (aliph. C-H *str*. asym.), 2939.20 (aliph. C-H *str*. sym.), 1589.48, 1521.59, 1496.21 (arom. ring skeleton), 1259.99 (C-N *str*.), 1156.56 (C-O *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.25 (s, 1H, CH of imidazole ring), 5.01-4.99 (m, 2H, propargyl -CH_2), 4.49 (broad, 4H, morpholine -CH_2), 3.99-3.91 (m, 4H, morpholine -CH_2), 4.38 (m, 1H, -CH of isopropyl), 1.52-1.51 (d, 6H, -CH_3 of iso-propyl); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 159.63, 155.11, 147.70, 142.35, 135.34, 65.59, 48.20, 45.92, 45.92, 18.84, 18.84; MS (*m/z*): 281.74 (M+); Anal. calcd. (found) % for $C_{12}H_{16}N_5OCl$: C, 51.16 (51.15); H, 5.72 (5.72); N, 24.86 (24.84); O, 5.68 (5.70); Cl, 12.58 (12.59).

2-Chloro-6-morpholino-9-propyl-9*H*-purine (4d): Off-whitish yellow, yield: 92 %; m.p.: 136 ºC; IR (KBr, cm^{-1}, ν_{max}): 3103.20 (arom. ring C-H *str*.), 2989.01 (aliph. C-H *str*. asym.), 2941.20 (aliph. C-H *str*. sym.), 1590.10, 1555.21, 1489.04 (arom. ring skeleton), 1269.23 (C-N *str*.), 1151.22 (C-O *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.29 (s, 1H, CH of imidazole ring), 4.45-4.39 (broad-m, 4H, morpholine -CH_2), 4.20-4.12 (m, 2H, morpholine-CH_2), 3.88-3.78 (m, 2H, -CH_2 alkyl chain), 3.70-3.63 (m, 2H, morpholine-CH_2), 1.83-1.79 (m, 2H, -CH_2 alkyl chain), 1.04-1.01 (t, 3H, -CH_3 alkyl chain); ^{13}C NMR (101



Scheme-I: Synthetic route for the Synthesis of N[9]-alkyl C[6]-purine morpholine **(4a-f)**

MHz, DMSO-d_6) δ ppm: 155.69, 153.82, 151.85, 142.59, 135.19, 66.52, 49.23, 46.56, 20.86, 11.99; MS (*m/z*): 281.74 (M+); Anal. calcd. (found) % for $C_{12}H_{16}N_5OCl$: C, 51.16 (51.12); H, 5.72 (5.73); N, 24.86 (24.87); O, 5.68 (5.69); Cl, 12.58 (12.59).

9-*sec*-Butyl-2-chloro-6-morpholino-9*H*-purine (4e): Dark yellow, yield: 93 %; m.p.: 165 ºC; IR (KBr, cm^{-1}, ν_{max}): 3019.96 (arom. ring C-H *str*.), 2960.88 (aliph. C-H *str*. asym.), 2946.06 (aliph. C-H *str*. asym.), 1601.22, 1556.55, 1452.42 (arom. ring skeleton), 1261.88 (C-N *str*.), 1170.55 (C-O *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.25 (s, 1H, CH of imidazole ring), 4.50-4.43 (m, 4H, morpholine-CH_2), 4.26-4.21 (m, 2H, morpholine -CH_2), 3.89-9.83 (m, 2H, morpholine -CH_2), 3.70-3.62 (m, 1H, -CH of alkyl chain), 1.69-1.65 (m, 2H, -CH_2 of alkyl chain), 1.53-1.51 (d, 3H, -CH_3 of alkyl chain), 0.99-1.02 (t, 3H, -CH_3 of alkyl chain); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 160.02, 157.54, 152.01, 147.85, 141.91, 68.12, 59.00, 29.62, 18.59, 15.62; MS (*m/z*): 295.76 (M+); Anal. calcd. (found) % for $C_{13}H_{18}N_5OCl$: C, 52.79 (52.78); H, 6.13 (6.13); N, 23.68 (23.69); O, 5.41 (5.41); Cl, 11.99 (11.99).

2-Chloro-9-cyclopropyl-6-morpholino-9*H*-purine (4f): Off-whitish yellow, yield: 86 %; m.p.: 181 ºC; IR (KBr, cm^{-1}, ν_{max}): 3102.33 (arom. ring C-H *str*.), 2989.02 (aliph. C-H *str*. asym.), 2939.22 (aliph. C-H *str*. sym.), 1598.16, 1519.98, 1455.72 (arom. ring skeleton), 1260.54 (C-N *str*.), 1170.22 (C-O *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.19 (s, 1H, CH of imidazole ring), 4.50-4.41 (broad-m, 4H, morpholine -CH_2), 4.00-3.96 (m, 2H, morpholine -CH_2), 3.70-3.62 (m, 2H, morpholine -CH_2), 2.42-2.38 (m, 1H, -CH of cyclopropane ring), 0.88-0.84 (m, 2H, -CH_2 of cyclopropane ring), 0.88-0.84 (m, 2H, -CH_2 of cyclopropane ring); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 165.59, 160.22, 155.23, 149.05, 140.00, 68.93, 47.22, 39.56, 12.97. MS (*m/z*): 279.72 (M+); Anal. calcd. (found) % for $C_{12}H_{14}N_5OCl$: C, 51.52 (51.49); H, 5.04 (5.05); N, 25.04 (25.06); O, 5.72 (5.71); Cl, 12.67 (12.68).

RESULTS AND DISCUSSION

For the purpose of functionalizing the 6th positions in one pot, 2,6-chloropurine was treated with one equivalent of morpholine in the presence of DIPEA. This led to various 6-substituted purines **3** as outlined in **Scheme-I**. It should be noticed that the yield decreased when using more than 1 equiv. of DIPEA. On the other hand, the same reaction with same mole proportion of purine and morpholine, in the presence of acidic catalyst was no longer gives product. The C[6] chlorine atom of purine was substituted by a -NH group of morpholine followed by reaction of **3** with various alkyl halides gives N[9] alkylation in 89 % yields [18]. The amination of compound **1** (**Scheme-I**) with morpholine (**2**), under changing the reaction condition *i.e*. using conc. HCl and isopropyl alcohol (IPA) were seems to be not very efficient and display poor yield.

Alkylation yields with 2-chloro-9-alkylated-6-morpholino-9*H*-purine (4a-f) (**Scheme-I**) were comparable to those with compound **3**. Selective alkylation at N^9 of purine was effected with 3.5 equiv. of alkyl halides. NMR and mass spectral data were consistent with the proposed structure. Dialkylated byproducts were not observed with less than 5.5 equiv. of other alkyl halides. Anhydrous K_2CO_3 is a much more convenient base than NaH dispersed in mineral oil and K_2CO_3/DMF was equivalent or superior to NaH/DMF for some alkylation [19].

Regiospecific amination by secondary amine group of step I products were optimized by varying the reaction condition. Literature review shows that K_2CO_3 is a better base than the other used bases as reported earlier [20]. As shown in analytical data, it exhibited excellent yield with 89 % regioselective way. The derived scaffolds can be summarized on the basis of their yield ratio and the moderate yield were found with cyclic system and excellent in case of straight chain aliphatic systems.

Conclusion

Treatment of 2,6-dichloropurine with DIPEA/*n*-butanol followed by alkyl halides (K_2CO_3/DMF) gave regiospecific C^6 amination and N^9 alkylation (N^7 isomers were not detected, even in crude reaction mixtures). Anhydrous K_2CO_3/DMF was a convenient alternative to NaH/DMF in some cases. Simultaneously, DIPEA/butanol was excellent than conc. HCl/IPA. We attribute the N^9 regiospecificity and C^6 regioselectivity on the basis of the literature. A broad array of regioisomerically pure 6-substituted-9-alkylpurines is now readily accessible.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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