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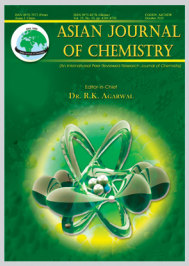

Synthesis and Biological Activity of 2-Aryloxyacetylamino-2-Deoxy-D-Glucoses

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D-Glucosamine possesses extensive bioactivities including antibacterial, insecticidal and plant growth-regulating activities. A series of 2-aryloxyacetylamino-2-deoxy-D-glucoses have been synthesized by acylation of D-glucosamine with aryloxyacetyl chlorides and their plant growth-regulating activities were tested. The results show that these compounds bearing chlorine atom at *para* position of benzene ring have notable inhibiting activities against cotyledon rootage of cucumber which are comparable with that of 2,4-dichlorophenoxyacetic acid.

Key Words: D-Glucosamine, Aryloxyacetic acid, Plant growth-regulating activity.

INTRODUCTION

Sugars act as both immediate substrates for intermediary metabolism and effective signaling molecules in plants and their availability is a powerful driver of plant growth[1,2]. During plant growth and development, sugars perform important hormone-like functions and modulate a range of vital processes such as seed germination[3], seedling development[4], root differentiation[5], floral transition[6], fruit ripening[7], embryogenesis[8] and senescence[9], as well as responses to light[10], stress[11] and pathogens[12]. D-Glucosamine, 2-amino-2-deoxy-D-glucose (D-GlcN), is an naturally occurring amino sugar that is synthesized by amidation of fructose-6-phosphate. Many reports have described the biological effects of D-GlcN in mammalian systems[13-15]. However, the physiological significance of D-GlcN in plants has only recently reported[16]. In fact, heterocycles[17-19] exhibits extensive bioactivities including antibacterial, insecticidal and plant growth-regulating activities, especially sugar. For example, D-GlcN and its acetyl derivative N-acetylglucosamine can induce the production of chitinase, an enzyme involved in defense mechanism and also in host/parasite interaction[20]. Hong *et al.*[16] reported that exogenous D-GlcN inhibits hypocotyls elongation in Arabidopsis and induces a significant increase in the production of reactive oxygen species (ROS).

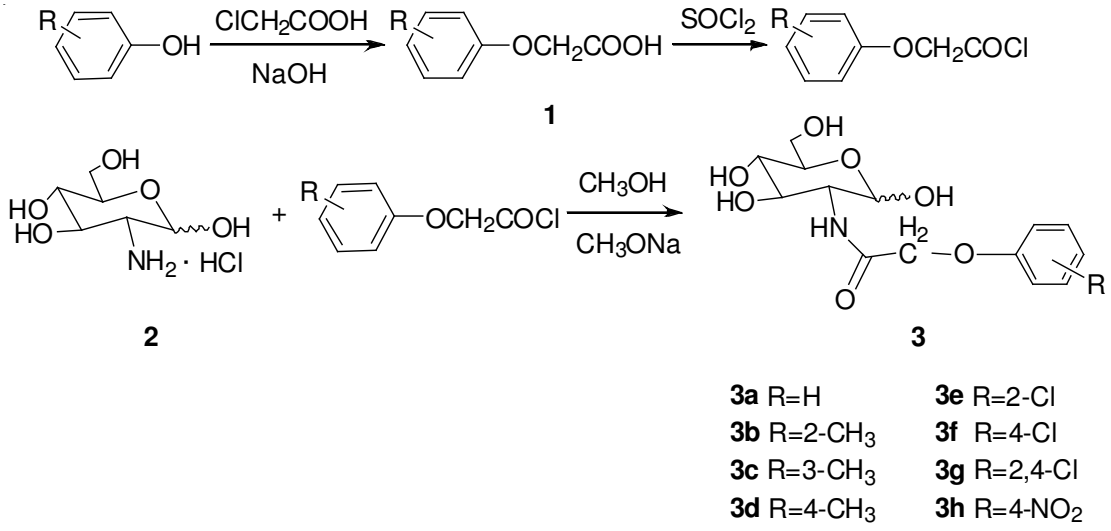
It is well known that aryloxyacetic acids exhibit high plant growth-regulating activity. However, they might induce unfavourable responses. For example, when 2,4-dichlorophenoxyacetic acid was used in citrus growing, pre-harvest fruit abscission happened[21]. The derivatives of aryloxyacetic acids were reported to not only improve the bioactivities but also reduce the extent of side effects[22], which encouraged us to incorporate the aryloxyacetic acid type regulator into D-glucosamine. Here, a series of 2-aryloxyacetylamino-2-deoxy-D-glucoses were prepared (**Scheme-I**) and their plant growth-regulating activities were also examined.

EXPERIMENTAL

Melting points were determined on a Taike X-4 melting point apparatus and were not corrected. Nuclear magnetic resonance spectra were recorded on Bruker Avance III 500 MHz and chemical shifts are expressed in ppm using TMS as an internal standard. Mass spectra were measured using a Thermo Finnigan LCQ Series, Agilent 6210 Series time-of-flight mass spectrometer (ESI/APCI), Thermo Scientific ITQ 1100 and Waters GCT Premier mass spectrometer (EI/CI).

General synthetic procedure for 2-aryloxyacetylamino-2-deoxy-D-glucoses

Synthesis of aryloxyacetyl chloride[23]: Phenol (0.1 mol) was dissolved in the solution of EtOH (15 mL) and aq. NaOH (8.6 g, 50 wt %) and mixed with the solution of sodium 2-chloroacetate (11.6 g, 0.1 mol) in H_2O (20 mL). The reactant was refluxed for 1 h. After cooling the reaction mixture, H_2O (20 mL) was added and the solution was acidified to give a precipitate. The solid was washed with water and recrystallized from EtOH-H_2O to obtain aryloxyacetic acid as a white solid: **1a** 98-99 ºC, 70.2 %; **1b** 153-154 ºC, 89.3 %; **1c** 103-105 ºC, 75.7 %; **1d** 142-143 ºC, 87.5 %; **1e** 148-150 ºC, 77.2 %; **1f** 158-160 ºC, 74.6 %; **1g** 137-139 ºC, 81.6 %; **1h** 175-176 ºC, 54.1 %.



Scheme-I

The prepared aryloxyacetic acid was dissolved in $SOCl_2$ (10 mL) and the mixture was refluxed for 6 h. Excess $SOCl_2$ was removed to obtain aryloxyacetyl chloride as brown liquid which was used directly without further purification.

Synthesis of 2-aryloxyacetylamino-2-deoxy-D-glucoses: D-Glucosamine hydrochloride (1 g, 5 mmol) was added into the solution of MeONa (0.81 g, 15 mmol) in MeOH (40 mL) and the mixture was stirred for 1 h to obtain a clear solution. After the dropwise addition of aryloxyacetyl chloride (7.5 mmol), the solution was stirred for 4 h at room temperature. The solid was filtrated and washed with CH_3OH and water to give pure product.

2-Phenoxyacetylamino-2-deoxy-D-glucose (3a): Yield 65 %; white solid; m.p. 176-178 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 7.64(d, *J* = 8.25 Hz, 1H, NH), 7.31-7.28 (m, 2H, ArH), 7.00-6.95 (m, 3H, ArH), 6.60-6.58 (m, 1H, OH), 5.00-4.94 (m, 2H, 1-H, OH), 4.60-4.58 (m, 1H, OH), 4.51 (s, 2H, OCH_2CO), 4.44 (s, 1H, OH), 3.70-3.49 (m, 5H, 2-H, 3-H, 5-H, 6-H, 6'-H), 3.20-3.17(m, 1H, 4-H); ESI-MS: 336.1 [M + Na]$^+$; HR-ESI-MS for $C_{14}H_{19}NO_7Na$: Found (%): 336.1049; calcd. (%) 336.1054.

2-(*o*-Tolyloxyacetylamino)-2-deoxy-D-glucose (3b): Yield 87 %; white solid; m.p. 169-170 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 7.41(d, *J* = 8.72 Hz, 1H, NH), 7.17-7.13 (m, 2H, ArH), 6.90-6.86 (m, 2H, ArH), 6.63 (d, *J* = 4.20 Hz, 1H, OH), 5.00-4.96 (m, 2H, 1-H, OH), 4.86 (d, *J* = 5.64 Hz, 1H, OH), 4.52 (d, *J* = 6.39 Hz, 2H, OCH_2CO), 3.71-3.60 (m, 3H, 2-H, 3-H, 6-H), 3.54-3.47 (m, 2H, 5-H, 6'-H), 3.19-3.14 (m, 1H, 4-H), 2.12 (s, 3H, $ArCH_3$); ESI-MS: 328.1 [M + H]$^+$; HR-ESI-MS for $C_{15}H_{22}NO_7$: Found (%): 328.1398; calcd. (%) 328.1396.

2-(*m*-Tolyloxyacetylamino)-2-deoxy-D-glucose (3c): Yield 68 %; light yellow solid; m.p. 176-177 ºC;^{1}H NMR (500 MHz, DMSO) δ: 7.52 (d, *J* = 8.44 Hz, 1H, NH), 7.18 (t, *J* = 7.86 Hz, 1H, ArH), 6.83-6.75 (m, 3H, ArH), 6.58-6.56 (m, 1H, OH), 5.00-4.97 (m, 1H, 1-H), 6.84 (s, 2H, OCH_2CO), 4.41 (s, 1H, OH), 3.71-3.58 (m, 2H, 3-H, 2-H), 3.57-3.42 (m, 2H, 6-H, 5-H), 3.19-3.04 (m, 2H, 6'-H, 4-H), 2.28(s, 3H, $ArCH_3$); ESI-MS: 350.1 [M + Na]$^+$; HR-ESI-MS for $C_{15}H_{21}NO_7Na$: Found (%): 350.1218; calcd. (%) 350.1216.

2-(*p*-Tolyloxyacetylamino)-2-deoxy-D-glucose (3d): Yield 74 %; white solid; m.p. 178-179 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 7.50 (d, *J* = 8.55 Hz, 1H, NH), 7.09 (d, *J* = 8.53 Hz, 2H, ArH), 6.90-6.85 (m, 2H, ArH), 6.58-6.56 (m, 1H, OH), 4.98-4.94 (m, 2H, 1-H, OH), 4.45 (d, *J* = 3.18 Hz, 2H, OCH_2CO), 4.38 (s, 1H, OH), 3.69-3.39(m, 5H, 2-H, 3-H, 5-H, 6-H, 6'-H), 3.17-3.06 (m, 1H, 4-H), 2.23(s, 3H, $ArCH_3$); ESI-MS: 328.1 [M + H]$^+$; HR-ESI-MS for $C_{15}H_{22}NO_7$: Found (%): 328.1401; calcd. (%) 328.1396.

2-(2'-Chlorophenoxyacetylamino)-2-deoxy-D-glucose (3e): Yield 81 %; white solid; m.p. 160-161 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 7.56 (d, *J* = 8.73 Hz, 1H, NH), 7.45 (dd, *J* = 1.68, 8.03Hz, 1H, ArH), 7.28 (td, *J* = 1.70, 7.45Hz, 1H, ArH), 7.11(dd, *J* = 1.26, 8.42Hz, 1H, ArH), 7.00(td, *J* = 1.33, 7.60 Hz, 1H, ArH), 6.64 (dd, *J* = 1.12, 4.51 Hz, 1H, OH), 4.99-4.97 (m, 2H, 1-H, OH), 4.88 (d, *J* = 5.54 Hz, 1H, OH), 4.64 (d, *J* = 1.56 Hz, 2H, OCH_2CO), 4.47(t, *J* = 5.48 Hz, 1H, OH), 3.71-3.66 (m, 1H, 3-H), 3.64-3.60 (m, 2H, 2-H, 6-H), 3.54-3.47 (m, 2H, 5-H, 6'-H), 3.19-3.14 (m, 1H, 4-H); ESI-MS: 348.1 [M + H]$^+$; HR-ESI-MS for $C_{14}H_{19}NO_7Cl$: Found (%): 348.0853; calcd. (%) 348.0850.

2-(4'-Chlorophenoxyacetylamino)-2-deoxy-D-glucose (3f): Yield 66 %; white solid; m.p. 162-163 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 7.72 (d, *J* = 8.35 Hz, 1H, NH), 7.35-7.33 (m, 2H, ArH), 7.03-6.98 (m, 2H, ArH), 6.62-6.59 (m, 1H, OH), 5.03-4.82 (m, 3H, 1-H, OH), 4.53 (s, 2H, OCH_2CO), 4.45 (s, 1H, OH), 3.69-3.40 (m, 5H, 2-H, 3-H, 5-H, 6-H, 6'-H), 3.20-3.17 (m, 1H, 4-H); ESI-MS: 370.1 [M + Na]$^+$; HR-ESI-MS for $C_{14}H_{18}NO_7ClNa$: Found (%): 370.0697; calcd. (%) 370.0669.

2-(2',4'-Dichlorophenoxyacetylamino)-2-deoxy-D-glucose (3g): Yield 71 %; white solid; m.p. 166-167 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 7.63-7.60 (m, 2H, ArH), 7.36 (dd, *J* = 2.58, 8.88 Hz, 1H, ArH), 7.12(d, *J* = 8.95 Hz, 1H, NH), 6.63 (d, *J* = 3.82 Hz, 1H, OH), 4.97(d, *J* = 4.25 Hz, 2H, 1-H, OH), 4.86 (d, *J* = 5.57 Hz, 1H, OH), 4.66 (s, 2H, OCH_2CO), 4.46 (t, *J* = 5.97 Hz, 1H, OH), 3.70-3.61 (m, 3H, 3-H, 2-H, 6-H), 3.53-3.47 (m, 2H, 5-H, 6'-H), 3.18-3.13 (m, 1H, 4-H); ESI-MS: 382.0 [M + H]$^+$; HR-ESI-MS for $C_{14}H_{18}NO_7Cl_2$: Found (%): 382.0464; calcd. (%) 382.0460.

2-(4'-Nitrophenoxyacetylamino)-2-deoxy-D-glucose (3h): Yield 67 %; white solid; m.p. 126-128 ºC; ^{1}H NMR (500 MHz, DMSO) δ: 8.23-8.21 (m, 2H, ArH), 8.04 (d, *J* = 8.95 Hz, 1H, NH), 7.20-7.15 (m, 2H, ArH), 6.63 (d, *J* = 6.20 Hz, 1H, OH), 5.01-4.96(m, 2H, 1-H, OH), 4.72(s, 1H, OH), 4.64 (s, 2H, OCH_2CO), 4.58-4.55 (m, 1H, OH), 3.72-3.60 (m, 2H, 2-H, 3-H), 3.52-3.41(m, 2H, 5-H, 6-H), 3.16-3.01 (m, 2H, 6'-H, 4-H); ESI-MS: 381.1 $[M + Na]^+$; HR-ESI-MS for $C_{14}H_{18}N_2O_9Na$: Found (%): 381.0928; calcd. (%) 381.0910.

Biological assay

Cotyledon rootage test of cucumber: After soaking, the seeds were germinated in covered enamelware containing 0.7 % agar and cultured at 26 ºC in dark for 3 days. The cotyledons of similar magnitude were chosen to use. Tested compounds were dissolved in DMF and later dropped evenly on 6 cm diameter filter paper. After air-volatilization of solvent, the filter paper was placed in 6 cm diameter glass utensil with distilled water to give 10 mg/L compound solution. Ten pieces of cotyledons were added and cultured in dark. After 3 days, the rooting number of cotyledon was measured and compared with those treated with distilled water to estimate the activity. Two replicates were included in the evaluation.

Cotyledon expansion test of cucumber: After soaking, the seeds were germinated in covered enamelware containing 0.7 % agar and cultured at 26 ºC in dark for 3 days. The cotyledons of similar magnitude were chosen to use. Tested compounds were dissolved in DMF and later dropped evenly on 6 cm diameter filter paper. After air-volatilization of solvent, the filter paper was placed in 6 cm diameter glass utensil with distilled water to give 10 mg/L compound solution. Ten pieces of cotyledons were added and cultured in light at 26 ºC (300 Lux). After 3 days, the total weight of cotyledon was measured and compared with those treated with distilled water to estimate the activity. Two replicates were included in the evaluation.

Hypocotyls inhibition test of rape: After soaking, the seeds with similar magnitude were chosen to use. Tested compounds were dissolved in DMF and later dropped evenly on 6 cm diameter filter paper. After air-volatilization of solvent, the filter paper was placed in 6 cm diameter glass utensil with distilled water to give 10 mg/L compound solution. Ten seeds were added and cultured at 25 ºC in dark. The length of hypocotyls was measured after 3 days and compared with those treated with distilled water to estimate the activity. Two replicates were included in the evaluation.

Coleoptiles growth test of wheat: After soaking, the seeds were germinated in covered enamelware containing 0.7 % agar and cultured at 25 ºC in dark for 3 days. When the seedling grew to 2.5-3.0 cm tall, the first 3 mm of coleoptile top was rejected. Coleoptile (5 mm) was truncated and dunked in distilled water for 1 h to remove endogenous hormone and then it was chopped into 10 segments. Tested compounds were dissolved in DMF and later dropped evenly on 6 cm diameter filter paper. After air-volatilization of solvent, the filter paper was placed in 10 mL beaker with 0.01 mol/L phosphoric acid-citric acid buffer solution (pH 5) containing 2 % sucrose to give 10 mg/L compound solution. Ten coleoptile segments were added and cultured at 25 ºC in dark for 18-20 h. The length of coleoptiles was measured and compared with those treated without tested compounds to estimate the activity. Two replicates were included in the evaluation.

RESULTS AND DISCUSSION

Synthesis and characterization of 2-aryloxyacetylamino-2-deoxy-D-glucoses: Aryloxyacetic acids were prepared from substituted phenol and chloroacetic acid. To inhibit the side reaction, both substituted phenol and chloroacetic acid were converted to the corresponding salt. The EtOH-H_2O co-solvent was used to improve the solubility of substituted sodium phenolate. Aryloxyacetyl chlorides were obtained from aryloxy-acetic acids upon the treatment of $SOCl_2$ and used directly in the next procedure without further purification.

D-Glucosamine hydrochloride was dissolved in the solution of MeONa in MeOH and then aryloxyacetyl chloride was added. During the reaction, the product was precipitated and it was pure enough after simple filtration and washing.

All title compounds were characterized by ^{1}H NMR and mass spectra. In the ^{1}H NMR, not all the hydrogen of hydroxyl group can be found even with deuterated DMSO. To avoid the interference of hydrogen of hydroxyl group and help to identify other protons, ^{1}H NMR of **3g** in deuterated DMSO with or without D_2O were selected to be investigated and the results were shown in Fig. 1. It was indicated that the peaks located at 6.63, 4.86 and 4.46 ppm disappear after the addition of a few drops of D_2O. Though the peak which resonates at 4.97 ppm doesn't disappear, the electronic integration of its peak area reveals that the relative number of protons was decreased from 2 to 1, which suggested that one of two protons was hydrogen atom bonded to oxygen atom and exchanged by D_2O. Therefore, the hydrogens of hydroxyl group in these title compounds generally resonate at 4-5 ppm, whereas the hydrogen of C-1 hydroxyl group resonates at lower field 6-7 ppm probably due to the electron-withdrawing inductive effect of oxygen atom in carbohydrate ring.

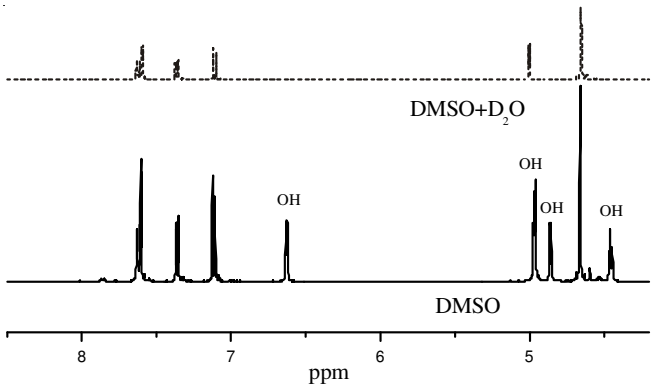


Fig. 1. ^{1}H NMR (8.5-4.2 ppm) of **3g** in DMSO with or without D_2O

Biological activity of 2-aryloxyacetylamino-2-deoxy-D-glucoses: Title compounds were tested for their plant growth-regulating activity in cotyledon rootage of cucumber, cotyledon expansion of cucumber, hypocotyls inhibition of rape and coleoptiles growth of wheat. The promotion or inhibition percents in these assays over control samples with no regulator were listed in Table-1. The conventional plant growth regulator 2,4-dichlorophenoxyacetic acid was chosen as standard sample

TABLE-1
PLANT GROWTH-REGULATING BIOACTIVITY OF TITLE COMPOUNDS *IN VITRO*

Compound	R	Bioactivity (%, 10 mg/L)			
		Cotyledon rootage of cucumber	Cotyledon expansion of cucumber	Hypocotyls inhibition of rape	Coleoptiles growth of wheat
3a	H	-0.6	-1.3	-6.8	0
3b	2-CH_3	41	-0.3	10.3	-4.8
3c	3-CH_3	28.2	-0.8	-1.9	-1.6
3d	4-CH_3	-19.8	-10.8	-2.4	-4.0
3e	2-Cl	8.9	-9.3	-3.1	-0.8
3f	4-Cl	-100	-2.1	57.8	4.8
3g	2,4-2Cl	-100	-3.7	81.7	-0.8
3h	4-NO_2	-3.8	-0.2	-0.5	-2.4
2,4-D	–	-100	-3.8	100	33.3

2,4-D = 2,4-Dichlorophenoxyacetic acid.

for comparison. In cotyledon rootage test of cucumber, compounds **3f** and **3g** bearing chlorine atom at *para* position of benzene ring show inhibiting activities and have the same inhibiting per cent 100 % at 10 mg/L as 2,4-dichlorophenoxyacetic acid. It is interesting to note the inhibiting activities seem to be strongly associated with the effects of substituent group on the benzene ring. Those without substituent or with methyl group on the benzene ring show very weak or none inhibiting activities. In addition, no inhibiting activity is observed in **3e** containing chlorine atom at *ortho* positon of benzene ring. Therefore, it seems that chlorine atom at *para* position of benzene ring play an important role to good bioactivities. The results of hypocotyls inhibition test of rape suggest that **3f** and **3g** have the obviously superior activities to other compounds, which supports this conclusion. Unfortunately, all title compounds exhibit no activities in cotyledon expansion test of cucumber and coleoptiles growth test of wheat.

Conclusion

A series of 2-aryloxyacetylamino-2-deoxy-D-glucoses were prepared in yields of 60-90 % by the N-acylation of D-glucosamine with aryloxyacetyl chloride. The plant growth-regulating activity of title compounds in cotyledon rootage of cucumber, cotyledon expansion of cucumber, hypocotyls inhibition of rape and coleoptiles growth of wheat were tested. Some compounds show good inhibiting activities against cotyledon rootage of cucumber. The good bioactivities are strongly associated with the existence of chlorine atom at *para* position on benzene ring.

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