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Facile Synthesis and Biological Evaluation of Novel *N*-Nitro Urea Derivatives Incorporating Amino Acid Ethyl Esters

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Abstract

A novel series of *N*-nitro urea derivatives **6a-w** containing various amino acid ethyl esters were conveniently synthesized via three steps including nitration, carbamic chlorination, and aminolysis reactions. The structures of all compounds prepared have been confirmed by ^{1}H NMR, IR spectroscopy and elemental analyses, and a part has been identified by ^{13}C NMR. The preliminary bioassay indicates that some of the target compounds possesses moderate herbicidal activity against *Echinochloa crusgalli* and *Amaranthus albus*. However, some of the title compounds presented high plant growth regulating activity against rice.

Keywords: Amino Acid Ethyl Ester; Synthesis · *N*-Nitro Urea Derivative; Herbicidal Activity

Introduction

Urea derivatives have played a pivotal role in pesticide chemistry due to their significant biological activities, such as herbicidal activities [1-2], antimicrobial [3], and antiviral [4-6]. There were many structural optimization studies on changing both sides of carbamide bridge's amines. It has been reported that substituted *N*-nitroanilines have a borad range of biological effects, including herbicidal properties [7-8], plant growth regulating activities and antifungal effects [9-10].

Additionally, in recent years, amino acid derivatives have received considerable attention because of their applications in pharmacological, food additives and agricultural field [11-12]. While the activities depended mainly on the effect of the amino acid groups [13], and it was also mentioned that attachment of an amino acid to a drug enhances its cellular uptake [14]. Furthermore, amino acid ester derivatives have wide market potential according to Chinese custom export products related statistics and have more and more extensive applications [15]. Our group are actively engaged in studying on the synthesis of *N*-nitrourea derivatives for a long time [16-17].

Aim of the Work

As a part of our extensive research, and in order to look for higher biological activity compounds, we herein introduce the amino acid ester group to the NH-CO-NH linkage with a nitro. We have linked the active group *N*-nitro aryl amine with plant growth regulating and herbicidal activities, amino acid ester group to urea substructure, retained a class of novel *N*-nitro amino acid ethyl ester urea derivatives.

Experimental

All reagents and chemicals were commercially available from Across, Aldrich, Shanghai or Beijing chemical company and all solvents and liquid reagents were dried by standard methods and distilled before use.

Melting points (uncorrected) were determined on an XRC-1 apparatus (Sichuan University Scientific Instruments Factory, Chengdu, China). MS were measured on a Finnigan Trace MS spectrometer. IR spectra were recorded on a PE-983 infrared spectrometer as KBr pellets with absorption in cm^{-1}. NMR were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker DPX 600 spectrometer and resonances are given in ppm (δ) relative to TMS. Elementary analyses were taken on a Vario EL III elementary analysis instrument.

General Synthetic Procedure for 2,4,6-trichlorophenyl-nitramine 2

The key intermediate 2,4,6-trichlorophenylnitramine **2** was obtained according to the reference [9]. Fuming nitric acid (1.01 mL, 24 mmol) was added dropwise to the stirred acetic anhydride (2.27 mL, 24 mmol) at 10 ~ 12°C. After the addition, the temperature of the reaction mixture was maintained between 10 and 12°C for 40 min. And a crude product of acetyl nitrate was obtained and was used directly for the next step. The obtained acetyl nitrate was added dropwise to a solution of substituted aniline (20 mmol) in dry acetic acid (20 mL) and acetic anhydride (2 mL) at 16 ~ 18°C. The reaction mixture was stirred for a further 45 ~ 90 min. The resulting purple solution was then poured into 60 mL of water (0 °C), and the resulting precipitate was filtered, washed with water (1L), and dissolved in aqueous 10% sodium carbonate, then acidified with ice-cold 2N hydrochloric acid to precipitate the substituted Phenylnitramine **2**, and recrystallized from cyclohexane. The Physico-chemical spectral data and melting point of compounds **2** was in agreement with the data reported in the literature [9].

Compounds **5** were prepared according to the reference [18]: $SOCl_2$ (1.8 ml, 26 mmol) was added dropwise to a solution of respective α-*L*-amino acids (20 mmol) **4** in EtOH (30 mL) at -8 ~ 10°C. After the addition, the mixture was refluxed for 5 ~ 7 h, and the solvent was evaporated under reduced pressure. The resulting residue was re dissolved in ethyl ether and the solvents were evaporated again to remove EtOH completely. The intermediate **5** were formed and were used in the next step without further purification.

General Procedure for the Preparation of the Target Compounds 6a-w

A solution of trisubstituted phenylnitramine **2** (10 mmol) in 50 mL of anhydrous toluene with 2 mL triethylamine was added dropwise to the solution of triphosgene (3.6 mmol) in dry toluene (50 mL) over a period of 2 h at 0 ~ 5°C. After a further 1 h of stirring at room temperature and 2 h at 50°C. The intermediate **3** was formed after quenching the unreacted phosgene with dry nitrogen, then a mixture of 10 mmol of triethylamine (2 or 3 molar equivalents) and a suitable α-amino acid ethyl ester hydrochloride **5** in CH_2Cl_2(or CH_3CN or $CHCl_3$ or DMF, details are in Table 1) (10 mL) was added. The mixture was stirred for 2 ~ 7 h at 30 ~ 80°C. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure, diluted with 80 ml ethyl acetate, washed with saturated salt solution (50 mL × 3) ， dried over anhydrous Na_2SO_4, filtered, the solvent was removed to yield the crude product **6a-w**, Crude products were purified by recrystallization with acetone/H_2O or on a silica gel column using petroleum/ethyl acetate (6:1 ~ 3:1) as a solvent system.

Ethyl 2-(3-Nitro-3-(2,4,6-Trichlorophenyl) Ureido) Acetate (6a)

White needle crystal. IR (KBr, cm^{-1}) ν: 3366(N-H), 1726(C=O ester), 1645(C=O urea), 1585(C=C Phenyl), 1257(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.67 (s, 2H, ArH), 6.71 (s, 1H, NH), 4.09 (q, *J* =10.5 Hz, 2H, OCH_2), 3.83 (d, *J* = 7.8 Hz, 2H, NCH_2), 1.19 (t, *J* = 9.9 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 14.0, 41.7, 60.2, 128.0, 131.2, 133.2, 134.7, 154.7, 170.4. Anal. calcd for $C_{11}H_{10}Cl_3N_3O_5$:C 35.65, H 2.72, N 11.34; found C 35. 73, H 2.85，N 11.27.

Ethyl 2-(3-Nitro-3-(2,4,6-Trichlorophenyl) Ureido)-3-Phenyl-propanoate (6b)

White solid. IR (KBr, cm^{-1}) ν: 3339(N-H), 2974, 2938(-CH_2), 1732(C=O ester), 1646(C=O urea), 1566 (C=C Phenyl), 1225(*N*-NO_2). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.36-7.10 (m, 7H, ArH), 6.39 (s, 1H, NH), 4.82-4.76 (m,1H, NCH), 4.17 (d, *J* = 10.2 Hz, 2H, OCH_2), 3.18-3.12 (m, 2H, $PhCH_2$), 1.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.1, 38.2, 54.1, 61.6, 127.0, 128.4, 128.5, 129.5, 131.4, 133.0, 134.6, 135.9, 153.9, 172.1; Anal. calcd for $C_{18}H_{16}Cl_3N_3O_5$:C 46.93, H 3.50, N 9.12; found C 46.98, H 3.57, N 9.03.

Ethyl 2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido)Propanoate (6c)

White crystal. IR (KBr, cm^{-1}) ν: 3327(N-H), 2997(-CH_3), 1731(C=O ester), 1643(C=O urea), 1572(C=C Phenyl), 1226(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.67 (s, 2H, ArH), 6.82 (d, *J* = 7.2 Hz, 1H, NH), 4.17-4.14 (m, 1H, NCH), 4.07 (q, *J* = 6.9 Hz, 2H, OCH_2), 1.27 (d, *J* = 7.2 Hz, 3H, CH_3), 1.16 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{12}H_{12}Cl_3N_3O_5$:C 37.47, H 3.14, N 10.93; found C 37.55, H 3.18, N 10.81.

Ethyl 3-Methyl-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido) Butanoate (6d)

White solid. IR (KBr, cm^{-1}) ν: 3345(N-H), 2964(-CH_3), 1735(C=O ester), 1646(C=O urea), 1577 (C=C Phenyl), 1202(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ7.69 (s, 2H, ArH), 6.79 (s, 1H, NH), 4.12-4.08 (m, 3H, OCH_2, NCH), 2.03 (q, *J* = 6.0 Hz, 1H, CH), 1.18 (t, *J* = 7.2 Hz, 3H, CH_3), 0.86 (t, *J* = 6.9 Hz, 6H, $2CH_3$). Anal. calcd for $C_{14}H_{16}Cl_3N_3O_5$:C 40.75, H 3.91, N 10.18; found C 40.81, H 3.97, N 10.13.

Ethyl 4-(Methylthio)-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido)Butanoate (6e)

White crystal. IR (KBr, cm^{-1}) ν: 3346(N-H), 1718(C=O ester), 1649(C=O urea), 1572 (C=C Phenyl), 1241(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.67 (s, 2H, ArH), 6.88 (d, *J* = 7.8 Hz, 1H, NH), 4.27 (q, *J* = 6.6 Hz, 1H, NCH), 4.08 (q, *J* =6.6 Hz, 2H, OCH_2), 2.02 (s, 3H, SCH_3), 1.96-1.80 (m, 4H, CH_2, SCH_2), 1.17 (t,

J = 6.9 Hz, 3H, CH_3). Anal. calcd for $C_{14}H_{16}Cl_3N_3O_5S$:C 37.81, H 3.63, N 9.45; found C 37.87, H 3.68, N 9.34.

Ethyl 1-(Nitro(2,4,6-Trichlorophenyl)Carbamoyl)Pyrrolidine-2-Carboxylate (6f)

Brown solid. IR (KBr, cm^{-1}) ν: 2978(-CH_2), 1754(C=O ester), 1648(C=O urea), 1513 (C=C Phenyl), 1395(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.68 (s, 2H, ArH), 4.26 (m, 1H, NCH), 4.03 (q, *J* =7.5 Hz, 2H, OCH_2), 3.54-3.46(m, 2H, NCH_2), 2.20-2.15 (m, 2H, CH_2), 1.95-1.92 (m, 2H, CH_2), 1.86-1.82 (m, 2H, CH_2), 1.14 (t, *J* = 6.9 Hz, 3H, CH_3). Anal. calcd for $C_{14}H_{14}Cl_3N_3O_5$:C 40.95, H 3.44, N 10.23; found C 41.02, H 3.48, N 10.17.

Diethyl 2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido)Succinate (6g)

White crystal. IR (KBr, cm^{-1}) ν: 3356(N-H), 1741, 1720(C=O ester), 1654(C=O urea), 1570 (C=C Phenyl), 1299(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.68 (s, 2H, ArH), 6.89 (d, *J* = 8.4 Hz, 1H, NH), 4.54 (q, *J* = 6.9 Hz, 1H, NCH), 4. 05-4.01 (m, 4H, 2OCH_2), 2.78 (d, *J* = 5.4 Hz, 2H, CH_2), 1.16 (q, *J* = 6.0 Hz, 6H, 2CH_3). Anal. calcd for $C_{15}H_{16}Cl_3N_3O_7$:C 39.45, H 3.53, N 9.20; found C 39.49, H 3.58, N 9.12.

Diethyl 2-(3-Nitro-3-(2,4,6-Trichlorophenyl) Ureido) Pentanedioate (6h)

White needle crystal. IR (KBr, cm^{-1}) ν: 3337(N-H), 1734, 1718(C=O ester), 1647(C=O urea), 1583 (C=C Phenyl), 1263(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.68 (s, 2H, ArH), 6.86 (d, *J* = 8.4 Hz, 1H, NH), 4.19 (q, *J* = 6.9 Hz, 1H, NCH), 4.05 (dd, *J* = 6.3 Hz, 2H, 2OCH_2), 2.36 (t, *J* = 7.2 Hz, 2H, CH_2), 1.98-1.79 (m, 2H, CH_2), 1.18~1.12 (m, 6H, 2CH_3). Anal. calcd for $C_{16}H_{18}Cl_3N_3O_7$:C 40.83, H 3.85, N 8.93; found C 40.88, H 3.89, N 8.87.

Ethyl 3-Methyl-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido) Pentanoate (6i)

White solid. IR (KBr, cm^{-1}) ν: 3345(N-H), 2966(-CH_3), 1735(C=O ester), 1646(C=O urea), 1577 (C=C Phenyl), 1260(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.69 (s, 2H, ArH), 6.81 (d, *J* = 7.8 Hz, 1H, NH), 4.19-4.16 (m, 1H, NCH), 4.07 (q, *J* = 6.6 Hz, 2H, OCH_2), 1.69-1.63 (m, 1H, CH), 1.52-1.47 (m, 2H, CH_2), 1.16 (t, *J* = 6.9 Hz, 3H, CH_3), 0.92~0.80 (m, 6H, 2CH_3). Anal. calcd for $C_{15}H_{18}Cl_3N_3O_5$:C 42.22, H 4.25, N 9.85; found C 42.28, H 4.31, N 9.81.

Ethyl 4-Methyl-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido) Pentanoate (6j)

White solid. IR (KBr, cm^{-1}) ν: 3314(N-H), 2952(-CH_3), 1728(C=O ester), 1641(C=O urea), 1562 (C=C Phenyl), 1224 (*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.66 (s, 2H, ArH), 6.78 (d, *J* = 7.8 Hz, 1H, NH), 4.15-4.07 (m, 3H, OCH_2, NCH), 1.69-1.63 (m, 1H, CH), 1.41-1.36 (m, 2H, CH_2), 1.17 (t, *J* = 8.4 Hz, 3H, CH_3), 0.86 (d, *J* = 7.2 Hz, 6H, 2CH_3). Anal. calcd for $C_{15}H_{18}Cl_3N_3O_5$:C 42.22, H 4.25, N 9.85; found C 42.27, H 4.31, N 9.80.

Ethyl 3-(1H-Indol-3-Yl)-2-(3-Nitro-3-(2,4,6-Trichlorophenyl) Ureido)Propanoate (6k)

White solid. IR (KBr, cm^{-1}) ν: 3363(N-H), 2921(-CH_2), 1725(C=O ester), 1647(C=O urea), 1560, 1544(C=C Phenyl), 1202 (*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.93 (s, 1H, indole NH), 7.67 (s, 2H, ArH), 7.47 (d, *J* = 8.4 Hz, 1H, ArH), 7.32 (d, *J* = 8.4 Hz, 1H, ArH), 7.13 (s, 1H, indole NCH), 7.04 (t, *J* = 7.5 Hz, 1H, ArH), 6.95 (t, *J* = 7.2 Hz, 1H, ArH), 6.69 (d, *J* = 7.8 Hz, 1H, NH), 4.47 (d, *J* = 6.6 Hz, 1H, NCH), 4.01 (q, *J* = 3.6 Hz, 2H, OCH_2), 3.12 (t, *J* = 3.0 Hz, 2H, CH_2), 1.09 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{20}H_{17}Cl_3N_4O_5$:C 48.07, H 3.43, N 11.21; found C 48.12, H 3.49, N 11.14.

Ethyl 3-(4-Hydroxyphenyl)-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido)Propanoate (6l)

White solid. IR (KBr, cm^{-1}) ν: 3355(N-H), 2977(-CH_2), 1726(C=O ester), 1654(C=O urea), 1570, 1513(C=C Phenyl), 1231 (*N*-NO_2). ^{1}H NMR (DMSO-d_6,600 MHz) δ 9.27 (s, 1H, OH), 7.70 (s, 2H, ArH), 6.99 (d, *J* = 8.4 Hz, 2H, ArH), 6.68 (t, *J* = 8.4 Hz, 3H, NH, ArH), 4.38 (q, *J* = 6.8 Hz, 1H, NCH), 4.08 (d, *J* = 7.2 Hz, 2H, OCH_2), 2.90 (q, *J* = 5.3 Hz, 2H, $PhCH_2$),1.16 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{18}H_{16}Cl_3N_3O_6$:C 45.35, H 3.38, N 8.81; found C 45.39, H 3.45, N 8.72.

Ethyl 3-Hydroxy-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido) Butanoate (6m)

White solid. IR (KBr, cm^{-1}) ν: 3475(-OH), 3328(N-H), 2984(-CH_3), 1718(C=O ester), 1653(C=O urea), 1576(C=C Phenyl), 1281 (*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.66 (s, 2H, ArH), 6.61 (d, *J* = 6.8 Hz, 1H, NH), 5.10 (d, *J* = 4.8 Hz, 1H, OH), 4.13-4.05 (m, 4H, OCH_2, CH, NCH), 1.17 (t, *J* = 6.9 Hz, 3H, CH_3), 1.09 (d, *J* = 6.0 Hz, 3H, CH_3). Anal. calcd for $C_{13}H_{14}Cl_3N_3O_6$: C 37.66, H 3.40, N 10.13; found C 37.74, H 3.45, N 10.09.

Ethyl 4-Amino-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido)-4-Oxobutanoate (6n)

White solid. IR (KBr, cm^{-1}) ν: 3356(N-H), 1741(C=O amide), 1735(C=O ester), 1648(C=O urea), 1560(C=C Phenyl), 1299(*N*-NO_2). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.68 (s, 2H, ArH), 6.90 (s, 1H, NH), 4.59-4.50 (m, 1H, NCH), 4.10-4.04 (m, 4H, OCH_2, NH_2), 2.78 (s, 2H, CH_2), 1.18 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{13}H_{13}Cl_3N_4O_6$:C 36.51, H 3.06, N 13.10; found C 36.57, H 3.03, N 13.02.

Ethyl 5-Amino-2-(3-Nitro-3-(2,4,6-Trichlorophenyl)Ureido)-5-Oxopentanoate (6o)

White solid. IR (KBr, cm^{-1}) ν: 3345(N-H), 1735(C=O ester), 1718(C=O amide), 1647(C=O urea), 1577(C=C Phenyl), 1264 (*N*-NO_2). ^1H NMR (DMSO-d_6, 600 MHz) δ 7.68 (s, 2H, ArH), 6.85 (d, *J* = 7.8 Hz, 1H, NH), 4.19 (d, *J* = 5.4 Hz, 1H, NCH), 4.09-4.02 (m, 4H, NH_2, OCH_2), 2.36 (d, *J* = 7.2 Hz, 2H, CH_2), 2.01-1.79 (m, 2H, CH_2), 1.18 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{14}H_{15}Cl_3N_4O_6$:C 38.07, H 3.42, N 12.69; found C 38.11, H 3.47, N 12.64.

Ethyl 2-(3-(2,6-Dibromo-4-Fluorophenyl)-3-Nitroureido)Acetate (6p)

White solid. IR (KBr, cm^{-1}) ν: 3355 (N-H), 2979 (CH_2), 1724 (C=O ester), 1637 (C=O urea), 1515 (C=C Phenyl), 1210 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.72 (d, *J* = 7.8 Hz, 2H, ArH), 6.66 (s, 1H, NH), 4.11 (q, *J* = 7.1 Hz, 2H, OCH_2), 3.83 (d, *J* = 4.1 Hz, 2H, NCH_2), 1.20 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{11}H_{10}Br_2FN_3O_5$: C 29.82, H 2.28, N 9.48; found C 29.67, H 2.35，N 9.30.

Ethyl 2-(3-Nitro-3-(2,6-Dibromo-4-Fluorophenyl)Ureido)-3-Phenylpropanoate (6q)

White solid. IR (KBr, cm^{-1}) ν: 3347 (N-H), 2976, 2936 (CH_2), 1734 (C=O ester),1645 (C=O urea), 1551 (C=C Phenyl), 1223 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.70 (d, *J* = 8.4 Hz, 2H, ArH), 7.33-7.29 (m, 2H, ArH), 7.25-7.21 (m, 3H, ArH), 6.69 (s, 1H, NH), 4.47 (d, *J* = 6.6 Hz, 1H, NCH), 4.08 (t, *J* = 7.2 Hz, 2H, OCH_2), 3.02 (q, *J* = 5.5 Hz, 2H, $PhCH_2$), 1.16 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{18}H_{16}Br_2FN_3O_5$:C 40.55, H 3.02, N 7.88; found C 40.63, H 3.11, N 7.76.

Ethyl 2-(3-(2,6-Dibromo-4-Fluorophenyl)-3-Nitroureido)Propanoate (6r)

White solid. IR (KBr) ν: 3333 (N-H), 2992 (CH_3), 1727 (C=O ester), 1641 (C=O urea),1570 (C=C Phenyl), 1223 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.71 (d, *J* = 8.4 Hz, 2H, ArH), 6.76 (s, 1H, NH), 4.19 (t, *J* = 7.5 Hz, 1H, NCH), 4.13-4.08 (m, 2H, OCH_2), 1.30 (d, *J* = 7.2 Hz, 3H, CH_3), 1.20 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{12}H_{12}Br_2FN_3O_5$:C 31.53, H 2.65, N 9.19; found C 31.45, H 2.55, N 9.31.

Ethyl 2-(3-(2,6-Dibromo-4-Fluorophenyl)-3 Nitroureido)-4-(Methylthio)Butanoate (6s)

White solid. IR (KBr, cm^{-1}) ν: 3328 (N-H), 1718 (C=O ester), 1647 (C=O urea), 1556 (C=C Phenyl), 1206 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.72 (d, *J* = 8.4 Hz, 2H, ArH), 6.83 (s, 1H, NH), 4.20 (q, *J* = 4.1 Hz, 1H, NCH), 4.12 (q, *J* =6.0 Hz, 2H, OCH_2), 2.06 (s, 3H, SCH_3), 1.97 (t, *J* = 6.0 Hz, 2H, SCH_2), 1.92-1.89 (m, 2H, CH_2), 1.21 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{14}H_{16}Br_2FN_3O_5S$:C 32.51, H 3.12, N 8.13; found C 32.70, H 3.19, N 8.02.

Diethyl 2-(3-(2,6-Dibromo-4-Fluorophenyl)-3-Nitroureido) Succinate (6t)

White solid. IR (KBr, cm^{-1}) ν: 3356 (N-H), 2980 (CH_2), 1735, 1718 (C=O ester), 1647 (C=O urea),1572 (C=C Phenyl), 1293 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.70 (d, *J* = 7.8 Hz, 2H, ArH), 6.83 (s, 1H, NH), 4.56-4.52 (m, 1H, NCH), 4.09-4.05 (m, 4H, $2OCH_2$), 2.78 (d, *J* = 6.0 Hz, 2H, CH_2), 1.18-1.15 (m, 6H, $2CH_3$). Anal. calcd for $C_{15}H_{16}Br_2FN_3O_7$:C 34.05, H 3.05, N 7.94; found C 34.17, H 3.11, N 7.80.

Ethyl 2-(3-(2,6-Dibromo-4-Fluorophenyl)-3-Nitroureido)-3-(4-Hydroxyphenyl)Propanoate (6u)

White solid. IR (KBr, cm^{-1}) ν: 3355 (N-H), 2979 (CH_2), 1724 (C=O ester), 1654 (C=O urea), 1589,1513 (C=C Phenyl), 1210 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 9.23 (s, 1H, OH), 7.69 (d, *J* = 7.8 Hz, 2H, ArH), 6.97 (d, *J* = 8.4 Hz, 2H, ArH), 6.66 (d, *J* = 8.4 Hz, 2H, ArH), 6.58 (s, 1H, NH), 4.39-4.34 (m, 1H, NCH), 4.05 (d, *J* = 7.2 Hz, 2H, OCH_2), 2.89-2.86 (m, 2H, $PhCH_2$), 1.13 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{18}H_{16}Br_2FN_3O_6$:C 39.37, H 2.94, N 7.65; found C 39.48, H 3.01, N 7.42.

Ethyl 2-(3-(2,6-Dibromo-4-Fluorophenyl)-3-Nitroureido)-3-Hydroxybutanoate *(6*v)

White solid. IR (KBr, cm^{-1}) ν: 3466 (OH), 3332 (N-H), 2984 (CH_3), 1715 (C=O ester), 1655 (C=O urea), 1576 (C=C Phenyl), 1286 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.72 (d, *J* = 7.8 Hz, 2H, ArH), 6.58 (s, 1H, NH), 5.12 (s, 1H, OH), 4.18-4.09 (m, 4H, OCH_2, CH, NCH), 1.21 (t, *J* = 6.9 Hz, 3H, CH_3), 1.13 (d, *J* = 6.6 Hz, 3H, CH_3). Anal. calcd for $C_{13}H_{14}Br_2FN_3O_6$:C 32.06, H 2.90, N 8.63; found C 32.18, H 2.97, N 8.42.

Ethyl4-Amino-2-(3-(2,6-Dibromo-4-Fluorophenyl)-3-Nitroureido)-4-Oxobutanoate (6w)

White solid. IR (KBr, cm^{-1}) ν: 3355 (N-H), 1735 (C=O ester), 1720 (C=O amide), 1640 (C=O urea), 1547 (C=C Phenyl), 1192 (*N*-NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.71 (d, *J* = 8.4 Hz, 2H, ArH), 6.84 (s, 1H, NH), 4.56-4.52 (m, 1H, NCH), 4.11-4.05 (m, 4H, OCH_2, NH_2), 2.77 (d, *J* = 6.0 Hz, 2H, CH_2), 1.17 (t, *J* = 7.2 Hz, 3H, CH_3). Anal. calcd for $C_{13}H_{13}Br_2FN_4O_6$:C 31.22, H 2.62, N 11.20.; found C 31.13, H 2.45, N 11.32.

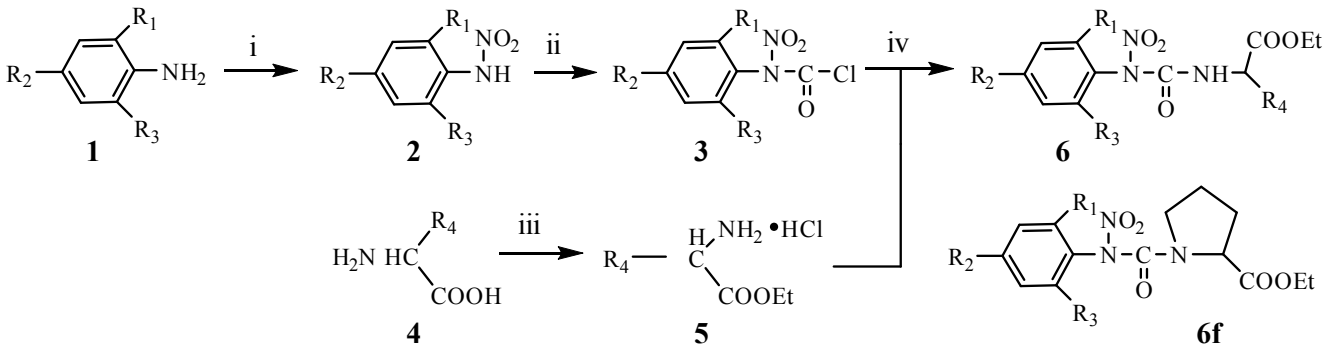
Results and Discussion

Synthesis of Novel *N*-Nitro Urea Derivatives Incorporating Amino Acid Ethyl Esters 6a-w

The reported substituted Phenylnitramines which exhibit varieties of bioactivities and stability, are often trisubstituted (ortho- and para-position) phenylnitramines. The preferred substituents

commonly are halogen, with the proviso that no more than two of the groups may represent iodine [9]. We preciously synthesized a series of unsymmetrical aryl ureas which contain 2,4,6-trisubstituted phenylnitramine. In continuation of our effort to develop *N*-nitro urea derivatives and in favor of forceful contrast, the synthetic route of title compounds is shown in Scheme 1. To synthesize urea derivatives, there are several known methods. Our choice is based on BTC (triphosgene), which was reported to possess advantages of easy quantitative and controlled, safe, mild reaction conditions, provide a convenient and safe 'one-pot' procedure for the synthesis of *N, N'*-unsymmetrically substituted urea [19-21].

The trisubstituted phenylnitramines **2** are prepared from **1** with acetyl nitrate by react with nitric acid in the presence of acetic anhydride. Then the key intermediates **2** were treated with Triphosgene to obtain various *N*-nitro-2,4,6-trisubstituted phenyl carbamic chloride **3**, which were directly reacted with various *L*-amino acid ethyl ester in different solvent to afford target compounds **6a-w** in satisfied yields (55-80%).



Scheme 1: General synthetic route for amino-acid containing derivatives **6a-w**. Reagents and conditions: (i) CH_3COOH, Ac_2O, nitric acid, 10 ~ 12˚C, 40 min, then 16 ~ 18˚C, 45 ~ 90 min; (ii) Triphosgene, toluene, NEt_3, 0 ~ 5˚C, 2h, then 25 ~ 50˚C, 3h; (iii) $SOCl_2$, EtOH, reflux, 5-7h; (iv) Substituted amino acid esters, NEt_3, CH_2Cl_2(or CH_3CN or $CHCl_3$ or DMF), 30 ~ 80˚C, 2-7h.

Amino acid is hardly dissolved in non-polarity solvents. However, the esters have preferable dissolution in organic solvents which are in favor of the synthesis. Amino acid ethyl ester hydrochloride was prepared from the esterification of corresponding amino acid (Gly; L-Phe; L-Ala; L-Val; L-Met; L-Pro; L-Asp; L-Glu; L-Ile; L-Leu; L-Trp; L-Tyr; L-Thr; L-Asn; L-Gln.) and dry ethanol in the presence of excess $SOCl_2$ [20]. Most of the esters were obtained as crystalline solids in excellent yield. We prepared amino acid ethyl esters as the starting materials for further reaction, which used in different solvents for respective amino acid ester due to the different R group (Table 1). The structures of these compounds were confirmed by spectral techniques. For example, the ^{1}H NMR spectrum data of **6g** showed the signals of the two CH_3 at 1.164 ppm as quaternary absorption and the signals of the two CH_2 at 4.063 ppm as mutiplets absorption due to the CH_3. The other signals appeared at δ 2.78 (d, J = 5.4 Hz, 2H, CH_2), 4.54 (q, 1H, J = 5.7 Hz, NCH), 6.89 (d, J = 8.4 Hz, 1H, NH), 7.68 (s, 2H, ArH).

Compd.	$R_{1,2,3}$	R_4	Condition	Yield (%)[a]	Mp (˚C)
6a	2,4,6-tri-Cl	-H	CH_2Cl_2, 30°C, 4h	80	158-159
6b	2,4,6-tri-Cl	$-CH_2Ph$	$CHCl_3$, 50°C, 4h	72	154-156
6c	2,4,6-tri-Cl	0	$CHCl_3$, 65°C, 4h	77	156-158
6d	2,4,6-tri-Cl	$-CH(CH_3)_2$	$CHCl_3$, 65°C, 4h	52	99-100
6e	2,4,6-tri-Cl	$-CH_2CH_2SCH_3$	$CHCl_3$, 60°C, 4h	75	136-138
6f	2,4,6-tri-Cl	$-(CH_2)_3-$	CH_3CN, 80°C, 6h	62	141-142
6g	2,4,6-tri-Cl	$-CH_2COOEt$	CH_3CN, 80°C, 6h	73	122-124
6h	2,4,6-tri-Cl	$-CH_2CH_2COOEt$	$CHCl_3$, 65°C, 5h	64	119-120
6i	2,4,6-tri-Cl	$-CH(CH_3)CH_2CH_3$	CH_3CN, 80°C, 5h	65	81-83
6j	2,4,6-tri-Cl	$-CH_2CH(CH_3)CH_3$	$CHCl_3$, 50°C, 5h	76	67-68

6k	2,4,6-tri-Cl	-indole-3-yl-methylene	DMF, 80°C, 4h	63	166-167
6l	2,4,6-tri-Cl	$-CH_2C_6H_4$-OH(p)	DMF, 60°C, 5h	72	159-160
6m	2,4,6-tri-Cl	$-CH(OH)CH_3$	$CHCl_3$, 65°C, 2h	78	179-180
6n	2,4,6-tri-Cl	$-CH_2CONH_2$	$CHCl_3$, 65°C, 5h	67	166-168
6o	2,4,6-tri-Cl	$-CH_2CH_2CONH_2$	$CHCl_3$, 65°C, 5h	63	156-157
6p	2,6-di-Br, 4-F	-H	CH_2Cl_2, 40°C, 4h	56	170-172
6q	2,6-di-Br, 4-F	$-CH_2Ph$	$CHCl_3$, 50°C, 4h	61	162-163
6r	2,6-di-Br, 4-F	0	$CHCl_3$, 65°C, 4h	61	158-159
6s	2,6-di-Br, 4-F	$-CH_2CH_2SCH_3$	$CHCl_3$, 65°C, 4h	67	138-139
6t	2,6-di-Br, 4-F	$-CH_2COOEt$	CH_3CN, 65°C, 4h	68	124-126
6u	2,6-di-Br, 4-F	$-CH_2C_6H_4$-OH(p)	CH_2Cl_2, 50°C, 6h	60	140-142
6v	2,6-di-Br, 4-F	$-CH(OH)CH_3$	$CHCl_3$, 65°C, 7h	55	160-162
6w	2,6-di-Br, 4-F	$-CH_2CONH_2$	CH_3CN, 80°C, 5h	63	122-124
[a] isolated yield based on 2,4,6-trichlorophenylnitramine **2**.					

Table 1: Preparation of compounds **6a-w** derivatives.

Biological Activity Evaluation

The herbicidal activities of the title compound **6a-w** against *Echinochloa crusgalli* and *Amaranthus albus* have been investigated at the dosages of 50 mg/L compared to distilled water and the commercially available herbicide Diuron according to the method that reported in our previous literatures [20-21]. The preliminary results of bioassay (Table 2) showed that most of the target compounds possessed higher herbicidal activity against hypocotyl than that of root to *Echinochloa crusgalli*. Respectively, compound **6q** exhibited the highest herbicidal activity against *Amaranthus albus*, which is close to the commercial herbicide Diuron. Compounds **6c**, **6p**, **6s**, and **6t** exhibited higher herbicidal activity against hypocotyls to *Echinochloa crusgalli* than Diuron. These data show that the presence of the electron-withdrawing group fluorine may increase their herbicidal activity.

The plant growth regulatory activity of title compounds against rice was also evaluated at the concentration of 10 mg/L, and the results are shown in Table 2. From Table 2, we can find that the compounds **6a**, **6c**, **6d**, **6k**, **6l**, **6r**, and **6s** exhibited higher plant growth regulating activity than the others.

Compd.	$R_{1,2,3}$	R_4	Relative inhibition at 50 mg/L (root % /hypocotyls %)	Active grade[a] of formation-promoting against rice root
			E. crusgalli *A. albus*	
6a	2,4,6-tri-Cl	-H	44.3/31.2 39.7/28.1	A
6b	2,4,6-tri-Cl	$-CH_2Ph$	25.4/24.5 16.3/11.4	B
6c	2,4,6-tri-Cl	$-CH_3$	14.7/53.3 23.9/33.2	A
6d	2,4,6-tri-Cl	$-CH(CH_3)_2$	17.1/32.5 41.9/23.7	A
6e	2,4,6-tri-Cl	$-CH_2CH_2SCH_3$	28.9/36.8 24.4/17.7	B
6f	2,4,6-tri-Cl	$-(CH_2)_3-$	26.7/40.2 26.7/20.2	C
6g	2,4,6-tri-Cl	$-CH_2COOEt$	23.7/31.2 31.5/10.9	B
6h	2,4,6-tri-Cl	$-CH_2CH_2COOEt$	26.6/38.2 11.0/22.0	C
6i	2,4,6-tri-Cl	$-CH(CH_3)\ CH_2CH_3$	37.0/34.1 34.1/34.9	B

6j	2,4,6-tri-Cl	-$CH_2CH(CH_3)$ CH_3	30.8/34.9 24.0/22.6	C
6k	2,4,6-tri-Cl	-indole-3-yl-methylene	23.1/30.0 38.2/15.3	A
6l	2,4,6-tri-Cl	-$CH_2C_6H_4$-OH(p)	19.8/39.9 30.2/18.8	A
6m	2,4,6-tri-Cl	-$CH(OH)CH_3$	24.3/43.7 20.5/22.1	C
6n	2,4,6-tri-Cl	-CH_2CONH_2	35.0/33.8 26.6/23.5	A
6o	2,4,6-tri-Cl	-$CH_2CH_2CONH_2$	36.1/38.9 22.3/12.3	A
6p	2,6-di-Br, 4-F	-H	25.7/45.9 23.4/22.1	B
6q	2,6-di-Br, 4-F	-CH_2Ph	26.9/31.0 55.9/66.5	D
6r	2,6-di-Br, 4-F	-CH_3	27.1/36.5 20.5/22.4	C
6s	2,6-di-Br, 4-F	-$CH_2CH_2SCH_3$	22.5/43.4 25.1/38.5	A
6t	2,6-di-Br, 4-F	-CH_2COOEt	24.5/42.8 33.0/25.3	A
6u	2,6-di-Br, 4-F	-$CH_2C_6H_4$-OH(p)	25.5/34.0 15.5/21.9	C
6v	2,6-di-Br, 4-F	-$CH(OH)CH_3$	29.8/31.0 29.9/31.2	D
6w	2,6-di-Br, 4-F	-CH_2CONH_2	26.0/32.1 24.3/22.2	C
Diuron	-	-	90.8/40.1 55.4/25.8	-
[a] Active grade: A > 70%, B > 50%, C > 30%, D > 30% at the concentration of 10 mg/L.				

Table 2: Herbicidal and plant growth regulating activities of targeted compounds **6a-w.**

Conclusion

Twenty-three novel *N*-nitro amino acid ethyl ester urea compounds were synthesized. The target compounds were confirmed by IR, ^{1}H NMR and elemental analyses. The preliminary result of biological activity test showed that the target compounds possesses moderate inhibitory activities on *E. crusgalli* and *A. albus* at test concentration.

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