Synthesis of Nitro- and Aminoisoflavones and their Effects on the Proliferation of Endothelial Cells

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Summary: The new nitroisofalvones **2a-k** were synthesized *via* nitration of the corresponding isoflavones **1a-f** using NH_4NO_3/TFAA in acetonitrile. The aminoisoflavones **3a-g**, also new, were produced by selective reduction of the corresponding nitroisoflavones with $SnCl_2.2H_2O$. The nitro- and aminoisoflavones, tested *in vitro* concerning their effects on the proliferation of endothelial cells showed modest activities. All the new products are fully characterized and the nitro compounds analyzed thoroughly by NMR to allow complete assignment of the proton and carbon resonance and to establish the orientation of nitration.

Keywords: Nitroisoflavone; Aminoisoflavone; Isoflavones; Endothelial Cells.

Introduction

Isoflavones are subclass of flavonoids found in many plants especially in Moraceae, Iridaceae and Leguminosae family members [1-3]. Around 1000 different isoflavones, mainly *O*-glycosides have been isolated and identified from plants [1-3]. Isoflavones have attracted attention of scientists during the past three decades due to their wide range of biological properties. It has been acknowledged that intake of diet containing isoflavones is associated with a reduction in the risk of hormone-dependent cancers [4,5], osteoporosis [6,7], and coronary heart disease [8,9]. Additionally, isoflavones have been found to display high antioxidant properties [10]. Structurally, these studies have been limited to oxygen-containing derivatives having hydroxyl, alkoxy, and carboxy substituents. As for nitrogen derivatives, very few *N*-containing isoflavonoids occur in nature. These include two 4-aminoisoflavones, piscerythramine and isopiscerythramine, and an oxazole derivative, piscerythoxazole, found in *Piscidia erytherina* roots bark [11]. Furthermore, 7,4'-dihydroxy-3'-nitroisoflavone has been reported from the culture broth of genetically engineered Streptomyces K_2 species [12]. Certain synthetic 4'-nitro-and 4'-amino-substituted isoflavones exhibited a range of beneficial biological effects including stimulative action on the central nervous system [13], anti-hypoxic [14] and weight enhancement activities [15]. On the other hand, certain amino- and nitroflavones appear to possess a high antitumor activity [16,17].

We now present a systematic study of nitration of the main soybeans isoflavones daidzein **1a,** genistein **1b**, formononetin **1c**, biochanin A **1d**, and some related compounds, with various nitrating agents to afford the nitrated products **2a-k.** The results established the pattern of substitution and provided intermediates for the synthesis of specific aminoisoflavones. For the latter task, a mild, selective, inexpensive and almost quantitative method using tin (II) chloride in ethanol was employed [18]. The synthetic products were analyzed thoroughly by NMR to allow complete assignments of the proton and carbon resonances, and to establish the orientation of nitration.

Finally, the nitro- and aminoisoflavones analogs were tested concerning their effects on the *in vitro* proliferation of endothelial cells. The latter plays a key role in angiogenesis and inhibition of their proliferation is a suitable *in-vitro* screening assay regarding antiangiogenic activity.

Experimental

General method

NMR spectra were recorded on a Varian Gemini 2000 spectrometer or an Inova 300 spectrometer, proton and carbon assignments being evaluated from gradient HMQC, gradient HMBC and gradient HMBC optimized for long range coupling, *J* values are given in Hz. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Low-resolution mass spectra (LRMS) and high-resolution mass spectra (HRMS) were obtained with JEOL, JMS SX102 mass spectrometer operating at 70 eV. TLC was conducted on Merck silica gel 60 F_{254} plates and Merck silica gel 60 (0,040-0.063 mm), and

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230-400 mesh was used for flash chromatography. The starting materials daidzein **1a**, genistein **1b**, formononctine **1c**, biohanin A **1d**, 7-hydroxyisoflavone **1e** and 5,7-dihydroxy-4'-nitroisoflavone **1f** were synthesized according to published procedures.

Calculations were performed with MOPAC 6.0 within the Cerius 2 program. Structures were optimized with AMI. The ESP contour plots were generated in the plane of the aromatic ring.

Cell Culture

Bovine brain capillary endothelial (BBCE) cells were maintained in Dulbecco's modified Eagle medium (DMEM) with low glucose concentration (1000 mg/L, 10% newborn calf serum, glutamine (2 mmol/L), and antibiotics. Cultures received Fibroblast Growth Factor-Basic (bFGF) (2.5 ng/ml) every other day until confluent.

Cell Proliferation assay

Cells were adjusted to a density of 5×10^3 per mL (or 2×10^4 per mL in the case of Human Umbilical Vein Endothelial Cells (HUVECs) in their respective media and seeded in 1 mL aliquots per well in to 12-well cluster dishes. After 16 hours, wells were given either 5 µL aliquots of solvent only, or solvent containing various concentrations of the compounds to be tested, or bFGF (2.5 ng/mL), and these processing were repeated every other day. Cells were counted at the times indicated with a Coulter particle counter. Values of cell densities represent the means of duplicate determinations which varied by less than 10% of the mean.

1-(2,4,6-Trihydroxyphenyl)-2-(2-methyl-4-nitrophenyl)ethanone

Phloroglucinol (1 g, 7.93 mmol), 2-methyl-4-nitophenylacetonitrile (0.9 g, 5.11 mmol), and $ZnCl_2$ (0.2g, 1.46 mmol) in dry ether (40 mL) were cooled in an ice water bath and saturated with dry HCl for 4 hrs. The reaction mixture was refrigerated for 12 hrs, saturated again with HCl gas for 2 hrs, and refrigerated for a further 12 hrs. The ether layer was decanted and the residue washed twice with ether (20 mL x 2). The remaining orange oil was refluxed in 2% aqueous HCL (30 mL) for 2 hrs and cooled to rt. The precipitate was recrystallized from aqueous ethanol to give the nitroethanone (2,4,6-trihydroxy-2'-methyl-4'-nitrodeoxybenzoin) as yellow solid (1.2 g, 77%): m.p. 237-239 ºC dec. ^{1}H NMR (200 MHz, acetone-d_6) δ 2.38 (s, 3H, CH_3) 4.61 (s, 2H, CH_2), 5.99 (s, 2H, H-3, H-5), 7.45 (d, *J* = 4.8 Hz, 1H, H-6'), 8.02 (d, *J* = 8.4 Hz, 1H, H-5'), 8.08 (s, 1H, H-3'); ^{13}C NMR (50 MHz, acetone-d_6) δ 19.8 (CH_3), 48.7 (CH_2), 96.0 (C-3, C-5), 105.7 (C-1), 121.4 (C-5'), 125.0 (C-3'), 132.2 (C-6'), 121.4 (C-5'),140.4 (C-2'), 144.3 (C-1'), 147.8 (C-4'), 165.5 (C-2, C-6), 165.9 (C-4), 201.9 (CO); *m/z* 303 (8%) 153 (100); HRMS: calcd for ($C_{15}H_{13}NO_6$) 303.0743, found 303.0749.

5,7-Dihydroxy-2'-methyl-4'-nitroisoflavone 1g

$BF_3.Et_2O$ (7.6 mL, 0.06 mol) was added to a solution of 2,4,6-trihydroxy-2'-methyl-4'-nitrodeoxybenzoin (3.3 g, 0.01 mol) in DMF (20 mL) under Ar. After 15 min a solution of methanesulfonyl chloride (3.1 mL, 0.04 mol) in DMF (5 mL) was added slowly. After heating at 70 ºC for 3 hrs the reaction mixture was cooled to ambient temperature and poured into ice-cold aqueous sodium acetate (12 g/100 mL). The solid precipitate was filtered off and recrystallized from aqueous ethanol to give **1g** as yellow crystals (2.66 g, 85%): m.p. 233-235 ºC, ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.34 (s, 3H, CH_3) 6.28 (d, *J* = 2.1 Hz, 1H, H-6), 6.46 (d, *J* = 2.1 Hz, 1H, H-8), 7.54 (d, *J* = 8.4 Hz, 1H, H-6'), 8.11 (dd, *J* = 8.4 Hz, 1H, H-5'), 8.20 (d, *J* = 2.4 Hz, 1H, H-3'), 8.36 (s, 1H, H-2), 10.98 (s, 1H, 7-OH), 12.59 (s, 1H, 5-OH); ^{13}C NMR (DMSO-d_6) δ 19.6 (CH_3), 94.0 (C-8), 99.3 (C-6), 104.3 (C-4a), 120.6 (C-5'), 121.7 (C-3), 124.2 (C-3'), 132.2 (C-6'), 138.2 (C-1'), 140.3 (C-2'), 147.4 (C-4'), 155.8 (C-2), 157.8 (C-8a), 161.8 (C-5), 164.7 (C-7), 179.0 (C-4); *m/z* 313 (100%) 295 (13), 266 (9), 153 (48), 133 (12), 115 (6); HRMS: calcd for ($C_{16}H_{11}NO_6$) 313.0587, found 313.0589. Anal. ($C_{16}H_{11}NO_6$) C, H, N.

7,4'-Dihydroxy-3'-nitroisoflavone 2a

TFAA (2.7 ml, 19.3 mmol) was added to a stirred suspension of daidzein **1a** (1.4 g, 5.51 mmol) and ammonium nitrate (0.44 g, 5.51 mmol) in dry acetonitrile (8 mL) at rt. After 1.5 hrs stirring, the dark-red solution was poured into ice-water (20 mL). The yellow precipitate was filtered, washed with water (50 mL), and dried. Recrystallization from EtOH:acetone (95:5) afforded **2a** (1.54 g, 94%) as yellow crystals, m.p. 309-310 ºC (from methanol), ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.89 (d, *J* = 2.2 Hz, 1H, H-8), 6.96 (dd, *J* = 8.8, 2.2 Hz, 1H, H-6), 7.20 (d, *J* = 8.8 Hz, 1H, H-5'), 7.78 (dd, *J* = 8.8, 2.2 Hz, 1H, H-6'), 7.99 (d, *J* = 8.8 Hz, 1H, H-5), 8.19 (d, *J* = 2.2 Hz, 1H, H-2'), 8.48 (s, 1H, H_2), 10.85 (s, 1H, 7-OH), 11.1 (s, 1H, 4'-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 102.1 (C-8), 115.3 (C-6), 116.3 (C-4a), 118.8 (C-5'), 121.2 (C-3), 123.2 (C-1'), 125.1 (C-2'), 127.2 (C-5), 135.4 (C-6'), 136.4 (C-3'), 151.6 (C-4'), 153.9(C-2), 157.4 (C-8a), 162.7 (C-7), 174.2 (C-4); *m/z* 299 (100%) 300 (20), 252 (25), 149 (8), 136 (18), 111(8), 97(11); HRMS: calcd for

($C_{15}H_9NO_6$) 299.0429, found 299.0421. Anal. ($C_{15}H_9NO_6$) C, H, N.

7,5,4'-Trihydroxy-3'-nitroisoflavone 2b

The procedure was the same as for compound **2a,** using genistein **1b** (1g, 3.7 mmol) ammonium nitrate (0.296 g, 3.7 mmol), trifluoroacetic anhydride (1.8 mL, 12,96 mmol) and anhydrous acetonitrile (3 mL). Recrystallization from EtOH:acetone (95:5) gave **2b** (1.13 g, 96%) as yellow crystals, m.p. 257-258 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.24 (d, *J* = 2.1 Hz, 1H, H-6), 6.42 (d, *J* = 2.1 Hz, 1H, H-8), 7.22 (d, *J* = 8.7 Hz, 1H, H-5'), 7.77 (dd, *J* = 8.7, 2.3 Hz, 1H, H-6'), 8.17 (d, *J* = 2.2 Hz, 1H, H-2'), 8.49 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 93.8 (C-8), 99.2 (C-6), 104.3 (C-4a), 118.9 (C-5'), 120.1 (C-3), 121.9 (C-1'), 125.2 (C-2'), 135.5 (C-3'), 151.8 (C-4'), 155.0 (C-2), 157.5 (C-8a), 161.9(C-5), 164.5 (C-7), 179.6 (C-4); *m/z* 315 (100%) 316 (18), 269 (6), 241 (7), 152 (17), 136 (3), 124(5); HRMS: calcd for ($C_{15}H_9NO_7$) 315.0379, found 315.0383. Anal. ($C_{15}H_9NO_7$) C, H, N.

7- Hydroxy-4'-methoxy-8-nitroisoflavone 2c

TFAA (1.84 mL, 13.05 mmol) was added to a stirred mixture of formononetin **1c** (1 g, 3.73 mmol) and ammonium nitrate (0.3 g, 3.73 mmol) in dry acetonitrile (8 mL) at rt. The dark-red mixture was stirred for an additional 5 hrs at 75 ºC. After cooling, the mixture was poured into ice-water and the precipitate was collected and dried. Purification using silica gel column chromatography (CH_2Cl_2: EtOAc 7:3) gave **2c, 2d** and **2g** (8:1:1). Compound **2c** (0.78 g, 66.7%) was obtained as yellow crystals, m.p. 277-278 ºC (from methanol), ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.79 (s, 3H, OCH_3), 7.01(d, *J* = 8.4 Hz, 2H, H-3', 5'), 7.21 (d, *J* = 9.1 Hz, 1H, H-6), 7.51 (d, *J* = 8.4 Hz, 2H, H-2', 6'), 8.15 (d, *J* = 9.1 Hz, 1H, H-5), 8.46 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.1 (OCH_3), 113.8 (C-3', 5'), 115.5 (C-6), 116.2 (C-4a), 123.3 (C-1'), 124.2 (C-3), 128.2 (C-8), 129.0 (C-5), 130.3 (C-2', 6'), 148.5 (C-8a), 152.9 (C-2), 154.6(C-7), 159.4 (C-4'), 173.7 (C-4); *m/z* 313 (100%) 314 (19), 298 (6), 267 (5), 156 (3), 132(13), 117(4); HRMS: calcd for ($C_{16}H_{11}NO_6$) 313.0587, found 313.0591. Anal. ($C_{16}H_{11}NO_6$) C, H, N.

Compound **2d** (0.075 g, 6.4%) was obtained as yellow crystals, m.p. 188-189 ºC (from methanol); ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.81 (s, 3H, OCH_3), 7.02(d, *J* = 8.8 Hz, 2H, H-3', 5'), 7.20 (s, 1H, H-8), 7.54 (d, *J* = 8.8 Hz, 2H, H-2', 6'), 8.46 (s, 1H, H-2), 8.57 (s, 1H, H-5); ^{13}C NMR (50 MHz, DMSO-d_6) δ 54.9 (OCH_3), 105.5 (C-8), 113.5 (C-3', 5'), 115.8 (C-4a), 123.1 (C-1'), 123.8 (C-3), 129.8 (C-5, 2', 6'), 136.1 (C-6), 153.7 (C-2), 155.4 (C-7), 158.2 (C-8a), 159.0 (C-4'), 174.9 (C-4); *m/z* 313 (100%) 314 (19), 298 (6), 267 (5), 156 (3), 132(13), 117(4); HRMS: calcd for ($C_{16}H_{11}NO_6$) 313.0587, found 313.0578.

8-nitrobiochanin A 2e

The procedure was the same as for **2c**, using biochanin A **1d** (1 g, 3.52 mmol). After a 7 hrs reaction time, a yield of 57% was obtained. Recrystallization from EtOH:acetone (95:5) gave **2e** as yellow crystals, m.p. 239-241 ºC (from methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.87 (s, 3H, OCH_3), 6.42 (s, 1H, H-6), 7.1 (d, *J* = 8.8 Hz, 2H, H-3', 5'), 7.52 (d, *J* = 8.8 Hz, 2H, H-2', 6'), 8.54 (s, 1H, H-2'); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.0 (OCH_3), 98.5 (C-6), 103.4 (C-4a), 113.6 (C-3', 5'), 121.7 (C-8, 1'), 122.9 (C-3), 130.1 (C-2', 6'), 149.5 (C-8a), 154.0 (C-2), 157.0 (C-7), 159.3 (C-4'), 162.7 (C-5), 179.5 (C-4); *m/z* 329 (100%) 300 (19), 298 (6), 267 (5), 132 (13), 117 (4); HRMS: calcd for ($C_{16}H_{11}NO_7$) 329.0535, found 329.0534. Anal. ($C_{16}H_{11}NO_7$) C, H, N.

7,4'-Dihydroxy-3'-5'-dinitroisoflavone 2f

Daidzein **1a** (0.3 g, 1.18 mmol), suspended in dioxane, (5 mL) was treated with concentrated sulfuric acid (2 mL) and nitric acid (d =1.52 kg/L, 2.6 mmol). After 6 hrs stirring at room temperature, the reaction mixture was concentrated under reduced pressure and poured into water (30 mL). The precipitate was filtered, washed with water, dried and recrystallized from aq. EtOH to give **2f** (0.27 g, 66.5%) as yellow crystals, m.p. 293-4 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.92 (d, *J* = 2.2 Hz, 1H, H-8), 6.95 (dd, *J* = 8.8, 2.2 Hz, 1H, H-6), 8.02 (d, *J* = 8.8 Hz, 1H, H-5), 8.49 (s, 1H, H-2',6'), 8.73 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) 102.3 (C-8), 115.8 (C-6), 116.1 (C-4a), 121.4 (C-3), 123.4 (C-1'), 125.4 (C-2'), 128.1 (C-8), 129.0 (C-5), 129.7 (C-2',6'), 139.7 (C-3',5'), 145.9 (C-4'), 148.5 (C-8a), 152.0 (C-4'), 154.8 (C-7), 173.3 (C-4); *m/z* 358 (100%) 359 (20), 310 (8), 282 (13), 270 (4), 252 (6), 236 (5), 180 (4), 139 (4); HRMS: calcd for ($C_{16}H_{10}N_2O_8$) 358.0437, found 358.0442. Anal. ($C_{16}H_{10}N_2O_8$) C: calcd, 53.64; found, 53.12; H, N.

7-Hydroxy-4'-methoxy-8,3'-dinitoisoflavone 2g

Formononetin **1c** (0.3 g, 1.12 mmol) and nitric acid (0.10 mL, d = 1.52 kg/L, 2.4 mmol) following the above procedure gave **2g** (0.29 g, 72%, reaction time 8 hrs) as yellow crystals, m.p. 265-267 ºC (from EtOH: acetone 95:5); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.96 (s, 3H, OCH_3), 7.25 (d, *J* = 9 Hz, 1H, H-6), 7.48 (d, *J* = 8.9 Hz, 1H, H-5'), 7.91 (dd, *J* = 8.9, 2.2 Hz, 1H, H-6'), 8.17 (d, *J* = 2.2 Hz, 1H, H-2'), 8.18 (d, *J* = 9 Hz, 1H, H-5), 8.64 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ

56.9 (OCH_3), 114.3 (C-5'), 115.8 (C-6), 116.0 (C-4a), 122.2 (C-3), 123.4 (C-1'), 125.4 (C-2'), 128.1 (C-8), 129.0 (C-5), 134.9 (C-6'), 139.0 (C-3'), 148.5 (C-8a), 152.0 (C-4'), 154.8 (C-7), 173.3 (C-4); *m/z* 358 (100%) 359 (20), 310 (8), 282 (13), 270 (4), 252 (6), 236 (5), 180 (4), 139 (4); HRMS: calcd for ($C_{16}H_{10}N_2O_8$) 358.0437, found 358.0442. Anal. ($C_{16}H_{10}N_2O_8$) C: calcd, 53.64; found, 53.12; H, N.

7,4'-Dihyroxy-8,3',5'-trinitroisflavone 2h

HNO_3 (0.2 mL, 4.8 mmol) in H_2SO_4 (0.5 mL) was added to a stirred mixture of daidzein **1a** (0.3 g, 1.18 mmol) in conc. H_2SO_4 (5 mL) at 0 ºC. After 5 hrs stirring, the mixture was poured into ice (50 g), the precipitate was filtered, washed with water, and recrystallized from EtOH:acetone (9:1) to give the **2h** (0.33 g, 72%) as yellow solid, m.p. 232-234 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.23 (d, *J* = 9.0 Hz, 1H, H-6), 8.23 (d, *J* = 9.0 Hz, 1H, H-5), 8.47 (s, 1H, H-2', 6'), 8.70 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.8 (C-6), 115.9 (C-4 a), 120.7 (C-1'), 121.8 (C-3), 128.1 (C-8), 128.9 (C-5), 129.7 (C-2', 6'), 139.8 (C-3', 5'), 145.7 (C-4'), 148.4 (C-8a), 154.6 (C-2), 154.9 (C-7), 172.9 (C-4); *m/z* 389 (100%) 390 (19), 342 (10), 296 (7), 252 (11), 235 (4), 181 (8), 165 (4), 139 (3); HRMS: calcd for ($C_{15}H_7N_3O_{10}$) 389.0132, found 389.0136. Anal. C, H; N: calcd, 10.80; found, 10.22.

7-Hydroxy-4'-methoxy-8,3',5'-trinitroisoflavone 2i

This compound was obtained in 59% as described above for **2h** (reaction time 8 hrs): m.p. 253-255 ºC (from aq EtOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.99 (s, 3H, OCH_3), 7.24 (d, *J* = 9.0 Hz, 1H, H-6), 8.15 (d, *J* = 9.0 Hz, 1H, H-5), 8.55 (s, 1H, H-2', 6'), 8.73 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 64.4 (OCH_3), 115.7 (C-6), 116.0 (C-4a), 120.4 (C-3), 127.9 (C-1'), 128.0 (C-8), 128.8 (C-5), 129.3 (C-2', 6'), 144.1 (C-3', 5'), 145.8 (C-4'), 148.3 (C-8a), 154.9 (C-7), 155.2 (C-2), 172.6 (C-4); *m/z* 403 (100%) 404 (15), 326 (8), 297 (3), 269 (10), 252 (28), 220 (7), 181 (8); HRMS: calcd for ($C_{16}H_9N_3O_{10}$) 403.0288, found 403.0283.

7-Hydroxy-8,4'-dinitroisoflavone 2j

The title compound was ontained as pale yellow crystals in 80% yield following the procedure decribed above (2.1 eq. of HNO_3) for **2h**; m.p. 258-9 ºC [Lit,[18] 265 ºC];^{1}H NMR (300 MHz, DMSO-d_6) δ 7.23 (d, *J* = 8.9 Hz, 1H, H-6), 7.88 (d, *J* = 8.7 Hz, 2H, H-2'), 8.16 (d, *J* = 8.9 Hz, 1H, H-5), 8.28 (d, *J* = 8.7 Hz, 2H, H-3', 5'), 8.66 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.9 (C-6), 116.1 (C-4a), 122.6 (C-3), 123.2 (C-3', 5'), 127.6 (C-8), 129.0 (C-5), 130.1 (C-2', 6'), 138.2 (C-1'), 147.1 (C-4'), 148.4 (C-8a), 154.8 (C-7), 154.9 (C-2), 172.8 (C-4); *m/z* 328 (100%), 329 (20), 298 (5), 282(14), 181 (19), 151 (5), 123 (4); HRMS: calcd for ($C_{15}H_8N_2O_7$) 328.0332, found 328.0338.

5,7-Dihydroxy-8,4',-dinitroisoflavone 2k

The title compound was obtained as described above for **2h**; 0.51 g (88.6 %) of yellow crystals, m.p. 246-247 ºC (from EtOH:acetone (95:5); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.44 (s, 1H, H-6), 7.87 (d, *J* = 8.4 Hz, 2H, H-2', 6'), 8.3 (d, *J* = 8.4 Hz, 2H, H-3', 5'), 8.74 (s, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 99.3 (C-6), 103.7 (C-4a), 121.7 (C-3, 8), 123.5 (C-3', 5'), 130.3 (C-2', 6'), 137.1 (C-1'), 147.3 (C-4'), 149.8 (C-8a), 156.3 (C-2), 157.6 (C-7), 162.9 (C-5), 179.0 (C-4); *m/z* 344 (100%) 345 (20), 314 (13), 299 (7), 286 (4), 252 (8), 197 (5), 167 (4), 139 (5); HRMS: calcd for ($C_{15}H_8N_2O_8$) 344.0281, found 344.0279. Anal. ($C_{15}H_8N_2O_8$) C, H, N.

General procedure for reduction of nitroisoflavones

A mixture of the isoflavone and an excess of stannous chloride in absolute ethanol (20 mL) was heated at 70 ºC under Ar. After the disappearance of starting material as indicated by TLC, cold water (20 mL) was added *via* syringe at 20 ºC. The pH was adjusted to 7-8 by addition of 5% aqueous $NaHCO_3$ and NaH_2PO_4 and the mixture was extracted four times with ethyl acetate (50 mL x 4). The combined organic layers were thoroughly washed with brine, treated with charcoal and dried over Na_2SO_4. Evaporation of the solvent leaves the amino compound that gave a single spot on TLC. The entire work-up was carried out under Ar owing to the lability of aminoisoflavones.

4'-Amino-5, 7-dihydroxisoflavone 3a

Compound **3a** was obtained from **1f** (0.5 g, 1.67 mmol) and $SnCl_2.2H_2O$ (2.38 g, 10.5 mmol); yield 97%, yellow crystals m.p. 249-250 ºC (from methanol); ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.23 (d, *J* = 2.1 Hz, 1H, H-6), 6.39 (d, *J* = 2.1 Hz, 1H, H-8), 6.62 (d, *J* = 8.6 Hz, 2H, H-3', 5'), 7.25 (d, *J* = 8.6 Hz, 2H, H-2', 6'), 8.28 (s, 1H, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 93.5 (C-8), 98.8 (C-6), 104.4 (C-4a), 113.4 (C-3', 5'), 117.5 (C-3), 122.7 (C-1'), 129.5 (C-2', C-6'), 148.7 (C-4'),153.2 (C-2), 157.5 (C-8a), 161.9 (C-5), 164.1 (C-7), 180.4 (C-4); *m/z* 269 (100%) 270 (19), 253 (6), 205 (5), 149 (4), 134 (10), 117 (32); HRMS: calcd for ($C_{15}H_{11}NO_4$) 269.0688, found 269.0679.

4'-Amino-5,7-dihydroxy-2-methylisoflavone 3b

Compound **3b** was obtained from **1g** (0.5 g, 1.59 mmol) and $SnCl_2.2H_2O$ (2.16 g, 9.58 mmol); yield

95%, yellow crystals m.p. 224 ºC decompo; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.03 (s, 3H, CH_3), 5.13 (br, 1H, NH_3), 6.23 (d, *J* = 2.2 Hz, 1H, H-6), 6.39 (d, *J* = 2.2 Hz, 1H, H-8), 6.41 (dd, *J* = 8.0, 2.2 Hz, 1H, H-5'), 6.45 (d, *J* = 2.2 Hz, 1H, H-3'), 6.81(d, *J* = 8.0 Hz, 1H, H-6'), 8.12 (s, 1H, H-2), 10.84 (s, 1H, 7-OH), 12.96(s, 1H, 5-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.8 (CH_3), 93.6 (C-8), 98.8 (C-6), 123.2 (C-3', 5'), 127.6 (C-8), 129.0 (C-5), 130.1 (C-2', 6'), 138.2 (C-1'), 147.1 (C-4'), 148.4 (C-8a), 104.6 (C-4a), 111.0 (C-5'), 115.2 (C-3'), 117.7 (C-1'), 123.7 (C-3),131.3 (C-6'), 137.8 (C-2'), 148.6 (C-4'), 154.8 (C-2), 156.7 (C-8a), 161.8 (C-5), 164.0 (C-7), 180.4 (C-4); *m/z* 283 (100%), 284 (20), 266 (9), 213(3), 207 (11), 153 (85), 141 (11), 130 (27), 105 (6); HRMS: calcd for ($C_{16}H_{13}NO_4$) 283.0845, found 283.0854.

3'- Amino-7, 4'-dihydroxyisoflavone 3c

Compound **3c** was obtained from **2a** (0.5 g, 1.67 mmol) and $SnCl_2.2H_2O$ (2.38 g, 10.5 mmol); yield 83%, yellow crystals m.p. 235 ºC decomp. (from methanol); ^{1}H NMR (200 MHz, DMSO-d_6) 5.13 (br, 2H, NH_2), 6.58 (dd, *J* = 8.5, 2.3 Hz, 1H, H-6'), 6.69 (d, *J* = 8.5 Hz, 1H, H-5'), 6.83 (d *J* = 2.3 Hz, 1H, H-2'), 6.85 (d, *J* = 2.2 Hz, 1H, H-8), 6.93(dd, *J* = 8.7, 2.2 Hz, 1H, H-6), 7.96 (d *J* = 8.7 Hz, 1H, H-5), 8.18 (s, 1H, H-2), 9.36 (s, 1H, 4'-OH), 10.79 (s, 1H, 7-OH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 102.0 (C-8), 114.0 (C-6), 114.9 (C-2'), 115.8 (C-5'), 116.6 (C-4a), 117.8 (C-6'), 123.1 (C-3), 124.1 (C-1'),127.2 (C-5), 134.9 (C-3'), 144.3 (C-4'), 152.4 (C-2), 157.2 (C-8a), 162.3 (C-7), 174.8 (C-4); *m/z* 269 (100%) 270 (25), 253 (6), 1137 (21), 133 (25), 112 (9); HRMS: calcd for ($C_{15}H_{11}NO_4$) 269.0688, found 269.0698.

3'-Amino-5,7,4'-trihydroxyisoflavone 3d

Compound **3d** was synthesized from **2b** (0.5 g, 1.58 mmol) and $SnCl_2.2H_2O$ (2.5 g, 11.1 mmol); yield 97%, yellow crystals m.p. 234 ºC decomp. (from methanol); ^{1}H NMR (200 MHz, DMSO-d_6) 4.67 (br, 2H, NH_2), 6.23 (d, *J* = 2.1 Hz, 1H, H-6), 6.39 (d, *J* = 2.1 Hz, 1H, H-8), 6.58 (dd *J* = 8.0, 2.1 Hz, 1H, H-6'), 6.70 (d, *J* = 8.0 Hz, 1H, H-5'), 6.82 (d, *J* = 2.0 Hz, 1H, H-2'), 8.25 (s, 1H, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 93.4 (C-8), 98.7 (C-6), 104.2 (C-4a), 113.8 (C-2'), 114.9 (C-5'), 116.9 (C-6'), 121.5 (C-3), 122.8 (C-1'), 136.1 (C-3'), 144.0 (C-4'), 153.4 (C-2), 157.3 (C-8a), 161.8 (C-5), 164.0 (C-7), 180.1 (C-4); *m/z* 285 (100%) 286 (20), 252 (17), 176 (7), 153 (12), 133 (14), 120 (6), 105 (4), 88 (13); HRMS: calcd for ($C_{15}H_{11}NO_5$) 285.0637, found 285.0641.

8-Amino-7-Hydroxy-4'methoxyisoflavone 3e

Compound **3e** was obtained from **2c** (0.5 g, 1.59 mmol) and $SnCl_2.2H_2O$ (1.8 g, 7.98 mmol); yield 83%, yellow crystals m.p. 211 ºC decomp. (from methanol); ^{1}H NMR (200 MHz, DMSO-d_6) 3.81(s, 3H, OCH_3), 4.87 (br, 2H, NH_2), 6.91 (d, *J* = 8.5 Hz, 1H, H-6), 7.01 (d, *J* = 8.5 Hz, 2H, H-3', 5'), 7.3 (d *J* = 8.5 Hz, 1H, H-5), 7.53 (d, *J* = 8.5 Hz, 2H, H-2', 6'), 8.36 (s, 1H, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 55.0 (OCH_3), 112.3 (C-5), 113.1 (C-6), 113.4 (C-3', 5'), 117.2 (C-4a), 122.4 (C-1'), 124.2 (C-3), 130.0 (C-2', 6'), 144.7 (C-8a), 147.5 (C-7), 152.6 (C-2), 158.8 (C-4'), 175 (C-4); *m/z* 283 (100%) 284 (24), 268 (11), 151 (27), 141 (5), 132 (7), 89 (3); HRMS: calcd for ($C_{16}H_{13}NO_4$) 283.0845, found 283.0837.

8,4'-Diamino-7-hydroxyisoflavone 3f

Compound **3f** was obtained from **2j** (0.5 g, 1.52 mmol) and $SnCl_2.2H_2O$ (3.78 g, 16.7 mmol); yield 78%, yellow crystals m.p. 245 ºC decomp; ^{1}H NMR (300 MHz, DMSO-d_6) 5.18 (br, 4H, NH_2), 6.62 (d, *J* = 8.5 Hz, 2H, H-3', 5'), 6.90 (d, *J* = 8.5 Hz, 1H, H-6), 7.24 (d *J* = 8.5 Hz, 2H, H-2', 6'), 7.29 (d, *J* = 8.5 Hz, 1H, H-5), 8.24 (s, 1H, H-2), 10.96 (s, 1H, 7-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 112.5 (C-5), 113.2 (C-6), 113.5 (C-3', 5'), 117.3 (C-4a), 119.4 (C-1'), 123.3 (C-3), 124.3 (C-8), 129.7 (C-2', 6'), 144.9 (C-8a), 148.5 (C-4'), 151.7 (C-2), 175.7 (C-4); *m/z* 268 (100%) 151 (12), 134 (6), 123 (5), 117 (16); HRMS: calcd for ($C_{15}H_{12}N_2O_3$) 268.0848, found 268.0845.

8,4'-Diamino-5,7-dihydroxyisoflavone 3g

Compound **3g** was synthesized from **2k** (0.5 g, 1.45 mmol) and $SnCl_2.2H_2O$ (3.56 g, 16.0 mmol); yield 78%, yellow crystals m.p. 194 ºC decomp; ^{1}H NMR (300 MHz, DMSO-d_6) 5.80 (br, 4H, NH_2), 6.28 (s, 1H, H-6), 6.61 (d, *J* = 8.5 Hz, 2H, H-3', 5'), 7.23 (d, *J* = 8.5 Hz, 2H, H-2', 6'), 8.25 (s, 1H, H-2), 12.25 (s, 1H, 7-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 112.5 (C-5), 98.2 (C-6), 104.1 (C-4a), 113.4 (C-3', 5'), 115.6 (C-8), 117.9 (C-1'), 122.1 (C-3), 143.7 (C-8a), 148.5 (C-4'), 150.7 (C-5), 151.9 (C-2), 152.9 (C-7), 180.8 (C-4); *m/z* 284 (100%) 285 (21), 167 (18), 142 (8), 117 (25); HRMS: calcd for ($C_{15}H_{12}N_2O_4$) 284.0795, found 284.0796.

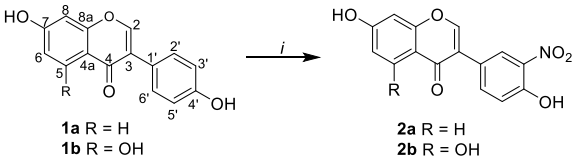
Results and Discussion

Three main routes towards the synthesis of nitroisoflavones are known in the literature. Nitro substituted deoxybenzoin [19-21], prepared in moderate yield from the corresponding phenols and nitrophenylacetic acid, were cyclized to nitroisoflavones. This procedure is restricted to cases where the nitro group is attached on the non-fused aromatic ring (ring B). Another route involves thallium (III) salts catalyzed oxidative rearrangement of nitroflavones to nitroisoflavones in a polar protic solvent

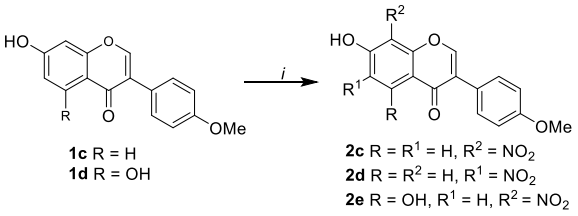
has also been used [22]. The disadvantages of this procedure are the high toxicity of the thallium reagent, long reaction times and poor yields obtained. Electrophilic nitration of isoflavones should be widely applicable, but has only been applied to 6-hydroxyisoflavone, 7-hydroxyisoflavone **1e**, 6-hydroxy-7-methoxyisoflavone and their methyl ethers using fuming HNO_3 in presence of glacial acetic acid or sulfuric acid [23-25]. Löwe *et al.* have demonstrated *in-vitro* that the pro-inflammatory oxidants peroxynitrates ($ONOO^-$) are capable for mononitrating the isoflavones (daidzein **1a** and genistein **1b**) and leaving the 4'-*O*-methyl ethers (formononetin and biochanin A) intact [26]. Because isoflavones are easily accessible by a one-pot synthesis from the corresponding phenols and phenylacetic acid, their nitration appeared to be the most promising general route to the various nitroflavones [27].

Nitroisoflavones are reduced to aminoisoflavones using either zinc [19,28], iron dust [28] or sodium hydrogen sulphite [23]. The reduction has also been achieved by hydrogenation over 10% Pd/C [29] or Rany nickel [30]. As in our hands, these reduction procedures were not entirely satisfactory, particularly for the *ortho*-aminohydroxy isoflavones, we adapted the use of $SnCl_2$ for this purpose as mentioned above [18].

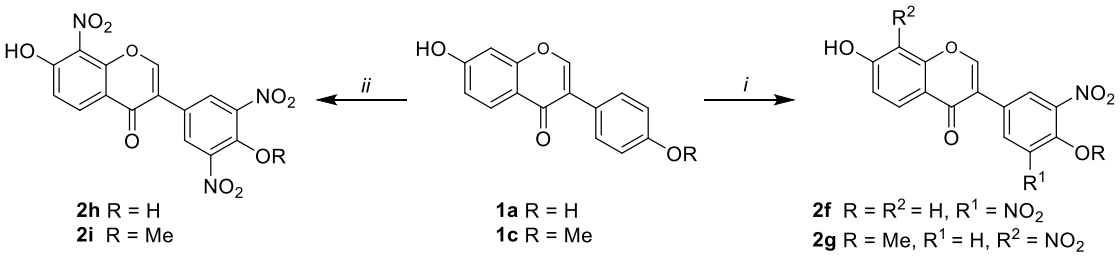
The nitration of daidzein **1a** and genistein **1b** with trifluoroacetyl nitrite [31], generated by mixing NH_4NO_3:TFAA (1:3.5), afforded 7,4'-dihydroxy-3'-nitroisoflavones **2a** and 5,7,4'-trihydroxy-3'-nitroisoflavone **2b,** respectively (Scheme-1). Under similar reaction conditions, the 4'-methyl ethers of **1a** and **1b** didn't react. Treatment of formononetin **1c** with NH_4NO_3:TFAA (1:3.5) in acetonitrile at 70 ºC for 5 hours furnished a mixture of 7-hydroxy-4'-methoxy-8-nitroisoflavone **2c** and 7-hydroxy-4'-methoxy-6-nitroisoflavone **2d** in an 8:1 ratio as revealed by ^1H NMR (Scheme-2). Refluxing a mixture of biochanin A **1d** and NH_4NO_3:TFAA in acetonitrile for 7 hours afforded 5,7- dihydroxy-4' methoxy-8-nitroisoflavone **2e** in 57% yield (Scheme 2). On the other hand, treatment of the isoflavones **1a-c** with either $Cu(NO_3)_2$ [32] or $AgNO_3$ [31] either in Ac_2O or in a solution of TFAA and acetonitrile at room temperature resulted in intractable mixtures. Treatment of daidzein **1a** and genistein **1b** with fuming HNO_3 (1eq.) in glacial acetic acid at room temperature, afforded the 3'-nitrated products **2a** and **2b** respectively in good yields. On the other hand, treatment of formononetin **1c** and biochanin A **1d** at 75 ºC provided the 8-nitro compounds **2c** and **2e** as major products in 78% and 64%, respectively. Byproducts from formononetin **1c** included 7-hydroxy-4'-methoxy-6-nitroisoflavone **2d** and 7-hydroxy-4'-methoxy8,3'-dinitroisoflavone **2g**. Using TFA or sulfuric acid as a solvent resulted in a significant decrease in the yield of the mono-products, whereas increasing the yields of the dinitro products. Nitration of daidzein **1a** with 2 equivalents of fuming HNO_3 in dioxane/H_2SO_4 afforded 7,4'-dihydroxy-3',5'-dinitroisoflavone **2f** in 66.5% yield. Formononetin **1c** under similar conditions afforded 7-hydroxy-4'-methoxy-8,3'- dinitroisoflavone **2g** in 72% yield (Scheme-3). Genistein **1b** and biochanin A **1d** on treatment with HNO_3 under similar conditions gave an intractable mixture. Treatment of daidzein **1a** and formononetin **1c** with 4 eq. of HNO_3 in sulfuric acid, furnished the corresponding 7,4-dihydroxy-8,3',5'-trinitroisoflavone **2h** and 7-hydroxy-4'-methoxy-8,3',5'-trinitroisoflavone **2i** in 73% and 59% yield, respectively (Scheme-3). Nitration of 7-hydroxyisoflavone **1e** with 2.1 eq. of HNO_3 in H_2SO_4 afforded the previously prepared 7-hydroxy-8,4'-dinitroisoflavone **2j** in good yield and purity (Scheme-4) [24]. Furthermore, treatment of 5,7- dihydroxy-4'-nitroisoflavone **1f** with 1.2 eq. of HNO_3 in H_2SO_4 gave 5,7-dihydroxy-8,4'-dinitroisoflavone **2k** in 80% yield.



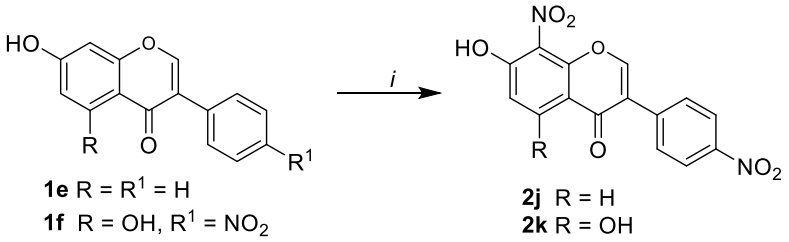
Scheme-1: Reagents: (i) NH_4NO_3, TFAA, acetonitrile, rt.



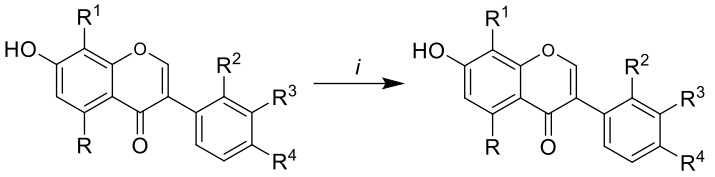
Scheme-2: Reagents: (i) NH_4NO_3, TFAA, acetonitrile, 70 ºC.



Scheme-3: Reagents: (i) fuming HNO_3, dioxane/H_2SO_4; ii) fuming HNO_3, H_2SO_4.



Scheme-4: Reagents: (i) fuming HNO_3, H_2SO_4.



Compounds	R	R^1	R^2	R^3	R^4	Compounds	R	R^1	R^2	R^3	R^4
1f	OH	H	H	H	NO_2	3a	OH	H	H	H	NH_2
1g	OH	H	Me	H	NO_2	3b	OH	H	Me	H	NH_2
2a	H	H	H	NO_2	OH	3c	H	H	H	NH_2	OH
2b	OH	H	H	NO_2	OH	3d	OH	H	H	NH_2	OH
2c	H	NO_2	H	H	OMe	3e	OH	NH_2	H	H	OMe
2j	H	NO_2	H	H	NO_2	3f	H	NH_2	H	H	NH_2
2k	OH	NO_2	H	H	NO_2	3k	OH	NH_2	H	H	NH_2

Scheme-5: Reagents: SnCl2.2H2O, EtOH, 70 oC, 4-9 hrs.

The experimental results presented above show that the order of reactivity in the nitration of daidzein is 3' (5') > 8 > 6. Since the prediction of the orientation on simple resonance arguments is not conclusive, we calculated the electrostatic potentials for daidzein **1a** using the AM1 method in the MOPAC 6.0 package in Cerius [33]. The ESP derived atom-centered monopole charges suggest the reactivity order 8 > 5' > 6 > 3' assuming that the most negative atom is the first to react. If one takes into account that the experimental reactivity at C-3'(5') includes both C-3' and C-5', and a possible steric hindrance at C-8, the calculated results agree with the experimental. In the case of formononetin ESP charges give a similar reactivity order 8 > 3' > 6 > 5' even though the experimentally observed order is 8 > 6 > 3'(5'). The calculations show that charge densities, especially in ring B, are dependent on the conformation of the hydroxyl or methoxy group. The results presented here are based on the minimum energy structures only.

The reduction of nitroisoflavones **1f, 1g** and **2** to aminoisoflavones **3** was best carried out using excess of tin (II) chloride dehydrate [18] under argon in 78-97% yield (Scheme 5). The work-up has also to be executed under argon atmosphere particularly when the catechol type aminoisoflavones are involved. Special care must be taken, since compounds **3c** and **3d** are very unstable and rapidly turn dark brown when they come in contact with air. Also, these two compounds are very difficult to extract from the reaction mixture.

Biological Activity

The half-maximal concentrations (IC_{50}) of the anti-proliferative effect of the synthetic nitro- and aminoisoflavones (Table-1) revealed that the introduction of nitro or amino groups in the isoflavones **1a-d** molecules lessens their anti-proliferative activity significantly. For example, replacing the 4'-hydroxyl in genistein (IC_{50} 5 μM) with a nitro (compound **1f**) or an amino (compound **3a**) group decreased the anti-proliferative activity on endothelial cells to 14.14 μM and >50, respectively. Further introduction of an amino group to compound **3a** (compound **3g**) resulted in a totally biologically inactive species.

Table-1: Half-maximal inhibitory activity of modified flavonoid on the *in vitro* proliferation of bovine brain capillary endothelial cells (BBCE).

Isoflavones	Formula	IC_{50} (μM), ±SD
5,7-Dihydroxy-4'-nitroisoflavone	**if**	**14.14±0.14**
5,7-Dihydroxy-2'-methyl-4'-nitroisoflavone	**1g**	**19.5±0.20**
7,4'-Dihydroxy-3'-nitroisoflavone	**2a**	**42.3±0.42**
5,7,4'-trihydroxy-3'-nitroisoflavone	**2b**	**35.2±0.40**
7-Hydroxy-4'-methoxy-8-nitroisoflavone	**2c**	**>50±0.50**
7-Hydroxy-4'-methoxy-8,3'-dinitroisoflavone	**2g**	**>50±0.49**
7,4'-dihydroxy-8,3',5'-trinitroisoflavone	**2h**	**>50±0.48**
7-Hydroxy-4'-methoxy-8,3',5'-trinitroisoflavone	**2i**	**45.9±0.50**
7-Hydroxy-4'-methoxy-8-nitroisoflavone	**2j**	**>50±0.49**
7,4'-Dihydroxy-3',5'-dinitroisoflavone	**2f**	**42.2±0.42**
4'-Amino-5,7-dihydroxyisoflvone	**3a**	**>50±0.47**
4'-Amino-5,7-dihydroxy-2'-methylisoflvone	**3b**	**>50±0.49**
3'-Amino-7,4'-dihydroxyisoflvone	**3c**	**37.5±0.38**
3'-Amino-5,7,4'-trihydroxyisoflvone	**3d**	**24.8±0.25**
8'-Amino-7-hydroxy-4'-methoxyisoflvone	**3e**	**31.7±0.32**
8,4'-Diamino-7-hydroxyisoflvone	**3f**	**>50±0.48**
8,4'-Diamino-5,7-dihydroxyisoflvone	**3g**	**>50±0.50**

Conclusion

In conclusion, the selective mono-nitration of polyhydroxyisoflavones **1a-d** was best achieved using NH_4NO_3: TFAA as a nitrating agent in 1:3.5 ratio and acetonitrile as a solvent. The mildness and region specificity of this method in case of daidzein **1a** and genistein **1b** along with the high purity of the products obtained should extend the scope of this nitrating system which is safe and inexpensive. Polynitration was best performed using HNO_3 in strong acids such as TFA or H_2SO_4. For the reduction of the nitroisoflavones to aminoisoflavones **3a-g**, the reagent of choice was tin (II) chloride in EtOH. The new nitro- and aminoisoflavones prepared displayed rather modest activities on proliferation of endothelial cells *in vitro.*

Acknowledgement

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References

1. N. Al-Maharik, Isolation of Naturally Occurring Novel Isoflavonoids: An Update. *J. Nat. Prod*. (2019) DOI: 10.1039/c8np00069g
2. N. Veitch, Isoflavonoids of the Leguminosae, *Nat. Prod. Rep.*, **24**, 417 (2007).
3. N. Veitch, Isoflavonoids of the Leguminosae, *Nat. Prod. Rep.*, **26**, 776 (2009).
4. L. Jian, Soy, Isoflavones, and Prostate Cancer, *Mol. Nutr. Food Res.*, **53**, 217 (2009).
5. S. Ziaei and R. Halaby, Dietary Isoflavones and Breast Cancer Risk, *Medicines*, **4**, 18 (2017).
6. X. Zheng, S.-K. Lee and O. K. Chun, Soy Isoflavones and Osteoporotic Bone Loss: A Review with an Emphasis on Modulation of Bone Remodeling, *J. Med. Food*, **19**, 1 (2016).
7. S. Ivanova and L. Vasileva, Current and Emerging Strategies in Osteoporosis Management. *Curr. pharm des.***, 23**, 6279 (2017).
8. A. Gil-Izquierdoa, J. L. Peñalvo, J. I. Gil, S. Medina, M. N. Horcajada, S. Lafay, M. Silberberg, R. Llorach, P. Zafrilla, P. García-Mora and F. Ferreres; Soy Isoflavones and Cardiovascular Disease Epidemiological, Clinical and -Omics Perspectives; *Curr. Pharm. Biotech*., **13**, 624 (2012).
9. K. Yamagata, M. Tagami and Y. Yamori, Dietary Polyphenols Regulate Endothelial Function and Prevent Cardiovascular Disease. *Nutrition*, **31**, 28 (2015).
10. E. Bartkiene, V. Sakiene, V. Bartkevics, J. Rusko, V. Lele, G. Juodeikiene, C. Wiacek and P. G. Braun, Lupinus Angustifolius L. Lactofermentation and Protein Isolation: Effects on Phenolic Compounds and Genistein, Antioxidant Properties, Trypsin Inhibitor Activity, and Protein Digestibility, *Eur. Food Res. Technol*., **1** (2018).
11. M. Moriyama, S. Tahara, J. L. Ingham and J. Mizutani, Isoflavonoid Alkaloids from *Piscidia Erythrina*, Phytochem.**, 32**, 1317 (1993).
12. R. Jiang, B. Li, C. Xiao, D. Yang and J. Wu, Production, Isolation and Structure Elucidation of New Isoflavonoid Compound K3-D1• Zhongguo Kangshegsu Zazhi, **22**, 81 (1997) (Ch).

13. A-G. Seuref, 2,3-Diphenylchromone Derivatives. *Belg*. **867**, 031 (Cl. C07D), (1978).
14. G-X. Shao, R-Y. Mo, C-Y. Wang, D-Y. Zhang, Z-Z. Yin, R. Ouyang and L-N. Xu Studies on the Synthesis and Structure-Antihypoxia Activity Relations of Daidzein, An Active Principle of Pueraria Pseudohimta, and its Derivatives, Yao Hsueh Hsueh Pao, **15**, 538 (1980).
15. L. Feurer, M. Nogradi, A. Gottsegen, B. Vermes, J. Streliszky, A. Wolfner, L. Farkas, S. Antusand M. Kovacs, Catabolic 7-Alkoxysoflavones as Feed Additives, *Ger. Offen.* **2**, 166, 458 (1974).
16. T. Akama, H. Ishida, Y. Shida, U. Kimura, K. Gomi, H. Saito, E. Fuse, S. Kobayashi, N. Yoda and M. Kasai, Design and Synthesis of Potent Antitumor 5,4'-Diaminoflavone Derivatives Based on Metabolic Considerations, *J. Med. Chem.*, **40**, 1894 (1997).
17. D. Dauzonne, B. Folleas, L. Martinez and G. G. Chabot, Synthesis and in *vitro* Cytotoxicity of a Series of 3-Aminoflavones, *Eur. J. Med. Chem*., **32**, 71 (1997).
18. F. D. Bellamy and K. Ou, Selective Reduction of Aromatic Nitro Compounds with Stannous Chloride in non Acidic and non Aqueous Medium, *Tetrahedron Lett.*, **25**, 839 (1984).
19. S. Karmarkar, Chromones. XXXIII. Further Applications of the Ethyl orthoformate Method for the Synthesis of Isoflavones, *J. Sci. Ind. Res*. B, **20**, 334 (1961).
20. A. Rusin, Z. Krawczyk, G. Grynkiewicz, A. Gogler, J. Zawisza-Puchałka and W. Szeja, Synthetic Derivatives of Genistein, Their Properties and Possible Applications, *Acta Biochimica Polonica***, 57**, 23 (2010).
21. D. Liu and C. A. Cheng, Facile and Practical Preparation of 5,7-Dihydroxy-3-(4-nitrophenyl)-4H-1-benzopyran-4-one, *J. Heterocyclic Chem*., **28**, 1641 (1991).
22. O. Singh and R. A. Kapil, General Method for the Synthesis of Isoflavones by Oxidative Rearrangement of Flavanones Using Thallium (III) Perchlorate, *Indian J. Chem.*, **24**, 100 (1993).
23. N. K. Chudgar, N. V. Mani and S. Sethna, Studies in Isoflavones. In Iodination, Bromination and Nitration of 6-Hydroxyisoflavone and Its Methyl Ether, *J. Inst. Chem*. (India), **42**, 194 (1970).
24. N. K. Chudgar, N. V. Mani and S. Sethna, Studies in Isoflavones. I. Bromination, Iodination, and Nitration of 7-hydroxy isoflavone, *J. Inst. Chem.* (India), **39**, 203 (1967).
25. T. J. Mabry, K. R. Markham and M. B. Thomas, The Ultraviolet Spectra of Isoflavones, Flavanones and Dihydroflavonols in The Systematic Identification of Flavonoids, The Systematic Identification of Flavonoids, *Springer*, p. 165 (1970).
26. W. Löwe, S. Witzel, S. Tappmeyer and R. Albuschat, An Aminoisoflavone-Salicyloylindole Ring Transformation, *J. Heterocyclic Chem*., **41**, 317 (2004).
27. S. Balasubramanian and M. G. Nair, An Efficient "One Pot" Synthesis of Isoflavones, *Synth. Commun.*, **30**, 469 (2000).
28. J. L. Bose and N. L. Dutta, lsoflavones: Part II- A New Synthesis of Isoformononetin and Daidzein, *J. Sci. Ind. Res*., **17B,** 266 (1958).
29. D. F. Diedrich, T. A. Scahill and S. Smith, Preparation and Physical Properties of Some Deoxybenzoins and Isoflavones. *J. Chem. Eng. Data***, 22**, 448 (1977).
30. J. Crivello, Nitrations and Oxidations with Inorganic Nitrate Salts in Trifluoroacetic Anhydride, *J. Org. Chem*., **46**, 3056 (1981).
31. M. Bagade and B. Ghiya, Nitration of Acetophenone, Chalcone, Flavanone and Flavone with Copper Nitrate-Acetic Anhydride, *Indian J. Chem.*, **22**, 71 (1991).
32. MOPAC: M. J. S. Dewar and J. J. P. Stewart, OQPE Bull., 6 (1986) 24. Stewart, J. J. P. QCPE# 455,1999. MOPAC6.0 in Cerius^2: ©Molecular Simulations Inc.
33. H. K. Sandhar, B. Kumar, S. Prasher, P. Tiwari, M. Salhan and P. Sharma, A Review of Phytochemistry and Pharmacology of Flavonoids, Internationale pharmaceutica sciencia, **1**, 25 (2011).