A convenient synthesis of chrysin-7-yl aryl *N*-bis(2-chloroethyl) phosphoramidate

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A series of novel chrysin-7-yl aryl *N*-bis(2-chloroethyl) phosphoramidates have been synthesised in good yield via phosphorylation reactions and their structures were elucidated by IR, NMR and elemental analysis.

Keywords: chrysin, phosphoramide mustard, phosphorylation

Chrysin (**4**), which is widely distributed in plants, has antioxidant,[1] antibacterial,[2] anticancer,[3] anti-inflammatory,[4] antiallergic,[5] and anxiolytic antivities.[6] Efforts to improve the biological activity of chrysin have led to the development of derivatives by appropriate modification of chrysin.[7,8] The concept of carrier molecules has been employed in the design of some of the earliest nitrogen mustard derivatives used in cancer chemotherapy.[9,10] In the past, large numbers of nitrogen mustards, containing the bis-(*β*-chloroethyl) amino group have been synthesised as potential antitumor agents. In the hope of increasing the biological specificity of these compounds, the mustard group has been attached to various carrier molecules such as antimalarial drugs,[11] amino acids,[12,13] steroids,[14] and carbohydrates.[15,16]

Given the importance of this functional group in cancer chemotherapy, our interest was to attach a phosphoramide mustard group to chrysin, as a carrier molecule. It was hoped that novel phosphoramide mustard analogues of chrysin could improve their physicochemical and biological properties. In the work described here, a series of novel chrysin-7-yl aryl *N*-bis(2-chloroethyl) phosphoramidates have been synthesised via a facile phosphorylation reaction (Scheme 1). The structures of compounds **3a–e** were elucidated by NMR, IR and elemental analysis. The study of their biological activity is in progress.

Results and discussion

In the synthesis of chrysin-7-yl aryl *N*-bis(2-chloroethyl) phosphoramidates **(5)** (Scheme 1), bis-(*β*-chloroethyl)-amine hydrochloride (**1**) was first reacted with phosphorus oxychloride under reflux to give di-(2-chloroethyl)-phosphoramidic dichloride (**2**). Then di-(2-chloroethyl)-phosphoramidic dichloride (**2**) was coupled to different substituted phenols to afford aryl-di-(2-chloroethyl) phosphoramidic chlorides **(3)**, which were purified further by chromatography. Chrysin (**4**) was reacted with aryl-di-(2-chloroethyl) phosphoramidic chloride (**3**) in tetrahydrofuran in the presence of triethylamine under reflux to form phosphoramide mustard analogues of chrysin (**5**). The structures of all the newly synthesised chrysin derivatives were confirmed by NMR, IR and elemental analysis.

Aryl-di-(2-chloroethyl) phosphoramidic chloride (**3**) can react with two different reaction positions (7-OH and 5-OH) of chrysin. The result showed that the phosphorylation reaction occurred chemoselectively at the 7-OH of chrysin. This conclusion was confirmed by ^{13}C NMR of compound **5a–e**. For example, ^{13}C NMR of compound **5a** showed 7-C at δ 155.50 (J = 6.2), 6-C at δ 103.64 (J = 6.0) and 8-C at δ 99.03 (J = 4.4) were split into doublet by the nearby phosphorus atom, respectively. In contrast, 5-C at δ 161.97 and 10-C at δ 108.18 were not split. These facts show that the phosphorylation reaction has proceeded chemoselectively at the 7-OH of chrysin. This may be due to the hydrogen bonding of 5-OH group with carbonyl group. Hydrogen bonding makes the phosphorylation reaction at 5-OH difficult.

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Experimental

IR spectra were recorded on a Shimadazu IR-408. ^{1}H, ^{13}C, ^{31}P NMR spectra were recorded on a Bruker Avance DPX spectrometer operating at 400.13, 100.62 and 161.98 MHz, respectively, with ^{13}C and ^{31}P spectra being recorded proton-decoupled. All NMR spectra were recorded in $CDCl_3$ at room temperature (20 ± 3 °C). ^{1}H and ^{13}C chemical shifts are quoted in parts per million downfield from TMS. ^{31}P chemical shifts are quoted in parts per million relative to an external 85% H_3PO_4 standard. J values refer to coupling constants, and signal splitting patterns are described as singlet(s), doublet(d), triplet(t), quartet(q), multiplet(m). Elemental analyses were carried out with a Flash EA 1112 elemental analyzer. TLC was performed on silica gel plates and preparative chromatography on columns of silica gel (200–300 mesh). All the reagents used were AR grade.

Preparation of di-(2-chloroethyl)-phosphoramidic dichloride **2***; general procedure*

A suspension of bis-(*β*-chloroethyl)-amine hydrochloride (40 g, 0.23 mmol) in distilled phosphorus oxychloride (174 mL, 1.78 mol, b.p. 105.5–107.5 °C), was refluxed for 24 h until complete solution resulted. The excess phosphorus oxychloride was removed by distillation and the residue was crystallised from acetone–petroleum ether. Di-(2-chloroethyl)-phosphoramidic dichloride (**2**) was obtained as white crystal (45 g, yield: 77.5%) m.p. 55–56 °C (lit. [17]: yield: 80%, m.p. 54–56 °C).

Preparation of aryl-di-(2-chloroethyl) phosphoramidic chloride **3***; general procedure*

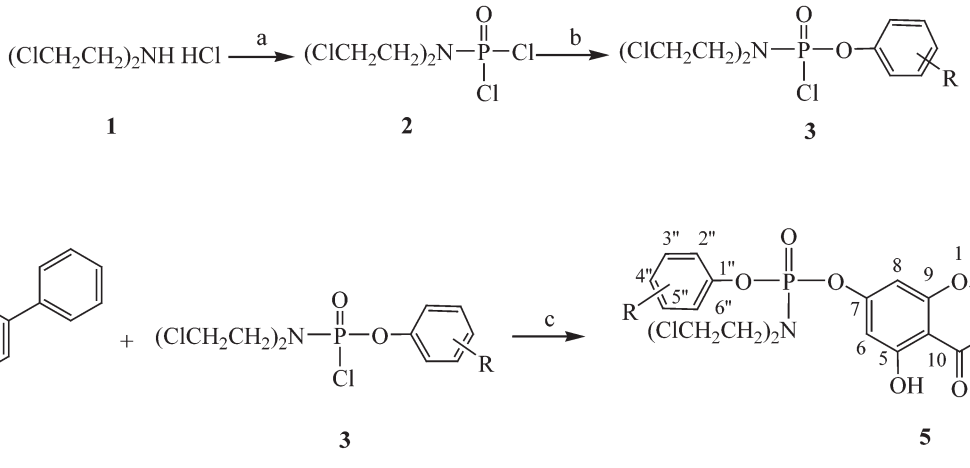
A solution of the dichlorophosphamide (**2**) (3 g, 11.58 mmol) and appropriate phenol (11.58 mmol) in dry toluene (30 mL) was stirred at 0 °C for 20 min, and then added dropwise a solution of dry triethylamine (2.4 mL, 17.37 mmol) and dry toluene (5 mL). The reaction mixture was stirred at room temperature for 1 h, and then was refluxed for 8 h. After cooling, the mixture was filtered to remove triethylamine hydrochloride. The toluene was distilled off under vacuum and the residue was purified by flash chromatography on silica gel and eluted using a mixture of dichloromethane and ethyl acetate (10:1 v/v) as the eluent. The product (**3**) was obtained as a light yellow oil.

3a: 2.7 g, yield: 75%, ^{1}H NMR ($CDCl_3$) δ: 7.40–7.18 (m, 5H, Ph-H), 3.55–3.27 (m, 8H, 2 CH_2CH_2Cl); ^{31}P NMR ($CDCl_3$) δ: –7.31.

3b: 3.5 g, yield: 90%, ^{1}H NMR ($CDCl_3$) δ: 7.28–6.97 (m, 4H, Ph-H), 3.55–3.26 (m, 8H, 2 CH_2CH_2Cl), 2.37 (s, 3H, CH_3); ^{31}P NMR ($CDCl_3$) δ: –6.61.

3c: 3.4 g, yield: 84%, ^{1}H NMR ($CDCl_3$) δ: 7.30–6.77 (m, 4H, Ph-H), 3.56–3.29 (m, 8H, 2 CH_2CH_2Cl), 3.81 (s, 3H, OCH_3); ^{31}P NMR ($CDCl_3$) δ: –6.71.

3d: 3.5 g, yield: 87%, ^{1}H NMR ($CDCl_3$) δ: 7.19–6.85 (m, 4H, Ph-H), 3.55–3.26 (m, 8H, 2 CH_2CH_2Cl), 3.78 (s, 3H, OCH_3); ^{31}P NMR ($CDCl_3$) δ: –6.05.



Conditions and reagent: (a) $POCl_3$; (b) $PhCH_3$, Et_3N, RC_6H_4OH (R=H, *m*-CH_3, *m*-OCH_3, *p*-OCH_3, *p*-Cl);

(c) THF, Et_3N, Chrysin

Entry	R		Yield /%
	3″	**4″**	
5a	H	H	52
5b	CH_3	H	67
5c	OCH_3	H	48
5d	H	OCH_3	58
5e	H	Cl	56

Scheme 1

3e: 3.1 g, yield: 70%, ^{1}H NMR ($CDCl_3$) δ: 7.36–7.15 (m, 4H, Ph-H), 3.58–3.33 (m, 8H, 2 CH_2CH_2Cl); ^{31}P NMR ($CDCl_3$) δ: –6.58. These data are consistent with the reported data.[18,19]

Preparation of chrysin-7-yl aryl N-bis(2-chloroethyl) phosphoramidates **5***;general procedure*

Triethylamine (0.3 mL, 2.0 mmol) was added to a solution appropriate aryl-di-(2-chloroethyl) phosphoramidic chloride **(3)** (2.0 mmol) and chrysin **(4)** (0.51 g, 2.0 mmol) in dry tetrahydrofuran. The reaction mixture was stirred and refluxed for 4–5 h. After cooling, the mixture was filtered to remove trethylamine hydrochloride. Tetrahydrofuran was distilled off under vacuum. The residue was purified with column chromatography on silica gel and eluted with dichloromethane: acetone (30:1, v/v), to give the title compounds (**5a–e**).

Chrysin-7-yl phenyl N-bis(2-chloroethyl) phosphoramidates (**5a**): Yellow sticky liquid. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ: 12.81 (s, 1H, 5-OH), 7.82 (d, 2H, J = 7.0 Hz, 2′, 6′-H), 7.53–7.45 (m, 3H, 3′, 4′, 5′-H), 7.39–7.33 (m, 2H, 3″, 5″-H), 7.31–7.27 (m, 2H, 2″, 6″-H), 7.25–7.15 (m, 1H, 4″-H), 7.00 (d, 1H, J = 1.7 Hz, 8-H), 6.68 (s, 1H, 3-H), 6.66 (d, 1H, J = 1.7 Hz, H-6), 3.67–3.55 (m, 8H, $N(CH_2CH_2Cl)_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ: 182.4 (4-C), 164.4 (2-C), 162.0 (5-C), 156.7 (9-C), 155.5 (d, J = 6.2 Hz, 7-C), 150.0 (d, J = 6.8 Hz, 1″-C), 132.0 (4′-C), 130.5 (1′-C), 129.7 (3″, 5″-C), 128.9 (3′, 5′-C), 126.1 (2′, 6′-C), 125.4 (4″-C), 119.8 (d, J = 5.0 Hz, 2″, 6″-C), 108.2 (10-C), 105.8 (3-C), 103.6 (d, J = 6.0 Hz, 6-C), 99.0 (d, J = 4.4 Hz, 8-C), 49.2 (d, J = 4.0 Hz, N-CH_2), 41.6 (Cl-CH_2). ^{31}P NMR (161.98 MHz, $CDCl_3$) δ: –0.37. IR(KBr) ν(cm^{-1}): 3454 (OH), 2961 (CH_2), 1657 (C=O), 1281 (P=O), 1030 (P-O-C). Anal. Calcd for $C_{25}H_{22}Cl_2NO_6P$: C, 56.20; H, 4.15; N, 2.62. Found: C, 56.04; H, 4.13; N, 2.63%.

Chrysin-7-yl 3′-(methyl)phenyl N-bis(2-chloroethyl) phosphoramidates (**5b**): Light yellow sticky liquid. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ: 12.78 (s, 1H, 5-OH), 7.87 (d, 2H, J = 7.8 Hz, 2′6′-H), 7.58–7.49 (m, 3H, 3′, 4′, 5′-H), 7.24 (t, 1H, J = 8.1 Hz, 5″-H), 7.09–7.06 (m, 2H, 2″, 8-H), 7.03–7.01 (m, 2H, 4″, 6″-H), 6.72 (s, 1H, 3-H), 6.66 (d, 1H, J = 1.7 Hz, 6-H), 7.06 (s, 1H, 2″-H), 3.67–3.56 (m, 8H, $N(CH_2CH_2Cl)_2$), 2.36 (s, 3H, 3″-CH_3). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ: 182.6 (4-C), 164.7 (2-C), 162.2 (5-C), 157.0 (9-C), 155.7 (d, J = 6.3 Hz, 7-C), 150.1 (d, J = 5.9 Hz, 1″-C), 140.3 (3″-C), 132.1 (4′-C), 130.8 (1′-C), 129.6 (5″-C), 129.1 (3′, 5′-C), 126.3 (2′, 6′, 4″-C), 120.5 (d, J = 4.9 Hz, 2″-C), 116.8 (d, J = 3.7 Hz, 6″-C), 108.4 (10-C), 106.0 (3-C), 103.8 (d, J = 6.4 Hz, 6-C), 99.2 (d, J = 4.5 Hz, 8-C), 49.5 (d, J = 4.1 Hz, N-CH_2), 41.7 (Cl-CH_2), 21.3 (3″-CH_3). ^{31}P NMR (161.98 MHz, $CDCl_3$) δ: –0.38. IR(KBr) ν(cm^{-1}): 3437 (OH), 2922 (CH_2), 1654 (C=O), 1277 (P=O), 1030 (P-O-C). Anal. Calcd for $C_{26}H_{24}Cl_2NO_6P$: C, 56.95; H, 4.41; N, 2.55. Found: C, 56.76; H, 4.39; N, 2.57%.

Chrysin-7-yl 3′-(methoxy)phenyl N-bis(2-chloroethyl) phosphoramidates (**5c**): Yellow sticky liquid. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ: 12.79 (s, 1H, 5-OH), 7.85 (d, 2H, J = 7.1 Hz, 2′, 6′-H), 7.54–7.47 (m, 3H, 3′, 4′, 5′-H), 7.28–7.24 (m, 1H, 5″-H), 7.02 (s, 1H, 8-H), 6.88 (d, 1H, J = 8.6 Hz, 4″-H), 6.83 (s, 1H, 2″-H), 6.76 (d, J = 8.3 Hz, 1H, 6″-H), 6.70 (s, 1H, 3-H), 6.67 (s, 1H, 6-H), 3.78 (s, 3H, 3″-OCH_3), 3.68–3.57 (m, 8H, $N(CH_2CH_2Cl)_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ: 182.4 (4-C), 164.4 (2-C), 162.0 (5-C), 160.6 (3″-C), 156.8 (9-C),

155.5 (d, J = 6.2 Hz, 7-C), 150.9 (d, J = 6.7 Hz, 1″-C), 132.0 (4′-C), 130.5 (1′-C), 130.1 (5″-C), 128.9 (3′, 5′-C), 126.1 (2′, 6′-C), 111.8 (d, J = 4.5 Hz, 6″-C), 111.2 (4″-C), 108.2 (10-C), 105.9 (d, J = 5.1 Hz, 2″-C), 105.7 (3-C), 103.7 (d, J = 5.9 Hz, 6-C), 99.1 (d, J = 4.2 Hz, 8-C), 55.3 (3″-OCH_3), 49.3 (d, J = 3.7 Hz, N-CH_2), 41.6 (Cl–CH_2). ^{31}P NMR (161.98 MHz, $CDCl_3$) δ: –0.40. IR(KBr) ν(cm^{-1}): 3439 (OH), 2922, 2853 (CH_3, CH_2), 1654 (C=O), 1278 (P=O), 1033 (P–O–C). Anal. Calcd for $C_{26}H_{24}Cl_2NO_7P$: C, 55.33; H, 4.29; N, 2.48. Found: C, 55.16; H, 4.26; N, 2.49%.

Chrysin-7-yl 4′-(methoxy)phenyl N-bis(2-chloroethyl) phosphoramidates (**5d**): Light yellow sticky liquid. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ: 12.78 (s, 1H, 5-OH), 7.87 (d, 2H, J = 8.0 Hz, 2′, 6′-H), 7.55–7.48 (m, 3H, 3′, 4′, 5′-H), 7.19 (d d, 2H, J = 9.1, 0.9 Hz, 2″, 6″-H), 7.00 (d, 1H, J = 1.7 Hz, 8-H), 6.89–6.85 (m, 2H, 3″, 5″-H), 6.70 (s, 1H, 3-H), 6.65 (d, 1H, J = 1.9 Hz, 6-H), 3.78 (s, 3H, 4″-OCH_3), 3.67–3.55 (m, 8H, N(CH_2CH_2Cl)$_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ: 182.6 (4-C), 164.7 (2-C), 162.1 (5-C), 157.0 (4″-C), 156.9 (9-C), 155.7 (d, J = 6.3 Hz, 7-C), 143.6 (d, J = 7.1 Hz, 1″-C), 132.1 (4′-C), 130.7 (1′-C), 129.0 (3′, 5′-C), 126.3 (2′, 6′-C), 120.8 (d, J = 4.6 Hz, 2″, 6″-C), 114.7 (3″, 5″-C), 108.3 (10-C), 105.9 (3-C), 103.8 (d, J = 6.2 Hz, 6-C), 99.1 (d, J = 4.4 Hz, 8-C), 55.5 (4″-OCH_3), 49.4 (d, J = 3.9 Hz, N-CH_2), 41.7 (Cl-CH_2). ^{31}P NMR (161.98 MHz, $CDCl_3$) δ: 0.15. IR(KBr) ν(cm^{-1}): 3443 (OH), 2924, 2849 (CH_3, CH_2), 1655 (C=O), 1275 (P=O), 1031 (P-O-C). Anal. Calcd for $C_{26}H_{24}Cl_2NO_7P$: C, 55.33; H, 4.29; N, 2.48. Found: C, 55.18; H, 4.27; N, 2.46%.

Chrysin-7-yl 4′-(chloro)phenyl N-bis(2-chloroethyl) phosphoramidates (**5e**): Yellow sticky liquid. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ: 12.79 (s, 1H, 5-OH), 7.87 (d, 2H, J = 8.0 Hz, 2′, 6′-H), 7.56–7.50 (m, 3H, 3′, 4′, 5′-H), 7.33 (d, 2H, J = 8.8 Hz, 3″, 5″-H), 7.22 (d d, 2H, J = 8.1, 0.7 Hz, 2″, 6″-H), 6.99 (d, 1H, J = 1.9 Hz, 8-H), 6.72 (s, 1H, 3-H), 6.65 (d, 1H, J = 1.9 Hz, 6-H), 3.66–3.59 (m, 8H, N(CH_2CH_2Cl)$_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ: 183.1 (4-C), 165.2 (2-C), 162.7 (5-C), 157.5 (9-C), 156.0 (d, J = 6.4 Hz, 7-C), 149.2 (d, J = 6.8 Hz, 1″-C), 132.6 (4′-C), 131.2 (4″-C), 130.4 (3″, 5″-C), 130.3 (1′-C), 129.6 (3′, 5′-C), 126.9 (2′, 6′-C), 121.8 (d, J = 4.9 Hz, 2″, 6″-C), 109.0 (10-C), 106.5 (3-C), 104.2 (d, J = 6.2 Hz, 6-C), 99.6 (d, J = 4.6 Hz, 8-C), 49.9 (d, J = 4.0 Hz, N-CH_2), 42.2 (C-CH_2). ^{31}P NMR (161.98 MHz, $CDCl_3$) δ: –0.27. IR(KBr) ν(cm^{-1}): 3431 (OH), 2962, 2930 (CH_2), 1645 (C=O), 1265 (P=O), 1037 (P-O-C). Anal. Calcd for $C_{25}H_{21}Cl_3NO_6P$: C, 52.79; H, 3.72; N, 2.46. Found: C, 52.63; H, 3.70; N, 2.44%.

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