SHORT PAPER

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The preparation of novel L-*iso*-glutamine derivatives as potential antitumor agents†

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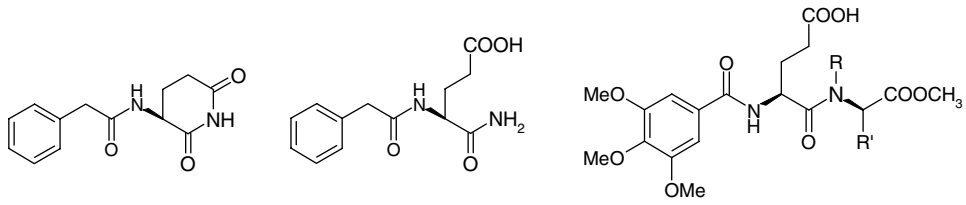
A series of novel L-*iso*-glutamine derivatives were prepared by condensing of compound **4** with various amino acid methyl esters. These compounds have not been reported in literature, and their chemical structures were confirmed by ESI-MS, IR and NMR.

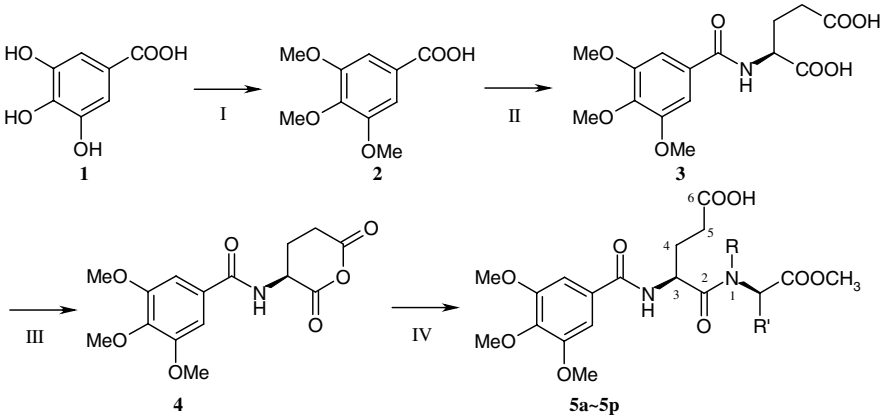
Keywords: preparation, L-*iso*-glutamine, derivatives

Antineoplastons are naturally occurring peptides and amino acid derivatives that were originally isolated from human urine.[1,2] They possess a broad-spectrum of antitumor activity and seem to be much safer and specific than many of the available chemotherapeutic agents.[1,2] Their selectivity is likely entailed to preferential interaction with cellular components or signaling mechanisms that predominate in cancer cells.[3] Antineoplastons A_{10} was the first characterised and synthesised in 1980. Because of its low bioavailability *in vivo*, its active metabolite, N2-phenylacetyl L-*iso*-glutamine, is an attractive synthetic target because of its low toxicity and adverse effects.[4,5] Structure-activity relationships showed that substitution of the two nitrogen atoms of L-*iso*-glutamine with lipophilic groups would improve their antitumor activity.[6] Here we report the preparation of a series of novel N-acyl L-*iso*-glutamine derivatives which were condensed with different amino acid methyl esters, in order to find new antitumor agents (Scheme 1).

In our synthesis, the acylated L-glutamic acid (**3**) was transformed into its corresponding anhydride (**4**) in Ac_2O. Only one paper has reported the reaction with compound **4** as reactant.[7] In that case, the cyclic anhydride was reacted with di-n-propylamine using water as a solvent at 5–10°C for 3h, and with the yield only 10.5%. Here the cyclic anhydride was attacked by free NH_2 groups which were from various L-amino acid methyl ester hydrochlorides with anhydrous CH_2Cl_2 as solvent at room temperature within 2h, and with the yields more than 40%. Aprotic polar solvents such as CH_2Cl_2 will be preferable for this reaction. The hydrochloride was neutralised by Et_3N in the reaction. The synthetic route is outlined in Scheme 2.



Scheme 1



Scheme 2 *Reagents and conditions:* (I) Me_2SO_4, NaOH/HCl; (II) $SOCl_2$/L-Glu-Na, Na_2CO_3/HCl; (III) Ac_2O/60°C; (IV) different L-amino acid methyl ester hydrochlorides, anhydrous CH_2Cl_2/Et_3N.

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

Starting from 3, 4, 5-trihydroxybenzoic acid (**1**) via a three-step reaction, the key intermediate N-(3, 4, 5-trimethoxy benzoyl)-glutamic acid anhydride (**4**) was obtained according to the literature.[8,9]

All the L-amino acid methyl hydrochlorides were commercially available, and some of them were pre-protected with Bzl and Cbz protective groups. The derivatives of natural amino acid methyl esters such as *p*-Cl-Phe and *p*-F-Phe were also used in our synthesis for molecular diversity and for structure-activity relationships study. It required a longer time to finish the reactions when longer side chains (R') and heterocyclic groups were used.

When compound **4** was treated with various L-amino acid methyl esters, the anhydride ring might be opened from two sides to get two different final products in each case. In fact, we only obtained one from products which were L-*iso*-glutamine derivatives characterised by the observation of cross-peaks in HMBC spectrum. As compound **5a** an example, in its HMBC spectrum cross-peaks between H-3 (δ 4.47) and C-2 (δ 171.9), and H-5 (δ 2.34) and C-6 (δ 173.9) respectively, indicated that the amino moiety was attached at C-2 position of L-*iso*-glutamine. In summary, 16 N-acyl L-*iso*-glutamine derivatives (**5a**–**5p**) were prepared and their chemical structures were confirmed by ESI-MS, IR and NMR. Their antitumor activity will be carried out further.

Experimental

General procedure for the preparation of compounds **5a–5p**: Compound **4** (1mmol) and various L-amino acid methyl ester hydrochlorides (1.1mmol) were dissolved in anhydrous CH_2Cl_2 10ml, and Et_3N 0.5ml was added. The mixture were stirred at room temperature and monitored by TLC. When finished, the organic layer was washed with distilled water, and then evaporated *in vacuo*. The final product was purified by flash column chromatography. The reaction conditions and results were listed in Table 1. All of their chemical structures were confirmed by ESI-MS, IR and ^1H NMR (Table 2).

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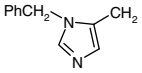
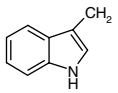
Table 1 Reaction conditions and results

Compd.	R	R′	Time (min)	M.p./°C	Yield/%*
5a	H	H	20	169~172	74
5b	H	CH_3	30	153~155	65
5c	H	$(CH_3)_2CHCH_2$	50	98~100	67
5d	H	$CH_3CH_2CH_2CH_2$	40	99~101	61
5e	H	$CH_3CH_2CH_2$	70	140~142	47
5f	H	$C_6H_5CH_2$	80	141~144	56
5g	H	*p*-Cl-$C_6H_4CH_2$	70	143~145	67
5h	H	*p*-F-$C_6H_4CH_2$	80	107~110	61
5i	H	$HOCH_2$	40	85~87	60
5j	H	*p*-HO-$C_6H_4CH_2$	70	87~89	49
5k	H	$C_6H_5CH_2SCH_2$	50	77~79	54
5l	H	$CH_3SCH_2CH_2$	60	124~127	59
5m	CH_3	H	80	127~129	60
5n	H	$C_6H_5CH_2OCONHCH_2CH_2CH_2$	60	108~112	43
5o	H		110	74~76	45
5p	H		120	120~122	42

*Calculated by the last step reaction.

Table 2 ESI-MS, IR and ^{1}H NMR data of compound **5a–5n**

Compd	ESI-MS (*m/z*)	IR (KBr, cm^{-1})	^{1}H NMR (600MHz, DMSO-d_6, δ, ppm)	$[a]^{20}_D$ (C=2,CH_2Cl_2)	Elemental analysis					
					Calculated			Found		
					C%	H%	N%	C%	H%	N%
5a	411.8 $[M-H]^+$	3325.1,2939.6, 1745.7, 1714.5, 1637.8,1584.2, 1131.3	8.45(d,1H,*J*=7.5Hz,NH),8.38(t,1H,*J*=5.4Hz,NH),7.23(s,2H,Ar-H),4.47(m,1H,CH),3.88(d,2H, *J*=5.4Hz,CH_2),3.83(s,6H,2-OCH_3),3.69(s,3H,OCH_3),3.62(s,3H,$COOCH_3$),2.34(t,2H,*J*=8.0Hz, CH_2), 2.06,1.92(2m,2H,CH_2)	+23.6	52.42	5.87	6.79	52.60	5.96	6.69
5b	425.1 $[M-H]^+$	3304.8,2933.5,1751.1, 1719.9,1647.7,1583.8, 1537.6,1129.9	8.74(d,1H,*J*=7.8Hz,NH),8.33(d,1H,*J*=6.0Hz,NH),7.24(s,2H,Ar-H),4.36(m,1H,CH),4.23(m,1H, CH) 3.85(s,6H,2-OCH_3),3.73(s,3H,OCH_3),3.60(s,3H,$COOCH_3$),2.23(t,2H,*J*=7.8Hz,CH_2), 2.06, 1.83 (2m,2H,CH_2),1.23(d,3H, *J*=7.2Hz,CH_3)	+25.7	53.52	6.15	6.57	53.38	6.42	6.74
5c	467.1 $[M-H]^+$	3300.4,2959.0,1749.2, 1718.9,1645.5,1584.2, 1128.8	7.75(d,1H,*J*=5.4Hz,NH),7.26(s,1H,Ar-H),7.20(s,1H,Ar-H),6.44(d,1H,*J*=7.5Hz,NH),4.95(m,1H, CH), 4.57(m,1H,CH),3.92(s,6H,2-OCH_3),3.81(s,3H,OCH_3),3.70(s,3H,$COOCH_3$),2.31(t,2H,*J*=7.4Hz,CH_2), 2.06(m,2H,CH_2),1.89(m,2H,CH_2),1.23(m,1H,CH),0.88(d,6H,*J*=7.2Hz,2-CH_3)*	+33.4	56.40	6.88	5.98	56.26	6.95	5.79
5d	467.1 $[M-H]^+$	3321.4,3256.9,2956.2, 1724.1,1647.6,1584.3, 1130.5	7.82(d,1H,*J*=7.6Hz,NH),7.15(s,1H,Ar-H),7.12(s,1H,Ar-H),6.44(d,1H,*J*=7.2Hz,NH), 4.93(m,1H, CH),4.60(m,1H,CH),3.92(s,6H,2-CH_3),3.80(s,3H,OCH_3),3.68(s,3H,$COOCH_3$)2.28 (t,2H,*J*=7.2Hz,CH_2),2.06(m,2H,CH_2),1.92(m,2H,CH_2),1.29(br,4H,2-CH_2),0.87(t,3H,*J*=7.8Hz,CH_3)*	+30.5	56.40	6.88	5.98	56.60	5.99	5.89
5e	453.1 $[M-H]^+$	3318.3,3256.6,2958.9, 1747.8,1720.7,1646.1, 1583.8,1535.6,1129.9	8.72(d,1H,*J*=7.7Hz,NH),8.26(d,1H,*J*=6.8Hz,NH),7.24(s,2H,Ar-H),4.36(m,1H,CH),4.24(m,1H, CH), 3.84(s,6H,2-OCH_3),3.71(s,3H,OCH_3),3.60(s,3H,$COOCH_3$),2.26(t,2H,*J*=7.4Hz,CH_2),2.06,1.83 (2m,2H,CH_2),1.55(m,2H,CH_2),1.26(m,2H,CH_2),0.84(t,3H,*J*=7.2Hz,CH_3)	+19.6	55.50	6.65	6.16	55.38	6.49	6.40
5f	501.1 $[M-H]^+$	3416.0,3303.7,2939.9, 1743.7,1718.8,1646.4, 1584.1,1536.7,1128.9	8.69(d,1H,*J*=4.3Hz,NH),8.38(d,1H,*J*=6.8Hz,NH),7.24(m,2H,Ar-H),7.18(m,5H,Ar-H),4.44(m,1H,CH), 4.34(m,1H,CH),3.85(s,6H,2-OCH_3),3.70(s,3H,OCH_3),3.57(s,3H,$COOCH_3$),2.18(t, 2H,*J*=7.6Hz,CH_2), 2.11(d,2H,*J*=7.4Hz,CH_2),1.98,1.76(2m,2H,CH_2)	+28.7	59.75	6.02	5.57	59.49	6.32	5.64
5g	535.1 $[M-H]^+$	3300.1,2942.3,1742.9, 1718.6,1647.0,1584.0, 1536.9,1129.0	7.74(d,1H,*J*=6.4Hz,NH),7.29(s,2H,Ar-H),7.22(m,2H,Ar-H),7.10(m,2H,Ar-H),6.54(d,1H,*J*= 7.8Hz,NH), 4.79(m,1H,CH),4.55(m,1H,CH),3.93(s,6H,2-OCH_3),3.89(s,3H,OCH_3),3.72(s,3H,$COOCH_3$), 3.12(d,2H,*J*=7.6Hz,CH_2),2.31(t,2H,*J*=7.6Hz,CH_2),1.95(m,2H,CH_2)*	+35.8	55.92	5.44	5.22	55.88	5.62	5.41
5h	519.1 $[M-H]^+$	3296.3,2942.1,1743.0, 1720.4,1648.6,1584.2, 1537.3,1128.9	7.78(d,1H,*J*=6.6Hz,NH),7.26(s,2H,Ar-H),7.12(m,2H,Ar-H),6.95(m,2H,Ar-H),6.47(d,1H,*J*=8.0Hz NH), 4.78(m,1H,CH),4.57(m,1H,CH),3.93(s,6H,2-OCH_3),3.86(s,3H,OCH_3),3.68(s,3H,$COOCH_3$) 3.10(d,2H,*J*=8.2Hz,CH_2),2.27(t,2H,*J*=7.2Hz,CH_2),1.89(m,2H,CH_2)*	+34.0	57.69	5.62	5.38	57.49	5.70	5.60
5i	441.1 $[M-H]^+$	3266.1,2941.7,1744.2, 1632.2,1584.3,1129.3	8.76(d,1H,*J*=6.5Hz,NH),8.26(d,1H,*J*=6.8Hz,NH),7.24(m,2H,Ar-H),5.02(m,1H,CH),4.33(m,1H, CH), 3.82(s,6H,2-OCH_3),3.71(s,3H,OCH_3),3.61(s,3H,$COOCH_3$),3.05(d,2H,*J*=5.5Hz,CH_2),2.28 (t,2H,*J*=6.0Hz,CH_2),2.05,1.83(2m,2H,CH_2)	+16.8	51.58	5.92	6.33	51.74	5.86	6.08
5j	517.1 $[M-H]^+$	3355.2,3300.7,2952.3, 1744.1,1647.7,1585.9, 1498.7,1257.7,1126.5	8.72(d,1H,*J*=7.0Hz,NH),8.33(d,1H,*J*=6.4Hz,NH),7.23(d,2H,*J*=3.4Hz,Ar-H),6.95(m,2H,Ar-H), 6.64(m,2H,Ar-H),4.34(m,1H,CH),3.83(s,6H,2-OCH_3),3.70(s,3H,OCH_3),3.56(s,3H,$COOCH_3$), 3.49(m,1H,CH),2.20(t,2H,*J*=7.5Hz,CH_2),2.11(d,2H,*J*=6.8Hz,CH_2),2.00,1.78(2m,2H,CH_2)	+19.8	57.91	5.83	5.40	57.68	5.92	5.42
5k	547.1 $[M-H]^+$	3272.9,2940.6,1746.5, 1722.0,1648.5,1584.0, 1128.4	8.77(d,1H,*J*=6.8Hz,NH),8.46(d,1H,*J*=7.2Hz,NH),7.34(d,2H,*J*=4.1Hz,Ar-H),7.30(m,5H,Ar-H), 4.47 (m,1H,CH),4.20(m,1H,CH),3.83(s,6H,2-OCH_3),3.75(s,3H,OCH_3),3.60(s,3H,$COOCH_3$),3.32 (s,2H,CH_2),3.06(d,2H,*J*=6.8Hz,CH_2),2.26(t,2H,*J*=7.8Hz,CH_2),2.07,1.84(2m,2H,CH_2)	+20.5	56.92	5.88	5.11	56.68	5.93	5.40
5l	485.1 $[M-H]^+$	3289.3,2941.8,1750.9, 1717.8,1647.2,1584.5, 1129.7	8.72(d,1H,*J*=5.7Hz,NH),8.32(d,1H,*J*=7.3Hz,NH),7.24(s,2H,Ar-H),4.47(m,1H,CH),4.37(m,1H, CH), 3.78(s,6H,2-OCH_3),3.70(s,3H,OCH_3),3.61(s,3H,$COOCH_3$),2.26(t,2H,*J*=7.8Hz,CH_2),2.13 (t,2H, *J*=7.4Hz,CH_2),2.06(m,4H,2-CH_2),1.83(s,3H,CH_3)	+17.6	51.84	6.21	5.76	51.68	6.50	5.84
5m	425.1 $[M-H]^+$	3295.9,2940.9,1750.8, 1729.9,1646.9,1683.3, 1582.9,1123.5	8.70(d,1H,*J*=6.8Hz,NH),7.22(s,2H,Ar-H),4.38(m,1H,CH),3.87(s,6H,2-OCH_3),3.71(s,3H,OCH_3), 3.62(s,3H,$COOCH_3$),2.99(s,2H,CH_2),2.56(s,3H,N-CH_3),2.12(t,2H,*J*=6.7Hz,CH_2),2.06,1.85 (2m,2H,CH_2)	+14.9	53.52	6.15	6.57	53.28	6.51	6.74
5n	602.1 $[M-H]^+$	3333.2,3276.9,2945.9, 1745.0,1718.6,1688.4, 1584.1,1128.2	8.74(d,1H,*J*=5.6Hz,NH),8.28(d,1H,*J*=6.9Hz,NH),7.34(m,5H,Ar-H),7.24(s,2H,Ar-H), 4.99(s,2H, CH_2), 4.36(m,1H,CH),4.19(m,1H,CH),3.83(s,6H,2-OCH_3),3.71(s,3H,OCH_3),3.59(s,3H,$COOCH_3$)2.96 (t,2H,*J*=5.2Hz,CH_2),2.25(t,2H,*J*=7.8Hz,CH_2),2.06,1.83(2m,2H,CH_2),1.66,1.54(2m,2H,CH_2), 1.45(m,2H,CH_2)	+36.8	57.70	6.18	6.96	57.56	6.41	6.69
5o	581.1 $[M-H]^+$	3241.5,2947.2,1744.7, 1651.9,1584.4,1498.0, 1233.9,1125.7	8.40(d,1H,*J*=5.7Hz,NH),8.27(d,1H,*J*=6.0Hz,NH),7.34(m,3H,Ar-H,=CH),7.52(d,1H,*J*=5.3Hz, Ar-H), 7.22(d,2H,Ar-H),7.12(d,2H,*J*=6.6Hz), 6.67(s,1H,=CH),5.03(m,2H,CH_2),4.74(m,1H,CH),4.59 (m,1H,CH), 3.92(s,3H,O-CH_3),3.91(s,3H,O-CH_3),3.89(s,3H,O-CH_3),3.64(s,1H,CH),3.57(s, 1H,CH), 3.07(m,2H,CH_2),2.53(m,1H,CH),2.46(m,1H,CH),2.27(s,3H,$COOCH_3$)*	+29.6	59.79	5.88	9.62	59.86	5.96	9.90
5p	540.1 $[M-H]^+$	3362.5,2948.5,1739.8, 1652.4,1584.7,1497.0, 1233.2,1126.4	8.14(s,1H,NH),8.09(s,1H,OH),7.91(d,1H,*J*=6.1Hz,NH),7.84(d,1H,*J*=6.3Hz,NH),7.51(t,2H, *J*=8.4Hz,Ar-H),7.16(m,4H,Ar-H),7.08(m,1H,=CH),4.76(m,1H,CH),4.55(m,1H,CH),3.92 (s,6H, 2-CH_3), 3.90(s,3H,CH_3),3.71(s,3H,$COOCH_3$),2.41(m,1H,CH),2.27(m,2H,CH_2), 2.20(m,2H, CH_2),1.96(m,1H,CH)*	+32.4	59.88	5.77	7.76	59.69	5.98	7.49

* $CDCl_3$ as solvent