Journal of Enzyme Inhibition and Medicinal Chemistry, June 2009; 24(3): 763–770

Synthesis of functionalized amino acid derivatives as new pharmacophores for designing anticancer agents

VIVEK KUMAR[1], MUKESH M. MUDGAL[1], NIDHI RANI[1], AMRITA JHA[1], MANU JAGGI[2], ANU T. SINGH[2], VINOD K. SANNA[2], PRATIBHA SINGH[2], PRAMOD K. SHARMA[3], RAGHUVEER IRCHHAIYA[3], & ANAND C. BURMAN[1,2]

[1]*Chemical Research, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad 201 010, UP, India,* [2]*Preclinical Research Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad 201 010, UP, India, and* [3]*Institute of Pharmacy, Bundelkhand University, Jhansi, India*

(*Received 2 April 2008; accepted 23 June 2008*)

Abstract

A new series of functionalized amino acid derivatives *N*-substituted 1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxamide (**1-17**) and 1-*N*-substituted-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide (**18-34**) were synthesized and evaluated for their *in vitro* cytotoxicity against human cancer cell lines. Compound **6** has shown interesting cytotoxicity ($IC_{50} = 5.67\ \mu m$) in ovarian cancer, while compound **10** exhibited promising cytotoxicity in ovarian ($IC_{50} = 6.1\ \mu m$) and oral ($IC_{50} = 4.17\ \mu m$) cancers. These compounds could be of use in designing new anti-cancer agents.

Keywords: *Anticancer, functionalized amino acid, carboxamide*

Introduction

Cancer is a disease of worldwide importance. According to World Health Organization (WHO) report, cancer is causing 7 million deaths every year or 12.5% of deaths worldwide [1,2]. Paclitaxel, a biggest-selling single anticancer drug, was discovered at Research Triangle Institute (RTI), USA in 1967 and brought to the market by BMS in 1993 as Taxol® [3]. During last one decade, a number of small organic molecules such as Imatinib (Gleevec), Gefitinib (Irresa), Erlotinib (Tarceva) and Canertinib have been discovered and reached to the market [4]. However, despite major breakthroughs in different areas of drug discovery, the successful treatment of the cancer still remains a significant challenge in the tewenty first century. The search for newer and safer anticancer agent associated with broader spectrum cytotoxicity is needed.

Designing of new chemical entities as anticancer agents, require simulation of a suitable bioactive pharmacophore. The pharmacophore should not only require potent but must also be safer on normal cell lines than tumor cells. Such types of information are, generally, not projected in publications. Both of the well known anticancer molecules Paclitaxel and Docetaxel have amino acids side chain, based on which we have synthesized these amino acids and tested them separately for their anticancer activity and obtained a few potent molecules which can be further used as a new anticancer pharmacophore or as side chains. The synthesis, *in vitro* cytotoxicity and their structure-activity relationship are presented here.

Materials and methods

All the solvents and reagents were purchased from different companies such as Aldrich, Lancaster, Across

Correspondence: V. Kumar, Chemical Research, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad 201 010, UP, India. Fax: 91 120 4376902. E-mail: kumarv@dabur.com. M. Jaggi, Preclinical Research Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad 201 010, UP, India. Fax: 91 120 4376902. E-mail: jaggim@dabur.com

ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd.
DOI: 10.1080/14756360802362975

& Rankem and were used as supplied. All TLC data (R_f values) were determined on aluminum sheets coated with silica gel 60 F_{254} (Merck). Visualization was achieved with UV light and iodine vapor. Column chromatography was performed using silica gel (100–200 mesh). Proton Magnetic Resonance (PMR) spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Micromass Quattro Micro^{TM} instrument. The purity of the synthesized compounds was determined on a Shimadzu HPLC LC-2010 C HT instrument using gradient system.

Chemistry

Synthesis of functionalized amino acid derivatives (**1–34**) is shown in Scheme 1. Coupling of *4S,5R*-1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxylic acid (**I**) with appropriate amines has been carried out to afford the respective *N*-substituted-1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxamide (**1-17**). The coupling reactions were performed by using either *N,N′*,-dicyclohexyl carbodiimide (DCC) and dimethylaminopyridine (DMAP) or *N*-ethyl-*N′*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) in DCM or DMF, which were used as solvent. The oxazolidine ring of compounds **1-17** was separately opened with 50% TFA/DCM to afford the corresponding 1-*N*-substituted-3-amino-2-hydroxy-3-phenylpropane-1-carboxamides (**18-34**). The functionalized amino acid derivatives (**1-34**) are listed in Table I.

5-N-*Isopropyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(1)***. Isopropyl amine (0.92 g, 15.5 mmol) was added, to a stirred solution of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid (5 g, 15.5 mmol) in dichloromethane (100 mL). The resulting solution was placed in ice bath for 15 min. at 0°C. To this reaction mixture 1-hydroxybezotriazole hydrate (HOBt, 2.10 g, 15.5 mmol) and *N*-methylmorpholine (NMM, 1.57 g, 15.5 mmol) were added. After stirring for 30 min at 0°C, *N*-ethyl-*N′*-3-dimethylaminopropyl carbodiimide hydrochloride (EDCI, 2.9 g, 15.1 mmol) was added and the reaction mixture was maintained at 0°C for 3 h, then stirred for 5 h at rt and left overnight. Water (100 mL) was added to reaction mixture and extracted with dichloromethane (100 mL). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was purified by column chromatography using dichloromethane/methanol as eluent, to provide the pure compound **1**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16-1.30 (m, 15H), 1.70 (s, 3H), 1.78 (s, 3H), 4.06-4.17 (m, 1H), 4.33 (d, 1H, $\mathcal{J} = 5.6$ Hz), 5.09 (bs, 1H), 6.33 (bs, 1H), 7.24-7.34 (m, 5H); MS (ES +) m/z (relative intensity) 363 (M + H) (10), 385 (M + H + Na) (100); HPLC purity = 95.7%.

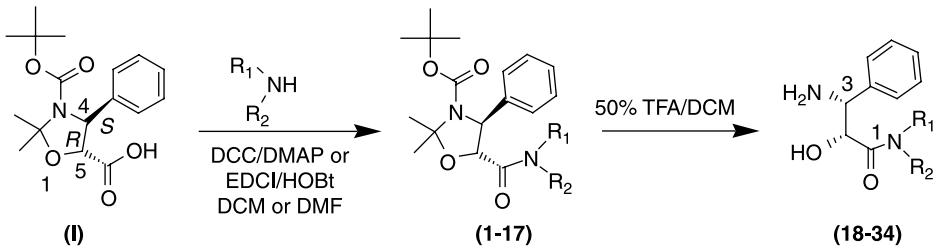
5-N-*Cyclopropyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(2)***. Compound **2** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclopropylamine, similar to procedure of compound **1**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.54-0.56 (m, 2H), 0.80-0.82 (m, 2H), 1.14 (bs, 9H), 1.68 (s, 3H), 1.75 (s, 3H), 2.73-2.76 (m, 1H), 4.32 (d, 1H, $\mathcal{J} = 5.8$ Hz), 5.06 (bs, 1H), 6.57 (bs, 1H), 7.26-7.42 (m, 5H); MS (ES +) m/z (relative intensity) 383 (M + H + Na) (100); HPLC purity = 99.7%.

5-N-*Cyclopentyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(3)***. Compound **3** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclopentyl amine, similar to procedure of compound **1**.

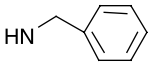
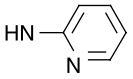
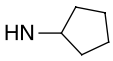
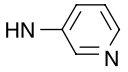
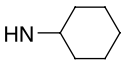
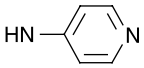
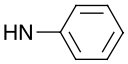
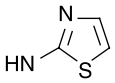
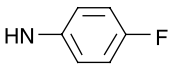
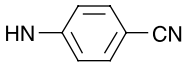
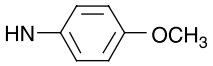
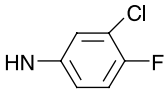
R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.15 (bs, 9H), 1.38-1.46 (m, 1H), 1.63-1.69 (m, 8H), 1.77 (s, 3H), 1.96-2.0 (m, 2H), 4.18 – 4.28 (m, 1H), 4.33 (d, 1H, $\mathcal{J} = 5.5$ Hz), 5.11 (bs, 1H), 6.43 (d, 1H, $\mathcal{J} = 6.9$ Hz), 7.34-7.24 (m, 5H); MS (ES +) m/z (relative intensity) 389 (M + H) (10), 411 (M + H + Na) (100); HPLC purity = 99.5%.

5-N-*Cyclohexyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(4)***. Compound **4** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carbo-



Scheme 1. Synthesis of tested compounds.

Table I. Functionalized amino acid derivatives (**1-34**).

Compound No.	R_1R_2N	Compound No.	R_1R_2N
1, 18		**10, 27**	
2, 19		**11, 28**	
3, 20		**12, 29**	
4, 21		**13, 30**	
5, 22		**14, 31**	
6, 23		**15, 32**	
7, 24		**16, 33**	
8, 25		**17, 34**	
9, 26			

xylic acid with cyclohexyl amine, similar to procedure of compound **1**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.04-1.27 (m, 11H), 1.31-1.44 (m, 3H), 1.60-1.82 (m, 9H), 1.88-1.95 (m, 2H), 3.78-3.81 (m, 1H), 4.32 (d, 1H, $\mathcal{J} = 5.5$ Hz), 5.09 (bs, 1H), 6.41 (d, 1H, $\mathcal{J} = 7.9$ Hz), 7.22-7.36 (m, 5H); MS (ES +) m/z (relative intensity) 403 (M + H) (30), 425 (M + H + Na) (100); HPLC purity = 99.3%.

5-N-*Phenyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(5)***. Compound **5** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with aniline, similar to procedure of compound **1**.

R_f 0.4 (20% EtOAc/Hexane); ^{1}HNMR ($CDCl_3$) δ 1.08 (bs, 9H), 1.72 (s, 3H), 1.76 (s, 3H), 4.44 (d, 1H, $\mathcal{J} = 5.6$ Hz), 5.12 (bs, 1H), 7.07 (t, 1H, $\mathcal{J} = 7.2$ Hz), 7.18-7.30 (m, 7H), 7.49-7.52 (m, 2H), 8.22 (bs, 1H); MS (ES +) m/z (relative intensity) 419 (M + H + Na) (100); HPLC purity = 93.8%.

5-N-*(4′-Fluoro) phenyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(6)***. Compound **6** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-fluoro aniline, similar to procedure of compound **1**.

R_f 0.3 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.78 (s, 3H), 1.82 (s, 3H), 4.51 (d, 1H, $\mathcal{J} = 5.9$ Hz), 5.16 (bs, 1H), 7.01-7.06 (m, 2H), 7.26-7.37 (m, 5H), 7.51-7.56 (m, 2H), 8.28 (bs, 1H); MS (ES +) m/z (relative intensity) 437 (M + H + Na) (100).

5-N-*(4′-cyano) phenyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(7)***. Compound **7** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-cyano aniline, similar to procedure of compound **1**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14-1.17 (m, 9H), 1.78-1.83 (m, 6H), 4.52 (d, 1H, J = 6.0 Hz), 5.15 (bs, 1H), 7.25-7.37 (m, 5H), 7.63-7.73 (m, 5H, 8.45 (bs, 1H); MS (ES-) m/z (relative intensity) 420 (M-H) (100); HPLC purity = 98.3%.

5-N-*(4′-Methoxy) phenyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(8)***. Compound **8** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-methoxy aniline, similar to procedure of compound **1**.

R_f 0.2 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.09 (bs, 9H), 1.71 (s, 3H), 1.75 (s, 3H), 3.78 (s, 3H), 4.43 (d, 1H, J = 5.7 Hz), 5.11 (bs, 1H), 6.79-6.82 (m, 2H), 7.18-7.32 (m, 5H), 7.39-7.42 (m, 2H), 8.11 (bs, 1H); MS (ES +) m/z (relative intensity) 427 (M + H) (10), 449 (M + H + Na) (100); HPLC purity = 95.9%.

5-N-*(3′-Chloro-4′-fluoro) phenyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(9)***. Compound **9** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 3-chloro-4-fluoro aniline, similar to procedure of compound **1**.

R_f 0.5 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.09 (bs, 9H), 1.70 (s, 3H), 1.74 (s, 3H), 4.42 (d, 1H, J = 5.9 Hz), 5.19 (bs, 1H), 7.03 (t, 1H, J = 8.7 Hz), 7.18-7.30 (m, 6H), 7.70-7.72 (m, 1H), 8.20 (bs, 1H); MS (ES +) m/z (relative intensity) 449 (M + H) (5), 471 (M + H + Na) (100); HPLC purity = 96.4%.

5-N-*Benzyl-3*-N′-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(10)***. Compound **10** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with benzylamine, similar to procedure of compound **1**.

R_f 0.5 (15% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14 (bs, 9H), 1.66 (s, 3H), 1.75 (s, 3H), 4.41-4.58 (m, 3H), 5.09 (bs, 1H), 6.84 (bs, 1H), 7.24-7.35 (m, 10H); MS (ES +) m/z (relative intensity) 433 (M + H + Na) (100); HPLC purity = 99.7%.

5-N-*2′-Pyridine-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(11)***. Compound **11** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 2-amino pyridine, similar to procedure of compound **1**.

R_f 0.6 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14 (bs, 9H), 1.79 (s, 3H), 1.82 (s, 3H), 4.51 (d, 1H, J = 5.9 Hz), 5.16 (bs, 1H), 7.06-7.1 (m, 1H), 7.26-7.46 (m, 5H), 7.7-7.75 (m, 1H), 8.24 (d, 1H, J = 8.2 Hz), 8.31-8.32 (m, 1H), 8.91 (bs, 1H); MS (ES +) m/z (relative intensity) 398 (M + H) (10), 420 (M + H + Na) (100); HPLC purity = 95.5%.

5-N-*3′-Pyridine-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(12)***. Compound **12** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 3-amino pyridine, similar to procedure of compound **1**.

R_f 0.7 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.70 (s, 3H), 1.76 (s, 3H), 4.53 (d, 1H, J = 6.0 Hz), 5.17 (bs, 1H), 7.26-7.46 (m, 6H), 8.23 (d, 1H, J = 8.0 Hz), 8.36-8.4 (m, 2H), 8.64 (bs, 1H); MS (ES +) m/z (relative intensity) 398 (M + H) (30), 420 (M + H + Na) (100); HPLC purity = 95.8%.

5-N-*4′-Pyridine-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(13)***. 4-Amino pyridine (1.46 g, 15.5 mmol) and 4-dimethylamino-pyridine (DMAP, 1.90 g, 15.5 mmol) were added to a stirred solution of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid (5 g, 15.5 mmol) in dichloromethane (100 mL). The reaction mixture was placed in ice bath and after 30 min at 0°C, to this *N,N′*-dicyclohexyl carbodiimide (DCC, 3.21 g, 15.5 mmol) was added under nitrogen condition. The reaction mixture was further stirred for 5 h at rt and left overnight. Water (100 mL) was added to reaction mixture and extracted with dichloromethane (100 mL). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was purified by column chromatography using dichloromethane/methanol as eluent. In several cases, solid was appeared during the addition of water in the reaction mixture. It was filtered, washed with water, dried and purified, as described above, to provide the pure compound.

R_f 0.7 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.17 (bs, 9H), 1.78 (s, 3H), 1.83 (s, 3H), 4.51 (d, 1H, J = 6.0 Hz), 5.16 (bs, 1H), 7.26-7.38 (m, 5H), 7.52-7.54 (m, 2H), 8.41 (bs, 1H), 8.53-8.55 (m, 2H); MS (ES +) m/z (relative intensity) 398 (M + H) (100), 420 (M + H + Na) (10); HPLC purity = 92.8%.

5-N-*2′-Thiazole-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(14)***. Compound **14** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 2-amino thiazole, similar to procedure of compound **1**.

R_f 0.6 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.75 (s, 3H), 1.81 (s, 3H), 4.60 (d, 1H, J = 5.9 Hz), 5.19 (bs, 1H), 7.03 (d, 1H, J = 3.5 Hz), 7.27-7.38 (m, 5H), 7.49 (d, 1H, J = 3.5 Hz), 9.92 (bs, 1H); MS (ES +) m/z (relative intensity) 404 (M + H) (5), 426 (M + H + Na) (100); HPLC purity = 98.3%.

5-N-*Piperidine-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(15)***. Compound **15** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with piperidine, similar to procedure of compound **1**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.13 (bs, 9H), 1.54-1.63 (m, 9H), 1.76 (s, 3H), 3.25-3.38 (m, 2H), 3.48-3.54 (m, 1H), 3.74-3.78 (m, 1H), 4.54 (d, 1H, J = 5.0 Hz), 5.53 (bs, 1H), 7.24-7.36 (m, 5H); MS (ES +) m/z (relative intensity) 389 (M + H) (10), 411 (M + H + Na) (100); HPLC purity = 99%.

5-N-*Morpholine-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(16)***. Compound **16** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with morpholine, similar to procedure of compound **1**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.07-1.37 (m, 9H), 1.51 (s, 3H), 1.67 (s, 3H), 3.39-3.52 (m, 8H), 4.76 (d, 1H, J = 4.6 Hz), 5.32 (bs, 1H), 7.26-7.37 (m, 5H); MS (ES +) m/z (relative intensity) 391 (M + H) (5), 413 (M + H + Na) (100); HPLC purity = 99.6%.

5-N-*Pyrrolodine-3*-N′-tert-*butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(17)***. Compound **17** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with pyrrolidine, similar to procedure of compound **13**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.11 (bs, 9H), 1.62 (s, 3H), 1.66 (s, 3H), 1.76-1.91 (m, 4H), 3.19-3.21 (m, 1H), 3.46-3.5 (m, 2H), 3.62-3.64 (m, 1H), 4.44 (d, 1H, J = 6.0 Hz), 5.41 (bs, 1H), 7.24-7.33 (m, 5H); MS (ES +) m/z (relative intensity) 397 (M + H + Na) (100), HPLC purity = 94.6%.

1-N-*Isopropyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(18)***. To 5-*N*-Isopropyl-3-*N′-tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide (**1**, 5 g, 13.79 mmol), 50% TFA/DCM (50 mL) was added at 0°C. Reaction mixture was stirred for 4 h at rt and then left overnight. Aqueous $NaHCO_3$ saturated solution was then added till neutralization. DCM layer was separated, dried over Na_2SO_4 and evaporated to afford the crude product. The product was further purified by column chromatography using 2% MeOH/DCM as eluent.

R_f 0.2 (15% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.02-1.18 (m, 6H), 2.20 (bs, 3H), 3.99-4.1 (m, 2H), 4.57 (d, 1H, J = 2.7 Hz), 6.62 (d, 1H, J = 7.3 Hz), 7.18-7.64 (m, 5H); MS (ES +) m/z (relative intensity) 223 (M + H) (100), 245 (M + H + Na) (90), HPLC purity = 80.7%.

Compounds **19–34** were prepared in the similar way to compound **18**.

1-N-*Cyclopropyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(19)***. R_f 0.2 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.47-0.50 (m, 2H), 0.75-0.78 (m, 2H), 1.98 (bs, 3H), 2.68-2.75 (m, 1H), 4.03 (d, 1H, J = 2.8 Hz), 4.58 (d, 1H, J = 2.8 Hz), 6.9 (bs, 1H), 7.28-7.45 (m, 5H); MS (ES +) m/z (relative intensity) 221 (M + H) (20), 243 (M + H + Na) (100), HPLC purity = 94.6%.

1-N-*Cyclopentyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(20)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.25-1.39 (m, 2H), 1.59-1.63 (m, 3H), 1.91-2.0 (m, 3H), 4.03 (d, 1H, J = 2.8 Hz), 4.14-4.23 (m, 1H), 4.57 (d, 1H, J = 2.8 Hz), 6.74 (bs, 1H), 7.26-7.41 (m, 5H); MS (ES +) m/z (relative intensity) 249 (M + H) (30), 271 (M + H + Na) (100), HPLC purity = ~ 100%.

1-N-*Cyclohexyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(21)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.05-1.42 (m, 5H), 1.59-1.84 (m, 5H), 2.32 (bs, 2H), 3.76-3.79 (m, 1H), 4.0 (s, 1H), 4.53 (s, 1H), 6.67 (d, 1H, J = 7.1 Hz), 7.26-7.40 (m, 5H); MS (ES +) m/z (relative intensity) 263 (M + H) (60), 285 (M + H + Na) (100), HPLC purity = 94.7%.

1-N-*Phenyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(22)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.90 (bs, 2H), 4.08 (s, 1H), 4.20 (s, 1H), 5.8 (bs, 1H), 7.01-7.06 (m, 1H), 7.17-7.19 (m, 1H), 7.20-7.30 (m, 4H), 7.39 (d, 2H, J = 7.4 Hz), 7.64 (d, 2H, J = 7.9 Hz), 9.63 (bs, 1H); MS (ES +) m/z (relative intensity) 257 (M + H) (5), 279 (M + H + Na) (100), HPLC purity = ~ 100%.

1-N-*(4′-Fluoro) phenyl-3-amino-2-hydroxy-3-phenyl-propane-1-carboxamide* ***(23)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.90 (bs, 2H), 4.07 (s, 1H), 4.20 (s, 1H), 5.75 (bs, 1H), 7.09-7.49 (m, 7H), 7.64-7.69 (m, 2H), 9.72 (bs, 1H); MS (ES +) m/z (relative intensity) 275 (M + H) (25), 297 (M + H + Na) (100), HPLC purity = 95%.

1-N-*(4′-Cyano) phenyl-3-amino-2-hydroxy-3-phenyl-propane-1-carboxamide* ***(24)***. R_f 0.2 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.13 (d, 1H, $\mathcal{J}$ = 3.9 Hz), 4.22 (d, 1H, $\mathcal{J}$ = 3.9 Hz), 7.18-7.40 (m, 5H), 7.73 (d, 2H, $\mathcal{J}$ = 8.6 Hz), 7.85 (d, 2H, $\mathcal{J}$ = 8.6 Hz); MS (ES +) m/z (relative intensity) 282 (M + H) (40), 304 (M + H + Na) (70), HPLC purity = 92.3%.

1-N-*(4′-Methoxy) phenyl-3-amino-2-hydroxy-3-phenyl-propane-1-carboxamide* ***(25)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.88 (bs, 2H), 3.71 (s, 3H), 4.04 (d, 1H, $\mathcal{J}$ = 3.0 Hz), 4.19 (s, 1H), 5.7 (bs, 1H), 6.86 (d, 2H, $\mathcal{J}$ = 8.7 Hz), 7.17-7.40 (m, 5H), 7.52-7.55 (m, 2H), 9.51 (bs, 1H); MS (ES +) m/z (relative intensity) 287 (M + H) (30), 309 (M + H + Na) (100), HPLC purity = 94.2%.

1-N-*(3′-Chloro-4′-fluoro) phenyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(26)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.07 (d, 1H, $\mathcal{J}$ = 3.6 Hz), 4.19 (d, 1H, $\mathcal{J}$ = 3.6 Hz), 7.17-7.39 (m, 6H), 7.58-7.63 (m, 1H), 7.99 (dd, 1H, $\mathcal{J}$ = 2.5, 6.9 Hz); MS (ES +) m/z (relative intensity) 309 (M + H) (40), 331 (M + H + Na) (100), HPLC purity = 90.8%.

1-N-*Benzyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(27)***. R_f 0.5 (15% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 8.23 (bs, 1H), 7.37-7.15 (m, 10H), 5.54 (bs, 1H), 4.27 (d, 2H, $\mathcal{J}$ = 6.0 Hz), 4.14 (d, 1H, $\mathcal{J}$ = 2.9 Hz), 3.99 (s, 1H), 1.81 (bs, 2H); MS (ES +) m/z (relative intensity) 271 (M + H) (5), 293 (M + H + Na) (100), HPLC purity = 98.7%.

1-N-*2′-Pyridine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(28)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 4.31 (d, 1H, $\mathcal{J}$ = 1.7 Hz), 4.71 (s, 1H), 6.88-6.92 (m, 1H), 7.26-7.47 (m, 6H), 7.65-7.7 (m, 1H), 8.27 (d, 1H, $\mathcal{J}$ = 8.3 Hz), 9.83 (bs, 1H); MS (ES +) m/z (relative intensity) 258 (M + H) (5), 280 (M + H + Na) (100), HPLC purity = 99.2%.

1-N-*3′-Pyridine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(29)***. R_f 0.2 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 4.26 (s, 1H), 4.72 (s, 1H), 7.28-7.44 (m, 6H), 8.20 (d, 1H, $\mathcal{J}$ = 8.4 Hz), 8.35 (d, 1H, $\mathcal{J}$ = 4.5 Hz), 8.61 (s, 1H), 9.16 (bs, 1H); MS (ES +) m/z (relative intensity) 258 (M + H) (80), 280 (M + H + Na) (100), HPLC purity = ∼ 100%.

1-N-*4′-Pyridine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(30)***. R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.16 (s, 1H, $\mathcal{J}$ = 4.2 Hz), 4.24 (d, 1H, $\mathcal{J}$ = 4.2 Hz), 7.19-7.40 (m, 5H), 7.64 (d, 2H, $\mathcal{J}$ = 6.2 Hz), 8.39 (d, 2H, $\mathcal{J}$ = 6.2 Hz); MS (ES +) m/z (relative intensity) 258 (M + H) (15), 280 (M + H + Na) (35).

1-N-*2′-Thiazole-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(31)***. R_f 0.3 (5% MeOH/DCM) ^{1}HNMR ($CDCl_3$) δ 1.25 (s, 2H), 4.37 (d, 1H, $\mathcal{J}$ = 1.8 Hz), 4.71 (d, 1H, $\mathcal{J}$ = 1.8 Hz), 6.97 (d, 1H, $\mathcal{J}$ = 3.5 Hz), 7.31-7.43 (m, 6H); MS (ES +) m/z (relative intensity) 264 (M + H) (100); HPLC purity = 96.8%.

1-N-*Piperidine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(32)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.19-1.25 (m, 1H), 1.38-1.5 (m, 5H), 2.48 (bs, 3H), 2.86-2.90 (m, 1H), 3.20-3.37 (m, 2H), 3.67-3.71 (m, 1H), 4.07 (d, 1H, $\mathcal{J}$ = 4.5 Hz), 4.44 (d, 1H, $\mathcal{J}$ = 4.5 Hz), 7.25-7.42 (m, 5H); MS (ES +) m/z (relative intensity) 249 (M + H) (50), 271 (M + H + Na) (100), HPLC purity = 94%.

1-N-*Morpholine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(33)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.29 (bs, 3H), 2.80-2.85 (m, 1H), 2.98-3.03 (m, 1H), 3.20-3.24 (m, 1H), 3.30-3.63 (m, 5H), 4.12 (d, 1H, $\mathcal{J}$ = 5.9 Hz), 4.35 (d, 1H, $\mathcal{J}$ = 5.9 Hz), 7.26-7.42 (m, 5H); MS (ES +) m/z (relative intensity) 251 (M^+H) (50), 273 (M + H + Na) (100), HPLC purity = 98.8%.

1-N-*Pyrrolodine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide* ***(34)***. R_f 0.2 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.52-1.77 (m, 4H), 2.31 (bs, 3H), 2.52-2.55 (m, 1H), 3.22-3.28 (m, 2H), 3.33-3.37 (m, 1H), 4.12-4.18 (m, 2H), 7.25-7.35 (m, 3H), 7.40−7.43 (m, 2H); MS (ES +) m/z (relative intensity) 235 (M + H) (50), 257 (M + H + Na) (100), HPLC purity = 97.8%.

Cytotoxicity

Various concentrations of functionalized amino acid derivatives (**1-34**) were screened for their cytotoxic activity *in vitro* on nine different human cancer cell lines tumor and one normal cell line. Briefly, a three day MTT *in vitro* cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically [5]. MTT was dissolved in phosphate buffered saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/mL; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. The cells were seeded in 96-well culture plates at a density of 5000−10,00 cells/well and incubated with various concentrations of functionalized amino acid derivatives

Table II. *In vitro* cytotoxicity data of functionalized amino acid derivatives (**1-34**).

No.	IC_{50} (μM)			
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	NIH3T3 (Normal fibroblast)
1	41.27	NA	45.70	NA
2	NA	NA	46.73	NA
3	NA	NA	24.89	NA
4	22.56	62.9	NA	74.5
5	9.41	64.41	NA	NA
6	5.67	27.75	12.6	86.6
7	85.17	NA	8.31	NA
8	13.13	87.72	16.12	NA
9	24.14	NA	NA	NA
10	6.1	24.34	4.17	26.2
11	89.32	NA	49.33	NA
12	37.46	69.59	NA	NA
13	NA	34.15	54.88	NA
15	57.32	NA	NA	NA
16	81.04	NA	47.88	NA
17	NA	NA	95.16	NA
21	NA	NA	22.02	NA
23	NA	NA	50.71	NA
24	69.53	25.9	12.5	NA
25	NA	NA	36.01	NA
26	56.9	84.06	36	NA
27	NA	NA	35.47	NA
29	89.54	NA	98.68	NA
30	NA	NA	37.82	NA
31	NA	NA	47.73	NA
32	NA	NA	96	NA
33	NA	NA	71.14	NA

Cytotoxicity was assessed by MTT assay as described in methods. The data shown represents the IC_{50} values obtained from the single independent experiment done in triplicates. NA represent an IC_{50} value $> 100\,\mu$M.

at 37°C (**1-34**) in a CO_2 incubator for 72 h. Control cells, treated with the appropriate vehicle were similarly incubated. The assay was terminated after 72 h by adding 25 μl of MTT to each well, then incubating for three hours, and finally adding 50 μL of 10% SDS-0.01 N HCl to each well to lyse the cells and dissolve formazan. After incubating for one hour, the plate was read spectrophotometrically at 540 nm and percentage inhibition of cell growth was calculated using the following formula: Cytotoxicity percentage $= 100 \times [1-(X/R_1)]$, where X = (absorbance of treated sample at 540 nm) R_1 = absorbance of control sample at 540 nm.

Results and discussion

Functionalized amino acid derivatives **(1-34)** were screened for their *in vitro* cytotoxicity on tumor as well as a non-tumorous cell lines and IC_{50} values were determined in micro molar (μM) concentrations. The human tumor cell lines used in the screening were ovary (PA-1), prostate (DU-145), oral (KB), colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2), leukemia (K562) and endotheial (ECV304) cancer cell lines. All the functionalized amino acid derivatives (**1-34**) and assay standard doxorubicin HCl (data not shown) were also tested against normal mouse fibroblast (NIH3T3) cell line to evaluate their tumor cell specificity (safety index). The cytotoxicity data is summarized in Table II. The compounds, which did not show cytotoxicity, are not listed in Table II. Structure activity relationship (SAR) of these derivatives has been described below. In the present discussion, compounds having $IC_{50} < 10$, 10–20 and $> 20\,\mu$M have been designated as high, moderate and low cytotoxic derivatives, respectively.

All the *N*-alkyl oxazolindine-5-carboxamide derivatives (**1-4**) showed low cytotoxicity, however, *N*-cyclohexyl-oxazolindine-5-carboxamide (**4**) was found slightly better than its *N*-isopropyl (**1**), *N*-cyclopropyl (**2**), *N*-cyclopentyl and (**3**) analogues. The *N*-aryl oxazolindine-5-carboxamide derivatives (**5-9**) have shown improved cytotoxicity than *N*-alkyl congeners (**1-4**). The oxazolindine-5-carboxamides, having *N*-phenyl (**5**) and *N*-(4′-fluoro)phenyl (**6**, $IC_{50} = 5.67\,\mu$M) substituents, have showed high cytotoxicity against ovarian (PA-1) cell line while its *N*-(4′-cyano)phenyl (**7**) derivative exhibited high cytotoxicity against oral (KB) cell line. Compound **6** has also shown safety index > 15. The *N*-(4′-methoxy)phenyl-oxazolindine-5-carboxamide (**8**) elicited moderate to low cytotoxicity against a number of cancer cell lines but low

order of cytotoxicity was observed in *N*-(3′-chloro-4′-fluoro)phenyl analog (**9**). It seemed that di-substituents or electron donating group present in the phenyl ring are not good choice for enhancing the cytotoxicity. However, *N*-benzyl-oxazolindine-5-carboxamide (**10**) exhibited high cytotoxictity aganist PA-1 ($IC_{50} = 6.1\,\mu M$) and KB ($IC_{50} = 4.17\,\mu M$) cell lines with safety index > 4. The *N*-heteroaryl-oxazolindine-5-carboxamide derivatives (**11–14**) were not found superior as compared to *N*-aryl (**5–9**) or *N*-benzyl (**10**) congeners. For example, all the *N*-pyridine derivatives (**11–13**) have shown low cytotoxicity, while *N*-thiazole (**14**) analog was essentially inactive. Similarly, oxazolindine-5-carboxamides having tertiary amines as substituents, like piperidine (**15**), morpholine (**16**) and pyrrolidine (**17**), have elicited low cytotoxicity. The lower cytotoxicity was exhibited when oxazolindine ring (**1–17**) was opened to corresponding amino-alcohol derivatives (**18–34**). All the amino-alcohol derivatives **(18–34)** either showed low cytotoxicity or were found inactive, except compound **24**, which possesses a *N*-(4′-cyano)phenyl substituent, showed moderate cytotoxicity against oral cancer cell line. Anticancer activity was also tested against an colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2), leukemia (K562) and endotheial (ECV304) cancer cell lines, but none of the compounds (**1–34**) showed "high" activity.

These results clearly indicated that oxazolindines (**1–17**), in general, were found superior to their corresponding amino-alcohol derivatives (**18–34**). Amongst oxazolindine-5-carboxamide derivatives, compounds containing a *N*-aryl (**5–9**) or *N*-benzyl (**10**) substituent, exhibited high cytototoxity against ovary and oral cancers with a good safety profile. These compounds could be of use in designing new anti-cancer agents.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja SPS. Plant based anti-cancer molecules: A chemical and biological proflie of some important leads. Bioorg Med Chem 2005;13: 5892–5908.
[2] Levi MS, Borne RF, Williamson JS. A review of cancer chemopreventive agents. Curr Med Chem 2001;8:1349–1362.
[3] http://en.wikipedia.org/wiki/Taxol.
[4] Mukherjee R, Kumar V, Srivastava SK, Agarwal SK, Burman AC. Betulinic acid derivatives as anticancer agents: Structure activity relationship. Anti Canc Agents Med Chem 2006;6(3):271–279.
[5] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Meth 1983;65:55–63.