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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, toxicity and chemo-sensitization of HeLa cells to etoposide, of some 2-methyl amino acid ester-substituted-1,3-benzoxazines

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Abstract A number of new L- or LD-2-amino acid ester-(substituted)-benz[1,3]oxazines **12–17** were synthesized from the reaction of free L- or LD-amino acid ester **9a–d** with 2-methylthio-1,3-benzoxazines **11a–g**. The structures of the new products **12–17** were confirmed from their ^{1}H, ^{13}CNMR and IR spectra and CHN microanalysis. Some of these compounds weakly inhibited DNA-PK and platelet aggregation. Studies of the toxicity for some of the new compounds showed mostly no inhibitory effects on HeLa cell growth at 1 and 10 μM and some up to 40 μM. The chemo-sensitization to etoposide by some of the compounds revealed that the most effective chemo-sensitizers at 10 μM were **14c** (1.83 fold), **12e** (1.42 fold), **15a** (0.8 fold), **16d** (0.76 fold) and **1c** (0.74). However, at 1 μM in the presence of etoposide, some compounds were shown to be more effective. No direct link was observed between the type of the L-amino acid methyl ester as well as the 7-, 8-, or 7, 8-substitution on the aromatic ring on the effectiveness of the chemo-sensitizers; however, the 7-hydroxy group did lower the effective chemo-sensitizers values.

Keywords 2-Amino acid ester-substituted-benz[1,3] oxazines · Toxicity · Chemo-sensitization to etoposide

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Introduction

The α-Amino acids are the fundamental building blocks in nature. *N*-aryl-α-amino acids are found as core structural components of antimicrobial, antiviral and pharmacologically active molecules (Ma and Yao, 1996). 2-Amino-1,3-benzoxazines can exhibit herbicidal, insecticidal, fungicidal and analgesic properties (Grigat *et al*., 1964; Tomita and Murakami, 1979; Schroth *et al*., 1989; Bereznak and Marshall, 2000). In addition, 2-amino-1,3-benzoxazines have been found to exhibit antiatherogenic activity giving them usefulness in the treatment and prophylaxis of atherosclerosis (Schroth *et al*., 1989). In particular, 2-morpholino-1,3-benzoxazine analogues **1** and **2**, 2-thiomorpholino-1,3-benzoxazine **3** and 2-(4-methylpiperazino)-1,3-benzoxazine **4** (Fig. 1) were reported to reduce arterial cholesterol accumulation in the SEA Japanese quail model (Gammill *et al*., 1990). Additionally, the 7-acetoxy-8-methyl analogue of **2** has been reported to inhibit cell proliferation as well as ADP-induced platelet aggregation (Gammill *et al*., 1991).

To date general method for methyl 2-α-amino acid-benz[1,3]oxazines synthesis has not been achieved. Previously 2-((8-methyl-4-oxo-4H-benz[*e*][1,3]oxazin-2-yl)amino)acetic acid was prepared from the reaction of ethyl 2-cyanato-3-methylbenzoate and glycine in low yield (≤30 %; Grigat *et al*., 1964). The development of a general synthetic method of 2-α-amino acid-benz[1,3]oxazines is challenging as the amino acids themselves contain nonpolar, polar neutral, acidic and basic side chains, which may complicate or interfere with the reactions.

However, it is of importance as these families of compounds have been found to enhance the efficacy of anticancer treatments through inhibition and activation of different pathways. For example, DNA-PK is a key enzyme

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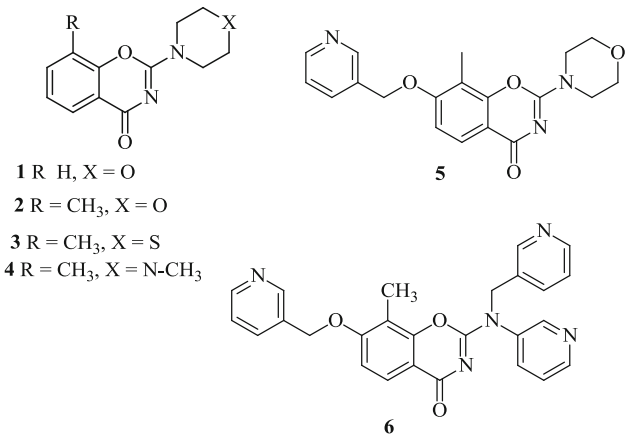


Fig. 1 Some structures of the biologically important 2-amino-1,3-benzoxazines **1–6**

involved in the non-homologous end joining (NHEJ) pathway repair of double strand DNA breaks, caused by some anticancer treatments (Matsuoka *et al*., 2007).

Recently, we found that some 2-morpholino-1,3-benzoxazines compounds showed high-to-moderate DNA-PK inhibition activity (ca. 0.28–6.80 μM IC_{50}) from which the most active compound was product **5** with an $IC_{50} = 0.28$ μM. We also found that the 2-(*N*-substituted (pyridin-3-ylmethyl)amino)-1,3-benzoxazines showed moderate-to-low (ca. 2.5–25.2 μM IC_{50}) DNA-PK inhibitory activity, with the most potent being **6** with an $IC_{50} = 2.5$ μM (Fig. 1; Ihmaid *et al*., 2012).

Radhamani *et al.* (2014) found compound **5** to be an effective radio-sensitizing agent that inhibits DSB repair and promotes apoptosis in both irradiated lung cancer and colon cancer cells. We proposed that its mechanism of action is through the inhibition of DNA-PK.

Furthermore, it was found (Fitzgibbon *et al*., 2013) that compound **12b** (**LTUSI54**), which was found to have no DNA-PK or PI3K inhibitory activity (>100 μM), still sensitized HeLa cells to the effects of etoposide. The study hypothesized that product **12b** promotes cell cycle arrest through activation of p38α pathways, independent of p53 mechanisms, results in a decrease of p53 stabilization and hence, restricted apoptosis and arrest in cell growth.

The aim of this study was to develop a simplified, reproducible method of 2-α-amino acid-benz[1,3]oxazines synthesis that can be used as a general method in future. Furthermore, through use of this new method, we aimed to develop a family of 2-α-amino acid-benz[1,3]oxazines and assess them for their chemo-sensitizing abilities against the ovarian cancer, HeLa cell line.

Results and discussion

Chemistry

Methyl esterification of α-amino acids

This step involves the protection of the carboxylic function of the α-amino acid **7** to prevent the formation of a zwitterion, which is a simple and reversible protection method. The α-amino acids **7** were converted to their corresponding methyl ester derivatives **8** then the hydrochloric salt compound 8 which was neutralized and gave product **9** using the previously reported procedure (McKerrow *et al*., 2010; Scheme 1).

*Synthesis of 2-amino acid ester-substituted-benz[1,3]oxazines **12–17***

The 2-thio-1,3-benzoxazines **10a–g** were synthesized according to the previously reported procedure (Scheme 1; Pritchard *et al*., 2005). Furthermore, the 2-methylthio-1,3-benzoxazines **11a–g** were synthesized according to the previously reported procedure (Scheme 1; Ihmaid *et al*., 2012).

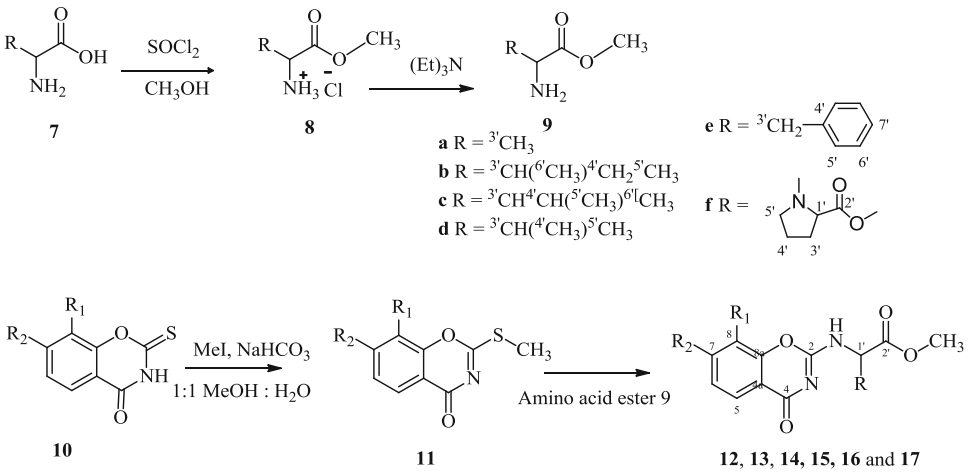
The reaction of compounds **11a–g** according to general procedure A with free amino acid ester **9a–e** gave 2-amino acid-benz[1,3]oxazines **12–17** (Scheme 1).

Structural conformation of 2-amino acid-1,3-benzoxazines methyl esters

The structures of the new 2-amino acid-1,3-benzoxazines methyl esters **12a–g**, **13a–c**, **14a–e**, **15a–c**, **16a–d** and **17a–d** were confirmed using IR, ^{1}H, ^{13}CNMR spectroscopy and microanalysis. The IR spectra of compounds **12–17** showed two carbonyl groups, one related to the amino acid ester C-2′ at ν_{max} 1,752, and the other carbonyl related to the benzoxazine C-4 at $\nu_{max} \sim 1{,}655$ (C=O). The ^{1}HNMR spectra showed the expected signals for the 2-amino acid ester-1,3-benzoxazine with characteristic signals appearing in the proton NMR of all the products **12–17**, as multiple signals $\delta \sim 4.5$ ppm for H1′ and the signal for $OCH_3 \sim 3.7$ ppm as a singlet.

It is well known that the phenomenon of geminal anisochrony (U_2 protons) can be observed in chiral species such as CXYZ–CU_2V and CXYZC ≡ CU_2V (Stiles, 1977). As a result of chirality, some of 2-amino acid esters-1,3-benzoxazines showed magnetic nonequivalent protons and carbon-13 chemical shift for the chemically equivalent groups (McKerrow *et al*., 2010).

Scheme 1 Esterification of amino acids, and the synthesis of substituted of 2-(amino acid)-1,3-benzoxazine **12–17**



Comp.	R_2	R_1	R	Yield	Comp.	R_2	R_1	R	Yield
11a, 12a	H	H	CH_3	75%	**14c**	H	Ph	$CH_2CH(CH_3)_2$	60%
11b, 12b	H	CH_3	CH_3	72%	**14d**	OH	H	$CH_2CH(CH_3)_2$	55%
11c, 12c	H	Ph	CH_3	67%	**14e**	OH	CH_3	$CH_2CH(CH_3)_2$	67%
11c*12c*	H	Ph	CH_3	63%	**15a**	H	CH_3	$CH(CH_3)_2$	63%
11d, 12d	OCH_2CH_3	H	CH_3	65%	**15b**	OH	H	$CH(CH_3)_2$	60%
11e, 12e	OCH_3	H	CH_3	70%	**15c**	OH	CH_3	$CH(CH_3)_2$	55%
11e,*12e*	OCH_3	H	CH_3	60%	**16a**	H	CH_3	CH_2Ph	55%
11f, 12f	OH	H	CH_3	70%	**16b**	H	Ph	CH_2Ph	55%
11g, 12g	OH	CH_3	CH_3	60%	**16c**	OH	H	CH_2Ph	50%
13a	H	CH_3	$CH(CH_3)CH_2CH_3$	55%	**16d**	OH	CH_3	CH_2Ph	53%
13b	OH	H	$CH(CH_3)CH_2CH_3$	51%	**17a**	H	H	L-Proline	60%
13c	OH	CH_3	$CH(CH_3)CH_2CH_3$	60%	**17b**	H	CH_3	L-Proline	68%
14a	H	H	$CH_2CH(CH_3)_2$	65%	**17c**	OH	H	L-Proline	60%
14b	H	CH_3	$CH_2CH(CH_3)_2$	70%	**17d**	OH	CH_3	L-Proline	63%

*DL amino acid is used to produce the product.

L-valine derivatives **15a–c** showed two different proton and carbon-13 chemical shifts for the 4′- and 5′-methyl groups. Similarly, the methyl groups 5′ and 6′ in leucine derivatives **14a–e** showed two different proton and carbon-13 chemical shifts. However, the CH_2 protons of C-4′ in isoleucine derivatives **13a–c** showed two nonequivalent protons. Similarly, the C-3′ CH_2 protons in phenylalanine derivatives **16a–d** were nonequivalent.

Chirality and anisochrony (magnetic nonequivalence)

It is worth noting t substitution of 2-methylthio group of compound **11** with α-amino acid methyl esters occurs through a 1,2-addition elimination reaction. Therefore, no racemization would be expected to take place as the C-3′ is not involved in the reaction.

*Hydrolysis of methyl 3-methyl-2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)butanoate **15a***

Product **15a** was hydrolyzed to the corresponding acid **18** using dilute NaOH solution under mild condition to keep the amino acid chirality unchanged in the final product (see "Experimental"). Structure of product **18** was confirmed from the CHN microanalysis, IR, ^1H and ^{13}C NMR spectroscopy.

Biological activity

*Inhibition of platelet aggregation of some 2-methyl amino acid-substituted-1,3-benzoxazines **12b**, **14b** and **14c***

Platelet aggregation was determined by the optical method in a two-channel platelet aggregometry (Chrono-Log) us-

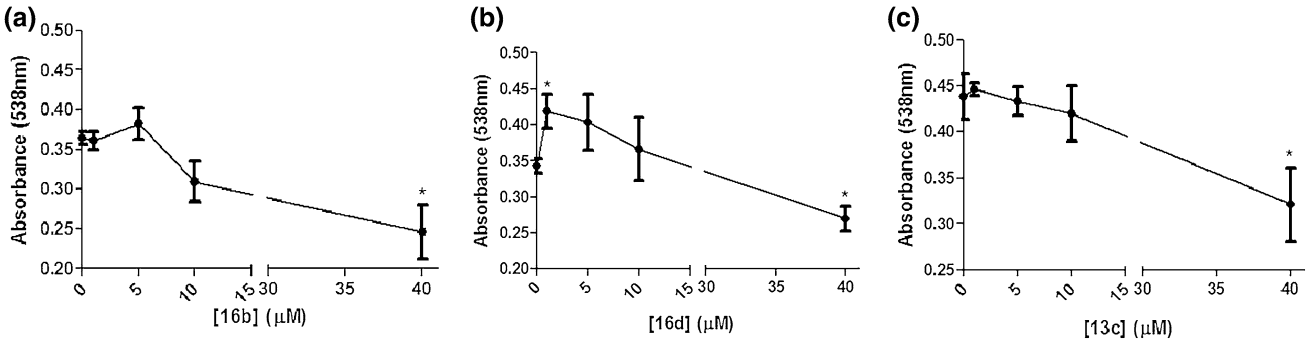


Fig. 2 SRB results of HeLa cells treated with increasing concentrations of **a 16b**, **b 16d** or **c 13c** for 48 h. *Graph* indicates mean of four replicates ±SEM. Statistical significance was determined using two-tailed *t* tests, *asterisk* indicates results statistically significant to the control

ing the previously reported protocol (Pritchard *et al*., 2007). Compounds **12b**, **14b** and **14c** showed no activity at 100 μM regardless of 8-substituent (Me or Ph) of the 2-methyl amino acid-1,3-benzoxazines (L-Alanine, L-Phenylalanine and L-Leucine **12b**, **14b**, and **14c,** respectively).

*DNA-PK IC_{50} for select 2-amino acid-substituted-1,3-benzoxazines methyl ester **12b**, **14c** and **16b***

The DNA-PK assay was performed on compounds **12b**, **14c** and **16b** by Reaction Biology Corporation, One Great Valley Parkway, Suite 2 Malvern, PA 19355 USA according to the previously reported method (Morrison *et al*., 2014). Compound **12b**, **14b**, and **14c** showed low-to-moderate DNA-PK activity with IC_{50} of 81.0, 25.7 and 10.0 μM, respectively.

*Toxicity of select 2-amino acid-substituted-1,3-benzoxazines methyl ester and the corresponding amino acid derivative **18***

When investigating the effects of the compounds **12d–g**, **13c**, **14a–c**, 15**a**, c, **16b**, **d**, **17b**, **d** and **18** alone on HeLa cervical cancer cells, no inhibitory effects were observed at low concentrations (1, and 10 μM). However, **13c**, **14b**, **16b**, **16d** and **17d** were found to significantly (p value <0.05) inhibit HeLa cell growth when used at the high concentration of 40 μM when compared to the DMSO control (Fig. 2). Figure 2 shows that **16b**, **16d** and **13c** have a dose-dependent effect on HeLa cell number, whereby there is no significant effect on cell growth until high concentrations of 40 μM. All other tested compounds were found to have no significant effect ($p > 0.05$) on cell number as indicated by the lack of change in absorbance across all tested concentrations.

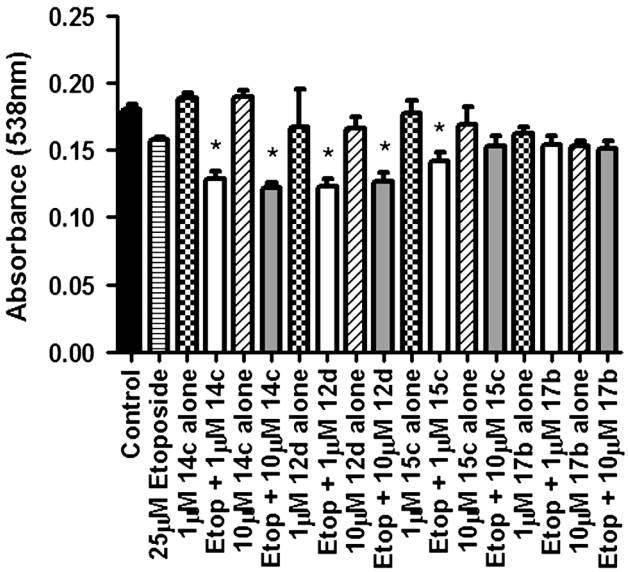


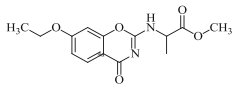
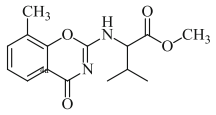
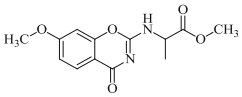
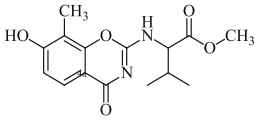
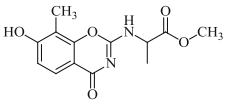
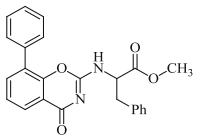
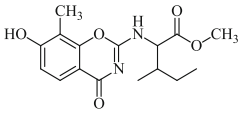
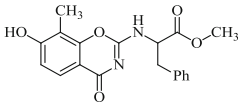
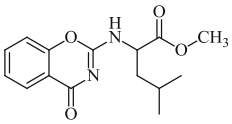
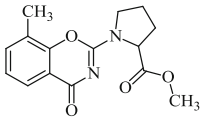
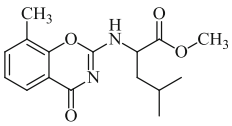
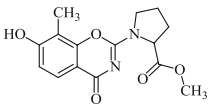
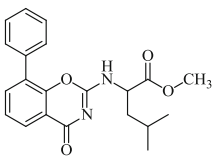
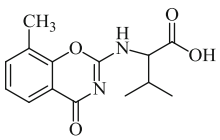
Fig. 3 SRB results of HeLa cells treated with 25 μM etoposide both in the presence and absence of test compounds at either 1 μM (*open bars*) or 10 μM (*shaded bars*) for 48 h. *Graph* indicates mean ± SEM of four replicates. Statistical significance was determined using two-tailed *t* tests, *asterisk* indicates combination treatments significantly different to the etoposide alone treatment group

*Chemo-sensitization to etoposide by selected 2-amino acid-substituted-1,3-benzoxazines methyl ester and the corresponding amino acid derivative **18***

We previously found (Fitzgibbon *et al*., 2013) that compound **12b** works synergistically with etoposide to inhibit growth of HeLa cells with no corresponding increase in cell death, as indicated by sub G1 analysis. This result is believed to be due to the observed enhancement of cell cycle arrest at both the S and G2 checkpoints.

In this study, we investigated the effect of select, newly synthesized compounds, on enhancing the chemo-toxic

Table 1 SRB results of HeLa cells treated with 25 μM etoposide both in the presence and absence of test compounds 1 or 10 μM for 48 h

		Etop + 1 μM	Etop + 10 μM			Etop + 1 μM	Etop + 10 μM
12d		1.56	1.37	**15a**		0.55	0.80
12e		1.25	1.42	**15c**		0.71	0.24
12g		0.58	0.68	**16b**		0.04	0.27
13c		0.31	0.74	**16d**		0.53	0.76
14a		0.37	0.40	**17b**		0.17	0.29
14b		0.49	0.44	**17d**		0.63	0.51
14c		1.31	1.83	**18**		0.99	1.35

Folds were calculated using etoposide alone at 25 μm as 12.5 % chemo-sensitizing effects on HeLa cells

effects of etoposide on the HeLa cervical cancer cell line. It was observed that 25 μM etoposide treatment resulted in a significant reduction in cell number from absorbance of 0.181–0.158 ($p < 0.05$). Figure 3 shows the effect observed by etoposide treatment as well as the chemo-sensitization effects of a selection of compounds. Table 1

summarizes chemo-sensitization effects observed for select compound relative to the chemo-sensitization of etoposide.

As can be seen in Fig. 3, 25 μM etoposide treatment resulted in a significant reduction in HeLa cell number from absorbance of 0.181–0.158 ($p < 0.05$). This observation was further enhanced by **14c**, which was found to be the most effective chemo-sensitizer at 10 μM (1.83 fold) as indicated by the lowest optical absorbance reading of 0.12. This was found to be a significant reduction in HeLa cell numbers when compared to the f etoposide-only treatment (1.83 fold, $p < 0.0001$). Similarly, when **14c** was used at the lower concentration (1 μM), there was a significant reduction observed in HeLa cell number with an absorbance of 0.129 when compared to etoposide-only treatment (1.31 fold, $p < 0.0001$). Compound **12d** was found to be the second most effective chemo-sensitizer when used at 1 μM in conjunction with etoposide resulting in an absorbance reading of 0.123, which is again a significant reduction in cell number compared to the etoposide-only treated HeLa cells (1.56 folds, $p < 0.0001$). However, when compound **12d** was used at a higher concentration of 10 μM in combination with etoposide, there was still a significant sensitization by comparison to the etoposide-only treated cells (1.37 fold, $p < 0.0001$) although this was not as effective as the lower 1 μM concentration. Similarly, compound **15c** was found to be more effective at enhancing the effects of etoposide on HeLa cells when used at a lower concentration. When 1 μM **15c** was used in the presence of etoposide, there was a significant reduction (0.71 fold) in cell numbers when compared to the etoposide alone treatment (absorbance 0.142, $p = 0.0102$). However, when 10 μM **15c** was used in combination with etoposide, there was no significant change (0.24 fold) in HeLa cell numbers observed. Conversely, **17b** was found to have no chemo-sensitizing effect on HeLa cells when used in combination with etoposide at 1 μM (0.17 fold) or 10 μM (0.29 fold) concentration with an absorbance of 0.154 and 0.152, respectively. The only other two compounds to share this observation were **14a** and **14b**, and all other compounds were found to have chemo-sensitizing effects on HeLa cells in combination with etoposide at one or both the concentrations tested (Table 1).

Additionally, hydrolysis of 2-amino acid-8-methyl-1,3-benzoxazines methyl ester **15a** to the corresponding acid compound **18** enhanced the chemo-sensitizing effects on HeLa cells in combination with etoposide at both the concentrations tested (1 and 10 μM) by 0.99- and 1.35-fold, respectively, compared with the methyl ester analogue **15a** (Table 1).

Structure activity relationship

It is worth noting that enhanced effects of etoposide on HeLa cells (effective chemo-sensitizer) showed no direct correlation with the type of L-amino acid methyl ester substitution at position 2 nor with the 7- or 8-substitution or 7, 8-bis-substitution on the aromatic ring. However, the 7-hydroxy group substitution appears to lower the efficacy of chemo-sensitization (Table 1).

It is worth noting that compound 14c enhances the chemo-sensitizing effects of etoposide on HeLa cells by 1.83-fold at 10 μM, a concentration at which it is found to have no effect on either DNA-PK activity or cell growth.

Conclusion

A number of new L- or LD-2-amino acid ester-substituted-benz[1,3]oxazines **12–17** and the corresponding acid analogue **18** were synthesized for the first time using simple synthetic methods. A selection of the novel compounds produced were assessed for their biological activity as chemo-sensitizers. Studies of the chemo-sensitization of HeLa cells to etoposide using selected new compounds revealed that some are more effective chemo-sensitizer at 10 μM, while others were more effective at 1 μM. Compound **14c** was the most effective chemo-sensitizer at 10 μM (1.83 fold), while compound **12d** was found to be the most effective chemo-sensitizer (1.56 folds) when used at 1 μM. 2-Amino acid-8-methyl-1,3-benzoxazines acid **18** enhanced the chemo-sensitizing effects on HeLa cells in combination with etoposide at both the concentrations tested (1 and 10 μM) by 0.99- and 1.35-fold, respectively.

Alone they showed no negative effect on HeLa cell growth at concentrations up to 10 μM.

Experimental

Chemistry

Infrared spectra were obtained using a Perkin Elmer FT-IR 1720 × spectrometer. ^{1}H and ^{13}CNMR spectra were obtained using a Bruker AC 200 NMR spectrometer at 200 and 50 MHz, respectively. All ^{1}H and ^{13}CNMR spectral results are recorded as chemical shifts (δ) relative to the internal TMS for proton and 77.0 ppm in $CDCl_3$ solvent and 39.4 ppm in DMSO-d_6 solvent ^{13}CNMR. Microanalysis was performed by Chemical and Micro Analytical Services (CMAS), Australia. Melting point determinations were carried out using a Stuart Scientific (SMP3) melting point apparatus, and all melting points are uncorrected.

Starting material

The starting reagents L, Dl α-amino-acids, sodium hydrogen carbonate, cesium carbonate, and methyl iodide and

bromine were purchased from Aldrich Chemical Company and were used as received.

Triphenylphosphine was purchased from Merck chemical company Germany.

Synthesis of 2 L, or DL-α-amino acid-1,3-benzoxazines

General procedure A

The appropriate 2-methylthio-1,3-benzoxazine **11a–g** (2.5 mmol) was suspended in dry 1,4-dioxane (10 mL) in a 50-mL round-bottomed flask. The appropriate α-amino acid methyl ester-free amine **9** (3 mmol) was then added, with stirring before the reaction mixture was heated to reflux for 4 h. At completion, the reaction mixture was evaporated to dryness under reduced pressure and triturated with minimal diethyl ether. The resulting solid was collected by vacuum filtration and recrystallized from an appropriate solvent.

L-Methyl 2-((4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-propanoate ***12a*** 2-(Methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11a** (0.48 g, 2.5 mmol) was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from toluene to give **12a** (0.46 g, 75 % yield), mp 211 °C. ν_{max} (KBr)/cm^{-1} 3,279, 2,923 (N–H), 1,771 (C=O), 1,665 (C=O), 1,624 (C=C), 1,489 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.61 (bs, 1H, N–H), 7.92 (d, 1H, $J = 6.5$ Hz, H-5), 7.71 (t, 1H, $J = 7.3$ Hz, H-7), 7.53 (t, 1H, $J = 7.0$ Hz, H-6), 7.32 (d, 1H, $J = 7.0$ Hz, H-8), 4.5 (q, 1H, $J = 7.3$ Hz, H-1′), 3.7 (s, 3H, OCH_3), 1.4 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 172.7 (C-2′), 166.1 (C-4), 154.4 (C-2), 151.6 (C-8a), 132.3 (C-7), 130.4 (C-5), 121.9 (C-6), 120.6 (C-8), 114.8 (C-4a), 51.3 (OCH_3), 48.1 (C-1′), 17.1 (C-3′); (Found C, 58.47; H, 4.95; N, 11.75; $C_{12}H_{12}N_2O_4$, requires C, 58.06; H, 4.87; N, 11.29).

L-Methyl 2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl) yl)amino)propanoate ***12b*** 8-Methyl-2-(Methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one (0.52 g, 2.5 mmol) **11b** was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from toluene to give **12b** (0.47 g, 72 % yield), mp 217–220 °C. ν_{max} (KBr)/cm^{-1} 3,279, 2,923 (N–H), 1,751 (C=O), 1,663 (C=O), 1,624 (C=C), 1,489 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.63 (bs, 1H, N–H), 7.72 (d, 1H, $J = 7.5$ Hz, H-5), 7.53 (d, 1H, $J = 7.5$ Hz, H-7), 7.21 (t, 1H, $J = 7.5$ Hz, H-6), 4.49 (q, 1H, $J = 7.3$ Hz, H-1′), 3.71 (s, 3H, OCH_3), 2.42 (s, 3H, 8-CH_3). 1.4 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.7 (C-2′), 165.1 (C-4), 157.4 (C-2), 151.6 (C-8a), 134.3 (C-7), 124.4 (C-8), 123.9 (C-5), 123.6 (C-6), 116.8 (C-4a), 51.3 (OCH_3), 49.1 (C-1′), 16.1 (C-3′), 13.4 (CH_3); (Found C, 59.47; H, 5.40; N, 10.75; $C_{13}H_{14}N_2O_4$, requires C, 59.54; H, 5.38; N, 10.68).

L-Methyl 2-((4-oxo-8-phenyl-4H-benz[e][1,3]oxazin-2-yl)-amino)propanoate ***12c*** 8-Phenyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one (0.67 g, 2.5 mmol) **11c** was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **12c** (0.54 g, 67 % yield), mp 204 °C. ν_{max} (KBr)/cm^{-1} 3,056, 2,911 (N–H), 1,751 (C=O), 1,655 (C=O), 1,603 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.60 (bs, 1H, N–H), 7.90 (dd, 1H, $J_{H5,H7} = 1.7$ Hz, $J_{H5,H6} = 7.72$ Hz, H-5), 7.63 (dd, 1H, $J_{H7,H5} = 1.7$ Hz, $J_{H7,H6} = 7.7$ Hz, H-7), 7.50–7.40 (m, 6H, H-6, H-10, H-11 & H-12), 4.40 (q, 1H, $J = 7.3$ Hz, H-1′), 3.62 (s, 3H, OCH_3), 1.43 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 172.2 (C-2′), 163.5 (C-4), 152.3 (C-2), 150.0 (C-8a), 134.5 (C-9), 134.3 (C-7), 128.1 (C-11), 127.7 (C-10), 127.2 (C-12), 125.8 (C-6), 124.9 (C-5), 109.5 (C-8), 117.6 (C-4a); 51.6 (C-1′), 49.4 (OCH_3), 16.1 (C-3′); (Found C, 66.36; H, 5.09; N, 8.56; $C_{18}H_{16}N_2O_4$, requires C, 66.66; H, 4.97; N, 8.64).

D,L-methyl 2-((4-oxo-8-phenyl-4H-benz[e][1,3]oxazin-2-yl)-amino)propanoate ***12c**** 8-Phenyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11c** (0.67 g, 2.5 mmol) was allowed to react with D, L-alanine methyl ester **9a***(0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **12c*** (0.51 g, 63 % yield), mp 210–213 °C. ν_{max} (KBr)/cm^{-1} 3,056, 2,911 (N–H), 1,751 (C=O), 1,655 (C=O), 1,603 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.65 (bs, 1H, N–H), 7.90 (dd, 1H, $J_{H5,H7} = 1.7$ Hz, $J_{H5,H6} = 7.7$ Hz, H-5), 7.62 (dd, 1H, $J_{H7,H5} = 1.7$ Hz, $J_{H7,H6} = 7.7$ Hz, H-7), 7.50–7.40 (m, 6H, H-6, H-10, H-11 & H-12), 4.43 (q, 1H, $J = 7.3$ Hz, H-1′), 3.62 (s, 3H, OCH_3), 1.4 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 172.2 (C-2′), 163.5 (C-4), 152.3 (C-2), 150.0 (C-8a), 134.5 (C-9), 134.3 (C-7), 128.1 (C-11), 127.7 (C-10), 127.2 (C-12), 125.8 (C-6), 124.9 (C-5), 109.5 (C-8), 117.6 (C-4a); 51.6 (C-1′), 49.4 (OCH_3), 16.1 (C-3′); (Found C, 66.36; H, 5.09; N, 8.56; $C_{18}H_{16}N_2O_4$, requires C, 66.66; H, 4.97; N, 8.64).

L-Methyl 2-((7-ethoxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)-amino)propanoate ***12d*** 7-Ethoxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11d** (**0.59 g**, **2.5 mmol**) was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethyl acetate to give **12d** (0.48 g, 65 % yield), mp 203 °C. ν_{max} (KBr)/cm^{-1} 3,234, 2,838 (N–H), 1,765 (C=O), 1,674 (C=O), 1,558 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.91 (s, 1H, N–

H), 7.73 (d, 1H, $J = 8.6$ Hz, H-5), 6.92 (dd, 1H, $J_{H6,H8} = 2.4$ Hz, $J_{H6,H5} = 8.7$ Hz, H-6), 6.81 (d, 1H, $J = 2.2$ Hz, H-8), 4.43 (q, 1H, $J = 7.3$ Hz, H-1′), 4.12 (q, 2H, $J = 5.6$, OCH_2), 3.72 (s, 3H, OCH_3), 1.40 (d, 3H, $J = 7.3$ Hz, H-3′), 1.30 (t, 3H, $J = 5.6$, CH_3); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.5 (C-2′), 169.1 (C-4), 162.7 (C-7), 157.0 (C-2), 154.4 (C-8a), 127.7 (C-5), 112.8 (C-6), 110.1 (C-4a), 99.8 (C-8), 63.7 (OCH_2), 51.3 (C-1′), 49.1 (OCH_3), 16.1 (C-3′), 13.6 (CH_3); (Found C, 57.34; H, 5.56; N, 9.72; $C_{14}H_{16}N_2O_5$, requires C, 57.53; H, 5.52; N, 9.58).

L-methyl 2-((7-methoxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl) amino)propanoate ***12e*** 7-Methoxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11e** (**0.56 g**, **2.5 mmol**) was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethyl acetate to give **12e** (0.49 g, 70 % yield), mp 176 °C. ν_{max} (KBr)/cm^{-1} 3,234, 2,838 (N–H), 1,765 (C=O), 1,674 (C=O), 1,558 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8,62 (bs, 1H, N–H), 7.83 (d, 1H, $J = 8.6$ Hz, H-5), 6.92 (dd, 1H, $J_{H6,H8} = 2.4$ Hz, $J_{H6,H5} = 8.7$ Hz, H-6), 6.81 (d, 1H, $J = 2.2$ Hz, H-8), 4.50 (q, 1H, $J = 7.3$ Hz, H-1′), 3.91 (s, 3H, 7-OCH_3), 3.72 (s, 3H, OCH_3), 1.40 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.4 (C-2′), 164.2 (C-4), 163.5 (C-7), 157.0 (C-2), 154.4 (C-8a), 127.7 (C-5), 112.4 (C-6), 110.2 (C-4a), 99.3 (C-8), 55.4 (7-OCH_3), 51.3 (C-1′), 49.1 (OCH_3), 16.1 (C-3′); (Found C, 55.95; H, 5.06; N, 10.21; $C_{13}H_{14}N_2O_5$, requires C, 56.11; H, 5.07; N, 10.07).

D,L-Methyl 2-((7-methoxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)propanoate ***12e**** 7-Methoxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11e** (**0.56 g**, **2.5 mmol**) was allowed to react with D,L-alanine methyl ester **9a*** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethyl acetate to give **12e*** (60 % yield), mp 176 °C. ν_{max} (KBr)/cm^{-1} 3,234, 2,838 (N–H), 1,765 (C=O), 1,674 (C=O), 1,558 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8,62 (bs, 1H, N–H), 7.82 (d, 1H, $J = 8.6$ Hz, H-5), 6.91 (dd, 1H, $J_{H6,H8} = 2.4$ Hz, $J_{H6,H5} = 8.73$ Hz, H-6), 6.82 (d, 1H, $J = 2.2$ Hz, H-8), 4.53 (q, 1H, $J = 7.3$ Hz, H-1′), 3.91 (s, 3H, 7-OCH_3), 3.72 (s, 3H, OCH_3), 1.40 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.4 (C-2′), 164.2 (C-4), 163.5 (C-7), 157.0 (C-2), 154.4 (C-8a), 127.7 (C-5), 112.4 (C-6), 110.2 (C-4a), 99.3 (C-8), 55.4 (7-OCH_3), 51.3 (C-1′), 49.1 (OCH_3), 16.1 (C-3′); (Found C, 55.95; H, 5.06; N, 10.21; $C_{13}H_{14}N_2O_5$, requires C, 56.11; H, 5.07; N, 10.07).

L-Methyl 2-((7-hydroxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)propanoate ***12f*** 7-Hydroxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11f** (0.52 g, 2.5 mmol)was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **12f** (0.46 g, 70 % yield), mp 199–201 °C. ν_{max} (KBr)/cm^{-1} 3,300–2,954 (O–H), 3,246, 2,867 (N–H), 1,743 (C=O), 1,655 (C=O), 1,573 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.53 (d, 1H, $J = 8.4$ Hz, H-5), 6.82 (d, 1H, $J = 8.4$ Hz, H-6), 6.6 (s, 1H, H-8), 4.5 (q, 1H, $J = 7.3$ Hz, H-1′), 3.7 (s, 3H, OCH_3),1.40 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.6 (C-2′), 164.9 (C-4), 159.7 (C-7), 157.1 (C-2), 152.7 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.5 (C-8), 108.9 (C-4a), 51.2 (C-1′), 49.1 (OCH_3), 16.2 (C-3′); (Found C, 54.66; H, 4.79; N, 10.79; $C_{12}H_{12}N_2O_5$, requires C, 54.55; H, 4.58; N, 10.60).

L-Methyl-2-((7-hydroxy-8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)propanoate ***12g*** 7-hydroxy-8-methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11g** (0.56 g, 2.5 mmol)was allowed to react with L-alanine methyl ester **9a** (0.31 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **12g** (0.45 g, 60 % yield), mp 204 °C. ν_{max} (KBr)/cm^{-1} 3,200–2,954 (OH), 3,246, 2,867 (N–H), 1,746 (C=O), 1,650 (C=O), 1,569 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.50 (d, 1H, $J = 8.4$ Hz, H-5), 6.83 (d, 1H, $J = 8.4$ Hz, H-6), 4.50 (q, 1H, $J = 7.3$ Hz, H-1′), 3.71 (s, 3H, OCH_3), 2.22 (s, 3H, 8-CH_3). 1.40 (d, 3H, $J = 7.3$ Hz, H-3′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.6 (C-2′), 164.9 (C-4), 159.7 (C-7), 157.1 (C-2), 152.7 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.5 (C-8), 108.9 (C-4a), 51.2 (C-1′), 49.1 (OCH_3), 16.2 (C-3′), 6.8 (8-CH_3); (Found C, 56.06; H, 5.09; N, 10.06; $C_{13}H_{14}N_2O_5$, requires C, 56.11; H, 5.07; N, 10.07).

L-Methyl 3-methyl-2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)pentanoate ***13a*** 8-Methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11b** (0.52 g, 2.5 mmol) was allowed to react with L-Isoleucine methyl ester according **9b** (0.44 g, 3 mmol)to general procedure A. The crude solid was collected and recrystallized from toluene to give **13a** (0.42 g, 55 % yield), mp 175–177 °C. ν_{max} (KBr)/cm^{-1} 3,280, 2,868 (N–H), 1,761 (C=O), 1,671 (C=O), 1,625 (C=C), 1,487 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.61 (bs, 1H, N–H), 7.73 (d, 1H, $J = 7.5$ Hz, H-5), 7.51 (d, 1H, $J = 7.5$ Hz, H-7), 7.22 (t, 1H, $J = 7.5$ Hz, H-6), 4.51 (m, 1H, H-1′), 3.70 (s, 3H, OCH_3), 2.40 (s, 3H, 8-CH_3), 1.72 (m, 3H, H-3′/H-4′), 0.97 (d, 3H, $J = 6.0$ Hz,C6′H_3), 0.94 (t, 3H, $J = 6.5$ Hz, C5′H_3); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.7 (C-2′), 165.0 (C-4), 157.7 (C-2), 151.6 (C-8a), 134.3 (C-7), 124.4 (C-8), 123.9 (C-6), 123.6 (C-5), 116.7 (C-4a), 52.2 (C-1′), 51.2 (OCH_3), 40.5 (C-4′), 23.9 (C-3′), 23.0, 21.3 (C-5′/C-6′), 13.4 (CH_3); (Found C, 63.06; H, 6.67; N, 9.14; $C_{16}H_{20}N_2O_4$, requires C, 63.14; H, 6.62; N, 9.20).

L-Methyl-2-((7-hydroxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-3-methylpentanoate ***13b*** 7-Hydroxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11f** (0.52 g, 2.5 mmol)was allowed to react with L-Isoleucine methyl ester **9b** (0.44 g, 3 mmol) according to general procedure A. The crude material was collected and recrystallized from ethanol to give **13b** (0.39 g, 51 % yield), mp 225 °C. ν_{max} (KBr)/cm^{-1} 3,300, 2,956 (O–H), 3,246, 2,867 (N–H), 1,752 (C=O), 1,655 (C=O), 1,583 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.62 (d, 1H, $J = 7.5$ Hz, H-5), 6.80 (d, 1H, $J = 7.5$ Hz, H-6), 4.52 (t, 1H, $J = 7.0$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.22 (s, 3H, 8-CH_3), 1.70 (m, 3H, H-3′/H-4′), 0.98 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.94 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.9 (C-2′), 167.3 (C-4), 160.3 (C-7), 152.7 (C-2), 151.8 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.4 (C-8), 108.9 (C-4a), 52.2 (C-1′), 51.2 (OCH_3), 41.5 (C-4′), 23.9 (C-3′), 21.8, 20.8 (C-5′/C-6′), 6.8 (8-CH_3); (Found C,58.75; H, 5.94; N, 8.80; $C_{15}H_{18}N_2O_5$, requires C, 58.82; H, 5.92; N, 9.15).

L-Methyl-2-((7-hydroxy-8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-3-methylpentanoate ***13c*** 7-Hydroxy-8-methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11g** (0.56 g, 2.5 mmol) was allowed to react with L-Isoleucine methyl ester **9b** (0.44 g, 3 mmol)according to general procedure A. The crude material was collected and recrystallized from ethanol to give **13c** (0.48 g, 60 % yield), mp 152 °C. ν_{max} (KBr)/cm^{-1} 3,300–2,956 (O–H), 3,246, 2,867 (N–H), 1,752 (C=O), 1,655 (C=O), 1,583 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.62 (d, 1H, $J = 7.5$ Hz, H-5), 6.81 (d, 1H, $J = 7.5$ Hz, H-6), 4.50 (t, 1H, $J = 7.0$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.22 (s, 3H, 8-CH_3), 1.70 (m, 3H, H-3′/H-4′), 0.98 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.94 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.9 (C-2′), 167.3 (C-4), 160.3 (C-7), 152.7 (C-2), 151.8 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.4 (C-8), 108.9 (C-4a), 52.2 (C-1′), 51.2 (OCH_3), 41.5 (C-4′), 23.9 (C-3′), 21.8, 20..8 (C-5′, 6′), 6.8 (8-CH_3); (Found C, 59.75; H, 6.10; N, 8.87; $C_{16}H_{20}N_2O_5$, requires C, 59.99; H, 6.29; N, 8.74).

L-Methyl 4-methyl-2-((4-oxo-4H-benz[e][1,3]oxazin-2-yl) amino)pentanoate ***14a*** 2-(Methylthio)-4*H*-benz[*e*][1,3] oxazin-4-one **11a** (0.48 g, 2.5 mmol) was allowed to react with L-Leucine methyl ester 9c (0.44 g, 3 mmol)according to general procedure A. The crude solid was collected and recrystallized from toluene to give **14a** (0.47 g, 65 % yield), mp 145–147 °C. ν_{max} (KBr)/cm^{-1} 3,240, 2,870 (N–H), 1,755 (C=O), 1,682 (C=O), 1,619 (C=C), 1,471 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.62 (s, 1H, N–H), 7.91 (d, 1H, $J = 6.5$ Hz, H-5), 7.70 (t, 1H, $J = 7.3$ Hz, H-7), 7.53 (t, 1H, $J = 7.0$ Hz, H-6), 7.30 (d, 1H, $J = 7.0$ Hz, H-8), 4.51 (t, 1H, $J = 7.0$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 1.70 (m, 3H, H-3′/H-4′), 0.99 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.94 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.3 (C-2′), 164.7 (C-4), 157.9 (C-2), 153.2 (C-8a), 133.5 (C-7), 126.2 (C-5), 124.7 (C-6), 117.1 (C-4a), 115.0 (C-8), 52.2 (C-1′), 51.2 (OCH_3), 40.5 (C-4′), 23.9 (C-3′), 21.8, 20.8 (C-5′/6′). (Found C, 62.06; H, 6.30; N, 9.50; $C_{15}H_{18}N_2O_4$, requires C, 62.06; H, 6.25; N, 9.65).

L-Methyl 4-methyl-2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)pentanoate ***14b*** 8-Methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11b** (0.52 g, 2.5 mmol) was allowed to react with L-Leucine methyl ester **9c** (0.44 g, 3 mmol)according to general procedure I. The crude solid was collected and recrystallized from toluene to give **14b** (0.53 g, 70 % yield), mp 177 °C. ν_{max} (KBr)/cm^{-1} 3,280, 2,868 (N–H), 1,761 (C=O), 1,671 (C=O), 1,625 (C=C), 1,487 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.70 (s, 1H, N–H), 7.70 (d, 1H, $J = 7.5$ Hz, H-5), 7.52 (d, 1H, $J = 7.5$ Hz, H-7), 7.2 (t, 1H, $J = 7.5$ Hz, H-6), 4.51 (t, 1H, $J = 7.0$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.42 (s, 3H, 8-CH_3), 1.71 (m, 3H, H-3′/H-4′), 0.99 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.94 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 172.6 (C-2′), 166.0 (C-4), 158.4 (C-2), 151.9 (C-8a), 135.5 (C-7), 124.8 (C-8), 125.2 (C-6), 124.5 (C-5), 117.2 (C-4a), 53.1 (C-1′), 52.4 (OCH_3), 40.5 (C-4′), 24.7 (C-3′), 23.0, 21..3 (C-5′, 6′), 14.6 (CH_3); (Found C, 63.06; H, 6.67; N, 9.14; $C_{16}H_{20}N_2O_4$, requires C, 63.14; H, 6.62; N, 9.20).

L-Methyl 4-methyl-2-((4-oxo-8-phenyl-4H-benz[e][1,3]oxazin-2-yl)amino)pentanoate ***14c*** 8-Phenyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11c** (0.67 g, 2.5 mmol) was allowed to react with L-Leucine methyl ester according **9c** (0.44 g, 3 mmol)to general procedure A. The crude solid was collected and recrystallized from toluene to give **14c** (0.55 g, 60 % yield), mp 184 °C. ν_{max} (KBr)/cm^{-1} 3,243, 2,867 (N–H), 1,745 (C=O), 1,689 (C=O), 1,622 (C=C), 1,475 (C=N); ^{1}H NMR (200 MHz, d_6-DMSO) δ ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.51 (s, 1H, N–H), 7.92 (dd, 1H, $J_{H5,H7} = 1.5$ Hz, $J_{H5,H6} = 7.7$ Hz, H-5), 7.70 (dd, 1H, $J_{H7,H5} = 1.5$ Hz, $J_{H7,H6} = 7.7$ Hz, H-7), 7.60–7.42 (m, 4H, H-6/H-10/H-11/H-12), 4.42 (m, 1H, H-1′), 3.62 (s, 3H, OCH_3), 1.70 (m, 3H, H-3′/H-4′), 0.93 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.87 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.6 (C-2′), 163.5 (C-4), 152.3 (C-2), 150.0 (C-8a), 134.6 (C-7), 134.3 (C-9), 128.8 (C-8), 128.6 (C-11), 127.7 (C-10), 127.2 (C-12), 125.5 (C-6), 124.5 (C-5), 117.0 (C-4a), 52.1 (C-1′), 51.2 (OCH_3), 41.5 (C-4′), 24.7 (C-3′), 23.0, 21.3 (C-5′/6′); (Found C, 68.81; H, 6.16; N, 7.40; $C_{21}H_{22}N_2O_4$, requires C,68.84; H, 6.05; N, 7.65).

L-Methyl-2-((7-hydroxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-4-methylpentanoate ***14d*** 7-Hydroxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11f** (0.52 g, 2.5 mmol) was allowed to react with L-Leucine methyl ester **9c** (0.44 g, 3 mmol)according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **14d** (0.42 g, 55 % yield), mp 210–213 °C. ν_{max} (KBr)/cm^{-1} 3,300–2,956 (O–H), 3,258, 2,867 (N–H), 1,749 (C=O), 1,654 (C=O), 1,583 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.61 (d, 1H, $J = 7.5$ Hz, H-5), 6.8 (d, 1H, $J = 7.5$ Hz, H-6), 4.50 (t, 1H, $J = 7.0$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.22 (s, 3H, 8-CH_3), 1.70 (m, 3H, H-3′/H-4′), 0.98 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.94 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.9 (C-2′), 167.3 (C-4), 160.3 (C-7), 152.7 (C-2), 151.8 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.4 (C-8), 108.9 (C-4a), 52.2 (C-1′), 51.2 (OCH_3), 41.5 (C-4′), 23.9 (C-3′), 21.8, 20.8 (C-5′, 6′), 6.8 (8-CH_3); (Found C, 59.85; H, 6.20; N, 8.33; $C_{16}H_{20}N_2O_5$, requires C, 59.99; H, 6.29; N, 8.74).

L-methyl-2-((7-hydroxy-8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-4-methylpentanoate ***14e*** 7-Hydroxy-8-methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11g** (0.56 g, 2.5 mmol) was allowed to react with L-Leucine methyl ester **9c** (0.44 g, 3 mmol)according to general procedure A. The crude material was collected and recrystallized from ethanol to give **14e** (0.54 g, 67 % yield), mp 195–197 °C. ν_{max} 3,300–2,956 (OH), 3,246, 2,867 (N–H), 1,752 (C=O), 1,655 (C=O), 1,583 (C=N) cm^{-1}; ^{1}HNMR (200 MHz, DMSO-d_6) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.61 (d, 1H, $J = 7.5$ Hz, H-5), 6.83 (d, 1H, $J = 7.5$ Hz, H-6), 4.52 (t, 1H, $J = 7.0$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.22 (s, 3H, 8-CH_3), 1.70 (m, 3H, H-3′/H-4′), 0.98 (d, 3H, $J = 6.0$ Hz, H-5′/H-6′), 0.94 (d, 3H, $J = 6.0$ Hz, H-6′/H-5′). ^{13}CNMR (50 MHz, DMSO-d_6) δ 171.9 (C-2′), 167.3 (C-4), 160.3 (C-7), 152.7 (C-2), 151.8 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.4 (C-8), 108.9 (C-4a), 52.2 (C-1′), 51.2 (OCH_3), 41.5 (C-4′), 23.9 (C-3′), 21.8, 20.8 (C-5′, 6′), 6.8 (8-CH_3); (Found C, 59.85; H, 6.20; N, 8.33; $C_{16}H_{20}N_2O_5$, requires C, 59.99; H, 6.29; N, 8.74).

L-Methyl 3-methyl-2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)butanoate ***15a*** 8-Methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11b** (0.52 g, 2.5 mmol) was allowed to react with L-valine methyl ester **9d** (0.39 g, 3 mmol)according to general procedure A. The crude solid was collected and recrystallized from ethyl acetate to give **15a** (0.46 g, 63 % yield), mp 207–209 °C. ν_{max} (KBr)/cm^{-1} 3,280, 2,868 (N–H), 1,761 (C=O), 1,671 (C=O), 1,625 (C=C), 1,487 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 8.61 (bs, 1H, N–H), 7.70 (d, 1H, $J = 7.5$ Hz, H-5), 7.51 (d, 1H, $J = 7.5$ Hz, H-7), 7.22 (t, 1H, $J = 7.5$ Hz, H-6), 4.32 (d, 1H, $J = 7.2$ Hz, H-1′), 3.71 (s, 3H, OCH_3), 2.2 (m, 1H, H-3′), 1.0 (d, 6H, $J = 7.3$ Hz, H-4′/H-5′), 0.99 (d, 3H, $J = 7.3$ Hz, H-4′/H-5′); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.2 (C-2′), 167.4 (C-4), 158.5 (C-8a), 154.5 (C-2), 141.5 (C-7), 128.8 (C-5), 123.4 (C-4a), 119.8 (C-6), 109.4 (C-8), 55.4 (C-1′), 51.9 (OCH_3), 29.3 (C-3′), 18.1, 17.7 (C4′,5′); (Found C, 62.56 %; H, 6.45 %; N, 9.64 %; $C_{15}H_{18}N_2O_4$, requires C, 62.06 %; H, 6.25 %; N, 9.65 %).

L-Methyl 2-((7-hydroxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl) amino)-3-methylbutanoate ***15b*** 7-Hydroxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11e** (0.52 g, 2.5 mmol) was allowed to react with L-valine methyl ester **9d** (0.39 g, 3 mmol)according to general procedure A. The crude material was collected and recrystallized from ethanol to give **15b** (0.44 g, 60 % yield), mp decomp 220 °C. ν_{max} 3,249–2,963 (OH), 3,246, 2,867 (N–H), 1,745 (C=O), 1,655 (C=O), 1,598 (C=N) cm^{-1}; ^{1}HNMR (200 MHz,DMSO-d_6) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.70 (d, 1H, $J = 8.4$ Hz, H-5), 6.82 (dd, 1H, $J_{H6,H8} = 2.4$ Hz, $J_{H6,H5} = 8.4$ Hz, H-6), 6.62 (d, 1H, $J = 2.2$ Hz, H-8), 4.32 (d, 1H, $J = 7.2$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.22 (m, 1H, H-3′), 1.00 (d, 3H, $J = 7.3$ Hz, H-4′/H-5′), 0.99 (d, 3H, $J = 7.3$ Hz, H-4′/H-5′). ^{13}CNMR (50 MHz,DMSO-d_6) δ 170.5 (C-2′), 164.4 (C-4), 162.2 (C-7), 157.5 (C-2), 154.5 (C-8a), 127.8 (C-5), 113.4 (C-6), 109.0 (C-4a), 100.4 (C-8), 59.4 (C-1′), 51.0 (OCH_3), 29.3 (C-3′), 18.1, 17.7 (C4′,5′); (Found C, 55.56; H, 5.45; N, 9.04; $C_{14}H_{16}N_2O_5$, 1/2 H_2O requires C, 57.53; H, 5.52; N, 9.58).

L-Methyl-2-((7-hydroxy-8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-3-methylbutanoate ***15c*** 7-Hydroxy-8-methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11g** (0.56 g, 2.5 mmol)was allowed to react with L-valine methyl ester **9d** (0.39 g, 3 mmol) according to general procedure A. The crude material was collected and recrystallized from ethanol to give **15c** (0.42 g, 55 % yield), mp 220 °C. ν_{max} 3,200–2,954 (OH), 3,246, 2,867 (N–H), 1,746 (C=O), 1,653 (C=O), 1,589 (C=N) cm^{-1}; ^{1}HNMR (200 MHz, DMSO-d_6) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.51 (d, 1H, $J = 8.6$ Hz, H-5), 6.8 (d, 1H, $J = 8.6$ Hz, H-6), 4.32 (d, 1H, $J = 7.2$ Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.32 (m, 1H, H-3′), 2.22 (s, 3H, 8-CH_3), 1.00 (d, 3H, $J = 7.3$ Hz, H-4′/H-5′), 0.99 (d, 3H, $J = 7.3$ Hz, H-4′/H-5′). ^{13}CNMR (50 MHz, DMSO-d_6) δ 170.6 (C-2′), 165.1 (C-4), 159.7 (C-7), 157.1 (C-2), 152.5 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.6 (C-8), 108.9 (C-4a), 59.4 (C-1′), 50.9 (OCH_3), 29.3 (C-3′), 18.1, 17.8 (C4′, 5′), 6.9 (8-CH_3); (Found C, 58.35; H, 5.83; N, 9.52; $C_{15}H_{18}N_2O_5$, requires C, 58.82; H, 5.92; N, 9.15).

L-Methyl 2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl) amino)-3-phenylpropanoate ***16a*** 8-Methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11b** (0.56 g, 2.5) mmol)was allowed to react with L-Phenylalanine methyl ester **9e** (0.54 g, 3 mmol) according to general procedure A. The

crude solid was collected and recrystallized from toluene/cyclohexane to give **16a** (0.46 g, 55 % yield), mp 205 °C. ν_{max} (KBr)/cm^{-1} 3,189, 2,867 (N–H), 1,755 (C=O), 1,675 (C=O), 1,625 (C=C), 1,476 (C=N); ^{1}HNMR (200 MHz, d$_6$-DMSO) δ 8.62 (s, 1H, N–H), 7.81 (d, 1H, Hz, J = 7.5 Hz, H-5), 7.51 (d, 1H, J = 7.6 Hz, H-7), 7.42 (t, 1H, J = 7.5 Hz, H-6), 7.22 (m, 5H, ArH5′/9′), 4.61 (m, 1H, H-1′), 3.62 (s, 3H, OCH_3), 3.12 (AB part of ABX system, 2H, 13.5 Hz, H-3′); ^{13}CNMR (50 MHz, d$_6$-DMSO) δ 171.2 (C-2′), 166.7 (C-4), 158.4 (C-2), 150.8 (C-8a), 136.4 (C-4′), 134.1 (C-7), 128.6, 128.3, 127.8, 127.5, 127.3, (CH, of C-5′/C-6′/C-7′/C-8′C-9′), 124.8 (C-8), 125.4 (C-6), 124.5 (C-5), 117.4 (C-4a), 55.0 (C-1′), 52.3 (OCH_3), 35.9 (C-3′); (Found C, 67.59; H, 5.54; N, 8.95; $C_{19}H_{18}N_2O_4$, requires C, 67.44; H, 5.36; N, 8.28).

L-Methyl 2-((4-oxo-8-phenyl-4H-benz[e][1,3]oxazin-2-yl)amino)-3-phenylpropanoate ***16b*** 8-Phenyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11c** (0.67 g, 2.5 mmol) was allowed to react with L-Phenylalanine methyl ester **9e** (0.54 g, 3 mmol) according to general procedure I. The crude solid was collected and recrystallized from toluene/cyclohexane to give **16b** (0.55 g, 55 % yield), mp 183 °C. ν_{max} (KBr)/cm^{-1} 3,189, 2,867 (N–H), 1,745 (C=O), 1,681 (C=O), 1,645 (C=C), 1,476 (C=N); ^{1}HNMR (200 MHz, d$_6$-DMSO) δ 8.61 (s, 1H, N–H), 7.90 (dd, 1H, $J_{H5,H7}$ = 1.6 Hz, $J_{H5,H6}$ = 7.6 Hz, H-5), 7.70 (dd, 1H, $J_{H7,H5}$ = 1.6 Hz, $J_{H7,H6}$ = 7.6 Hz, H-7), 7.60–7.40 (m, 4H, H-6/H-10/H-11/H-12), 7.2 (m, 5H, ArH5′/9′), 4.61 (m, 1H, H-1′), 3.63 (s, 3H, OCH_3), 3.12 (AB part of ABX system, 2H, 13.5 Hz, H-3′); ^{13}CNMR (50 MHz, d$_6$-DMSO) δ 170.2 (C-2′), 164.7 (C-4), 157.7 (C-2), 149.8 (C-8a), 136.4 (C-4′), 134.4 (C-9), 134.1 (C-7), 128.6, 128.3, 127.8, 127.5, 127.3, 125.9 (CH, of C10, 11, 12 and C5′, 6′, 7′, 8′, 9′), 125.4 (C-6), 124.5 (C-5), 117.4 (C-4a), 55.0 (C-1′), 51.3 (OCH_3), 35.9 (C-3′); (Found C, 72.09; H, 5.14; N, 6.95; $C_{24}H_{20}N_2O_4$, requires C, 71.99; H, 5.03; N, 7.00).

L-Methyl-2-((7-hydroxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-3-phenylpropanoate ***16c*** 7-Hydroxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11f** (0.5,2 g, 2.5 mmol) was allowed to react with L-Phenylalanine methyl ester **9e** (0.54 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **16c** (0.43 g, 50 % yield), mp 195–197 °C. ν_{max} (KBr)/cm^{-1} 3,300–2,954 (O–H), 3,246, 2,867 (N–H), 1,743 (C=O), 1,655 (C=O), 1,573 (C=N); ^{1}HNMR (200 MHz, d$_6$-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.51 (d, 1H, J = 8.5 Hz, H-5), 7.22 (m, 5H, ArH5′/9′), 6.83 (d, 1H, J = 8.5 Hz, H-6), 6.62 (s, 1H, H-8), 4.7 (m, 1H, H-1′), 3.7 0 (s, 3H, OCH_3), 3.12 (AB part of ABX system, 2H, 13.5 Hz, H-3′). 2.12 (s, 3H, 8-CH_3); ^{13}CNMR (50 MHz, d$_6$-DMSO) δ 170.8 (C-2′), 165.1 (C-4), 159.8 (C-7), 157.5 (C-2), 152.4 (C-8a), 136.7 (C-4′), 128,3 (C6′/C-8′), 127,6 (C5′/C-9′), 125.9 (C-7′), 124.3 (C-5), 112.2 (C-6), 109.5 (C-8), 108.9 (C-4a), 55.0 (C-1′), 51.2 (OCH_3), 36.2 (C-3′); (Found C, 63.80; H, 4.90; N, 8.59; $C_{18}H_{16}N_2O_5$, requires C, 63.52; H, 4.74; N, 8.23).

L-Methyl-2-((7-hydroxy-8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)-3-phenylpropanoate ***16d*** 7-Hydroxy-8-methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11g** (0.5,6 g, 2.5 mmol) was allowed to react with L-Phenylalanine methyl ester **9e** (0.54 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **16d** (0.49 g, 53 % yield), mp 195–197 °C. ν_{max} (KBr)/cm^{-1} 3,300–2,954 (O–H), 3,246, 2,867 (N–H), 1,746 (C=O), 1,650 (C=O), 1,569 (C=N); ^{1}HNMR (200 MHz, d$_6$-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), δ 7.61 (d, 1H, J = 8.4 Hz, H-5), 6.81 (d, 1H, J = 8.4 Hz, H-6), 4.52 (q, 1H, J = 7.3 Hz, H-1′), 3.70 (s, 3H, OCH_3), 2.22 (s, 3H, 8-CH_3). 1.43 (d, 3H, J = 7.3 Hz, H-3′); ^{13}CNMR (50 MHz, d$_6$-DMSO) δ 171.6 (C-2′), 164.9 (C-4), 159.7 (C-7), 157.1 (C-2), 152.7 (C-8a), 124.3 (C-5), 112.2 (C-6), 109.5 (C-8), 108.9 (C-4a), 51.2 (C-1′), 49.1 (OCH_3), 16.2 (C-3′), 6.8 (8-CH_3); (Found C, 64.65; H, 5.10; N, 8.05; $C_{19}H_{18}N_2O_5$, requires C, 64.40; H, 5.12; N, 7.91 %).

L-Methyl 1-(4-oxo-4H-benz[e][1,3]oxazin-2-yl)pyrrolidine-2-carboxylate ***17a*** 2-(Methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11a** (0.48 g, 2.5 mmol) was allowed to react with L-Proline methyl ester **9f** (0.39 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from toluene to give **17a** (0.41 g, 60 % yield), mp 139–141 °C. ν_{max} (KBr)/cm^{-1} 1,741 (C=O), 1,675 (C=O), 1,619 (C=C), 1,466 (C=N); ^{1}HNMR (200 MHz, d$_6$-DMSO) δ 8.61 (bs, 1H, N–H), 7.92 (d, 1H, J = 6.5 Hz, H-5), 7.70 (t, 1H, J = 7.3 Hz, H-7), 7.51 (t, 1H, J = 7.0 Hz, H-6), 7.32 (d, 1H, J = 7.0 Hz, H-8), 4.70 (m, 1H, H-1′), 3.70 (s, 3H, OCH_3), 3.70 (m, 1H and m, 1H, C5′H$_2$), 2.2 (m, 4H, C3′H$_2$ and C4′H$_2$); ^{13}CNMR (50 MHz, d$_6$-DMSO) δ 171.2 (C-2′), 164.4 (C-4), 155.7 (C-2), 152.9 (C-8a), 133.4 (C-7), 126.2 (C-5), 124.9 (C-6), 116.8 (C-4a), 115.1 (C-8), 58.9 (C-1′), 51.4 (OCH_3), 46.5 (C-5′), 29.0 (C-3′), 22.6 (C-4′).

L-Methyl 1-(8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)pyrrolidine-2-carboxylate ***17b*** 8-Methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11b** (0.56 g, 2.5) mmol)was allowed to react with L-Proline methyl ester **9f** (0.39 g, 3 mmol) according to general procedure I. The crude solid was collected and recrystallized from toluene\cyclohexane to give **17b** (0.49 g, 68 % yield), mp 164 °C. ν_{max} (KBr)/cm^{-1} 1,756 (C=O), 1,676 (C=O), 1,621 (C=C), 1,483 (C=N); ^{1}HNMR (200 MHz, d$_6$-DMSO) δ 8.64 (bs, 1H, N–H), 7.70 (d, 1H, J = 7.5 Hz, H-5), 7.51 (d, 1H, J = 7.5 Hz, H-7), 7.32 (t, 1H, J = 7.5 Hz, H-6), 4.70 (m,

1H, H-1′), 3.70 (s, 3H, OCH_3), 3.73 (m, 1H and m, 1H, C5′H_2), 2.32 (s, 3H, 8-CH_3), 2.12 (m, 4H, C3′H_2 and C4′H_2); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.0 (C-2′), 164.9 (C-4), 155.4 (C-2), 151.3 (C-8a), 134.2 (C-7), 124.4 (C-8), 124.1 (C-6), 123.6 (C-5), 116.6 (C-4a), 58.7 (C-1′), 51.4 (OCH_3), 46.5 (C-5′), 29.1 (C-3′), 22.6 (C-4′), 13.2 (CH_3); (Found C, 62.46; H, 5.38; N, 9.86; $C_{15}H_{16}N_2O_4$, requires C, 62.49; H, 5.59; N, 9.72).

L-Methyl 1-(7-hydroxy-4-oxo-4H-benz[e][1,3]oxazin-2-yl)pyrrolidine-2-carboxylate ***17c*** 7-Hydroxy-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11f** (0.52 g, 2.5) was allowed to react with L-Proline methyl ester **9f** (0.39 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **17c** (0.44 g, 60 % yield), mp 201 °C. ν_{max} (KBr)/cm^{-1} 3,058–2,851 (O–H), 1,736 (C=O), 1,660 (C=O), 1,539 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.70 (1H, $J = 8.6$ Hz, H-5), 6.82 (d, 1H, $J = 8.6$ Hz, H-6), 6.61 (s, 1H, H-8), 4.61 (m, 1H, H-1′), 3.70 (s, 3H, OCH_3), 3.73 (m, 1H and m, 1H, C5′H_2), 2.22 (m, 4H, C3′H_2 and C4′H_2); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.1 (C-2′), 164.4 (C-4), 163.5 (C-7), 155.2 (C-2), 154.5 (C-8a), 127.5 (C-5), 114.1 (C-6), 108.0 (C-4a), 100.5 (C-8), 58.8 (C-1′), 51.4 (OCH_3), 46.5 (C-5′), 29.0 (C-3′), 22.6 (C-4′); (Found C, 58.05; H, 4.54; N, 9.49;$C_{14}H_{14}N_2O_5$, requires C, 57.93; H, 4.86; N, 9.65).

L-Methyl-1-(7-hydroxy-8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)pyrrolidine-2-carboxylate ***17d*** 7-Hydroxy-8-methyl-2-(methylthio)-4*H*-benz[*e*][1,3]oxazin-4-one **11g** (0.5,6 g, 2.5 mmol) was allowed to react with L-Proline methyl ester **9f** (0.39 g, 3 mmol) according to general procedure A. The crude solid was collected and recrystallized from ethanol to give **17d** 0.46 g, (60 % yield), mp decomp, 264 °C. ν_{max} (KBr)/cm^{-1} 3,300–2,956 (O–H), 1,737 (C=O), 1,656 (C=O), 1,541 (C=N); ^{1}HNMR (200 MHz, d_6-DMSO) δ 10.22 (bs, 1H, O–H), 8,61 (bs, 1H, N–H), 7.61 (d, 1H, $J = 7.5$ Hz, H-5), 6.82 (d, 1H, $J = 7.5$ Hz, H-6), 4.70 (m, 1H, H-1′), 3.70 (s, 3H, OCH_3), 3.69 (m, 1H and m, 1H, C5′H_2), 2.22 (m, 4H, C3′H_2 and C4′H_2), 2.12 (s, 3H, 8-CH_3); ^{13}CNMR (50 MHz, d_6-DMSO) δ 171.6 (C-2′), 164.9 (C-4), 159.6 (C-7), 155.7 (C-2), 152.7 (C-8a), 124.3 (C-5), 112.4 (C-6), 109.5 (C-8), 108.8 (C-4a), 58.7 (C-1′), 51.4 (OCH_3), 46.5 (C-5′), 29.1 (C-3′), 22.6 (C-4′), 6.6 (8-CH_3); (Found C, 58.96; H, 5.49; N, 9.13; $C_{15}H_{16}N_2O_5$, requires C, 59.21; H, 5.30; N, 9.21).

L-3-methyl-2-((8-methyl-4-oxo-4H-benz[e][1,3]oxazin-2-yl)amino)butanoic acid ***18*** The L-Methyl 3-methyl-2-((8-methyl-4-oxo-4H-benz[*e*][1,3]oxazin-2-yl)amino)butanoate 15a (0.29 g, 1 mmol) was dissolved in anhydrous methanol (5 mL),to this solution 1 M NaOH (1.1 ml, 1.1 mmol) was added dropwise. The mixture was stirred overnight at room temperature. At the completion, the reaction mixture was neutralized with 10 % hydrochloric acid and the solid was separated by vacuum filtration and recrystallized from ethanol and gave white solid **18** (0.16 g, 58 %), mp decomp 195–198. ν_{max} (KBr)/cm^{-1} 3,286, (N–H), 1,706 (carboxylic C=O), 1,654 (C=O), 1,624 (C=C), 1,443 (C=N); ^{1}HNMR (300 MHz, d_6-DMSO) δ 10.80 (bs, 1H, OH), 8.69 (d, 1H, $J = 8.1$ Hz N–H), 7.84 (d, 1H, $J = 7.2$ Hz, H-5), 7.34 (d, 1H, $J = 7.2$ Hz, H-7), 6.83 (t, 1H, $J = 7.7$ Hz, H-6),), 4.24 (d,d, 1H, $J_{NH} = 8.4$ Hz, $J_{H3'} = 4.8$, H-1′), 2.22 (s, 3H, 8-CH_3). 2.19 (m, 1H, H-3′) 0.94, 0.92 (2d, 6H 2 × CH_3, $J = 6.9$ and 6.6*)*; ^{13}CNMR (75 MHz, d_6-DMSO) δ 172.4 (C-2′), 169.8 (C-4), 157.6 (C-2), 152.8 (C-8a), 136.0 (C-7), 127.2 (C-5), 126.8 (C-8), 119.1 (C-6), 115.9 (C-4a), 57.9 (C-1′), 30.3 (C-3′), 19.1 (8-CH_3)17.7, 15.9 (2 × CH_3), (Found C, 60.96; H, 5.79; N, 10.23; $C_{15}H_{16}N_2O_5$, requires C, 60.86; H, 5.84; N, 10.14).

HeLa cervical cancer cell line used was obtained from the American Type Culture Collection and was cultured in Dulbecco's Modified Eagle Medium (DMEM), Invitrogen, Grand Island) containing phenol red, supplemented with 10 % fetal bovine (FBS, Sigma-Aldrich, St Louis).

Preparation of stock solutions

All stock solutions were made up using DMSO (Sigma-Aldrich, St. Louis), and Compounds and etoposide (Sigma-Aldrich, St. Louis) were made up to a 10 mM stock. It should be noted that in accordance with Lee *et al*. (2006), the amount of DMSO added to both test and control media never exceeded 0.5 % of the total volume, and control cultures were treated with equivalent volumes of DMSO that was used to prepare stock solutions of the drug to eliminate adverse effects of DMSO.

Toxicity and chemo-sensitization (SRB assay)

In order to determine the toxicity of the compounds alone as well as their sensitizing effects in combination with etoposide, the sulphonamide blue assay (SRB assay) was utilized. The method put forward by Freshney (1992) was used to carry out the assay with slight variations. Cells were seeded on 24-well plates at a concentration of 2 × 103 cells/well. After cells had adhered overnight, culture media was removed and replaced by 1 ml of treatment media. Treatment media was removed after 48 h before cells were fixed, stained and absorbance was measured at 538 nm using a Flex Station 3 (Molecular Devices, California). Absorbance at 538 nm is directly proportional to overall cell number.

DNA-PK inhibition assay

The DNA-PK assays were performed by Reaction Biology Corporation, One Great Valley Parkway, Suite 2 Malvern, PA 19355 USA.

All Compounds were dissolved in DMSO and tested for their ability to inhibit human DNA-PK. Compounds were tested in a 10-dose IC_{50} profile with fourfold serial dilution starting at 100 μM. The Control compound, LY294002, was tested in a 10-dose IC_{50} profile with threefold serial dilutions starting at 10 μM. Reactions were carried out using 20 μM Peptide substrate [EPPLSQEAFADLWKK], 10 μg/ml DNA and 10 μM ATP using the HTRF assay format.

Platelet aggregometry

Venous blood was collected from ostensibly healthy, drug-free volunteers into trisodium citrate 22.0 g/L. Ethics approval was obtained from La Trobe University Human Ethics Committee (HEC approval No. 07-127). Platelet aggregation was determined by the optical method in a two-channel platelet aggregometer (Chrono-Log) using the previously reported protocol (Pritchard *et al*., 2007).

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