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Novel synthesis of 3,4-dihydro-5-bromo[1,4]oxazin-2-one derivatives, new protease inhibitor scaffold

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We designed and synthesized a new class of serine protease inhibitors based on the oxazinone core. To this end, we first developed a short and efficient route to synthesize a new 3,4-dihydro[1,4]oxazin-2-one ring. Then we successfully synthesised the corresponding 5-bromo derivatives which have never been reported before, and demonstrated their inhibitory activities on α-chymotrypsin.

Introduction

Oxazin-2-ones and related structures have been extensively used as precursors for the synthesis of chiral compounds, mainly amino acids. Their synthesis as well as their biological properties are well documented.[1,2] Nevertheless an exhaustive literature survey reveals that 3,4-dihydro-5-halogeno[1,4]oxazin-2-ones **I** (Fig. 1) have never been previously described.

From a biological point of view, it appears to us that such chiral building blocks could be suitable for the design of new protease inhibitors. The presence of a leaving group (halogen atom) at the 5 position of the oxazinone ring could participate in the protease inhibitory process. Indeed as depicted in Fig. 1, nucleophilic attack on the lactone function by the hydroxyl function of the serine residue of a serine protease such as α-chymotrypsin, could lead to enzyme alkylation *via* substitution of the leaving group by the nucleophile residue (His-57) of the enzyme catalytic triad. Such a protease inhibition mechanism has been already reported in the case of serine protease inhibition by the 3-alkoxy-7-amino-4-chloroisocoumarin core (Fig. 1).[3–9]

The challenge we faced was to carry out a new original and efficient synthesis of this versatile oxazinone building block, bearing a halogen atom at the 5 position and various substituents at the 3, 4, and 6 positions. We report in this paper, the first synthesis of 3,4-dihydro-5-halogenooxazin-2-ones **I** and their corresponding α-chymotrypsin inhibitory activities.

Results and discussion

The protected amino acids H-(L)-leucine-OtBu and H-(L)-phenylalanine-OtBu were selected in order to achieve the first oxazinone cores; nevertheless, it will be possible to extend this work to other commercially available amino acids in order to introduce a wide range of substituents at the 3 position of the oxazinone core. First, we tried to directly alkylate the amine function *via* a nucleophilic substitution through the use of ethyl bromoacetate as electrophilic reagent. Unfortunately, this synthetic pathway was not efficient, since it appeared difficult to limit the substitution reaction to monoalkylation of the amine function in reasonable yields. To circumvent this difficulty, we used the method of Fukuyama who reported an efficient monoalkylation method of the amine function *via* the formation of a sulfonamide intermediate (Scheme 1).[10] For this purpose, (H-(L)-leucine-OtBu and H-(L)-phenylalanine-OtBu were first sulfonylated to give the corresponding sulfonamides **1** and **2** in 96% and 84% yields respectively, through the use of 4-nitrobenzenesulfonyl chloride in the presence of TEA in methylene chloride. The next step was the alkylation of the sulfonamide group which was carried out under mild conditions according to two procedures, either under Mitsunobu conditions (Method A: ROH, DEAD, Ph_3P, CH_2Cl_2, rt) or under conventional nucleophilic substitution conditions (Method B: RX, K_2CO_3, DMF, rt).[10] The *N*,*N*-disubstituted 4-nitrobenzenesulfonamide derivatives **3a**–**c** and **4a**–**c** (Table 1)

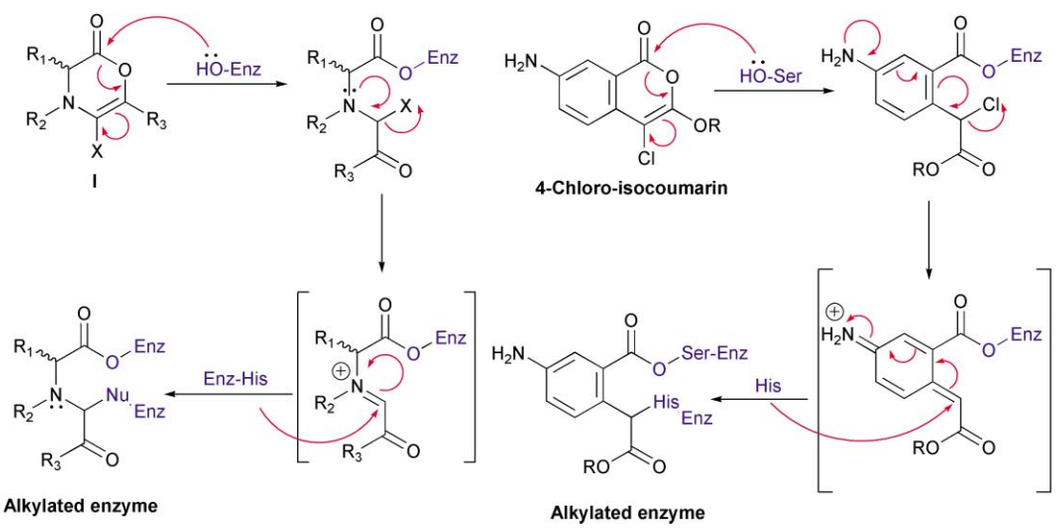

Fig. 1

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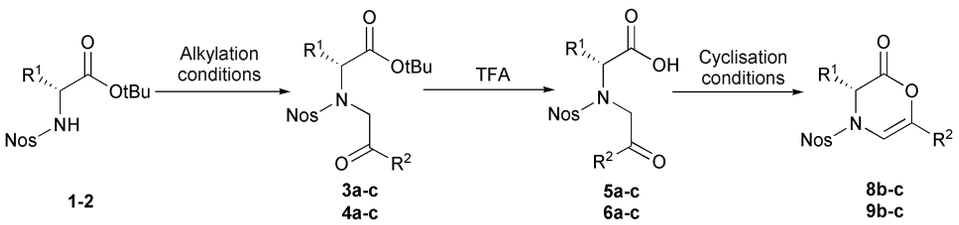
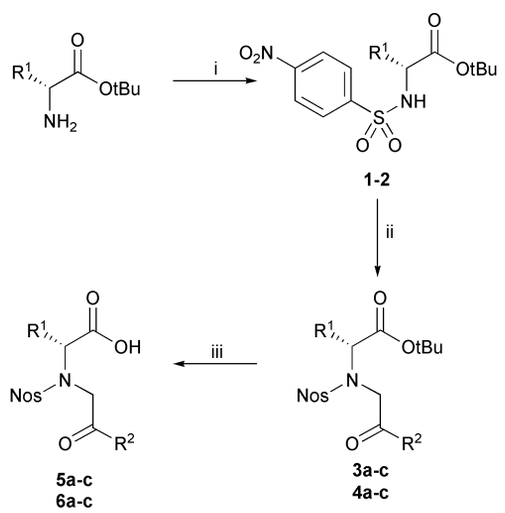
Table 1



		Alkylation conditions			Cyclisation conditions					
R^1	R^2	Method [a]	Product	Yield (%)	Coupling reagent	Mol eq.	Solvent	*T*/°C	Product	Yield (%)
(L)–Leu	OEt	A	**3a**	55	—	—	—	—	—	—
	Ph	A	**3b**	17	$SOCl_2$	1	PhMe	70	**8b**	55
	(4-MeO)–Ph	B	**3c**	95	PyBOP	1.1	CH_2Cl_2	25	**8c**	92
(L)–Phe	OEt	A	**4a**	85	PCl_5	2.5	PhMe	110	**7**	46
	Ph	A	**4b**	24	PCl_5	1	PhMe	25	**9b**	24
	Naphthyl	B	**4c**	87	$SOCl_2$	1	CH_2Cl_2	25	**9c**	84

[a] Method A: R^2OH (2.5 eq.), DEAD (2.5 eq.), PPh_3 (2.5 eq.), CH_2Cl_2, rt, 1 h; Method B: R^2X (1.7 eq.), K_2CO_3 (2.0 eq.), MeCN, rt, 5 h.

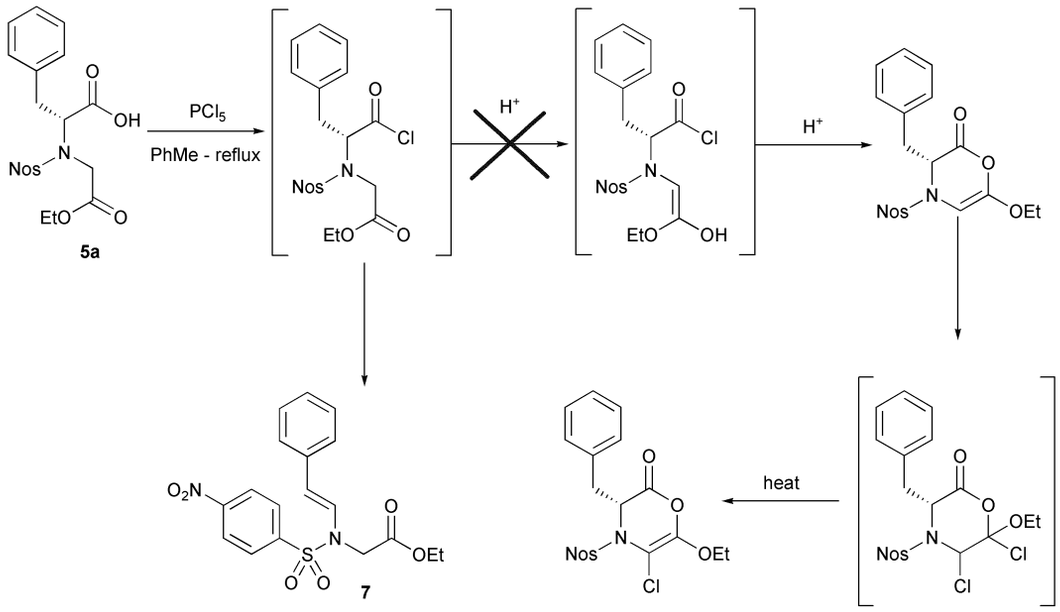


Scheme 1 *Reagents and conditions*: (i) *p*-nitrobenzenesulfonyl chloride, TEA, CH_2Cl_2, rt; (ii) Method A: $R^2C(O)CH_2OH$, DEAD, PPh_3, CH_2Cl_2, rt; or Method B: $R^2C(O)CH_2Br$, K_2CO_3, DMF, rt; (iii) TFA–CH_2Cl_2 (1 : 1), rt.

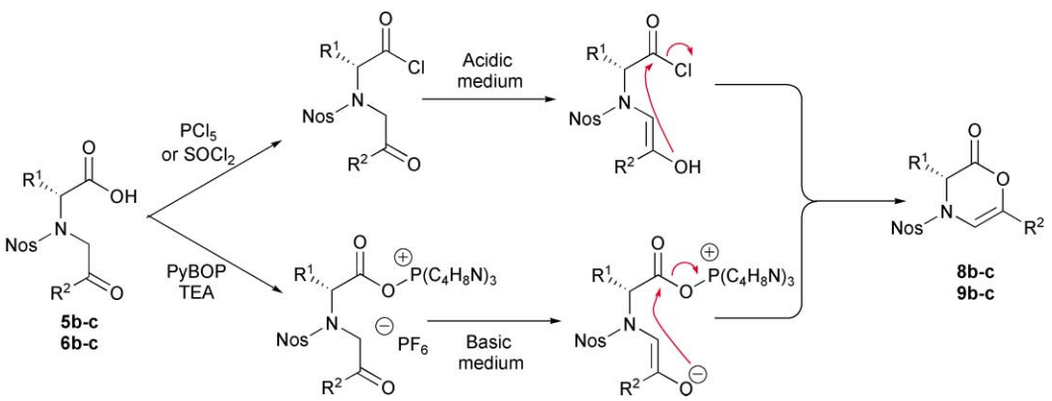
were obtained in variable yields depending on the reaction conditions and on the ketone or ester substituent R^2. The results obtained indicate that alkylation appears more efficient under standard nucleophilic substitution conditions rather than under Mitsunobu reaction conditions. Upon treatment with TFA in CH_2Cl_2, *tert*-butyl esters **3a**–**c** and **4a**–**c** were easily deprotected into the corresponding carboxylic acids **5a**–**c** and **6a**–**c** in quantitative yields.[11] The key step of this synthesis was the cyclisation of carboxylic acid **5a**–**c** and **6a**–**c** into the corresponding 3,4-dihydro[1,4]oxazin-2-one core. For this purpose, we have investigated different cyclisation procedures using various chemical reagents. First, we tried to carry out the cyclisation on prototype **5a** through the use of phosphorus pentachloride (PCl_5) in refluxing toluene (Scheme 2). Using an excess of PCl_5, it seemed possible to perform both steps, *i.e.* the cyclisation then the chlorination at the 5-position of the resulting oxazinone ring, in a one-pot process.[12] Indeed, we hoped to carry out this two-step reaction according to the mechanism depicted in Scheme 2, *i.e.* PCl_5 first reacts with the carboxylic acid function leading to the corresponding acyl chloride; simultanously, the experimental conditions, *i.e.* an acidic medium (HCl released) in refluxed toluene, favor the enol formation from the corresponding ester function. The enol form then reacts with the acyl chloride moiety leading to the 3,4-dihydrooxazin-2-ones. In a second step, excess PCl_5 can react with the double bond of the oxazinone ring leading to the corresponding 5,6-dihalogeno intermediate, which after a dehydrohalogenation reaction, leads to the desired compounds 3,4-dihydro-5-halogenooxazin-2-ones. Surprisingly, in the described conditions, we only isolated compound **7** in 46% yield, whose structure clearly indicated that the cyclisation reaction did not occur. Fully characterized by LCMS, ^1H, ^{13}C NMR and elemental analysis, compound **7** was exclusively found under a *trans* configuration which was assigned on the basis of the characteristic coupling constant of 14.5 Hz between the two olefinic protons. It can be underlined that formation of **7** resulted from both processes, decarboxylation and dehydrogenation. According to the mechanism reported in Scheme 2, we cannot exclude the acid chloride intermediate formation resulting from the conversion of the corresponding carboxylic acid moiety in the presence of PCl_5. Nevertheless it can be suggested that, under the experimental conditions, the formation of the enol function from the corresponding ester was not favored. Consequently, cyclisation did not occur, and only compound **7** is formed. To avoid this side-reaction, we decided first to perform the cyclisation process and to isolate the cyclic intermediate, and in a second separate step to achieve the halogenation reaction of the oxazinone ring. To reach the oxazinone ring, we have seen that the cyclisation depended on two independent conditions; on the one hand, the carboxylic acid function must be activated with a coupling reagent to allow the esterification, and on the other hand, the experimental conditions have to favor enol formation from the corresponding carbonyl group. As far as we had seen that enol formation did not occur in the case of ester functions (R = OEt), we carried out this cyclisation starting with the aromatic ketone compound (R = Ph, Ph(OMe), Naphthyl). Indeed, it is known that enol formation from aromatic ketones can be favored under acidic or basic conditions. Consequently, we have tried to achieve the cyclisation through the use of several coupling reagents in acidic or basic media (Scheme 3).

Cyclisation in acidic conditions

First, we investigated the cyclisation on compound **6b** at room temperature in the presence of only one equivalent of PCl_5. Under these experimental conditions, *i.e.* in an acidic medium, the enol formation from the ketone group is favored and consequently it readily reacted with the acyl chloride moiety resulting from the action of PCl_5, leading to the oxazinone core. Following this method, the expected 3,4-dihydro[1,4]-oxazin-2-one **9b** was isolated in low yield (24%). To optimize



Scheme 2



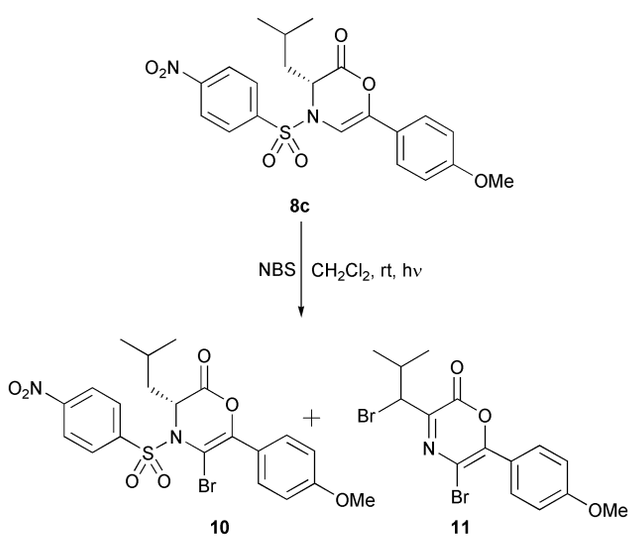
Scheme 3

the cyclisation yield, we also investigated other reagents such as thionyl chloride ($SOCl_2$) which is known to react with carboxylic acid functions similarly to PCl_5, but is unable to halogenate double bonds.[13] When analogues **5b** or **6c** were stirred in the presence of $SOCl_2$ (1 equivalent) in toluene at 70 °C or in CH_2Cl_2 at room temperature, the expected oxazinones **8b** and **9c** were isolated in 55 and 84% yield respectively, and fully characterized (LCMS, ^1H and ^{13}C NMR and elemental analysis). ^1H NMR spectra of the compounds **8b** and **9c** show a singlet signal at 6.6 ppm, characteristic of the proton at the 5-position of the oxazinone core.

Cyclisation in basic conditions

Cyclisation was performed on compound **5c** which is stirred with the peptidic coupling reagent PyBOP (1.1 equivalent)[14] in the presence of TEA, leading to the corresponding oxazinone **8c** in almost quantitative yield. To summarize, it can be observed that oxazinone **8c** can be synthesized in 4 steps starting from commercial protected leucine in 84% overall yield, which represents a real synthetic improvement compared to previously reported oxazinone syntheses.[15,16] The whole results, including the different experimental conditions, are summarized in Table 1.

The next step of the synthesis was to investigate the most efficient methods of introducing a halogen atom at the 5-position of the oxazinone ring. Compound **8c** was first used as an oxazinone model. Since PCl_5 was not suitable for the introduction of a chlorine atom at the 5 position of the oxazinone ring,[17] we first tried to achieve this halogenation using bromine under various conditions (solvents, temperature, and reaction time);[18] unfortunately, halogenation did not occur, and we observed the formation of compound **5c** which resulted from the opening of the oxazinone core. Next we used NBS as bromination reagent in CH_2Cl_2 at room temperature (Scheme 4).[19] Under these experimental conditions, we isolated two compounds brominated at the 5 position, *i.e.*



Scheme 4

Table 2

Compounds	α-Chymotrypsin inhibition (%)		
	1 μM	10 μM	100 μM
8b	nd[a]	21	70
8c	nd	0	51
9c	nd	26	97
10	nd	nd	52
11	40	100	nd

[a] nd : not determined.

bromooxazinone compound **10** and dibromooxazinone compound **11**. 5-Bromo-3,4-dihydrooxazin-2-one **10** was obtained in 30% yield and fully characterized (LCMS, ^{1}H and ^{13}C NMR and elemental analysis). Compound **10** ^{1}H NMR spectrum shows the disappearance of the singlet signal at 6.6 ppm, characteristic of the proton at the 5-position of the oxazinone derivative **8c**. Compound **11** was obtained in 18% yield and resulted from the reaction of NBS with compound **10** according to the following sequence: formation of a double bond C=N at the α-position of the lactone function, leading to the elimination of the 4-nitrosulfonamido moiety, followed by bromination of the allylic carbon C_β of the side chain.[20] When the same reaction was carried out in the dark, formation of the dibromo compound **11** was not observed.

The last part of this study reports the preliminary results related to the protease inhibitory properties of the new synthesized scaffolds. As shown in Table 2, compounds **8b**, **8c**, **9c**, **10** and **11**, assayed as α-chymotrypsin inhibitors according to standard procedures,[21] have demonstrated interesting protease inhibitory properties, notably compound **11** which is the most potent inhibitor with an IC_{50} value around 1 μM. These encouraging preliminary results gave credit to our hypothesis relative to the design of protease inhibitors based on the 5-halogenooxazin-2-one scaffold. Synthesis of a focused library based on this scaffold is under way and will be tested on various classes of proteases known to be involved in different pathologies.

In conclusion, we have designed and synthesized a new class of serine protease inhibitors based on the oxazinone core. Indeed, we have developed a short original and efficient method to synthesize a new 3,4-dihydro[1,4]oxazin-2-one ring. Moreover we succeeded in synthesizing novel 5-bromo-3,4-dihydro[1,4]oxazin-2-one and 5-bromo[1,4]oxazin-2-one derivatives and in demonstrating their inhibitory activities on α-chymotrypsin.

Experimental section

All common chemicals and solvents were reagent grade or better. The purity of each compound was checked by ^{1}H and ^{13}C NMR spectroscopy, mass spectroscopy, thin-layer chromatography, melting point, and elemental analysis and results are consistent with the proposed structures. The ^{1}H NMR spectra were recorded on either a Bruker AC-300 MHz or a Bruker AMX-250 instrument, and ^{13}C NMR on a Bruker AMX-62 MHz. Elemental analyses were performed by Service Central d'Analyses du CNRS (Vernaison, France) and were within ± 0.3% of the theoretical values. Thin-layer chromatographic analyses (TLC) were conducted on silica gel plates 0.2mm thick ($60F_{254}$ Merck) with various mixtures as eluent. Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck). Analytical purity was assessed by LC-MS using a Waters 2790 system. Low resolution mass spectra were recorded on a Micromass ZMD mass spectrometer. Mass spectra were acquired under electrospray ionisation (ESI).

Sulfonylation. General procedure leading to compounds 1–2

To a solution of the amino acid *tert*-butyl ester (1 eq.) in CH_2Cl_2 were added TEA (2.2 eq.) then 4-nitrobenzenesulfonyl chloride (1.1 eq.). The reaction mixture was stirred at room temperature for 12 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give compounds **1** and **2**

***N*-(4-Nitrobenzenesulfonyl)-L-leucine *tert*-butyl ester 1**

96% yield; R_f (EtOAc–cyclohexane [1 : 2]) 0.57; mp 74 °C; δ_H (250 MHz, $CDCl_3$) 8.38 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 8.20 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 4.31 (t, 1H, *J* 7.5, C*H*–CO_2tBu), 1.70 (m, 1H, CH_3–C*H*–CH_3), 1.57 (m, 2H, CH–CH_2–CH), 1.35 (s, 9H, tBu), 0.85 (m, 6H, Me); δ_C (62 MHz, $CDCl_3$) 171.53, 150.49, 146.35, 129.02, 124.59, 83.19, 55.54, 42.79, 28.06, 24.82, 23.15, 21.78; *m*/*z* (ESI) : $(M + H)^+$ 373.

***N*-(4-Nitrobenzenesulfonyl)-L-phenylalanine *tert*-butyl ester 2**

84% yield; R_f (EtOAc–cyclohexane [1 : 2]) 0.51; mp 54 °C; δ_H (250 MHz, $CDCl_3$) 8.16 (d, 2H, *J* 9.0, CH=C*H*–C–NO_2), 8.06 (d, 2H, *J* 9.0, C*H*=CH–C–NO_2), 7.16 (m, 3H, Ph), 7.03 (m, 2H, Ph), 4.06 (m, 1H, C*H*–CO_2tBu), 2.96 (m, 2H, CH_2–Ph), 1.20 (s, 9H, tBu); δ_C (62 MHz, $CDCl_3$) 168.49, 148.78, 144.63, 133.88, 128.33, 127.38, 127.15, 126.13, 122.92, 82.13, 56.21, 38.26, 26.55; *m*/*z* (ESI) : $(M + Na)^+$ 429.

Mitsunobu alkylation. Procedure A leading to compounds 3a, 3b, 4a, 4b

To a solution of *N*-(4-nitrobenzenesulfonyl)amino acid *tert*-butyl ester (1 eq.) in CH_2Cl_2 at 0 °C were added R^2OH (2.5 eq), PPh_3 (2.5 eq.) and then DEAD (2.5 eq.). The reaction mixture was stirred at room temperature for 1 hour, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compounds **3a**, **3b**, **4a** and **4b**.

***N*-(Ethoxycarbonylmethyl)-*N*-(4-nitrobenzenesulfonyl)-L-leucine *tert*-butyl ester 3a**

55% yield; R_f (EtOAc–cyclohexane [1 : 4]) 0,52; δ_H (250 MHz, $CDCl_3$) 8.09 (d, 2H, *J* 9.0, CH=C*H*–C–NO_2), 7.88 (d, 2H, *J* 9.0, C*H*=CH–C–NO_2), 4.26 (s, 2H, CH_2–COOEt), 4.03 (t, 1H, *J* 7.7, C*H*–CO_2tBu), 3.93 (q, 2H, *J* 7.3, O–CH_2–CH_3), 1.50 (m, 1H, CH_3–C*H*–CH_3), 1.29 (m, 2H, CH–CH_2–CH), 1.05 (s, 9H, tBu), 1.02 (t, 3H, *J* 7.3, O–CH_2–CH_3), 0.62 (d, 6H, *J* 6.7, Me); δ_C (62 MHz, $CDCl_3$) 169.84, 169.41, 150.16, 145.40, 129.33, 124.02, 82.64, 61.56, 58.62, 45.82, 39.66, 27.82, 24.41, 21.76, 14.16; *m*/*z* (ESI) $(M + H)^+$ 459.

***N*-(2-Phenyl-2-oxoethyl)-*N*-(4-nitrobenzenesulfonyl)-L-leucine *tert*-butyl ester 3b**

17% yield; R_f (EtOAc–cyclohexane [1 : 6]) 0.45; δ_H (250 MHz, $CDCl_3$) 8.36 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 8.19 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 7.82 (m, 2H, Ph), 7.65 (m, 3H, Ph), 5.08 (s, 2H, CH_2–C(O)–Ph), 4.31 (t, 1H, *J* 7.4, C*H*–CO_2tBu), 1.71 (m, 1H, CH_3–C*H*–CH_3), 1.57 (m, 2H, CH–CH_2–CH), 1.16 (s, 9H, tBu), 0.85 (m, 6H, Me); δ_C (62 MHz, $CDCl_3$) 194.28, 170.46, 150.22, 146.54, 135.66, 130.46, 129.85, 129.43, 128.64, 128.10, 125.71, 124.23, 83.18, 55.33, 50.04, 40.12, 28.15, 23.76, 22.81, 21.71; *m*/*z* (ESI) $(M + Na)^+$ 513.

N-(Ethoxycarbonylmethyl)-N-(4-nitrobenzenesulfonyl)-L-phenylalanine *tert*-butyl ester 4a

85% yield; R_f (EtOAc–cyclohexane [1 : 4]) 0.50; δ_H (250 MHz, $CDCl_3$) 8.18 (d, 2H, *J* 8.9, CH=C*H*–C–NO_2), 8.00 (d, 2H, *J* 8.9, C*H*=CH–C–NO_2), 7.15 (m, 3H, Ph), 7.04 (m, 2H, Ph), 4.40 (m, 1H, C*H*–CO_2tBu), 4.27 (s, 2H, CH_2–CO_2Et), 4.12 (q, 2H, *J* 7.2, O–CH_2–CH_3), 2.96 (m, 2H, CH_2–Ph), 1.23 (t, 3H, *J* 7.2, O–CH_2–CH_3), 1.11 (s, 9H, tBu); δ_C (62 MHz, $CDCl_3$) 167.84, 167.28, 149.14, 144.21, 134.34, 127.94, 127.88, 127.17, 125.75, 122.44, 81.49, 60.33, 58.41, 44.19, 35.74, 26.29, 12.76; *m*/*z* (ESI) $(M + Na)^+$ 515.

N-(2-Phenyl-2-oxoethyl)-N-(4-nitrobenzenesulfonyl)-L-phenylalanine *tert*-butyl ester 4b

24% yield; R_f (EtOAc–CH_2Cl_2–cyclohexane [1 : 4 : 12]) 0.43; δ_H (300 MHz, $CDCl_3$) 8.27 (d, 2H, *J* 8.7, CH=C*H*–C–NO_2), 8.10 (d, 2H, *J* 8.7, C*H*=CH–C–NO_2), 7.88 (m, 2H, Ph), 7.60 (m, 3H, Ph), 7.19 (m, 5H, Ph), 5.11 (s, 2H, CH_2–C(O)–Ph), 4.49 (q, 1H, *J* 8.7, C*H*–CO_2tBu), 3.00 (d, 2H, *J* 8.7, CH_2–Ph), 1.12 (s, 9H, tBu); δ_C (62 MHz, $CDCl_3$) 194.36, 169.64, 150.71, 146.76, 136.41, 135.48, 134.65, 130.17, 129.70, 129.31, 128.66, 127.88, 124.56, 83.55, 62.40, 51.15, 38.19, 28.38; *m*/*z* (ESI) $(M + H)^+$ 525.

Alkylation using nucleophile substitution. Procedure B leading to compounds 3c and 4c

To a solution of *N*-(4-nitrobenzenesulfonyl)-amino acid *tert*-butyl ester (1 eq.) in MeCN were added R^2X (1.7 eq), then K_2CO_3 (2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compounds **3c** and **4c**.

N-(2-(4-Methoxyphenyl)-2-oxoethyl)-N-(4-nitrobenzenesulfonyl)-L-leucine *tert*-butyl ester 3c

95% yield; R_f (EtOAc–toluene [1 : 6]) 0.63; δ_H (300 MHz, $CDCl_3$) 8.38 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 8.20 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 7.92 (d, 2H, *J* 9.0, C*H*=CH–C–OMe), 6.97 (d, 2H, *J* 9.0, CH=C*H*–C–OMe), 5.02 (AB, 2H, *J* 18.9, CH_2–C(O)–Ph), 4.31 (t, 1H, *J* 7.5, C*H*–CO_2tBu), 3.90 (s, 3H, O–CH_3), 1.70 (m, 1H, CH_3–C*H*–CH_3), 1.57 (m, 2H, CH–CH_2–CH), 1.35 (s, 9H, tBu), 0.87 (d, 3H, *J* 6.6, Me), 0.84 (d, 3H, *J* 6.6, Me); δ_C (62 MHz, $CDCl_3$) 192.52, 170.53, 164.47, 150.31, 146.49, 130.57, 129.85, 129.48, 128.60, 128.13, 125.75, 124.21, 114.44, 82.99, 58.87, 55.94, 50.37, 40.20, 28.25, 24.98, 22.61, 21.85; *m*/*z* (ESI) $(M + H)^+$ 521, $(M + Na)^+$ 543.

N-(2-(Naphthalen-2-yl)-2-oxoethyl)-N-(4-nitrobenzenesulfonyl)-L-phenylalanine *tert*-butyl ester 4c

87% yield; R_f (EtOAc–cyclohexane [1 : 6]) 0.28; mp 59 °C; δ_H (250 MHz, $CDCl_3$) 8.12 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 7.80 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 7.73 (m, 4H, Naphthyl), 7.42 (m, 3H, Naphthyl), 7.02 (m, 5H, Ph), 5.09 (s, 2H, CH_2–C(O)–Naphthyl), 4.37 (m, 1H, C*H*–CO_2tBu), 2.90 (m, 2H, CH_2–Ph), 0.99 (s, 9H, tBu); δ_C (62 MHz, $CDCl_3$) 193.57, 168.95, 149.93, 146.02, 135.85, 132.38, 132.13, 129.82, 129.41, 129.01, 128.72, 128.55, 128.31, 127.86, 126.97, 123.75, 123.18, 82.76, 61.73, 50.54, 37.48, 27.65; *m*/*z* (ESI) $(M + H)^+$ 575.

Cleavage of the *tert*-butyl ester function. General procedure leading to compounds 5a–c and 6a–c

To a solution of *N*,*N*-disubstituted-amino acid *tert*-butyl ester (1 eq.) in CH_2Cl_2 at 0 °C was added TFA (20 eq.). The reaction mixture was stirred at room temperature for 1 hour, then concentrated under reduced pressure to give the compounds **5a–c** and **6a–c**.

N-(Ethoxycarbonylmethyl)-N-(4-nitrobenzenesulfonyl)-L-leucine 5a

Quantitative yield; R_f (EtOAc–cyclohexane [1 : 2]) 0.40; δ_H (250 MHz, $CDCl_3$) 8.15 (dd, 2H, *J* 9.1, CH=C*H*–C–NO_2), 7.90 (dd, 2H, *J* 9.1, C*H*=CH–C–NO_2), 4.56 (t, 1H, *J* 7.3, C*H*–CO_2H), 3.93 (m, 4H, CH_2–CO_2–CH_2–CH_3), 1.51 (m, 1H, CH_3–C*H*–CH_3), 1.31 (m, 2H, CH–CH_2–CH), 1.01 (m, 3H, O–CH_2–CH_3), 0.64 (d, 3H, *J* 6.6, Me), 0.62 (d, 3H, *J* 6.6, Me); δ_C (62 MHz, $CDCl_3$) 176.92, 170.54, 150.66, 144.57, 129.49, 124.46, 62.70, 58.21, 46.24, 39.27, 24.47, 22.68, 21.48, 14.16; *m*/*z* (ESI) $(M + H)^+$ 403, $(M + Na)^+$ 425.

N-(2-Phenyl-2-oxoethyl)-N-(4-nitrobenzenesulfonyl)-L-leucine 5b

Quantitative yield; R_f (EtOAc–cyclohexane [1 : 2]) 0.38; δ_H (300 MHz, $CDCl_3$) 8.40 (d, 2H, *J* 8.7, CH=C*H*–C–NO_2), 8.20 (d, 2H, *J* 8.7, C*H*=CH–C–NO_2), 7.96 (d, 2H, *J* 8.1, Ph), 7.65 (t, 1H, *J* 8.1, Ph), 7.53 (t, 2H, *J* 8.1, Ph), 5.00 (AB, 2H, *J* 18.9, CH_2–C(O)–Ph), 4.44 (m, 1H, C*H*–CO_2H), 1.65 (m, 1H, CH_3–C*H*–CH_3), 1.27 (m, 2H, CH–CH_2–CH), 0.84 (d, 3H, *J* 6.3, Me), 0.82 (d, 3H, *J* 6.3, Me); δ_C (62 MHz, $CDCl_3$) 194.74, 174.21, 150.48, 145.86, 134.70, 130.81, 129.77, 129.45, 128.43, 127.36, 124.49, 58.39, 52.34, 39.76, 24.82, 22.79, 22.06; *m*/*z* (ESI) $(M + H)^+$ 435.

N-(2-(4-Methoxyphenyl)-2-oxoethyl)-N-(4-nitrobenzenesulfonyl)-L-leucine 5c

Quantitative yield; R_f (EtOAc–toluene [1 : 6]) 0.33; mp 173 °C; δ_H (250 MHz, DMSO) 10.50 (s, 1H, COO*H*), 8.41 (d, 2H, *J* 8.7, CH=C*H*–C–NO_2), 8.19 (d, 2H, *J* 8.7, C*H*=CH–C–NO_2), 8.00 (d, 2H, *J* 9.0, C*H*=CH–C–OMe), 7.09 (d, 2H, *J* 9.0, CH=C*H*–C–OMe), 4.95 (AB, 2H, *J* 19.2, CH_2–C(O)–Ph), 4.32 (m, 1H, C*H*–CO_2H), 3.87 (s, 3H, O–CH_3), 1.87 (m, 1H, CH_3–C*H*–CH_3), 1.47 (m, 2H, CH–CH_2–CH), 0.84 (d, 3H, *J* 6.6, Me), 0.80 (d, 3H, *J* 6.6, Me); δ_C (62 MHz, DMSO) 195.54, 171.58, 163.38, 149.70, 145.00, 131.50, 130.14, 129.63, 128.99, 128.14, 125.34, 122.92, 114.83, 58.75, 56.34, 53.68, 36.58, 23.68, 22.54, 21.66; *m*/*z* (ESI) $(M - H)^-$ 463.

N-(Ethoxycarbonylmethyl)-N-(4-nitrobenzenesulfonyl)-L-phenylalanine 6a

Quantitative yield; R_f (EtOAc–cyclohexane [1 : 2]) 0.37; δ_H (300 MHz, $CDCl_3$) 8.20 (d, 2H, *J* 8.9, CH=C*H*–C–NO_2), 7.95 (d, 2H, *J* 8.9, C*H*=CH–C–NO_2), 7.22 (m, 3H, Ph), 7.12 (m, 2H, Ph), 4.64 (m, 1H, C*H*–CO_2H), 4.17 (m, 4H, CH_2–CO_2–CH_2–CH_3), 3.04 (m, 2H, CH_2–Ph), 1.28 (t, 3H, *J* 7.2, O–CH_2–CH_3); δ_C (62 MHz, $CDCl_3$) 174.31, 170.02, 150.54, 146.17, 136.78, 135.92, 129.45, 129.21, 128.84, 127.66, 124.44, 62.49, 58.61, 46.72, 36.67, 14.42; *m*/*z* (ESI) $(M + Na)^+$ 459.

N-(2-Phenyl-2-oxoethyl)-N-(4-nitrobenzenesulfonyl)-L-phenylalanine 6b

Quantitative yield; R_f (EtOAc–cyclohexane [1 : 4]) 0.17; mp 144 °C; δ_H (300 MHz, $CDCl_3$) 8.25 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 8.00 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 7.89 (d, 2H, *J* 7.2, Ph), 7.68 (t, 1H, *J* 7.2, Ph), 7.52 (t, 2H, *J* 7.2, Ph), 7.24 (m, 3H, Ph), 7.13 (m, 2H, Ph), 4.91 (AB, 2H, *J* 18.9, CH_2–C(O)–Ph), 4.53 (m, 1H, C*H*–CO_2H), 3.18 (m, 2H, CH_2–Ph); δ_C (62 MHz, $CDCl_3$) 195.74, 172.97, 150.91, 145.62, 136.55, 135.47, 134.72, 129.90, 129.84, 129.59, 128.92, 128.09, 124.81, 62.26, 51.12, 37.53; *m*/*z* (ESI) : $(M + H)^+$ 469, $(M + Na)^+$ 491.

_N_-(2-Naphthalen-2-yl-2-oxoethyl)-_N_-(4-nitrobenzenesulfonyl)-L-phenylalanine 6c

Quantitative yield; R_f (EtOAc–MeOH [9 : 1]) 0.43; mp 122 °C; δ_H (250 MHz, DMSO) 10.40 (s, 1H, COO*H*), 8.21 (d, 2H, *J* 8.8, CH=C*H*–C–NO_2), 7.85 (d, 2H, *J* 8.8, C*H*=CH–C–NO_2), 7.43 (m, 4H, Naphthyl), 7.10 (m, 3H, Naphthyl), 6.88 (m, 5H, Ph), 4.95 (AB, 2H, *J* 19.2, CH_2–C(O)–Ph), 4.48 (m, 1H, C*H*–CO_2H), 3.18 (m, 2H, CH_2–Ph); δ_C (62 MHz, DMSO) 193.78, 172.78, 150.52, 145.74, 135.76, 132.11, 132.05, 129.54, 129.32, 129.12, 128.66, 128.32, 127.80, 127.00, 123.63, 123.41, 63.43, 50.84, 37.65; *m*/*z* (ESI) $(M + H)^+$ 519.

_N_-(4-Nitrobenzenesulfonyl)-_N_-(I-styryl)glycine ethyl ester 7

To a solution of **6a** (114 mg, 0.26 mmol) in toluene was added PCl_5 (136 mg, 0.65 mmol). The reaction mixture was stirred at reflux for 48 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compound **7** (47 mg, 46% yield); Found: C, 55.3; H, 4.7; N, 7.2. $C_{18}H_{18}N_2O_6S$ requires C, 55.4; H, 4.65; N, 7.2%; R_f (EtOAc–cyclohexane [1 : 4]) 0.38; δ_H (250 MHz, $CDCl_3$) 8.26 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 7.96 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 7.20 (m, 6H, C*H*=CH–*Ph*), 5.63 (d, 1H, *J* 14.5, CH=C*H*–Ph), 4.35 (s, 2H, CH_2–CO_2Et), 4.03 (q, 2H, *J* 7.1, O–CH_2–CH_3), 1.10 (t, 3H, *J* 7.1, O–CH_2–C*H*$_3$); δ_C (62 MHz, $CDCl_3$) 167.45, 150.56, 144.76, 135.50, 129.05, 128.92, 127.49, 126.02, 125.79, 124.59, 113.47, 62.21, 47.96, 14.35; *m*/*z* (ESI) $(M + Na)^+$ 413.

3,4-Dihydro-(3*R*)-isobutyl-4-_N_-(4-nitrobenzenesulfonyl)-6-phenyl[1,4]oxazin-2-one 8b

To a solution of **5b** (90 mg, 0.21 mmol) in toluene was added $SOCl_2$ (15 µl, 0.21 mmol). The reaction mixture was stirred at 70 °C for 2 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compound **8b** (48 mg, 55% yield); Found: C, 57.7; H, 4.9; N, 6.6. $C_{20}H_{20}N_2O_6S$ requires C, 57.7; H, 4.8; N, 6.7%; R_f (EtOAc–cyclohexane [1 : 2]) 0.72; mp 104 °C; $[\alpha]_D^{20}$ (c = 1, DCM) +20; δ_H (250 MHz, $CDCl_3$) 8.27 (d, 2H, *J* 8.5, CH=C*H*–C–NO_2), 7.90 (d, 2H, *J* 8.5, C*H*=CH–C–NO_2), 7.44 (m, 2H, Ph), 7.34 (m, 3H, Ph), 6.68 (s, 1H, C*H*=C(O)–Ph), 4.61 (m, 1H, C*H*–CO_2), 1.80 (m, 1H, CH_3–C*H*–CH_3), 1.50 (m, 2H, CH–CH_2–CH), 0.97 (d, 3H, *J* 6.2, Me), 0.94 (d, 3H, *J* 6.2, Me); δ_C (62 MHz, $CDCl_3$) 163.12, 144.41, 135.09, 130.89, 129.81, 129.73, 129.01, 128.81, 125.54, 125.03, 124.87, 101.89, 55.10, 39.06, 24.83, 23.44, 22.17; *m*/*z* (ESI) $(M + H)^+$ 417.

3,4-Dihydro-(3*R*)-isobutyl-6-(4-methoxyphenyl)-4-_N_-(4-nitrobenzenesulfonyl)[1,4]oxazin-2-one 8c

To a solution of **5c** (1g, 2.2 mmol) in CH_2Cl_2 at 0 °C were added PyBOP (1.3 g, 2.5 mmol) then TEA (650 µl, 4.7 mmol). The reaction mixture was stirred at room temperature for 14 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compound **8c** (0.9 g, 92% yield); Found: C, 56.4; H, 5.0; N, 6.3. $C_{21}H_{22}N_2O_7S$ requires C, 56.5; H, 5.0; N, 6.3%; R_f (EtOAc–cyclohexane [1 : 3]) 0.42; mp 55 °C; $[\alpha]_D^{20}$ (c = 1, DCM) −86; δ_H (300 MHz, $CDCl_3$) 8.34 (d, 2H, *J* 8.7, CH=C*H*–C–NO_2), 7.97 (d, 2H, *J* 8.7, C*H*=CH–C–NO_2), 7.45 (d, 2H, *J* 9.0, C*H*=CH–C–OMe), 6.93 (d, 2H, *J* 9.0, CH=C*H*–C–OMe), 6.62 (s, 1H, C*H*=C(O)–Ph), 4.69 (m, 1H, C*H*–CO_2), 3.85 (s, 3H, O–C*H*$_3$), 1.88 (m, 1H, CH_3–C*H*–CH_3), 1.48 (m, 2H, CH–CH_2–CH), 1.06 (d, 3H, *J* 6.2, Me), 1.02 (d, 3H, *J* 6.2, Me); δ_C (62 MHz, $CDCl_3$) 162.96, 161.55, 151.02, 142.32, 128.65, 126.26, 125.07, 121.85, 114.75, 99.66, 55.84, 54.68, 38.57, 24.43, 23.08, 21.73; *m*/*z* (ESI) $(M + H)^+$ 447.

(3*R*)-Benzyl-3,4-dihydro-4-_N_-(4-nitrobenzenesulfonyl)-6-phenyl-[1,4]oxazin-2-one 9b

To a solution of **6b** (105 mg, 0.22 mmol) in toluene was added PCl_5 (46 mg, 0.27 mmol). The reaction mixture was stirred at room temperature for 48 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compound **9b** (24 mg, 24% yield); Found: C, 61.3; H, 4.1; N, 6.2. $C_{23}H_{18}N_2O_6S$ requires C, 61.3; H, 4.0; N, 6.2%; R_f (EtOAc–cyclohexane [1 : 2]) 0.68; mp 94 °C; $[\alpha]_D^{20}$ (c = 1, DCM) = +38; δ_H (250 MHz, $CDCl_3$) 8.25 (d, 2H, *J* 9.1, CH=C*H*–C–NO_2), 8.00 (d, 2H, *J* 9.1, C*H*=CH–C–NO_2), 7.19 (m, 10H, Ph), 6.66 (s, 1H, C*H*=C(O)–Ph), 4.73 (t, 1H, *J* 6.9, C*H*–CO_2), 2.96 (d, 2H, *J* 6.9, CH_2–Ph); δ_C (62 MHz, $CDCl_3$) 163.02, 151.04, 144.87, 135.48, 134.65, 130.17, 129.70, 129.31, 128.66, 127.88, 126.15, 125.07, 124.56, 101.16, 61.43, 37.21; *m*/*z* (ESI) $(M + H)^+$ 451.

(3*R*)-Benzyl-3,4-dihydro-6-(naphthalen-2-yl)-4-_N_-(4-nitrobenzenesulfonyl)[1,4]oxazin-2-one 9c

To a solution of **6c** (156 mg, 0.30 mmol) in CH_2Cl_2 was added $SOCl_2$ (22 µl, 0.30 mmol). The reaction mixture was stirred at room temperature for 20 hours, then concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was successively washed with a 5% $NaHCO_3$ aqueous solution, brine and 5% citric acid aqueous solution, then dried over $MgSO_4$. The organic solvent was removed *in vacuo* to give a residue which was purified by flash chromatography on silica gel to give the compound **9c** (126 mg, 84% yield); Found: C, 64.7; H, 4.1; N, 5.6. $C_{27}H_{20}N_2O_6S$ requires C, 64.8; H, 4.0; N, 5.6%; R_f (EtOAc–cyclohexane [1 : 3]) 0.61; mp 122 °C; $[\alpha]_D^{25}$ (c = 1, DCM) +11; δ_H (250 MHz, $CDCl_3$) 8.11 (d, 2H, *J* 9.3, CH=C*H*–C–NO_2), 7.72 (d, 2H, *J* 9.3, C*H*=CH–C–NO_2), 7.64 (m, 4H, Naphthyl), 7.35 (m, 3H, Naphthyl), 7.06 (m, 5H, Ph), 6.65 (s, 1H, C*H*=C(O)–Naphthyl), 4.73 (t, 1H, *J* 6.9, C*H*–CO_2), 2.96 (d, 2H, *J* 6.9, CH_2–Ph); δ_C (62 MHz, $CDCl_3$) 162.97, 150.93, 144.32, 135.11, 134.65, 132.38, 132.13, 129.82, 129.41, 129.01, 128.72, 128.55, 128.31, 127.86, 126.97, 126.15, 125.07, 124.56, 123.75, 101.53, 62.05, 37.46; *m*/*z* (ESI) $(M + H)^+$ 501.

5-Bromo-3,4-dihydro-(3*R*)-isobutyl-6-(4-methoxyphenyl)-4-_N_-(4-nitrobenzenesulfonyl)-[1,4]oxazin-2-one 10

To a solution of **8c** (200 mg, 0.4 mmol) in CH_2Cl_2 was added NBS (80 mg, 0.4 mmol). The reaction mixture was stirred at room temperature for 3 days, then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to give the compound **10** (70 mg, 30% yield); Found: C, 48.1; H, 4.0; N, 5.4. $C_{21}H_{21}BrN_2O_7S$ requires C, 48.0; H, 4.0; N, 5.3%; R_f (EtOAc–cyclohexane [1 : 3]) 0.45; $[\alpha]_D^{25}$ (c = 1, DCM) −42; δ_H (250 MHz, $CDCl_3$) 8.32 (d, 2H, *J* 8.9, CH=C*H*–C–NO_2), 8.02 (d, 2H, *J* 8.9, C*H*=CH–C–NO_2), 7.46 (d, 2H, *J* 8.9, C*H*=CH–C–OMe), 6.86 (d, 2H, *J* 8.9, CH=C*H*–C–OMe), 4.78 (m, 1H, C*H*–CO_2), 3.77 (s, 3H, O–C*H*$_3$), 1.88 (m, 1H, CH_3–

C*H*–CH_3), 1.48 (m, 2H, CH–C*H*$_2$–CH), 1.05 (d, 3H, *J* 6.2, Me), 1.02 (d, 3H, *J* 6.2, Me); δ_C (62 MHz, $CDCl_3$) 163.76, 161.51, 151.03, 143.22, 130.23, 128.96, 124.55, 121.85, 113.84, 93.24, 56.71, 55.43, 36.86, 24.52, 22.84, 20.90; *m*/*z* (ESI) $(M + H)^+$ 526 (528, 530).

5-Bromo-3-(1-bromo-2-methylpropyl)-6-(4-methoxyphenyl)[1,4]-oxazin-2-one 11

To a solution of **8c** (200 mg, 0.4 mmol) in CH_2Cl_2 was added NBS (80 mg, 0.4 mmol). The reaction mixture was stirred at room temperature for 3 days, then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to give the compound **11** (30 mg, 18% yield); Found: C, 43.2; H, 3.6; N, 3.3. $C_{15}H_{15}NO_3$ requires C, 43.2; H, 3.6; N, 3.4%; R_f (EtOAc–cyclohexane [1 : 3]) 0.61; δ_H (250 MHz, $CDCl_3$) 7.85 (d, 2H, *J* 9.0, C*H*=CH–C–OMe), 6.92 (d, 2H, *J* 9.0, CH=C*H*–C–OMe), 4.82 (d, 1H, *J* 9.5, Br–C*H*–CH$(Me)_2$), 3.82 (s, 3H, O–C*H*$_3$), 2.59 (m, 1H, C*H*$(Me)_2$), 1.20 (d, 3H, *J* 6.6, Me), 0.96 (d, 3H, *J* 6.6, Me); δ_C (62 MHz, $CDCl_3$) 161.58, 151.34, 149.54, 130.41, 120.98, 113.40, 110.93, 54.88, 31.33, 26.43, 20.78, 19.80; *m*/*z* (ESI) $(M + H)^+$ 418 (416, 420).

α-Chymotrypsin inhibition

The enzyme was preincubated for 10 minutes at 37 °C with inhibitor by adding an aliquot (10 μl) of the inhibitor in DMSO to the enzyme in buffer (0.1 M Hepes, 0.5M NaCl, pH 6). Percentage of inhibition was spectrophotometrically measured ($\lambda = 410$ nm) on a Uvikon 930 spectrophotometer by adding an aliquot of the substrate solution (N-Succinyl-Ala-Ala-Pro-Phe-*p*-Nitroanilide) to the enzyme–inhibitor solution into a cuvette.

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