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Original article

Design and synthesis of novel platelet fibrinogen receptor antagonists with 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold. A systematic study

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Abstract

New platelet glycoprotein IIb/IIIa (GP IIb/IIIa, integrin $\alpha_{IIb}\beta_3$) antagonists were prepared on a 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold. Their anti-aggregatory activities in human platelet rich plasma and their affinity towards $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrins were assessed. Various substitution positions and side chain variations were studied. In contrast to the generally accepted model, compounds containing ethyl esters as aspartate mimetics were in general more active than the corresponding free acids. We suggest an explanation for the observed behaviour of these new compounds.

Keywords: 2*H*-1,4-benzoxazine-3(4*H*)-one; Fibrinogen receptor; Fibrinogen receptor antagonists; Platelet aggregation

1. Introduction

Platelet aggregation plays a vital role in primary haemostasis, but under pathological conditions, such as those following an atherosclerotic plaque rupture, this process may lead to an arterial thrombosis that can result in myocardial infarction, ischaemic stroke, and peripheral artery disease [1]. The final common step in platelet aggregation is the binding of fibrinogen to its glycoprotein IIb/IIIa (GPIIb/IIIa, integrin $\alpha_{IIb}\beta_3$) receptor, which is located on the surface of activated platelets. Development of $\alpha_{IIb}\beta_3$ antagonists has been one of the main focuses in antithrombotic research over the last decade [2]. They are based predominantly on the RGD (Arg-Gly-Asp) sequence found in many natural ligands of the receptor. According to the generally accepted model, a free carboxylic group and a basic functionality, in appropriate spatial positions, are the key pharmacophore elements of the $\alpha_{IIb}\beta_3$ antagonists, mimicking the aspartate β-carboxylate and the arginine guanidinium group in the RGD sequence. In addition, an alkyl- or aryl-sulphonamide or carbamate functional group at the position α- to the carboxy terminus of the antagonist constitutes an additional, exosite-binding group [3]. This approach has led to the discovery of tirofiban (Scheme 1), one of three parenteral $\alpha_{IIb}\beta_3$ antagonists currently being used for the management of acute coronary syndrome and for therapy adjunctive to coronary surgical procedures [4].

However, several orally active drug candidates targeted against integrin $\alpha_{IIb}\beta_3$ have so far proved to be ineffective in clinical trials. This could be due to their poor pharmacokinetic properties; modest bioavailability and relatively short half-life, resulting in high variation of the plasma drug concentration (peak to trough ratio) and thus preventing the sustained inhibition required for a beneficial pharmacological effect [5]. In addition, several results suggest that, at low doses, $\alpha_{IIb}\beta_3$ antagonists may activate the receptor, leading to

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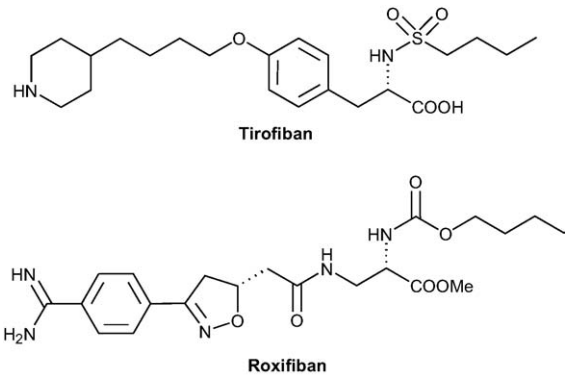
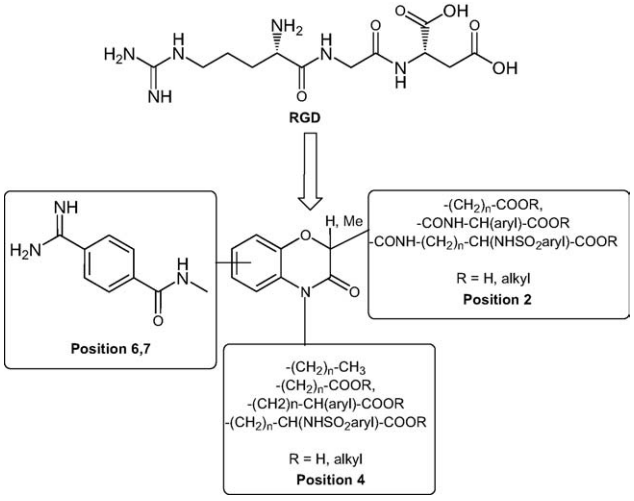


Scheme 1. Structures of Tirofiban and Roxifiban.

increased aggregation [6]. Orally active, low molecular weight $\alpha_{IIb}\beta_3$ antagonists, like roxifiban (Scheme 1), with higher affinity towards the receptor and consequently more favourable pharmacokinetic properties, are therefore still of interest [7].

We report the design and synthesis of a new series of $\alpha_{IIb}\beta_3$ antagonists. Various 6,6-bicyclic heterocycles have already been used as templates in developing RGD mimetics [8,9]. In our work, we focused on 2*H*-1,4-benzoxazine-3(4*H*)-one as a peptidomimetic building block. This parent bicyclic heterocycle was chosen because of its ready synthetic accessibility and, more importantly, the various substitution options that allow different spatial positions of the pharmacophore functional groups to be studied (Scheme 2).

The compounds synthesized were tested for their anti-aggregatory activity against ADP-induced aggregation in human platelet rich plasma. Their in vitro affinities towards the isolated $\alpha_{IIb}\beta_3$ and the closely related $\alpha_V\beta_3$ integrin were also assessed. While antagonists with a free carboxylic group expressed higher affinity towards the $\alpha_{IIb}\beta_3$ in vitro, the corresponding carboxylic esters showed higher anti-aggregatory activity against ADP-induced aggregation. These results are somehow paradoxical and have not been reported before, but were consistent through almost the



Scheme 2. RGD tripeptide as the lead compound and strategy for the synthesis of 2*H*-1,4-benzoxazine-3(4*H*)-one-based RGD mimetics.

whole series of compounds. We attempt to resolve this paradox with a hypothesis that could contribute to an explanation of the failure in clinical trials of several drug candidates.

2. Chemistry

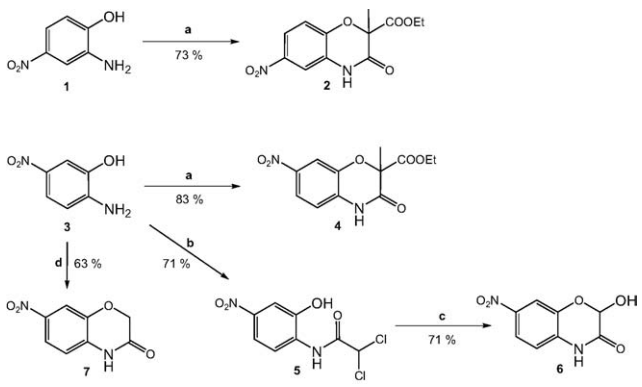
2.1. Preparation of 2H-*1,4-benzoxazine-3(4*H*)-one scaffolds*

Our strategy for the preparation of potential $\alpha_{IIb}\beta_3$ antagonists was to attach various substituents with a pharmacophore group to the 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold. In order to achieve different substitution patterns on the scaffold, four starting heterocycles were synthesized, as shown in Scheme 3.

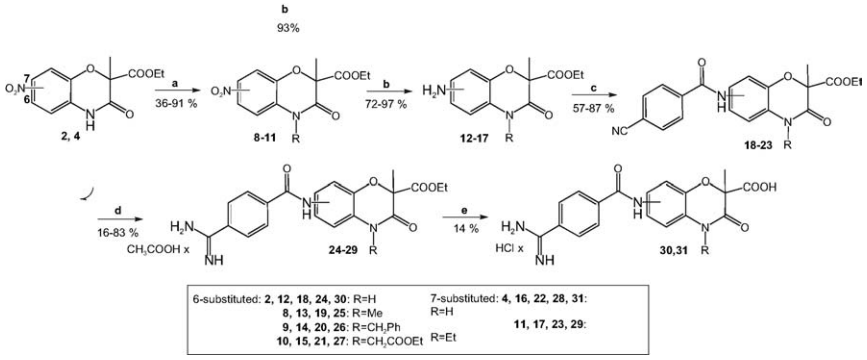
Initially, 2-amino-4-nitrophenol **1** was used to perform one-step *O*-alkylation and, following cyclization with diethyl 2-bromo-2-methylmalonate, to give ethyl 2-methyl-6-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-carboxylate (**2**) [10]. The corresponding 7-nitro derivative **4** was prepared from 2-amino-5-nitrophenol (**3**). Alternatively, introduction of a hydroxyl group at position 2 was achieved by *N*-acylation of **3** with 2,2-dichloroacetylchloride to give **5** and subsequent cyclization under basic conditions to give **6** [11]. 2*H*-1,4-benzoxazine-3(4*H*)-one derivative bearing no substituents at position 2 (**7**) was obtained from **3** and ethyl 2-bromoacetate [12].

2.2. 2,6- and 2,7-Di-substituted 2H-*1,4-benzoxazine-3(4*H*)-ones with an ethoxycarbonyl or carboxy substituent at position 2*

Scaffolds **2** and **4** were used as starting materials for the synthesis of the target compounds **24–31** (Scheme 4). Initially, a variety of residues were attached to position 4 of the benzoxazinone ring by NaH assisted alkylation with different alkyl halides. The nitro group was then reduced by catalytic hydrogenation and the resulting amines (**12–17**) were acylated with *p*-cyanobenzoyl chloride to give **18–23**. Nitriles **18–23** were subsequently subjected to Pinner reaction



Scheme 3. Synthetic route to 2*H*-1,4-benzoxazine-3(4*H*)-ones **2**, **4**, **6** and **7**. (a) $EtOOCC(CH_3)BrCOOEt$, KF, DMF, 60 °C; (b) $Cl_2CHCOCl$, Et_3N, Et_2O; (c) $NaHCO_3$, H_2O, 100 °C; (d) $BrCH_2COOEt$, KF, DMF, 60 °C.



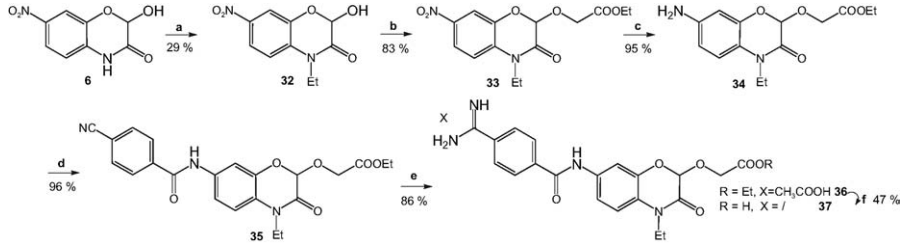
Scheme 4. (a) NaH, MeI (**8**), $PhCH_2Br$ (**9**), $BrCH_2COOEt$ (**10**), EtBr (**11**), toluene; (b) H_2, Pd/C, EtOH; (c) 4-CNC_6H_4COCl, Et_3N, CH_2Cl_2; (d) 1. $HCl_{(g)}$, EtOH; 2. $CH_3COO^-NH_4^+$, EtOH; (e) 1.$NaOH_{(aq)}$, dioxane; 2. $HCl_{(aq)}$.

[8]; treatment with dry gaseous HCl in absolute ethanol and reaction of the resulting imidates with ammonium acetate yielded amidines **24**–**29** in the form of their acetate salts. Finally, saponification of ethyl esters **24** and **29** was performed in order to obtain free carboxylic acids **30** and **31**.

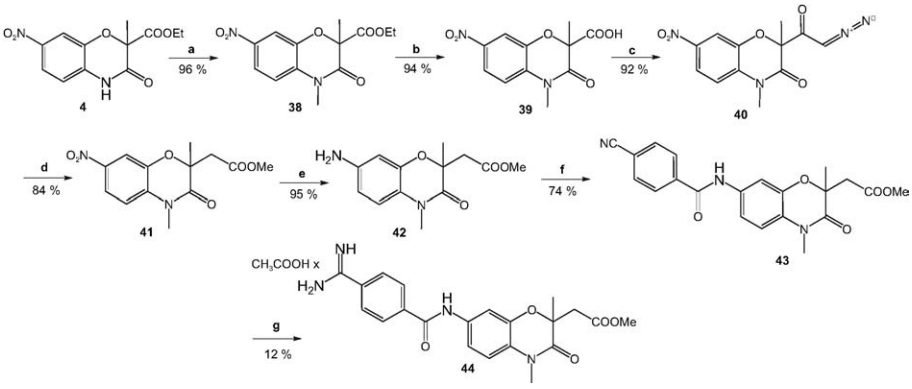
*2.3. 2,7-Di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones with ethylcarbonylmethoxy or carboxymethoxy substituent at position 2*

In our next series, alkylation of scaffold **6** bearing a hydroxyl group at position 2 of the benzoxazine ring gave a mixture of *N*-alkylated and *N,O*-dialkylated products that could be separated by flash chromatography (**32**; *N*-alkylated product: *N,O*-dialkylated product ≅ 3:1). Therefore an ethyl group was introduced to position 4 by KF-mediated alkylation followed by *O*-alkylation of **32** with ethyl bromoacetate under the same conditions. The resulting nitro compound **33** was converted by catalytic hydrogenation to amine **34**, which was acylated with *p*-cyanobenzoyl chloride to give **35**. The amidine **36** was obtained by Pinner conversion of nitrile **35** and further saponification of **36** to give the free acid **37** (Scheme 5).

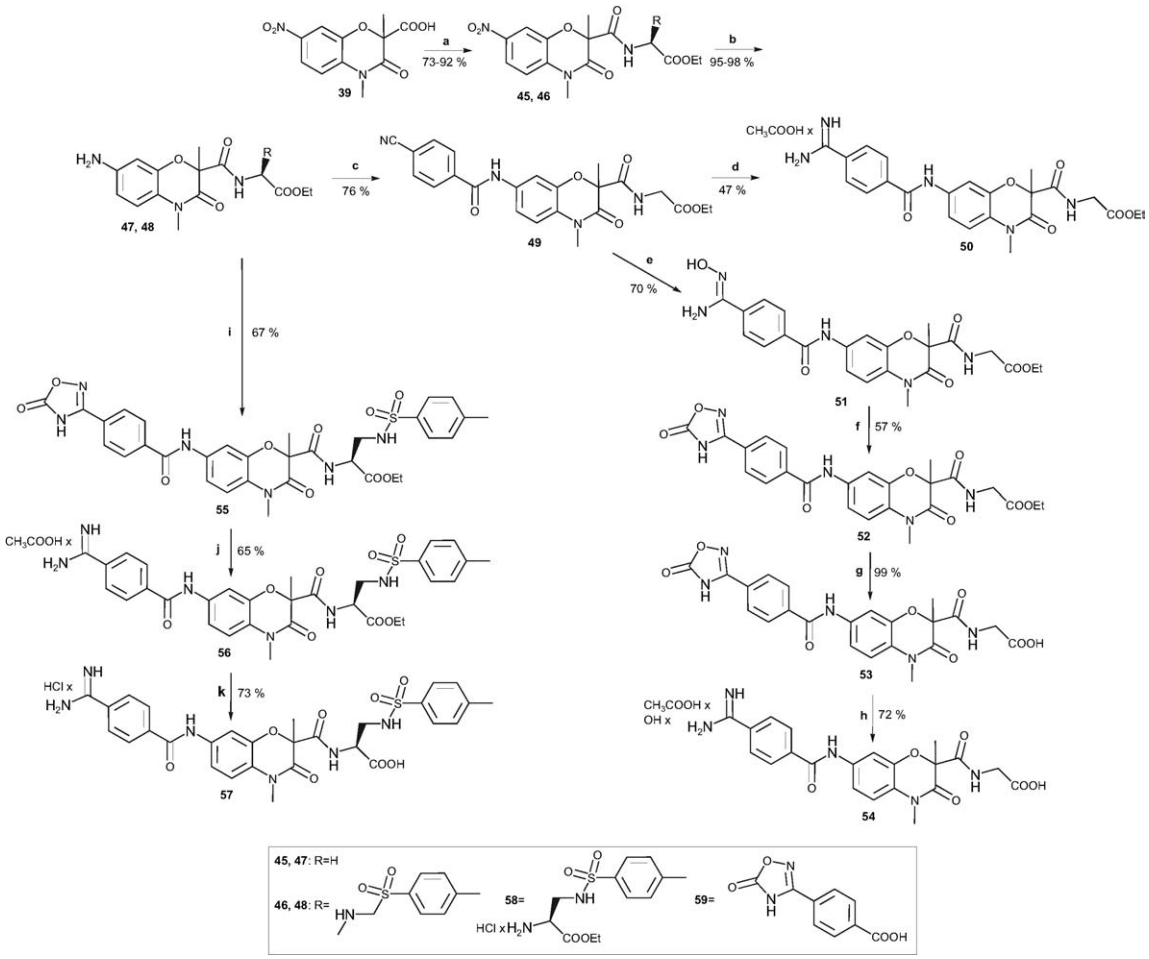
The synthesis of target compound **44** is outlined in Scheme 6. The initial benzoxazinone **4** was *N*-alkylated to give **38** in which the ethyl ester group was cleaved to give **39** [13]. In order to introduce a methylene spacer between the benzoxazine ring and the carboxyl moiety at position 2, a modified Arndt-Eistert synthesis was conducted on carboxylic acid **39** [14]. After mixed anhydride activation of the acid, reaction with trimethylsilyldiazomethane ($TMSCHN_2$) as a substitute for diazomethane yielded diazoketone **40**, which was then subjected to a Wolff rearrangement. The resulting homologated ester **41** was reduced to give amine **42**. The



Scheme 5. (a) EtBr, KF, DMF; (b) $BrCH_2COOEt$, KF, DMF; (c) H_2, Pd/C, EtOH; (d) 4-CNC_6H_4COCl, Et_3N, CH_2Cl_2; (e) 1. HCl (g), EtOH; 2. $CH_3COO^-NH_4^+$, EtOH; (f) 1. $NaOH_{(aq)}$, EtOH, 2.CH_3COOH.



Scheme 6. (a) MeI, KF, DMF; (b) NaOH, dioxane; (c) 1. EtOCOCl, Et_3N, THF; 2. $TMSCHN_2$, MeCN; (d) $PhCOO^-Ag^+$, Et_3N, MeOH, ultrasound; (e) H_2, Pd/C, MeOH; (f) 4-CNC_6H_4COCl, Et_3N, CH_2Cl_2; (g) 1. $HCl_{(g)}$, MeOH; 2. $CH_3COO^-NH_4^+$, MeOH.

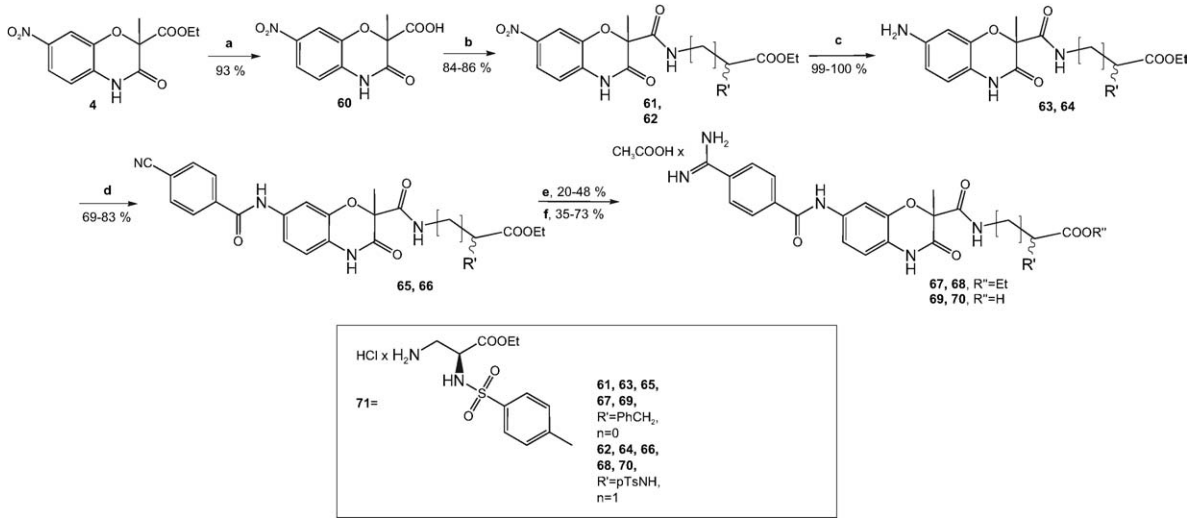


Scheme 7. (a) GlyOEt, **58**, EDC, HOBt, NMM, DMF; (b) H_2, Pd/C, EtOH; (c) 4-CNC_6H_4COCl, Et_3N, CH_2Cl_2, 0 °C; (d) 1. $HCl_{(g)}$, EtOH; 2. $CH_3COO^-NH_4^+$, EtOH; (e) $NH_2OH \times HCl$, Et_3N, EtOH, 50 °C; (f) 1. EtOCOCl, pyridine, –15 °C; 2. 120 °C; (g) $NaOH_{(aq)}$, EtOH; (h) H_2, Pd/C, CH_3COOH, DMF; (i) **59**, EDC, HOBt, NMM, DMF; (j) H_2, Pd/C, CH_3COOH, DMF; (k) 1. $NaOH_{(aq)}$, EtOH; 2.HCl.

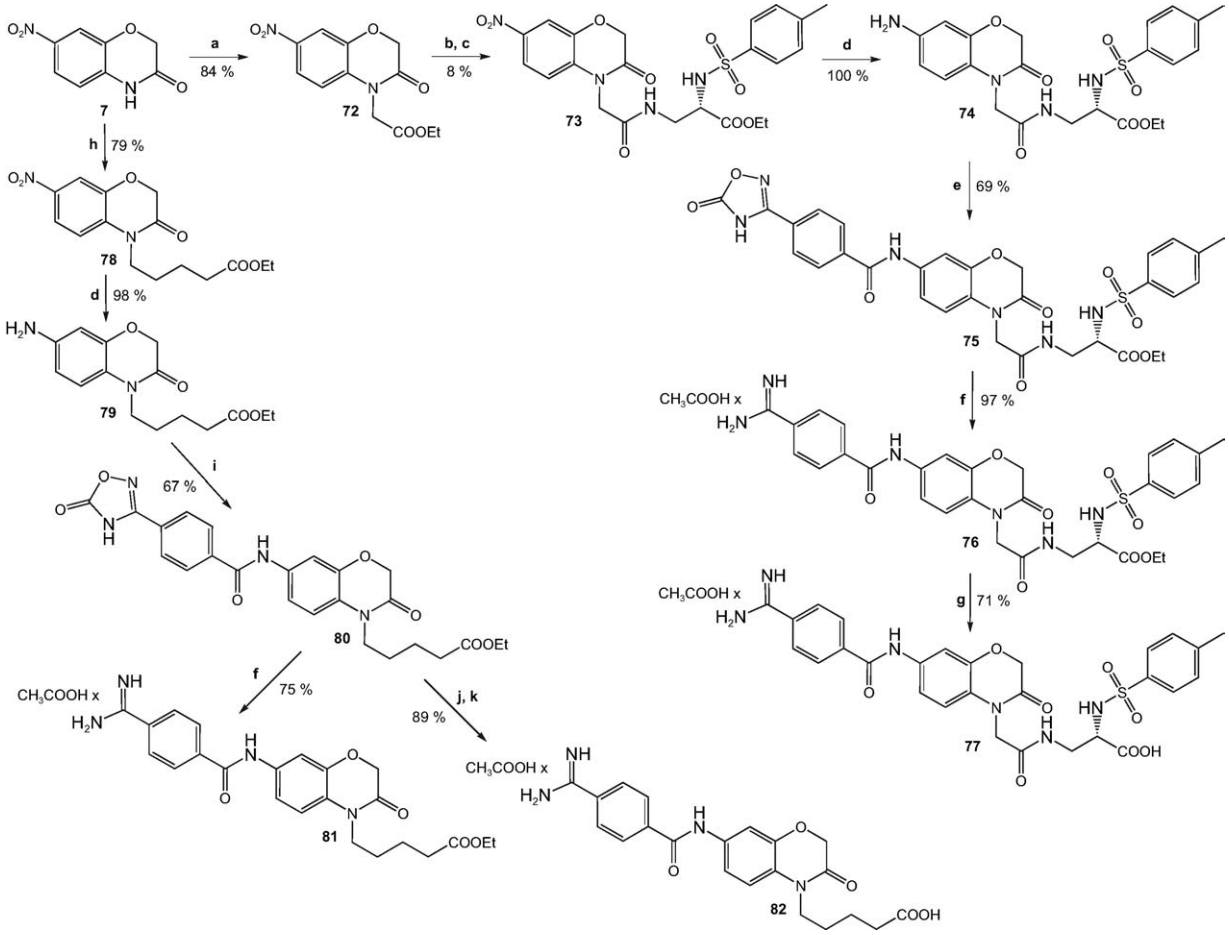
amidine **44** was obtained by acylation of **42** with *p*-cyanobenzoyl chloride and subsequent Pinner conversion of the nitrile **43**.

In the next series we decided to further lengthen the substituent at position 2 of the benzoxazinone ring, as depicted in Scheme 7. For this purpose, glycine ethyl ester was coupled to the acid **39** by the standard EDC/HOBt coupling procedure to give **45**. The nitro group was then reduced by catalytic hydrogenation and the resulting amine **47** was acylated with *p*-cyanobenzoyl chloride to give **49**. Nitrile **49** was subjected to Pinner reaction to yield amidine **50**. Since the 1,2,4-oxadiazol-5(4*H*)-one ring has been described as an easily accessible and versatile precursor of the amidino moiety [15], we utilized this approach for preparing target compound **55** from **49**. Nitrile **49** was first converted to amidoxime **51** with hydroxylammonium chloride in basic ethanolic media. This was then *O*-acylated with ethyl chloroformate and subsequently cyclized under reflux in pyridine to yield the 1,2,4–oxadiazol-5(4*H*)-one derivative (**52**). The ester group was then saponified with NaOH in ethanol to give **53** and the cyclic carbamate protection group removed by catalytic hydrogenation to give **54**. As an additional lipophilic group neighbouring the carboxylic acid residue has been proved to be beneficial for increasing $\alpha_{IIb}\beta_3$ binding affinity in numerous studies [3], we prepared L-2-amino-3-(toluene-4-sulfonylamino)-propionic acid ethyl ester hydrochloride (**58**), starting from L-asparagine [16]. This was coupled to **39** by the standard EDC/HOBt method to give **46**. The nitro group was then reduced to give amine **48**. 4-(1,2,4-oxadiazol-5(4*H*)-one)benzoic acid (**59** [15]) was coupled to the resulting amine **48** to give **55.** Removal of the cyclic carbamate protection group by catalytic hydrogenation yielded amidine **56**. Saponification of the ester group with NaOH in ethanol yielded free acid **57**.

The synthesis of target compounds **67, 68, 69** and **70** is presented in Scheme 8. The initial acid **4** was coupled to L-phenylalanine ethyl ester hydrochloride or L-3-amino-2-(toluene-4-sulfonylamino)-propionic acid ethyl ester hydrochloride (**71** [17]). Reduction of **61** and **62** by catalytic hydrogenation furnished key intermediate amines **63** and **64**, which were acylated with *p*-cyanobenzoyl chloride. The resulting nitriles **65** and **66** were converted to amidines **67** and **68** by Pinner's procedure and ester groups were removed to yield **69** and **70**.



Scheme 8. (a) $NaOH_{(aq)}$, dioxane; (b) L-PheOEt, **71**, EDC, HOBt, NMM, DMF; (c) H_2, Pd/C, EtOH; (d) 4-CNC_6H_4COCl, Et_3N, CH_2Cl_2; (e) 1. $HCl_{(g)}$, EtOH; 2. $CH_3COO^-NH_4^+$, EtOH; (f) 1. $NaOH_{(aq)}$, EtOH; 2. CH_3COOH.



Scheme 9. (a) $BrCH_2COOEt$, KF, DMF, 60 °C; (b) $NaOH_{(aq)}$, dioxane; (c) **71**, EDC, HOBt, NMM, DMF; (d) H_2, Pd/C, EtOH; (e) **59**, EDC, HOBt, NMM, DMF; (f) 1. H_2, Pd/C, CH_3COOH, DMF; 2. $CH_3COO^-NH_4^+$, EtOH; (g) 1. $NaOH_{(aq)}$, EtOH; 2. CH_3COOH; (h) $Br(CH_2)_4COOEt$, $BnN^+(Et)_3Cl^-$, MeCN, 60 °C; (i) **59**, EDC, HOBt, NMM, DMF; (j) $NaOH_{(aq)}$, EtOH; (k) H_2, Pd/C, CH_3COOH, DMF.

*2.4. Preparation of 4,7-di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones*

4,7-Di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones **76**, **77**, **81** and **82** were obtained from benzoxazine derivative **7** (Scheme 9). This was first *N*-alkylated with ethyl 2-bromoacetate, using KF as a base, to give **72**. Saponification of **72** with NaOH in dioxane, and coupling of the resulting acid with **71** gave **73**. Subsequent catalytic hydrogenation of the nitro group yielded amine **74**, which was acylated with carboxylic acid **59** to give **75**. Removal of the cyclic carbamate protection group by catalytic hydrogenation gave **76**. Saponification of **76** with NaOH in ethanol yielded **77**.

Alternatively, **7** was *N*-alkylated with ethyl 5-bromopentanoate, using the phase-transfer procedure with K_2CO_3 as base and benzyltriethylammonium chloride as the catalyst, to give **78** [13]. Catalytic hydrogenation of the nitro group and further coupling of **59** to the resulting amine **79** gave **80**. 1,2,4-Oxadiazol-5(4*H*)-one group was converted to amidinium **81** salt by catalytic hydrogenation in acetic acid/DMF. Analogously, saponification of the ester group and cleavage of the 1,2,4-oxadiazol-5(4*H*)-one group in **80** yielded **82**.

3. Results and discussion

The ability of the synthesized compounds to inhibit platelet aggregation and the binding of fibrinogen to $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrins was characterized in a platelet aggregation assay and a solid-phase competitive displacement assay, respectively. The results are presented in Table 1.

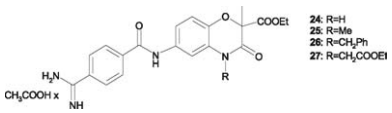
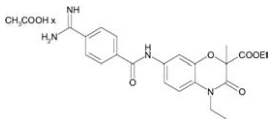
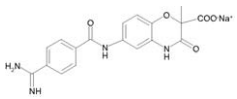
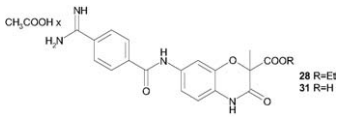
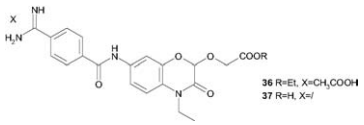
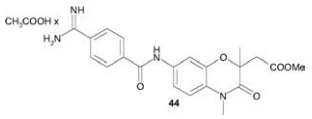
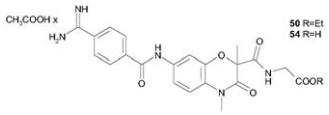
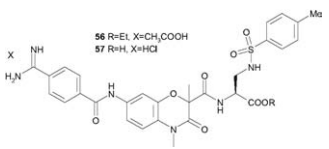
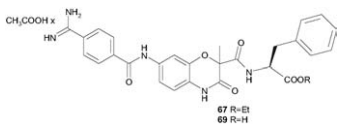
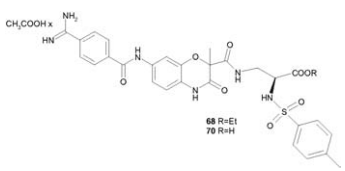
The first series of compounds were 2,6-di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones (**24**–**27, 30**) containing the *p*-benzoylamidine moiety as an arginine mimetic, since it has been proposed that a benzamidine moiety should interact most favourably with the carboxylate moiety at the binding site [18]. A carboxylic acid (or ester) moiety at position 2 was supposed to mimic the aspartate residue. Since **24** showed modest inhibition of platelet aggregation, the free acid derivative **30** was synthesized. To our surprise, it showed no anti-agreggatory activity at all and this stimulated us to optimise the ester **24**. Different substituents were attached at the ring nitrogen (position 4, compounds **25**–**27**) to increase non-polar interaction with the binding site, as proposed previously [8]. Only a methyl group was tolerated (compound **25**), while bulkier substituents resulted in significant loss of activity (compounds **26** and **27**). All the compounds mentioned showed low affinity for both $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrins (>100 μM), which is consistent with low anti-aggregatory activity.

2,7-Di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones were more promising (**28** compared to **24**). Again, the corresponding free acid **31** was practically inactive in inhibiting aggregation while showing higher affinity for $\alpha_{IIb}\beta_3$ (and, interestingly, for $\alpha_V\beta_3$). *N*-ethylation of **28** (**29**) resulted in negligible improvement in inhibition of platelet aggregation. In the next step, the 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold was lengthened at position 2 with an oxymethylene moiety (**36** and **37**) while retaining the same substituent at position 7. Surprisingly, good affinity for $\alpha_{IIb}\beta_3$, but poor anti-aggregatory effect was observed. Because of the unfavourable effect of the oxymethylene group on activity, we decided to replace it with a methylene spacer between the benzoxazine ring and the carboxyl moiety. The resulting ester **44** was far more efficient in inhibiting aggregation but had surprisingly low affinity for integrin $\alpha_{IIb}\beta_3$. Further improvement of anti-aggregatory activity was observed with derivatives lengthened at position 2 with a carboxylglycine moiety (**50** and **54**). As seen before with free acid derivatives, free acid (**54**) had poor anti-aggregatory activity but strong affinity for integrin $\alpha_{IIb}\beta_3$.

Several groups have reported that introduction of an aryl- or alkyl- sulphonamide group to the position α- to the carboxyl moiety resulted in higher activity of $\alpha_{IIb}\beta_3$ antagonists [3,17]. Importantly, additional binding groups should not only enhance the affinity towards the receptor, but also prolong the drug's plasma half-life [19]. For these reasons, glycine in **54** was replaced by L-Phe, N_α- and N_β-*p*-toluenesulfonyl-2,3-diaminopropionates. In our series, introduction of the *p*-toluensulfonamide group did not increase the potency of the compounds. The β-sulfonamido derivatives (**56**, **57**) showed weaker anti-aggregatory activity and the $\alpha_{IIb}\beta_3$ affinity was comparable to that of the glycine derivatives. The α-sulfonamido derivative **68** was a slightly more potent anti-aggregatory agent than the β-sulfonamido analogue **56** with free acid (**70**) which was totally insoluble in aqueous media. L-Phe substituted derivatives (**67**, **69**) inhibited aggregation in a modestly improved manner, and the ester and free acid derivatives had almost the same affinity for $\alpha_{IIb}\beta_3$, with IC_{50} reaching the nanomolar range. Compounds **67** and **69** were selective versus $\alpha_V\beta_3$; in fact, only **31**, **69**, **76** and **77** had any affinity for $\alpha_V\beta_3$.

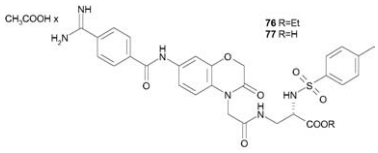
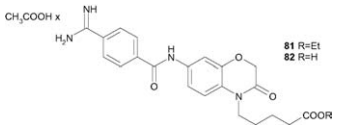
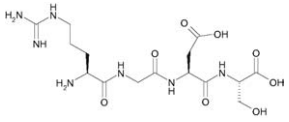
Of the 2,7-di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones, only a few were good inhibitors of platelet aggregation. The probable explanation is that they fail to form the "cup-shaped" conformation, which is presumably the one that fits best the binding site of integrin $\alpha_{IIb}\beta_3$ [2(a)]. Furthermore, a series of 4,7-di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones were synthesized. Molecular modelling (HyperChem 7.5, Hypercube, Inc.: conformations not included in the article) revealed that 4,7-di-substituted analogues should have more "cup-shaped" minimum conformations while retaining the generally accepted distance requirement between carboxylate and amidine moieties (Table 1) [2(a)]. Compound **76** was a fairly potent inhibitor of aggregation, but did not reach the nanomolar range. Its free acid counterpart **77** had a high affinity for $\alpha_{IIb}\beta_3$, but was not tested for inhibition of aggregation due to lack of solubility in aqueous media. Finally, derivatives with more flexible conformations were synthesized, **81** and **82**. In contrast to the previously described compounds, ester **81** inhibited aggregation only in the mid

Table 1
Inhibition of platelet aggregation and inhibition of binding of fibrinogen to integrins $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ expressed as IC_{50} values (N.D. not determined because of poor solubility in aqueous media)

Compound	Structural formula	IC_{50} (μM) (20 μM ADP)	IC_{50} (μM) ($\alpha_{IIb}\beta_3$ affinity)	IC_{50} (μM) ($\alpha_V\beta_3$ affinity)	Distance between carboxylate and amidine C-atoms (Å) [a]
24	 24: R=H 25: R=Me 26: R=CH_2Ph 27: R=CH_2COOEt	200	>100	>100	14.296
25		145	>100	>100	14.370
26		790	>100	>100	14.111
27		591	>100	>100	14.120
29		40	>100	>100	13.322
30		>250	>100	>100	14.302
28	 28 R=Et 31 R=H	45	71.595	>100	12.948
31		>250	43.840	63.585	11.648
36	 36 R=Et, X=CH_2COOH 37 R=H, X=	78	76.375	>100	15.376
37		650	0.257	>100	14.393
44		5.0	>100	>100	14.116
50	 50 R=Et 54 R=H	1.8	10.242	>100	15.765
54		>25	0.128	>100	16.783
56	 56 R=Et, X=CH_2COOH 57 R=H, X=HCl	21	33.276	>100	17.724
57		25	6.023	>100	15.580
67	 67 R=Et 69 R=H	0.6	7.721	>100	16.125
69		6.9	7.202	83.477	15.533
68	 68 R=Et 70 R=H	5.6	12.685	>100	15.523
70		N.D.	N.D.	N.D.	16.522

(continued on next page)

Table 1 *(continued)*

Compound	Structural formula	IC_{50} (μM) (20 μM ADP)	IC_{50} (μM) ($\alpha_{IIb}\beta_3$ affinity)	IC_{50} (μM) ($\alpha_V\beta_3$ affinity)	Distance between carboxylate and amidine C-atoms (Å) [a]
76		9.5	4.606	20.562	19.081
77		N.D.	0.308	9.213	19.026
81		18.9	0.123	>100	18.074
82		2.5	0.0167	>100	17.732
RGDS		260	1.145	3.296×10^{-3}	12.729

[a] Distance between pharmacophore centres: conformations of potential energy minima were generated with HyperChem 7.5, Hypercube, Inc.

micromolar range, while the free acid **82** was able to bind to $\alpha_{IIb}\beta_3$ with dramatically improved potency. The astonishing fact was the high affinity observed for the ester analogue.

In most cases, there is seen to be a reasonable correlation between the anti-aggregatory potency of the ester analogues and the affinity of free acids for $\alpha_{IIb}\beta_3$. Compounds **37** (and ester analogue **36**) and **82** (and ester analogue **81**) however, showed high affinity for $\alpha_{IIb}\beta_3$ but failed to adequately inhibit platelet aggregation. These discrepancies are believed to be the consequence of compounds **36**, **37**, **81** and **82** not being full antagonists of the $\alpha_{IIb}\beta_3$ receptor; despite their high affinity for the receptor, they would not be able to inhibit aggregation, due to remaining intrinsic activity (see below).

Notably, in almost all cases, esters were more potent than the free acids in inhibiting platelet aggregation, in contrast with the generally accepted model. Grumel et al. [20] reported an equivocal observation, where ester analogues of synthesized RGD mimetics showed similar or better anti-aggregatory activity than the corresponding free acids. This could be easily explained by the fact that free acid analogues are zwitterions which are known to dissolve poorly in aqueous media. The activity of ester analogues could be the consequence of in situ hydrolysis of the ester bond in the assay, as numerous esterases are present in the blood (and therefore in the assay plasma). However, cleavage of the ester bond would form the same free acids that are not soluble enough to allow measurement of their anti-aggregatory activity. Eventually, free acids formed after hydrolysis would precipitate in the aqueous assay medium. But again, this does not explain the anti-aggregatory activity of the esters.

We propose that some or all of the free acid analogues might be partial agonists of the $\alpha_{IIb}\beta_3$ receptor, while esters might be full antagonists of the fibrinogen receptor in terms of receptor activation, even though both are inhibitors of platelet aggregation. Cox et al. also reported a similar hypothesis [21]. Fibrinogen binding to $\alpha_{IIb}\beta_3$ receptor triggers outside-in signalling, which leads to receptor activation and further platelet activation [22]. The ability of fibrinogen to activate its receptor is intrinsic activity. The same effect can be observed by a small RGD peptide and, presumably, all small binding molecules [23]. This results in release of intracellular Ca^{2+} (positive influence on platelet release reaction) and exposure of ligand-induced binding sites (LIBS), which are presumed to play an important role in receptor clustering after fibrinogen binding in the last stage of platelet aggregation [23,24]. Alternatively, the $\alpha_{IIb}\beta_3$ receptors might remain in an activated conformation after partial agonist dissociation, thus leaving free the fibrinogen binding site [6]. Consequently, aggregation of platelets could occur after fibrinogen binding to free $\alpha_{IIb}\beta_3$ receptors, even though the majority of $\alpha_{IIb}\beta_3$ receptors are occupied (Fig. 1). The full antagonists, however, should be devoid of this activity, as inhibition of fibrinogen binding should prevent, not only aggregation, but also all pro-aggregatory processes. We believe that compounds having small alkyl ester groups instead of carboxylic ones could be regarded as full antagonists. In support of the above hypothesis, Dickfeld et al. [25] reported that some known fibrinogen receptor "antagonists" (tirofiban, integrelin, lamifiban; free acid compounds) do inhibit ADP-induced platelet aggregation but fail to inhibit TRAP-induced platelet aggregation and platelet release (ATP and P-selectin secretion), and even induce LIBS exposure. Therefore, these "antagonists" act also in a pro-aggregatory manner, which is a consequence of their intrinsic activity. Based on these results, Dickfeld et al. proposed that development of $\alpha_{IIb}\beta_3$ receptor antagonists with less or zero LIBS activity and enhanced release blocking might be advantageous. Nevertheless, currently used "antagonists" (tirofiban, integrelin) inhibit ADP-mediated platelet aggregation potently by merely binding to $\alpha_{IIb}\beta_3$ receptors with high affinity. The major therapeutic

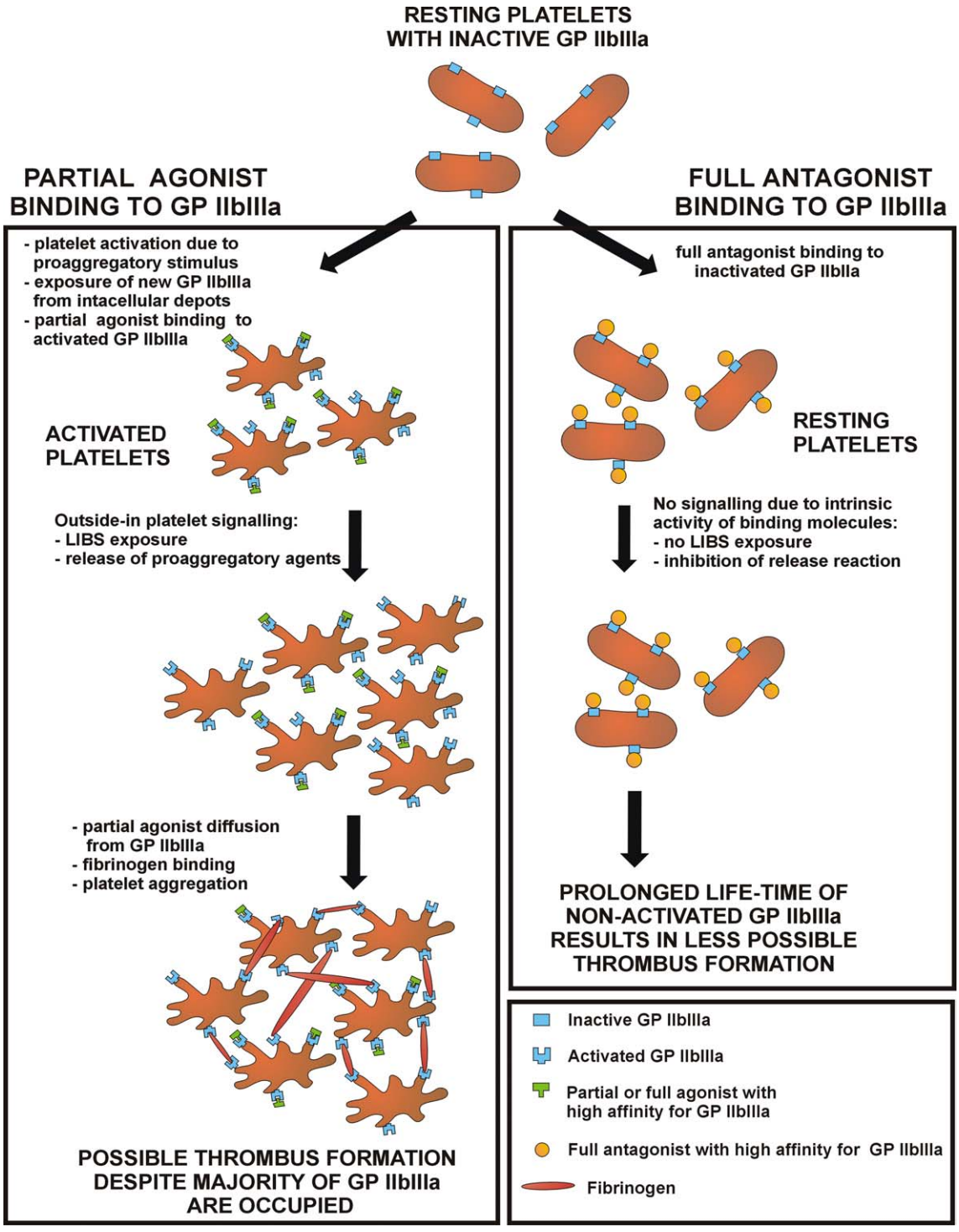


Fig. 1. Proposed mechanism of fibrinogen receptor partial agonist/antagonist activity.

problem with these compounds is that at least 80% of all $\alpha_{IIb}\beta_3$ receptors must be occupied to achieve therapeutically useful anti-aggregatory activity [21], so antagonists with extremely high affinity are required. High receptor occupancy, however, correlates with high bleeding risk, so plasma levels of such agents must be kept within a relatively narrow range. We assume that full antagonists would not provoke bleeding risks to any great extent, since they are expected to inhibit platelet aggregation with less than 80% receptor occupancy.

What can be said about the molecular mechanism of this "ester-free acid paradox"? $\alpha_{IIb}\beta_3$ receptors exist in active and inactive conformations [25]. In the natural pathway, fibrinogen binds only to activated receptors, leading to platelet aggregation. In contrast, it has been shown that RGD peptides and their mimetics can bind to the inactive conforma-

tion of $\alpha_{IIb}\beta_3$ [26]. Later, Xiong et al. [27,28] elucidated the crystal structure of the extracellular segment of integrin $\alpha_V\beta_3$ with the RGD-containing ligand. The active conformation of $\alpha_V\beta_3$ was shown to complex Mn^{2+} ions in the β_3-subunit carboxylate-binding site, while the inactive conformation lacks Mn^{2+} ions in this site. These ions are supposed to form an ionic bond with the carboxylate moiety of the binding molecule, which would not be possible in the inactive conformation. Since integrins $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ share the same β_3-subunit, we propose that the same could be true of the integrin $\alpha_{IIb}\beta_3$. Namely, free acids bind to the active form of integrin $\alpha_{IIb}\beta_3$ by forming an ionic bond with the metal ion in the binding site. This explains the high binding affinity of the free acids. Since fibrinogen binds to the active conformation of the receptor and triggers further signalling, the active conformation must be responsible for the outside-in signalling. The inactive conformation of $\alpha_{IIb}\beta_3$ lacks the metal ion and has more free space in the "carboxylate-binding hole". It could therefore accommodate an even larger group than the carboxylate, i.e. the small alkyl ester group (see Fig. 2).

Since, in the inactive conformation of integrin $\alpha_V\beta_3$ (and presumably in the inactive conformation of $\alpha_{IIb}\beta_3$), there is no metal ion in the binding site, there is no requirement for a binding group to form an ionic bond. Instead, hydrogen bonds could be formed between ester oxygens and the metal-ion chelating groups of the β_3-subunit of the integrin $\alpha_{IIb}\beta_3$ (for more details see [28]). Since formation of an ionic bond releases more free energy than hydrogen bonding, a lower affinity is observed for esters. It seems reasonable to predict that the inactive conformation of $\alpha_{IIb}\beta_3$ is incapable of outside-in signalling and should therefore not contribute to pro-aggregatory processes. Compounds which bind to the inactive conformation of the receptor could diminish the capacity of total fibrinogen-binding sites, not only by mere occupation, but also by prolonging the lifetime of the inactive conformation of the receptor. Being bound to the inactive conformation of $\alpha_{IIb}\beta_3$ receptor means that they should also be incapable of triggering outside-in signalling. Compounds with an ester moiety could, therefore, be envisaged as full fibrinogen receptor antagonists.

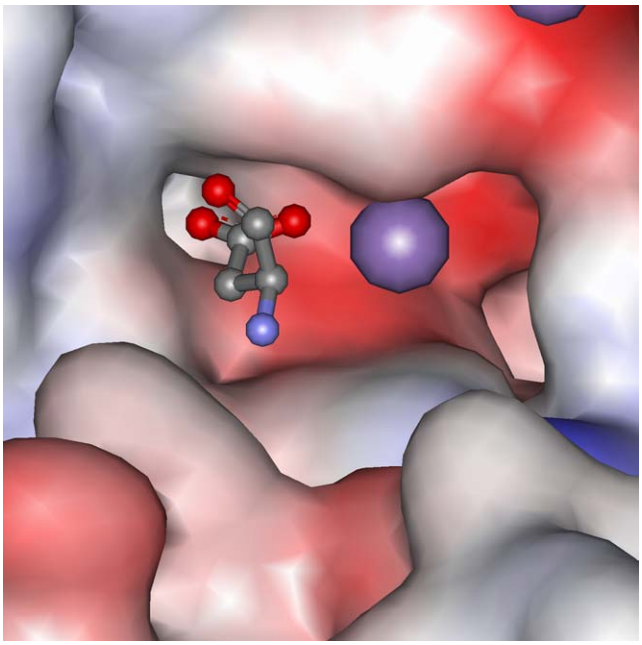


Fig. 2. ΔThe aspartate binding site in the β_3-subunit (vitronectin receptor, presented as protein surface) with Mn^{2+} ions (violet spheres) and aspartate residue ("ball and stick" rendering) [28]. For clarity, only the aspartate residue of the c(RGDfV) ligand is shown. The absence of a metal ion (to the right of the carboxyl group) would clearly leave enough space for a small alkyl group.

The proposed hypothesis of fibrinogen partial agonists/antagonists action could offer an answer to the previously described fact that low doses of fibrinogen receptor "antagonists" having a carboxylic acid moiety do not inhibit but, rather, promote platelet aggregation [29]. Considering free acid analogues as partial agonists of the $\alpha_{IIb}\beta_3$ receptor, one might expect that, in lower doses, a free acid compound would bind to the receptor to an insufficient extent to inhibit aggregation, but sufficient enough to trigger platelet activation and LIBS exposure. In higher doses, compounds with high affinity for the receptor would occupy almost all platelet $\alpha_{IIb}\beta_3$ receptors, thus preventing the binding of fibrinogen. The proposed hypothesis could offer some new insight into a molecular explanation for previously reported phenomena that some fibrinogen receptor "antagonists" failed to reach the desired therapeutic effect after administration to patients in clinical trials [5,30,31]. Though Scarborough and Gretler [30], reported that poor pharmacokinetics have a major impact on therapeutic insufficiency of some fibrinogen receptor "antagonists" a combination of poor pharmacokinetics and the proposed mechanism appears reasonable.

4. Conclusions

In conclusion, we have prepared novel 2*H*-1,4-benzoxazine-3(4*H*)-one template-based $\alpha_{IIb}\beta_3$ receptor ligands. Although aspartate mimetic moieties and substitution positions were varied, almost all the compounds synthesized showed rather unusual binding behaviour, ethyl esters being more active than free acids against ADP-induced human platelets aggregation. Although the impact of this phenomenon on in vivo antagonist properties is yet to be clarified, we propose a new hypothesis. Compounds bearing small alkyl ester moieties may be full antagonists of $\alpha_{IIb}\beta_3$ receptor, while the corresponding free acids could act as partial agonists. The proposed hypothesis could offer a new insight into the several $\alpha_{IIb}\beta_3$ antagonists that fail to elicit appropriate therapeutic activity. The ester compounds could not be used as therapeutic agents, as hydrolysis under in vivo conditions would form the corresponding free acids, but could be useful as a starting points for the design of new, therapeutically active, fibrinogen receptor antagonists.

5. Experimental protocols

5.1. General methods and materials

Chemicals from Aldrich Chemical Co., Fluka, and Acros Organics were used without further purification. Anhydrous solvents were prepared according to standard procedures [32]. Analytical TLC was performed on Merck silica gel (60 GF 254) plates (0.25 mm) and components were visualized with ultraviolet light (254 nm wavelength). Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker AVANCE DPX$_{300}$ spectrometer in $CDCl_3$ or DMSO-d_6 solution with TMS as the internal standard. IR spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrometer. Microanalyses were performed on a Perkin-Elmer C, H, N analyzer 240 C. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. All yields relate to purified products.

Human integrin $\alpha_V\beta_3$ was obtained from Chemicon. Human integrin $\alpha_{IIb}\beta_3$, purified human fibrinogen, biotin *N*-hydroxysuccinimide ester and anti-biotin (goat) peroxidase conjugate were obtained from Calbiochem. Tris(hydroxymethyl)aminomethane (Tris) buffer, Tween 20 $CaCl_2$, $MgCl_2$ and $MnCl_2$ were obtained from Acros Organics. Bovine serum albumin (BSA) was purchased from Aldrich Chemical Co. BM chemiluminescence ELISA Substrate (POD) was obtained from Roche Diagnostics, Mannheim. RGDS was purchased from Bachem.

5.2. Synthesis

5.2.1. General procedures

*5.2.1.1. General procedure for the alkylation with alkyl halides in the presence of KF (**2, 4, 7, 32, 33, 38, 72**) [10].* To a suspension of 100 mmol KF in 25 ml of anhydrous *N,N*-dimethylformamide (DMF), 40 mmol of alkylhalide and 40 mmol of starting compound were added. The mixture was stirred for 30 min at room temperature (RT) after which it was heated to 60 °C and stirred until complete conversion to the final product was established with thin-layer chromatography (TLC). The reaction mixture was cooled to RT and poured on 100 g of ice. The ice was allowed to melt and the precipitate was filtered off, washed with water and, unless stated otherwise, recrystallized from ethanol.

5.2.1.2. General procedure for N*-alkylation of amides with NaH and alkylhalides (**8, 9, 10, 11, 38**) [13].* To a suspension of 1.0 mmol of amide in 5 ml of anhydrous toluene NaH (1.0 mmol) was added and the suspension stirred for 30 min at RT. Alkyl halide (1.1 mmol) was added and the mixture heated at 100 °C until complete conversion to the final product was established with TLC. The reaction mixture was cooled to RT and washed with water (3 × 5 ml) and brine (5 ml). The toluene phase was dried over Na_2SO_4, filtered and evaporated under vacuum. Cold ethanol was poured onto the oily residue and mixed vigorously until the crude product precipitated. The precipitate was filtered off and dried under vacuum.

*5.2.1.3. General procedure for reduction of nitro compounds to amines by catalytic hydrogenation (**12–17, 34, 42, 47, 48, 63, 64, 74, 79**).* Argon was bubbled into a solution of nitro compound (5 mmol) in anhydrous ethanol (50 ml) and palladium (10% by weight, 10% on active charcoal) was added stepwise. Hydrogen was bubbled into the suspension and stirring continued under 1 atm of hydrogen at RT until no nitro compound was detected with TLC. The catalyst was filtered off and the solvent evaporated under vacuum.

*5.2.1.4. General procedure for acylation with carboxylic acid chlorides (**18–23, 35, 43, 49, 65, 66**).* To a cooled (–10 °C) solution of amine (1.0 mmol) and triethylamine (1.1 mmol) in distilled dichloromethane (10 ml), carboxylic acid chloride was added stepwise and the mixture stirred for 6 h. During stirring, the temperature was allowed to rise to RT. If the product precipitated from the reaction mixture, it was filtered off and washed with a small portion of dichloromethane. Otherwise, the solvent was evaporated and the residue dissolved in ethyl acetate (20 ml) and washed with water (20 ml), 10% aqueous citric acid (2 × 20 ml), saturated aqueous $NaHCO_3$ (2 × 20 ml) and brine (20 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated under vacuum.

*5.2.1.5. General procedure for conversion of nitriles into amidines (Pinner reaction) (**24–29, 36, 44, 50, 67, 68**) [33].* Into an ice-cooled solution of nitrile (1.0 mmol) in anhydrous ethanol (20 ml), gaseous HCl was bubbled until complete saturation was achieved (~30 min). The mixture was stirred for 24 h at RT and solvent then evaporated under vacuum. The residue was dispersed in diethyl ether (20 ml), filtered and washed with diethyl ether (2 × 20 ml). The crude residue was further dried with a membrane pump and dissolved in anhydrous ethanol (10 ml). Ammonium acetate (3.0 mmol) was added to the solution and stirred for another 24 h at RT. Diethyl ether was added dropwise into the reaction mixture until all the solid product was precipitated. The crude product was filtered off and dried under vacuum.

*5.2.1.6. General procedure for saponification of the ester group with $NaOH_{(aq)}$/dioxane (**30, 31, 39, 60, 73**).* Into a stirred solution of ester (20 mmol) in dioxane (70 ml), 2 M $NaOH_{(aq)}$ (30 mmol) was added. The reaction was allowed to continue for the next 24 h at RT and the solvent was then evaporated. The crude residue was dissolved in water (60 ml) and the solution washed with ethyl acetate (2 × 50 ml). The

water phase was acidified with 4 M HCl to pH~3, extracted with ethyl acetate (2 × 50 ml) and dried over Na_2SO_4. The solvent was evaporated under vacuum to yield crude product.

*5.2.1.7. General procedure for saponification of the ester group with $NaOH_{(aq)}$/ethanol (**37, 53, 57, 69, 70, 77, 82**).* To a dispersion of ethyl ester (0.29 mmol) in 96% ethanol (10 ml), 2 M $NaOH_{(aq)}$ (0.3 ml, 0.6 mmol) was added and stirred at RT until no starting compound was detected by TLC. Unless otherwise stated, the mixture was treated with acetic acid (1.0 ml) and cooled at 4 °C overnight. Precipitated product was filtered off, washed with ice-cooled ethanol (10 ml) and dried under vacuum.

*5.2.1.8. General procedure for coupling with EDC/HOBt/NMM (**45, 46, 55, 61, 62, 73, 75, 80**).* To a cooled (–10 °C) solution of carboxylic acid (1.0 mmol) and amine (1.0 mmol) in anhydrous *N,N*-dimethylformamide (5 ml), 1-hydroxybenzotriazole (HOBT) monohydrate and *N*-methylmorpholine (2.3 mmol) were added. The pH of the mixture was checked with wet pH-indicator strip (~8) and *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) (1.3 mmol) was added. The temperature was allowed to rise to RT and the mixture was stirred until complete conversion to the final product was detected with TLC. The solvent was evaporated and the residue was dissolved in ethyl acetate (20 ml), washed with aqueous citric acid (10%, 2 × 20 ml), saturated aqueous $NaHCO_3$ (2 × 20 ml) and brine (20 ml) and dried over Na_2SO_4. The solvent was evaporated under vacuum to yield crude product.

*5.2.1.9. General procedure for conversion of 5-oxo-4,5-dihydro-1,2,4-oxadiazoles to amidines by catalytic hydrogenation (**54, 56, 76, 81, 82**) [15].* Argon was bubbled into a solution of 5-oxo-4,5-dihydro-1,2,4-oxadiazole derivative (0.23 mmol) in anhydrous DMF (10 ml) and acetic acid (5 ml). Palladium (10% in weight, 10% on active charcoal) was then added stepwise. Hydrogen was bubbled into the suspension and stirring continued under 1 atm of hydrogen at RT until no initial compound was detected. The catalyst was filtered off and the solvent evaporated under vacuum. The oily residue was treated with diethyl ether and the crude product dried under vacuum.

*5.2.2. Ethyl 2-methyl-6-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-carboxylate (**2**) [10]*

This was prepared according to the general procedure as in Section 5.2.1.1 from 2-amino-4-nitrophenol (6.17 g, 40.0 mmol) and diethyl 2-bromo-2-methylmalonate (7.64 ml, 40.0 mmol). *m* = 8.15 g (73%). M.p. 194–198 °C. IR (KBr, cm^{-1}): 3462, 1736, 1696, 1528, 1490, 1384, 1348, 1261, 1132, 1082, 1018, 966. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.08 (t, 3H, J = 7.2 Hz, $CH_2\underline{CH_3}$), 1.76 (s, 3H, 2-CH_3), 4.14 (m, 2H, CH_2), 7.25 (d, 1H, J = 9.0 Hz, Ar–H_8), 7.76 (d, 1H, J = 2.6 Hz, Ar–H_5), 7.89 (dd, 1H, J = 9.0, 2.6 Hz, Ar–H_7), 11.36 (s, 1H, –CONH–). MS (EI) = 280 (M^+).

*5.2.3. Ethyl 2-methyl-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-carboxylate (**4**) [10]*

Prepared according to the general procedure as in Section 5.2.1.1 from 2-amino-5-nitrophenol (6.17 g, 40.0 mmol) and diethyl 2-bromo-2-methylmalonate (7.64 ml, 40.0 mmol). *m* = 9.30 g (83%). M.p. 191–193 °C. IR (KBr, cm^{-1}): *3088, 1749, 1697, 1605, 1540, 1508, 1340, 1237, 1123, 1012, 826, 744. *Primary amines usually show two IR signals in the 3300–3400 cm^{-1} region differing by approximately 100 cm^{-1}. This region is however covered with "low" base line. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.07 (t, 3H, J = 7.2 Hz, $CH_2\underline{CH_3}$), 1.75 (s, 3H, 2-CH_3), 4.12 (m, 2H, CH_2), 7.10 (d, 1H, J = 8.9 Hz, Ar–H_5), 7.84 (d, 1H, J = 2.6 Hz, Ar–H_8), 7.95 (dd, 1H, J = 8.9, 2.6 Hz, Ar–H_6), 11.60 (s, 1H, –CONH–). MS (FAB) = 281 (MH^+).

*5.2.4. 2,2-Dichloro-*N*-(2-hydroxy-4-nitrophenyl)acetamide (**5**)*

Dichloroacetyl chloride (4.91 ml, 50 mmol) was dissolved in diethylether (20 ml) and added dropwise to a cooled (–10 °C) suspension of 2-amino-5-nitrophenol (8.11 g, 50 mmol) and triethylamine (6.96 ml, 50 mmol) in diethylether (100 ml). After 1 h of stirring, the temperature was raised to RT and stirring continued for another 4 h. Precipitated triethylamonium chloride was filtered off and the filtrate washed with water (100 ml), 0.5 M HCl (2 × 100 ml), brine (100 ml) and then dried over Na_2SO_4. The solvent was evaporated under vacuum and the residue recrystallized from chloroform. *m* = 9.45 g (71%). M.p. 149–151 °C. IR (KBr, cm^{-1}): 3178, 1681, 1592, 1557, 1511, 1430, 1340, 1267, 1079, 942. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 6.12 (s, 1H, $CHCl_2$), 6.95 (s, 1H, OH), 7.81 (d, 1H, J = 2.3 Hz, Ar–H_6), 7.92 (dd, 1H, J = 9.1, 2.3 Hz, Ar–H_4), 8.25 (d, 1H, J = 9.1 Hz, Ar–H_3), 8.91 (s, 1H, –CONH–). MS (EI) = 264 (M^+). Anal. CHN $C_8H_6Cl_2N_2O_4$.

*5.2.5. 2-Hydroxy-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one (**6**)*

5 (5.30 g, 20 mmol) was suspended in an aqueous solution (150 ml) of $NaHCO_3$ (3.36 g, 40 mmol) and boiled for 30 min. The reaction mixture was cooled to RT, acidified with 1 M HCl (pH ~3) and extracted with ethyl acetate (2 × 80 ml). The organic phase was washed with 1 M HCl (2 × 100 ml), saturated aqueous $NaHCO_3$ (2 × 100 ml), brine (100 ml) and dried over Na_2SO_4. The solvent was evaporated, the residue suspended in a mixture of diethylether (20 ml) and petroleum ether (10 ml) and filtered off. *m* = 2.96 g (71%). M.p. 183–187 °C. IR (KBr, cm^{-1}): 3088, 1690, 1605, 1506, 1342, 1216, 1014. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 5.62 (d, 1H, J = 5.8 Hz, 2-H), 7.11 (d, 1H, J = 8.9 Hz, Ar–H_5), 7.82 (d, 1H, J = 2.5 Hz, Ar–H_8), 7.95 (dd, 1H, J = 8.9, 2.5 Hz, Ar–H_6), 8.32 (d, 1H, J = 5.8 Hz, 2-OH), 11.42 (s, 1H, –CONH–). MS (EI) = 210 (M^+). Anal. CHN $C_8H_6N_2O_5$.

*5.2.6. 2-Methyl-7-nitro-2H-1,4-benzoxazine-3(4H)-one (**7**) [10]*

Prepared according to the general procedure as in Section 5.2.1.1 from 2-amino-5-nitrophenol (10.0 g, 60 mmol) and ethyl bromoacetate (6.85 ml, 60 mmol). *m* = 7.37 g (63%). M.p. 235–239 °C. IR (KBr, cm^{-1}): 3080, 1698, 1598, 1509, 1392, 1341, 1222, 1044, 887, 742. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 4.72 (s, 2H, CH_2), 7.06 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.75 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.89 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar–H_6), 11.30 (s, 1H, –CONH–). MS (FAB) = 195 (MH^+).

*5.2.7. Ethyl 2,4-dimethyl-6-nitro-2H-1,4-benzoxazin-3(4H)-one-2-carboxylate (**8**)*

Prepared according to the general procedure as in Section 5.2.1.2 from 2.10 g (7.5 mmol) of **2** and 0.69 ml (11.0 mmol) methyl iodide. *m* = 0.77 g (36%). M.p. 101–103 °C. IR (KBr, cm^{-1}): 3472, 1745, 1636, 1522, 1474, 1381, 1348, 1277, 1152, 1019, 895. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.06 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.79 (s, 3H, 2-CH_3), 3.41 (s, 3H, N–CH_3), 4.09 (m, 2H, CH_2); 7.32 (d, 1H, *J* = 9.0 Hz, Ar–H_8), 7.99 (m, 2H, Ar–H_7, Ar–H_5). MS (EI) = 294 (M^+). Anal. CHN $C_{13}H_{14}N_2O_6$.

*5.2.8. Ethyl 4-benzyl-2-methyl-6-nitro-2H-1,4-benzoxazin-3(4H)-one-2-carboxylate (**9**)*

Prepared according to the general procedure as in Section 5.2.1.2 from 2.38 g (8.5 mmol) of **2** and 1.19 ml (10.0 mmol) benzyl bromide. *m* = 2.48 g (79%). M.p. 87–90 °C. IR (KBr, cm^{-1}): 3484, 1749, 1636, 1525, 1456, 1385, 1342, 1172, 1128, 1002, 878, 727. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.08 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.88 (s, 3H, 2-CH_3), 4.15 (m, 2H, –$\underline{CH_2}$–CH_3), 5.05 (d, 1H, *J* = 16.6 Hz, –$\underline{CH_2}$–Ph), 5.50 (d, 1H, *J* = 16.6 Hz, –$\underline{CH_2}$–Ph), 7.27–7.41 (m, 6H, Ph, 5H, Ar–H_8), 7.82 (d, 1H, *J* = 2.5 Hz, Ar–H_5), 7.95 (dd, 1H, *J* = 8.6, 2.5 Hz, Ar–H_7). MS (EI) = 370 (M^+). Anal. CHN $C_{19}H_{18}N_2O_6$.

*5.2.9. Ethyl 4-(2-ethoxy-2-oxoethyl)-2-methyl-6-nitro-2H-1,4-benzoxazin-3(4H)-one-2-carboxylate (**10**)*

Prepared according to the general procedure as in Section 5.2.1.2 from 1.68 g (6.0 mmol) of **2** and 0.95 ml (8.5 mmol) ethyl bromoacetate. *m* = 1.65 g (75%). M.p. 108–110 °C. IR (KBr, cm^{-1}): 3414, 2997, 1749, 1707, 1617, 1522, 1379, 1344, 1210, 1018, 885, 749. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.07 (t, 3H, *J* = 7.2 Hz, 2-$COOCH_2\underline{CH_3}$), 1.20 (t, 3H, *J* = 7.2 Hz, $NCH_2COOCH_2\underline{CH_3}$), 1.80 (s, 3H, 2-$CH_3$), 4.05–4.20 (m, 4H, 2 × $\underline{CH_2}$–CH_3), 4.81 (d, 1H, *J* = 17.7 Hz, N–$\underline{CH_2}$–), 4.97 (d, 1H, *J* = 17.7 Hz, N–$\underline{CH_2}$–), 7.37 (d, 1H, *J* = 9.0 Hz, Ar–H_8), 8.00 (m, 2H, Ar–H_7, Ar–H_5). MS (FAB) = 367 (MH^+). Anal. CHN $C_{16}H_{18}N_2O_8$.

*5.2.10. Ethyl 4-ethyl-2-methyl-7-nitro-2H-1,4-benzoxazin-3(4H)-one-2-carboxylate (**11**)*

Prepared according to the general procedure as in Section 5.2.1.2 from 2.00 g (7.1 mmol) of **4** and 0.99 ml (8.6 mmol) ethyl bromide. *m* = 2.01 g (91%). M.p. 75–79 °C. IR (KBr, cm^{-1}): 2983, 1747, 1715, 1602, 1522, 1391, 1338, 1263, 1140, 1022, 884, 793, 745. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.16 (t, 3H, *J* = 7.2 Hz, $NCH_2\underline{CH_3}$), 1.32 (t, 3H, *J* = 7.2 Hz, $OCH_2\underline{CH_3}$), 1.92 (s, 3H, 2-CH_3), 4.17 (m, 4H, 2 × CH_2), 7.06 (d, 1H, *J* = 8.6 Hz, Ar–H_5), 7.98 (m, 2H, Ar–H_6, Ar–H_8). MS (EI) = 308 (M^+). Anal. CHN $C_{14}H_{16}N_2O_6$.

*5.2.11. Ethyl 6-amino-2-methyl-2H-1,4-benzoxazin-3(4H)-one-2-carboxylate (**12**) [10]*

Prepared according to the general procedure as in Section 5.2.1.3 from 2.50 g (8.93 mmol) of **2**. *m* = 2.07 g (93%). M.p. 132–133 °C. IR (KBr, cm^{-1}): 3383, 1753, 1703, 1617, 1521, 1494, 1421, 1351, 1228, 1125, 1015, 867. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.06 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.60 (s, 3H, 2-CH_3), 4.06 (m, 2H, CH_2), 4.86 (s, 2H, NH_2), 6.11–6.15 (m, 2H, Ar–H_5, Ar–H_7), 6.67 (d, 1H, *J* = 9.4 Hz, Ar–H_8), 10.62 (s, 1H, –CONH–). MS (EI) = 250 (M^+).

*5.2.12. Ethyl 6-amino-2,4-dimethyl-2H-1,4-benzoxazin-3(4H)-one -2-carboxylate (**13**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 0.82 g (2.8 mmol) of **8**. *m* = 0.53 g (72%). M.p. 116–119 °C. IR (KBr, cm^{-1}): 3447, 3364, 2981, 1724, 1675, 1613, 1516, 1383, 1278, 1234, 1134, 1014, 858. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.03 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.63 (s, 3H, 2-CH_3), 3.23 (s, 3H, N–CH_3), 4.05 (m, 2H, CH_2), 4.93 (s, 2H, NH_2), 6.23 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar–H_7), 6.37 (d, 1H, *J* = 2.3 Hz, Ar–H_5), 6.73 (d, 1H, *J* = 8.7 Hz, Ar–H_8). MS (EI) = 264 (M^+). Anal. CHN $C_{13}H_{16}N_2O_4$.

*5.2.13. Ethyl 6-amino-4-benzyl-2-methyl-2H-1,4-benzoxazin-3(4H)-one -2-carboxylate (**14**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 1.46 g (3.95 mmol) of **9.** The crude product was recrystallized from ethanol. *m* = 1.10 g (82%). M.p. 153–156 °C. IR (KBr, cm^{-1}): 3458 (wide signal), 1723, 1674, 1609, 1513, 1446, 1398, 1340, 1234, 1125, 1006, 838. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.06 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.71 (s, 3H, 2-CH_3), 4.10 (q, 2H, *J* = 7.2 Hz, –$\underline{CH_2}$–CH_3), 4.79 (d, 1H, *J* = 16.4 Hz, –$\underline{CH_2}$–Ph), 4.94 (s, 2H, NH_2), 5.27 (d, 1H, *J* = 16.4 Hz, –$\underline{CH_2}$–Ph), 6.20 (m, 2H, Ar–H_5, Ar–H_7), 6.76 (d, 1H, *J* = 9.0 Hz, Ar–H_8), 7.27–7.38 (m, 5H, Ph). MS (EI) = 340 (M^+). Anal. CHN $C_{19}H_{20}N_2O_4$.

*5.2.14. Ethyl 6-amino-4-(2-ethoxy-2-oxoethyl)-2-methyl-2H-1,4-benzoxazin-3(4H)-one -2-carboxylate (**15**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 1.83 g (5.0 mmol) of **10**. The crude product was recrystallized from ethanol. *m* = 1.36 g (80%). M.p. 155–160 °C. IR (KBr, cm^{-1}): 3448 (wide signal), 1734, 1684, 1625, 1512, 1387, 1236, 1127, 1016, 940, 849. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.06 (t, 3H, *J* = 7.2 Hz,

2-COOCH$_2$CH$_3$), 1.21 (t, 3H, J = 7.2 Hz, NCH$_2$COOCH$_2$CH$_3$), 1.64 (s, 3H, 2-CH$_3$), 4.02–4.19 (m, 4H, 2 × CH$_2$–CH$_3$), 4.54 (d, 1H, J = 17.5 Hz, N–CH$_2$–), 4.65 (d, 1H, J = 17.5 Hz, N–CH$_2$–), 4.90 (s, 2H, NH$_2$), 6.21–6.26 (m, 2H, Ar–H$_5$, Ar–H$_7$), 6.76 (d, 1H, J = 8.3 Hz, Ar–H$_8$). MS (EI) = 336 (M^+). Anal. CHN $C_{16}H_{20}N_2O_6$.

*5.2.15. Ethyl 7-amino-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**16**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 3.00 g (10.7 mmol) of **4**. m = 2.30 g (86%). M.p. 139–141 °C. IR (KBr, cm^{-1}): 3401, 3228, 2986, 1746, 1698, 1658, 1518, 1426, 1386, 1316, 1242, 1126, 1009, 843. ^{1}H-NMR (DMSO-d$_6$, 300 MHz): δ (ppm) 1.08 (t, 3H, J = 7.2 Hz, CH$_2$CH$_3$), 1.62 (s, 3H, 2-CH$_3$), 4.07 (q, J = 7.2 Hz, 2H, CH$_2$), 4.92 (s, 2H, NH$_2$), 6.17–6.58 (m, 3H, Ar–3H), 10.43 (s, 1H, –CONH–). MS (EI) = 250 (M^+). EI-HRMS calcd. for $C_{12}H_{14}N_2O_4$: 250.096030. Found: 250.095357.

*5.2.16. Ethyl 7-amino-4-ethyl-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**17**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 1.80 g (5.84 mmol) of **11**. m = 1.57 g (97%). M.p. 105–112 °C. IR (KBr, cm^{-1}): 3467, 3371, 2988, 1727, 1671, 1630, 1513, 1416, 1266, 1124, 1007, 834. ^{1}H-NMR (CDCl$_3$, 300 MHz): δ (ppm) 1.18 (t, 3H, J = 7.2 Hz, NCH$_2$CH$_3$), 1.26 (s, 3H, 2-CH$_3$), 1.32 (t, 3H, J = 7.2 Hz, OCH$_2$CH$_3$), 3.62 (s, 2H, NH$_2$), 4.02 (m, 4H, 2 × CH$_2$), 6.35–6.78 (m, 3H, Ar–3H). MS (FAB) = 279 (MH^+). Anal. CHN $C_{14}H_{18}N_2O_4$.

*5.2.17. Ethyl 6-[(4-cyanobenzoyl)amino]-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**18**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 1.00 g (4.0 mmol) of amine **12** and 715 mg (4.2 mmol) 4-cyanobenzoyl chloride. m = 1.20 g (80%). M.p. 198–199 °C. IR (KBr, cm^{-1}): 3303, 2992, 2230, 1741, 1686, 1621, 1520, 1380, 1253, 1124, 1016, 857. ^{1}H-NMR (DMSO-d$_6$, 300 MHz): δ (ppm) 1.08 (t, 3H, J = 7.2 Hz, CH$_2$CH$_3$), 1.69 (s, 3H, 2-CH$_3$), 4.11 (m, 2H, CH$_2$), 7.02 (d, 1H, J = 8.7 Hz, Ar–H$_8$), 7.29 (dd, 1H, J = 8.7, 2.5 Hz, Ar–H$_7$), 7.54 (d, 1H, J = 2.5 Hz, Ar–H$_5$), 8.00–8.10 (m, 4H, Ar–4H), 10.44 (s, 1H, 6-NH), 11.01 (s, 1H, –CONH–). MS (FAB) = 380 (MH^+). Anal. CHN $C_{20}H_{17}N_3O_5$.

*5.2.18. Ethyl 6-[(4-cyanobenzoyl)amino]-2,4-dimethyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**19**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 460 mg (1.74 mmol) of amine **13** and 312 mg (1.83 mmol) 4-cyanobenzoyl chloride. The crude product was suspended in ethanol and filtered off. m = 570 mg (83%). M.p. 201–204 °C. IR (KBr, cm^{-1}): 3450, 2229, 1732, 1695, 1663, 1618, 1542, 1437, 1365, 1244, 1155, 1129, 1014, 859, 758. ^{1}H-NMR (DMSO-d$_6$, 300 MHz): δ (ppm) 1.05 (t, 3H, J = 7.2 Hz, CH$_2$CH$_3$), 1.72 (s, 3H, 2-CH$_3$), 3.32 (s, 3H, N–CH$_3$), 4.08 (m, 2H, CH$_2$), 7.09 (d, 1H, J = 8.7 Hz, Ar–H$_8$), 7.44 (dd, 1H, J = 8.7, 2.3 Hz, Ar–H$_7$), 7.66 (d, 1H, J = 2.3 Hz, Ar–H$_5$), 7.99–8.12 (m, 4H, Ar–4H), 10.50 (s, 1H, –CONH–). MS (EI) = 393 (M^+). Anal. CHN $C_{21}H_{19}N_3O_5$.

*5.2.19. Ethyl 4-benzyl-6-[(4-cyanobenzoyl)amino]-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**20**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 1.02 g (3.0 mmol) of amine **14** and 540 mg (3.15 mmol) 4-cyanobenzoyl chloride. The crude product was suspended in ethanol and filtered off. m = 1.20 g (85%). M.p. 168–171 °C. IR (KBr, cm^{-1}): 3451, 2231, 1732, 1697, 1654, 1620, 1559, 1513, 1439, 1374, 1340, 1263, 1125, 1017, 862, 734. ^{1}H-NMR (DMSO-d$_6$, 300 MHz): δ (ppm) 1.07 (t, 3H, J = 7.2 Hz, CH$_2$CH$_3$), 1.79 (s, 3H, 2-CH$_3$), 4.12 (q, 2H, J = 7.2 Hz, –CH$_2$–CH$_3$), 4.87 (d, 1H, J = 16.4 Hz, –CH$_2$–Ph), 5.34 (d, 1H, J = 16.4 Hz, –CH$_2$–Ph), 7.12 (d, 1H, J = 8.9 Hz, Ar–H$_8$), 7.27–7.39 (m, 5H, Ph), 7.43 (dd, 1H, J = 8.9, 2.3 Hz, Ar–H$_7$), 7.58 (d, 1H, J = 2.3 Hz, Ar–H$_5$), 7.96–8.04 (m, 4H, Ar–4H), 10.43 (s, 1H, –CONH–). MS (EI) = 469 (M^+). Anal. CHN $C_{27}H_{23}N_3O_5$.

*5.2.20. Ethyl 6-[(4-cyanobenzoyl)amino]-4-(2-ethoxy-2-oxoethyl)-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**21**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 1.34 g (4.0 mmol) of amine **15** and 710 mg (4.2 mmol) 4-cyanobenzoyl chloride. The crude product was suspended in ethanol and filtered off. m = 1.61 g (87%). M.p. 130–131 °C. IR (KBr, cm^{-1}): 3426, 2230, 1734, 1646, 1558, 1386, 1253, 1014, 860, 758. ^{1}H-NMR (DMSO-d$_6$, 300 MHz): δ (ppm) 1.08 (t, 3H, J = 7.2 Hz, 2-COOCH$_2$CH$_3$), 1.22 (t, 3H, J = 7.2 Hz, NCH$_2$COOCH$_2$CH$_3$), 1.73 (s, 3H, 2-CH$_3$), 4.02–4.21 (m, 4H, 2 × CH$_2$–CH$_3$), 4.61 (d, 1H, J = 17.7 Hz, N–CH$_2$–), 4.77 (d, 1H, J = 17.7 Hz, N–CH$_2$–), 7.13 (d, 1H, J = 8.7 Hz, Ar–H$_8$), 7.46 (dd, 1H, J = 8.7, 2.3 Hz, Ar–H$_7$), 7.50 (d, 1H, J = 2.3 Hz, Ar–H$_5$), 8.01–8.10 (m, 4H, Ar–4H), 10.46 (s, 1H, –CONH–). MS (EI) = 465 (M^+). Anal. CHN $C_{24}H_{23}N_3O_7$.

*5.2.21. Ethyl 7-[(4-cyanobenzoyl)amino]-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**22**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 1.00 g (4.0 mmol) of amine **16** and 664 mg (4.0 mmol) 4-cyanobenzoyl chloride. m = 1.27 g (83%). M.p. 212–214 °C. IR (KBr, cm^{-1}): 3391, 2231, 1692, 1557, 1518, 1413, 1325, 1131, 1014, 854, 753. ^{1}H-NMR (DMSO-d$_6$, 300 MHz): δ (ppm) 1.08 (t, 3H, J = 7.2 Hz, CH$_2$CH$_3$), 1.70 (s, 3H, 2-CH$_3$), 4.10 (q, 2H, J = 7.2 Hz, CH$_2$), 6.87–7.56 (m, 3H, Ar–3H), 8.00–8.10 (m, 4H, Ar–4H), 10.44 (s, 1H, 7-NH), 10.92 (s, 1H, –CONH–). MS (FAB) = 380 (MH^+). Anal. CHN $C_{20}H_{17}N_3O_5$.

*5.2.22. Ethyl 7-[(4-cyanobenzoyl)amino]-4-ethyl-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**23**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 1.20 g (4.31 mmol) of amine **17** and 716 mg

(4.31 mmol) 4-cyanobenzoyl chloride. *m* = 1.00 g (57%). M.p. 143–147 °C. IR (KBr, cm^{-1}): 3293, 2982, 2228, 1756, 1696, 1655, 1514, 1395, 1326, 1188, 1130, 1017, 862, 763. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.17 (t, 3H, *J* = 7.2 Hz, $NCH_2\underline{CH_3}$), 1.29 (t, 3H, *J* = 7.2 Hz, $OCH_2\underline{CH_3}$), 1.86 (s, 3H, 2-CH_3), 4.07 (m, 4H, 2xCH_2), 7.00–7.46 (m, 3H, Ar–3H), 7.82–7.97 (m, 4H, Ar–4H), 10.46 (s, 1H, –CONH–). MS (FAB) = 408 (MH^+). Anal. CHN $C_{22}H_{21}N_3O_5$.

*5.2.23. Ethyl 6-({4-[amino(imino)methyl]benzoyl}amino)-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate in the form of acetate (**24**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 1.00 g (2.6 mmol) of nitrile **18**. *m* = 965 mg (80%). M.p. 191–195 °C. IR (KBr, cm^{-1}): 3316, 2987, 1713, 1654, 1621, 1558, 1493, 1407, 1291, 1233, 1124, 1017, 861, 700. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.08 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.69 (s, 3H, 2-CH_3), 1.74 (s, 3H, $\underline{CH_3}COOH$), 4.10 (m, 2H, CH_2), 7.01 (d, 1H, *J* = 8.7 Hz, Ar–H_8), 7.30 (dd, 1H, *J* = 8.7, 2.5 Hz, Ar–H_7), 7.55 (d, 1H, *J* = 2.5 Hz, Ar–H_5), 7.92 (d, 2H, *J* = 8.7 Hz, Ar–2H), 8.08 (d, 2H, *J* = 8.7 Hz, Ar–2H), 10.40 (broad, 3H, –CONH–, HN=C–$\underline{NH_2}$). MS (EI) = 396 (M^+-free base). EI-HRMS calcd. for $C_{20}H_{20}N_4O_5$: 396.1434. Found: 396.1445.

*5.2.24. Ethyl 6-({4-[amino(imino)methyl]benzoyl}amino)-2,4-dimethyl-2H-1,4-benzoxazin-3(4H)-one-2-carboxylate in the form of hydrochloride (**25**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 490 mg (1.25 mmol) of nitrile **19**. The crude product was dissolved in a saturated ethanolic solution of HCl and precipitated by the addition of diethyl ether. *m* = 90 mg (16%). M.p. 188–190 °C. IR (KBr, cm^{-1}): 3424, 1731, 1662, 1618, 1555, 1516, 1472, 1425, 1302, 1249, 1120, 861. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.05 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.72 (s, 3H, 2-CH_3), 3.32 (s, 3H, N–CH_3), 4.08 (m, 2H, CH_2), 7.09 (d, 1H, *J* = 8.9 Hz, Ar–H_8), 7.54 (dd, 1H, *J* = 8.9, 2.3 Hz, Ar–H_7), 7.76 (d, 1H, *J* = 2.3 Hz, Ar–H_5), 7.99 (d, 2H, *J* = 8.5 Hz, Ar–2H), 8.20 (d, 2H, *J* = 8.5 Hz, Ar–2H), 9.35 (broad, 2H, ^+H_2N=C–$\underline{NH_2}$), 9.57 (broad, 2H, $^+\underline{H_2}$N=C–NH_2), 10.64 (s, 1H, –CONH–). MS (FAB) = 411 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.25. Ethyl 6-({4-[amino(imino)methyl]benzoyl}amino)-4-benzyl-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate in the form of acetate (**26**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 705 mg (1.50 mmol) of nitrile **20**. *m* = 625 mg (76%). M.p. 163–166 °C. IR (KBr, cm^{-1}): 3456, 1748, 1639, 1559, 1551, 1402, 1334, 1265, 1131, 1012, 936, 862. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.07 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.79 (s, 3H, 2-CH_3), 1.80 (s, 3H, $\underline{CH_3}COOH$), 4.12 (m, 2H, –$\underline{CH_2}$–CH_3), 4.89 (d, 1H, *J* = 16.2 Hz, –$\underline{CH_2}$–Ph), 5.33 (d, 1H, *J* = 16.2 Hz, –$\underline{CH_2}$–Ph), 7.11 (d, 1H, *J* = 8.7 Hz, Ar–H_8), 7.25–7.39 (m, 5H, Ph), 7.49 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar–H_7), 7.70 (d, 1H, *J* = 2.3 Hz, Ar–H_5), 7.94 (d, 2H, *J* = 8.5 Hz, Ar–2H), 8.10 (d, 2H, *J* = 8.5 Hz, Ar–2H), 10.56 (s, 1H, Ar–$\underline{NH}$CO). MS (FAB) = 487 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.26. Ethyl 6-({4-[amino(imino)methyl]benzoyl}amino)-4-(2-ethoxy-2-oxoethyl)-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate in the form of acetate (**27**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 1.16 g (2.50 mmol) of nitrile **21**. *m* = 1.13 g (83%). M.p. 137–139 °C. IR (KBr, cm^{-1}): 3366, 2986, 1748, 1677, 1512, 1378, 1262, 1126, 1017, 862, 708. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.08 (t, 3H, *J* = 7.1 Hz, 2-$COOCH_2\underline{CH_3}$), 1.22 (t, 3H, *J* = 7.1 Hz, $NCH_2COOCH_2\underline{CH_3}$), 1.73 (s, 3H, 2-$CH_3$), 1.78 (s, 3H, $\underline{CH_3}COOH$), 4.04–4.22 (m, 4H, 2 × $\underline{CH_2}$–CH_3), 4.61 (d, 1H, *J* = 17.8 Hz, N–$\underline{CH_2}$–), 4.77 (d, 1H, *J* = 17.8 Hz, N–$\underline{CH_2}$–), 7.12 (d, 1H, *J* = 8.7 Hz, Ar–H_8), 7.46–7.54 (m, 2H, Ar–H_7, Ar–H_5), 7.90–8.09 (m, 4H, Ar–4H), 10.42 (broad, 4H, CONH, NH=C–NH_2). MS (FAB) = 483 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.27. Ethyl 7-({4-[amino(imino)methyl]benzoyl}amino)-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate in the form of acetate (**28**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 500 mg (1.21 mmol) of nitrile **22**. *m* = 477 mg (78%). M.p. 193–196 °C. IR (KBr, cm^{-1}): 3454, 1637, 1560, 1522, 1406, 1118, 863. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.08 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.70 (s, 3H, 2-CH_3), 1.80 (s, 3H, $\underline{CH_3}COOH$), 4.11 (q, 2H, *J* = 7.2 Hz, CH_2), 6.87–7.58 (m, 3H, Ar–3H), 8.09–8.11 (m, 4H, Ar–4H), 10.43 (s, 1H, 7-NH). MS (FAB) = 397 (MH^+-free base). Anal. CHN $C_{20}H_{20}N_4O_5 \times CH_3COOH \times 2\ H_2O$.

*5.2.28. Ethyl 7-({4-[amino(imino)methyl]benzoyl}amino)-4-ethyl-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate in the form of acetate (**29**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 300 mg (0.74 mmol) of nitrile **23**. *m* = 151 mg (48%). M.p. 146–158 °C. IR (KBr, cm^{-1}): 3136, 3046, 1737, 1679, 1606, 1548, 1514, 1407, 1317, 1280, 1123, 1011, 861, 705. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.05 (t, 3H, *J* = 7.2 Hz, $OCH_2\underline{CH_3}$), 1.15 (t, 3H, *J* = 7.2 Hz, $NCH_2\underline{CH_3}$), 1.73 (s, 3H, 2-CH_3), 1.79 (s, 3H, $\underline{CH_3}COOH$), 4.08 (m, 4H, 2 × CH_2), 7.22–7.67 (m, 3H, Ar–3H), 7.94–8.13 (m, 4H, Ar–4H), 10.54 (s, 1H, NH). MS (FAB) = 425 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.29. Sodium 6-({4-[Amino(imino)methyl]benzoyl}-amino)-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate (**30**)*

Prepared according to the general procedure as in Section 5.2.1.6 from **24** (770 mg, 1.69 mmol) without acidification. The compound precipitated from the reaction mixture. *m* = 0.11 g (14%). M.p. 216–222 °C. IR (KBr, cm^{-1}): 3354,

1697, 1618, 1518, 1398, 1235, 1132, 866, 780. ^{1}H-NMR (DMSO-d_6 + D_2O, 300 MHz): δ (ppm) 1.49 (s, 3H, 2-CH_3), 6.78 (d, 1H, J = 8.7 Hz, Ar–H_8), 7.11 (dd, 1H, J = 8.7, 2.3 Hz, Ar–H_7), 7.31 (d, 1H, J = 2.3 Hz, Ar–H_5), 7.87–7.99 (m, 4H, 4 × Ar–H). MS (EI) = 368 (M^+-free acid). Compound was too hygroscopic for CHN analysis.

*5.2.30. 7-({4-[Amino(imino)methyl]benzoyl}amino)-2-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-carboxylate in the form of hydrochloride(**31**)*

Prepared according to the general procedure as in Section 5.2.1.6 from **28** (400 mg, 0.92 mmol). m = 0.089 g (14%). M.p. 185–189 °C. IR (KBr, cm^{-1}): 3447, 1653, 1557, 1514, 1406, 1350, 1178, 1026, 904, 756, 670.^{1}H-NMR (DMSO-d_6 + D_2SO_4, 300 MHz): δ (ppm) 1.24 (s, 3H, 2-CH_3), 6.87–6.91 (dd, 1H, J = 8.7, 2.6 Hz, Ar–H_7), 7.35 (d, 1H, J = 8.7 Hz, Ar–H_8), 6.87–6.91 (d, 1H, J = 2.6 Hz, Ar–H_5), 7.96 (s, 1H, –CONH–), 7.90–8.15 (dd, 4H, J = 8.3 Hz, Ar–4H) 9.12–9.45 (2 s, broad, 4H, NH_2=C^+–NH_2), 10.40 (s, 1H, –CONH–). MS (FAB) = 369 (MH^+-free acid). Compound was too hygroscopic for CHN analysis.

*5.2.31. 4-Ethyl-2-hydroxy-7-nitro-2*H*-1,4-benzoxazin-3(4*H*)-one (**32**)*

Prepared according to the general procedure as in Section 5.2.1.1 from 2.10 g (10.0 mmol) of **6** and 0.84 ml (11.0 mmol) ethyl bromide. The final product was first separated from the starting compound by column chromatography using ethyl acetate/hexane = 3/2 as an eluent and afterwards from *N*,*O*-dialkylated byproduct by the same method using ether/petrolether = 2/1 as an eluent. m = 0.69 g (29%). M.p. 133–136 °C. IR (KBr, cm^{-1}): 3296, 3064, 2968, 1774, 1667, 1601, 1527, 1449, 1346, 1275, 1092, 1033, 918, 810, 746. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.16 (t, 3H, J = 6.8 Hz, CH_3), 3.85–4.17 (m, 2H, CH_2), 5.71 (s, 1H, Hz, 2-H), 7.50 (d, 1H, J = 9.0 Hz, Ar–H_5), 7.85 (d, 1H, J = 2.6 Hz, Ar–H_8), 8.00 (dd, 1H, J = 9.0, 2.6 Hz, Ar–H_6), 8.38 (s, 1H, 2-OH). Coupling between the signals at 5.71 in 8.38 ppm was determined by COSY NMR. MS (EI) = 238 (M^+). Anal. CHN $C_{10}H_{10}N_2O_5$.

*5.2.32. Ethyl 2-[(4-ethyl-7-nitro-2*H*-1,4-benzoxazin-3(4*H*)-one-2-yl 2-yl)oxy]acetate (**33**)*

Prepared according to the general procedure as in Section 5.2.1.1 from 524 mg (2.2 mmol) of **32** and 0.23 ml (2.3 mmol) ethyl bromide. m = 595 mg (83%). M.p. 112–113 °C. IR (KBr, cm^{-1}): 2990, 1744, 1701, 1603, 1519, 1400, 1341, 1215, 1105, 1025, 883, 807, 745. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.17 (m, 6H, 2 × CH_3), 3.97–4.16 (m, 4H, 2 × $\underline{CH_2}$–CH_3), 4.39 (d, 1H, J = 16.2 Hz, OCH_2CO), 4.51 (d, 1H, J = 16.2 Hz, OCH_2CO), 5.81 (s, 1H, Hz, 2-H), 7.56 (d, 1H, J = 9.0 Hz, Ar–H_5), 7.98 (d, 1H, J = 2.6 Hz, Ar–H_8), 8.05 (dd, 1H, J = 9.0, 2.6 Hz, Ar–H_6). MS (EI) = 324 (M^+). EI-HRMS calcd. for $C_{14}H_{16}N_2O_7$: 324.0958. Found: 324.0963.

*5.2.33. Ethyl 2-[(7-amino-4-ethyl -2*H*-1,4-benzoxazin-3(4*H*)-one-2-yl -2-yl)oxy]acetate (**34**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 465 mg (1.4 mmol) of **33**. The oily residue was immediately used for the next step without further characterization. m = 390 mg (95%).

*5.2.34. Ethyl 2-({7-[(4-cyanobenzoyl)amino]-4-ethyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-yl}oxy)acetate (**35**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 380 mg (1.3 mmol) of amine **34** and 233 mg (1.4 mmol) 4-cyanobenzoyl chloride. m = 530 mg (96%). M.p. 57–61 °C. IR (KBr, cm^{-1}): 3565, 2230, 1748, 1683, 1607, 1514, 1439, 1274, 1108, 1046, 856, 790. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.16 (t, 6H, J = 7.2 Hz, 2CH_3), 3.97 (m, 2H, N–$\underline{CH_2}CH_3$), 4.11 (m, 2H, O–$\underline{CH_2}CH_3$), 4.30 (d, 1H, J = 16.2 Hz, O–CH_2–CO), 4.44 (d, 1H, J = 16.2 Hz, O–CH_2–CO), 7.31 (d, 1H, J = 9.0 Hz, Ar–H_5), 7.52 (dd, 1H, J = 9.0, 2.3 Hz, Ar–H_6), 7.67 (d, 1H, J = 2.3 Hz, Ar–H_8), 8.04 (d, 2H, J = 8.3 Hz, Ar–2H), 8.11 (d, 2H, J = 8.3 Hz, Ar–2H), 10.53 (s, 1H, –CONH–). MS (FAB) = 424 (MH^+). Anal. CHN $C_{22}H_{21}N_3O_6$ × 0.5 H_2O.

*5.2.35. Ethyl 2-{[7-({4-[amino(imino)methyl]benzoyl}-amino)-4-ethyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-yl]oxy}-acetate in the form of acetate (**36**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 444 mg (1.05 mmol) of nitrile **35**. m = 450 mg (86%). M.p. 202–206 °C. IR (KBr, cm^{-1}): 3384, 2982, 1742, 1687, 1608, 1514, 1438, 1351, 1223, 1108, 1031, 800, 705. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.17 (m, 6H, 2CH_3), 1.76 (s, 3H, $\underline{CH_3}$–COOH), 3.97 (q, 2H, J = 7.2 Hz, N–$\underline{CH_2}CH_3$), 4.11 (m, 2H, O–$\underline{CH_2}CH_3$), 4.30 (d, 1H, J = 16.0 Hz, O–CH_2–CO), 4.45 (d, 1H, J = 16.0 Hz, O–CH_2–CO), 7.31 (d, 1H, J = 8.9 Hz, Ar–H_5), 7.55 (dd, 1H, J = 8.9, 2.3 Hz, Ar–H_6), 7.70 (d, 1H, J = 2.3 Hz, Ar–H_8), 7.94 (d, 2H, J = 8.5 Hz, Ar–2H), 8.11 (d, 2H, J = 8.5 Hz, Ar–2H), 9.60–10.85 (broad, 3H, –CONH–, NH_2). MS (FAB) = 441 (MH^+-free base). Anal. CHN $C_{22}H_{25}N_4O_6$ × CH_3COOH × 0.5 H_2O.

*5.2.36. 2-{[7-({4-[amino(imino)methyl]benzoyl}amino)-4-ethyl-2*H*-1,4-benzoxazin-3(4*H*)-one-2-yl]oxy}acetic acid (**37**)*

Prepared according to the general procedure as n Section 5.2.1.7 from 100 mg (0.20 mmol) of ester **36**. The crude product was purified by column chromatography utilizing methanol/water/acetonitrile/acetic acid = 2/2/6/1 as an eluent. m = 45 mg (48%). M.p. 235–240 °C. IR (KBr, cm^{-1}): 3394, 1666, 1598, 1513, 1412, 1337, 1274, 1100, 1043, 863, 704. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.12 (t, 3H, J = 7.2 Hz, CH_3), 3.93 (q, 2H, J = 7.2 Hz, N–CH_2–), 4.17 (d, 1H, J = 16.2 Hz, O–CH_2–CO), 4.31 (d, 1H, J = 16.2 Hz, O–CH_2–CO), 7.28 (d, 1H, J = 8.9 Hz, Ar–H_5), 7.53 (dd, 1H, J = 8.9, 2.5 Hz, Ar–H_6), 7.69 (d, 1H, J = 2.5 Hz, Ar–H_8), 7.95 (d, 2H, J = 8.5 Hz, Ar–2H), 8.13 (d, 2H, J = 8.5 Hz, Ar–2H). MS (FAB) = 413 (MH^+). Anal. CHN $C_{20}H_{20}N_4O_6$ × 2 H_2O.

*5.2.37. Ethyl 2,4-dimethyl-7-nitro-2H-1,4-benzoxazine-3(4H)-one-2-carboxylate (**38**) [10]*

Prepared according to the general procedure as in Section 5.2.1.1 from 5.60 g (20.0 mmol) of **4** and 1.32 ml (21.0 mmol) methyl iodide. *m* = 5.86 g (96%). M.p. 118–120 °C. IR (KBr, cm^{-1}): 3069, 2984, 1751, 1698, 1602, 1522, 1506, 1377, 1341, 1245, 1126, 1073, 1024, 910, 817, 748, 550, 505. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.05 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.78 (s, 3H, 2-CH_3), 3.40 (s, 3H, N–CH_3), 4.10 (m, 2H, CH_2); 7.43 (d, 1H, *J* = 8.9 Hz, Ar–H_5), 7.87 (d, 1H, *J* = 2.5 Hz, Ar–H_8), 8.02 (dd, 1H, *J* = 8.9, 2.5 Hz, Ar–H_6). MS (FAB) = 295 (MH^+).

*5.2.38. 2,4-Dimethyl-7-nitro-2H-1,4-benzoxazine-3(4H)-one-2-carboxylic acid (**39**)*

Prepared according to the general procedure as in Section 5.2.1.6 from 5.88 g (20.0 mmol) of ester **38**. *m* = 5.00 g (94%). M.p. 158–160 °C. IR (KBr, cm^{-1}): 3540, 3381, 1760, 1678, 1599, 1527, 1341, 1250, 1114, 1026, 871, 745. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.76 (s, 3H, 2-CH_3), 3.39 (s, 3H, N–CH_3), 7.41 (d, 1H, *J* = 9.1 Hz, Ar–H_5), 7.84 (d, 1H, *J* = 2.6 Hz, Ar–H_8), 7.98–8.02 (dd, 1H, *J* = 9.1, 2.6 Hz, Ar–H_6). MS (EI) = 266 (M^+). EI-HRMS calcd. for $C_8H_6Cl_2N_2O_4$: 266.0539. Found: 266.0549.

*5.2.39. 2-(2-Diazoacetyl)-2,4-dimethyl-7-nitro-2H-1,4-benzoxazin-3(4H)-one (**40**)*

A 1.6 g (6.0 mmol) of acid **39** was dissolved in 30 ml of freshly distilled THF and 0.86 ml (6.2 mmol) triethylamine was added. The mixture was flushed with argon and cooled to –15 °C. A solution of ethyl chloroformate (0.59 ml, 6.2 mmol) in 5 ml of THF was added and the resulting mixture stirred for 30 min at –5 °C. The precipitated triethylammonium chloride was filtered off. Acetonitrile (20 ml) was added to the filtrate, followed by 6.0 ml (12.0 mmol) of trimethylsilyldiazomethane (2.0 M solution in hexane, Aldrich). The mixture was stirred for 24 h at 4 °C. Afterwards 80 ml of diethyl ether was added and extracted with 10% aqueous citric acid (2 × 30 ml), saturated aqueous $NaHCO_3$ (2 × 30 ml) and brine (30 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated under vacuum. The crude product was used for the next step. *m* = 1.59 g (92%). M.p. 128–130 °C. IR (KBr, cm^{-1}): 3452, 3085, 2126, 1691, 1636, 1600, 1525, 1341, 1108, 1022, 876, 744, 531. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.73 (s, 3H, 2-CH_3), 3.38 (s, 3H, N–CH_3), 6.48 (s, 1H, CH–N_2), 7.40 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.89–8.02 (m, 2H, Ar–H_8,H_6). MS (EI) = 290 (M^+). EI-HRMS calcd. for $C_{12}H_{10}N_4O_5$: 290.0651. Found: 290.0662.

*5.2.40. Methyl 2-(2,4-dimethyl-7-nitro-2H-1,4-benzoxazin-3(4H)-one-2-yl)acetate (**41**)*

A 1.45 g (5.0 mmol) of diazoketone **40** was suspended in 50 ml of anhydrous methanol and a solution of 0.23 g (1.0 mmol) of silver benzoate in 2.8 ml of triethylamine was gradually added while the mixture was sonicated in an ultrasound bath. After 30 min the solvent was evaporated and the residue dissolved in 60 ml of ethyl acetate and extracted with 10% aqueous citric acid (2 × 20 ml), saturated aqueous $NaHCO_3$ (2 × 20 ml) and brine (30 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified by column chromatography utilizing ethyl acetate/hexane = 3/2 as the eluent. *m* = 1.32 g (84%). IR (NaCl, cm^{-1}): 2953, 1741, 1693, 1600, 1524, 1322, 1209, 1074, 881, 744. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.54 (s, 3H, 2-CH_3), 2.86 (d, 1H, *J* = 16.6 Hz, CH_2), 3.34 (d, 1H, *J* = 16.6 Hz, CH_2), 3.46 (s, 3H, N–CH_3), 3.68 (s, 3H, O–CH_3), 7.03 (d, 1H, *J* = 9.0 Hz, Ar–H_5), 7.81 (d, 1H, *J* = 2.6 Hz, Ar–H_8), 7.94–7.98 (dd, 1H, *J* = 9.0, 2.6 Hz, Ar–H_6). MS (EI) = 294 (M^+). EI-HRMS calcd. for $C_{13}H_{14}N_2O_6$: 294.0852. Found: 294.0862.

*5.2.41. Methyl 2-(7-amino-2,4-dimethyl-2H-1,4-benzoxazin-3(4H)-one-2-yl)acetate (**42**)*

Prepared according to the general procedure as in Section 5.2.1.3 from 382 mg (1.3 mmol) of **41** with methanol as solvent. The oily residue was immediately used for the next step without further characterization. *m* = 324 mg (95%).

*5.2.42. Methyl 2-{7-[(4-cyanobenzoyl)amino]-2,4-dimethyl-2H-1,4-benzoxazin-3(4H)-one-2-yl} acetate (**43**)*

Prepared according to the general procedure as in Section 5.2.1.4 from 320 mg (1.21 mmol) of amine **42** and 228 mg (1.31 mmol) 4-cyanobenzoyl chloride. *m* = 352 mg (74%). M.p. 138–142 °C. IR (KBr, cm^{-1}): 3372, 2953, 2228, 1732, 1673, 1517, 1394, 1215, 1153, 1000, 866, 635. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.41 (s, 3H, 2-CH_3), 2.83 (d, 1H, *J* = 16.2 Hz, CH_2), 3.15 (d, 1H, *J* = 16.2 Hz, CH_2), 3.29 (s, 3H, N–CH_3), 3.57 (s, 3H, O–CH_3), 7.14 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.41–7.45 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar–H_6), 7.49 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 8.02 (d, 2H, *J* = 8.7 Hz, Ar–2H), 8.09 (d, 2H, *J* = 8.7 Hz, Ar–2H), 10.45 (s, 1H, –CONH–). MS (FAB) = 394 (MH^+). Anal. CHN $C_{21}H_{19}N_3O_5 \times 0.5\ H_2O$.

*5.2.43. Methyl 2-[7-({4-[amino(imino)methyl]benzoyl}amino)-2,4-dimethyl-2H-1,4-benzoxazin-3(4H)-one-2-yl]acetate in the form of acetate (**44**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 330 mg (0.84 mmol) of nitrile **43** utilizing anhydrous methanol as the solvent. *m* = 47 mg (12%). M.p. 180–184 °C. IR (KBr, cm^{-1}): 3391, 2946, 1713, 1670, 1606, 1516, 1413, 1314, 1238, 1149, 1009, 860, 705. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.41 (s, 3H, 2-CH_3), 1.80 (s, 3H, $\underline{CH_3}$COOH), 2.83 (d, 1H, *J* = 16.2 Hz, CH_2), 3.15 (d, 1H, *J* = 16.2 Hz, CH_2), 3.30 (s, 3H, N–CH_3), 3.57 (s, 3H, O–CH_3), 7.14 (d, 1H, *J* = 8.9 Hz, Ar–H_5), 7.44–7.48 (dd, 1H, *J* = 8.9, 2.1 Hz, Ar–H_6), 7.51 (d, 1H, *J* = 2.1 Hz, Ar–H_8), 7.93 (d, 2H, *J* = 8.3 Hz, Ar–2H), 8.10 (d, 2H, *J* = 8.3 Hz, Ar–2H), 10.43 (broad, 1H, –CONH–). MS (FAB) = 411 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.44. Ethyl 2-{[(2,4-dimethyl-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}acetate (**45**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **39** (0.96 g, 3.6 mmol) and glycine ethyl ester (0.92 g, 3.8 mmol). *m* = 0.92 g (73%). M.p. 147–150 °C. IR (KBr, cm^{-1}): 3340, 2993, 1746, 1697, 1602, 1531, 1342, 1207, 1128, 1070, 1027, 898, 816, 746, 589. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.07 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.78 (s, 3H, 2-CH_3), 3.37 (s, 3H, N–CH_3), 3.72 (d, 2H, *J* = 6.0 Hz, NH–$\underline{CH_2}$–), 3.96 (q, 2H, *J* = 7.2 Hz, –$\underline{CH_2}CH_3$), 7.39 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.98–8.02 (m, 2H, Ar–H_6, H_8), 8.73 (t, 1H, *J* = 6.0 Hz, NH). MS (FAB) = 352 (MH^+). Anal. CHN $C_{15}H_{17}N_3O_7 \times 0.25\ H_2O$.

*5.2.45. Ethyl (2*S*)-2-{[(2,4-dimethyl-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}-3-{[(4-methylphenyl)sulphonyl]amino}propanoate (**46**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **39** (0.64 g, 2.4 mmol) and **58** (0.76 g, 2.4 mmol). *m* = 1.18 g (92%). M.p. 75–80 °C. IR (KBr, cm^{-1}): 3472, 1700, 1602, 1526, 1457, 1382, 1342, 1159, 1093, 814, 663. $[\alpha]_D^{20}$ = –8.6° (c 0.81; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.93 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.07 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.78 (s, 3H, 2-CH_3), 1.79 (s, 3H, 2-CH_3), 2.39 (s, 6H, 2 × Ph–$\underline{CH_3}$), 2.92 (m, 2H, NH–$\underline{CH_2}$–CH), 3.02 (m, 2H, NH–$\underline{CH_2}$–CH), 3.37 (s, 3H, N–CH_3), 3.38 (s, 3H, N–CH_3), 3.80 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 3.96 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 4.18 (m, 1H, CH), 4.26 (m, 1H, CH), 7.34–7.42 (m, 6H, 2 × SO_2–$\underline{Ph}$–CH_3, 2H, 2 × Ar–H_5), 7.53–7.66 (m, 5H, 2 × SO_2–$\underline{Ph}$–CH_3, 2H, SO_2NH), 7.70 (t, 1H, *J* = 6.0 Hz, SO_2NH), 7.97–8.04 (m, 4H, 2 × Ar–H_6, 2 × Ar–H_8), 8.53 (d, 1H, *J* = 8.3 Hz, CONH), 8.59 (d, 1H, *J* = 8.3 Hz, –CONH–). MS (FAB) = 535 (MH^+). Anal. CHN $C_{23}H_{26}N_4O_9S$.

*5.2.46. Ethyl 2-{[(7-amino-2,4-dimethyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}acetate (**47**)*

Prepared according to the general procedure as in Section 5.2.1.3 from **45** (0.76 g, 2.15 mmol). *m* = 0.68 g (98%). M.p. 146–148 °C. IR (KBr, cm^{-1}): 3380, 2989, 1743, 1681, 1639, 1518, 1394, 1312, 1187, 1018, 836, 615. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.13 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.66 (s, 3H, 2-CH_3), 3.21 (s, 3H, N–CH_3), 3.66–3.74 (m, 2H, NH–$\underline{CH_2}$–), 4.02 (m, 2H, –$\underline{CH_2}CH_3$), 5.56 (s, 2H, NH_2), 6.31–6.35 (dd, 1H, *J* = 8.5, 2.3 Hz, Ar–H_6), 6.39 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 6.82 (d, 1H, *J* = 8.5 Hz, Ar–H_5), 8.37 (t, 1H, *J* = 6.0 Hz, NH). MS (EI) = 321 (M^+).

*5.2.47. Ethyl (2*S*)-2-{[(7-amino-2,4-dimethyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]-amino}-3-{[(4-methylphenyl)sulphonyl]amino}propanoate (**48**)*

Prepared according to the general procedure as in Section 5.2.1.3 from **46** (1.07 g, 2.0 mmol). *m* = 0.96 g (95%). M.p. 82–88 °C. IR (KBr, cm^{-1}): 3366, 2981, 1734, 1685, 1519, 1397, 1309, 1159, 1093, 1029. $[\alpha]_D^{20}$= + 5.9° (c 0.63; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.05–1.14 (m, 6H, 2 × $CH_2\underline{CH_3}$), 1.62 (s, 3H, 2-CH_3), 1.63 (s, 3H, 2-CH_3), 2.39 (s, 6H, 2 × Ph–$\underline{CH_3}$), 3.00 (m, 4H, 2 × NH–$\underline{CH_2}$–CH), 3.19 (s, 3H, N–CH_3), 3.20 (s, 3H, N–CH_3), 3.88–4.04 (m, 4H, 2 × O–$\underline{CH_2}$–CH_3), 4.19 (m, 1H, CH), 4.30 (m, 1H, CH), 5.01 (s, 2H, NH_2), 5.02 (s, 2H, NH_2), 6.28 (m, 2H, 2 × Ar–H_6), 6.31 (d, 1H, *J* = 2.5 Hz, Ar–H_8), 6.37 (d, 1H, *J* = 2.5 Hz, Ar–H_8), 6.75–6.82 (m, 2H, 2 × Ar–H_5), 7.40 (d, 4H, *J* = 8.3 Hz, 2 × SO_2–$\underline{Ph}$–CH_3, 2H), 7.59–7.75 (m, 6H, 2 × SO_2–$\underline{Ph}$–CH_3, 2H, 2 × SO_2NH), 8.07 (d, 1H, *J* = 7.9 Hz, –CONH–), 8.22 (d, 1H, *J* = 7.5 Hz, –CONH–). MS (EI) = 504 (M^+). EI-HRMS calcd. for $C_{23}H_{28}N_4O_7S$: 504,1679. Found: 504,1695.

*5.2.48. Ethyl 2-{[(7-{[(4-cyanophenyl)carbonyl]amino}-2,4-dimehyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]-amino}acetate (**49**)*

Prepared according to the general procedure as in Section 5.2.1.4 from **47** (0.65 g, 2.0 mmol) and 4-cyanobenzoyl chloride (0.36 g, 2.1 mmol). *m* = 0.68 g (76%). M.p. 171–174 °C. IR (KBr, cm^{-1}): 3376, 2986, 2228, 1734, 1697, 1514, 1431, 1389, 1221, 1151, 1018, 865, 761, 634. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.11 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.73 (s, 3H, 2-CH_3), 3.30 (s, 3H, N–CH_3), 3.62–3.80 (m, 2H, NH–$\underline{CH_2}$–), 4.01 (m, 2H, –$\underline{CH_2}CH_3$), 7.15 (d, 1H, *J* = 8.9 Hz, Ar–H_5), 7.39–7.42 (dd, 1H, *J* = 8.9, 2.3 Hz, Ar–H_6), 7.74 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 8.02 (d, 2H, *J* = 8.5 Hz, Ar–2H), 8.10 (d, 2H, *J* = 8.5 Hz, Ar–2H), 8.54 (t, 1H, *J* = 6.0 Hz, CO–$\underline{NH}$–CH_2), 10.51 (s, 1H, –CONH–). MS (FAB) = 451 (MH^+). Anal. CHN $C_{23}H_{22}N_4O_6$.

*5.2.49. Ethyl 2-({[7-({4-[amino(imino)methyl]benzoyl}-amino)-2,4-dimehyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)-carbonyl]amino}acetate in the form of acetate (**50**)*

Prepared according to the general procedure as in Section 5.2.1.5 from **49** (0.60 g, 1.33 mmol). *m* = 0.33 g (47%). M.p. 146–149 °C. IR (KBr, cm^{-1}): 3318, 2988, 1688, 1512, 1384, 1260, 1153, 1018, 863, 649. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.11 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.73 (s, 3H, 2-CH_3), 1.76 (s, 3H, $\underline{CH_3}COOH$), 3.30 (s, 3H, N–CH_3), 3.63–3.79 (m, 2H, NH–$\underline{CH_2}$–), 4.00 (q, 2H, *J* = 7.2 Hz, –$\underline{CH_2}CH_3$), 7.15 (d, 1H, *J* = 8.9 Hz, Ar–H_5), 7.41–7.45 (dd, 1H, *J* = 8.9, 2.1 Hz, Ar–H_6), 7.76 (d, 1H, *J* = 2.1 Hz, Ar–H_8), 7.94 (d, 2H, *J* = 8.3 Hz, Ar–2H), 8.11 (d, 2H, *J* = 8.3 Hz, Ar–2H), 8.54 (s, 1H, CO–$\underline{NH}$–CH_2), 10.48 (s, 1H, –CONH–). MS (FAB) = 468 ($\underline{MH}^+$-free base). Anal. CHN $C_{23}H_{25}N_5O_6 \times 1.2\ CH_3COOH \times 1\ H_2O$.

*5.2.50. Ethyl 2-({[7-({4-[amino(hydroxyimino)methyl]-benzoyl}amino)-2,4-dimehyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}acetate (**51**)*

To a suspension of hydroxylammonium chloride (0.22 g, 3.0 mmol) in anhydrous ethanol (20 ml), triethylamine (0.42 ml, 3.00 mmol) was added and the resulting solution stirred for 20 min, after which a solution of **49** (0.45 g, 1.0 mmol) in anhydrous ethanol (10 ml) was added and the mixture heated at 50 °C overnight. The product was precipi-

tated by cooling in the refrigerator and filtered off. $m = 0.34$ g (70%). M.p. 150–153 °C. IR (KBr, cm^{-1}): 3346, 2986, 2936, 1732, 1697, 1608, 1516, 1430, 1389, 1317, 1253, 1161, 1088, 1015, 928, 859, 814, 705. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.11 (t, 3H, $J = 7.2$ Hz, $CH_2\underline{CH_3}$), 1.73 (s, 3H, 2-CH_3), 3.32 (s, 3H, N–CH_3), 3.63–3.80 (m, 2H, NH–$\underline{CH_2}$–), 4.00 (q, 2H, $J = 7.2$ Hz, –$\underline{CH_2}CH_3$), 5.93 (s, 2H, NH_2), 7.13 (d, 1H, $J = 8.7$ Hz, Ar–H_5), 7.40–7.44 (dd, 1H, $J = 8.7$, 2.3 Hz, Ar–H_6), 7.76 (d, 1H, $J = 2.3$ Hz, Ar–H_8), 7.83 (d, 2H, $J = 8.5$ Hz, Ar–2H), 7.95 (d, 2H, $J = 8.5$ Hz, Ar–2H), 8.53 (t, 1H, $J = 6.0$ Hz, CO–$\underline{NH}$–CH_2), 9.83 (s, 1H, OH), 10.29 (s, 1H, –CONH–). MS (FAB) = 484 (MH^+). Anal. CHN $C_{23}H_{25}N_5O_7 \times 0.5\ H_2O$.

*5.2.51. Ethyl 2-{[(2,4-dimethyl-7-{[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino}- 2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]amino}acetate (**52**)*

Into an ice-cooled solution of **51** (0.3 g, 0.62 mmol) in freshly distilled pyridine (15 ml) argon was bubbled for 5 min. Afterwards, ethyl chloroformate (0.06 ml, 0.66 mmol) was added dropwise, stirred for 45 min in an ice-bath and then 30 min at RT. Again, the argon was bubbled in and the mixture heated under reflux (~120 °C) for a further 6 h. The solvent was evaporated under vacuum and the crude residue recrystallized from methanol. $m = 0.18$ g (57%). M.p. 221–225 °C. IR (KBr, cm^{-1}): 3366, 2987, 1760, 1693, 1513, 1430, 1250, 1150, 1018, 862, 758. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.11 (t, 3H, $J = 7.2$ Hz, $CH_2\underline{CH_3}$), 1.73 (s, 3H, 2-CH_3), 3.34 (s, 3H, N–CH_3), 3.62–3.80 (m, 2H, NH–$\underline{CH_2}$–), 4.00 (q, 2H, $J = 7.2$ Hz, –$\underline{CH_2}CH_3$), 7.15 (d, 1H, $J = 8.9$ Hz, Ar–H_5), 7.40–7.44 (dd, 1H, $J = 8.9$, 2.3 Hz, Ar–H_6), 7.76 (d, 1H, $J = 2.3$ Hz, Ar–H_8), 7.96 (d, 2H, $J = 8.7$ Hz, Ar–2H), 8.12 (d, 2H, $J = 8.7$ Hz, Ar–2H), 8.54 (t, 1H, $J = 6.0$ Hz, CO–$\underline{NH}$–CH_2), 10.45 (s, 1H, –CONH–), 13.11 (s, 1H, –CONH–). MS (FAB) = 509 (MH^+). Anal. CHN $C_{24}H_{23}N_5O_8 \times 0.25\ H_2O$.

*5.2.52. 2-{[(2,4-Dimethyl-7-{[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]amino}acetic acid (**53**)*

Prepared according to the general procedure as in Section 5.2.1.7 from **52** (0.15 g, 0.29 mmol). $m = 0.14$ g (99%). M.p. 206–209 °C. IR (KBr, cm^{-1}): 3447, 1779, 1680, 1552, 1513, 1436, 1290, 1205, 1032, 946, 816, 756, 670. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.72 (s, 3H, 2-CH_3), 3.30 (s, 3H, N–CH_3), 3.52–3.75 (m, 2H, NH–$\underline{CH_2}$–), 7.13 (d, 1H, $J = 8.9$ Hz, Ar–H_5), 7.40–7.44 (dd, 1H, $J = 8.9$, 2.3 Hz, Ar–H_6), 7.72 (d, 1H, $J = 2.3$ Hz, Ar–H_8), 7.96 (d, 2H, $J = 8.5$ Hz, Ar–2H), 8.11 (d, 2H, $J = 8.5$ Hz, Ar–2H), 8.38 (t, 1H, $J = 6.0$ Hz, CO–$\underline{NH}$–CH_2), 10.42 (s, 1H, –CONH–), 12.66 (s, 1H, –CONH–). MS (FAB) = 482 (MH^+). Anal. CHN $C_{22}H_{19}N_5O_8 \times 1.75\ H_2O$.

*5.2.53. 2-({[7-({4-[Amino(imino)methyl]benzoyl}amino)-2,4-dimethyl-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]-amino}acetic acid in the form of acetate (**54**)*

Prepared according to the general procedure as in Section 5.2.1.9 from **53** (0.11 g, 0.23 mmol). $m = 0.08$ g (72%). M.p. 242–245 °C. IR (KBr, cm^{-1}): 3338, 1659, 1514, 1389, 1314, 1150, 1032, 863, 703. ^{1}H-NMR (DMSO-d_6 + D_2O, 300 MHz)*: δ (ppm) 1.74 (s, 3H, 2-CH_3), 7.2 (d, 1H, $J = 9.1$ Hz, Ar–H_5), 7.32–7.37 (dd, 1H, $J = 9.1$, 1.9 Hz, Ar–H_6), 7.49 (t, 1H, $J = 4.9$ Hz, CO–$\underline{NH}$–CH_2), 7.68 (d, 2H, $J = 8.7$ Hz, Ar–2H), 7.86 (d, 1H, $J = 1.9$ Hz, Ar–H_8), 8.01 (d, 2H, $J = 8.7$ Hz, Ar–2H), 10.42 (s, 1H, –CONH–). Signals between 3.1 in 3.9 ppm (N–CH_3, NH–$\underline{CH_2}$–) are covered with the signal for H_2O. MS (FAB) = 440 (MH^+-free base). Anal. CHN $C_{21}H_{21}N_5O_6 \times CH_3COOH \times 2.25\ H_2O$.

*5.2.54. Ethyl (2S)-2-{[(2,4-dimethyl-7-{[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]amino}-3-{[(4-methylphenyl)sulphonyl]amino}propanoate (**55**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **48** (0.91 g, 1.8 mmol) and **59** (0.39 g, 1.9 mmol). $m = 0.84$ g (67%). The crystalline product was immediately used for the next step without further characterization.

*5.2.55. Ethyl (2S)-2-{[(2,4-dimethyl-7-{[4-(amino(imino)-methyl)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]amino}-3-{[(4-methylphenyl)sulphonyl]-amino}propanoate in the form of acetate (**56**)*

Prepared according to the general procedure as in Section 5.2.1.9 from **55** (0.80 g, 1.16 mmol). $m = 0.53$ g (65%). M.p. 151–155 °C. IR (KBr, cm^{-1}): 3333, 1676, 1515, 1390, 1316, 1157, 1092, 1030, 816, 661. $[\alpha]_D^{20} = -3.1°$ (c 0.66; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.97 (t, 3H, $J = 7.2$ Hz, $CH_2\underline{CH_3}$), 1.10 (t, 3H, $J = 7.2$ Hz, $CH_2\underline{CH_3}$), 1.71 (s, 3H, 2-CH_3), 1.72 (s, 3H, 2-CH_3), 1.75 (s, 6H, 2 × $\underline{CH_3}$–COOH), 2.30 (s, 3H, Ph–$\underline{CH_3}$), 2.38 (s, 3H, Ph–$\underline{CH_3}$), 3.01 (m, 4H, 2 × NH–$\underline{CH_2}$–CH), 3.30 (s, 6H, 2 × N–CH_3), 3.85 (m, 2H, O–$\underline{CH_2}$–CH_3), 3.99 (q, 2H, $J = 7.2$ Hz, O–$\underline{CH_2}$–CH_3), 4.16 (m, 1H, CH), 4.29 (m, 1H, CH), 7.15 (m, 2H, 2 × Ar–H_5), 7.30 (d, 2H, $J = 8.29$ Hz, SO_2–$\underline{Ph}$–CH_3), 7.39 (d, 2H, $J = 7.9$ Hz, SO_2–$\underline{Ph}$–CH_3), 7.45 (m, 2H, 2 × Ar–H_6), 7.58 (d, 2H, $J = 7.9$ Hz, SO_2–$\underline{Ph}$–CH_3), 7.64 (d, 2H, $J = 8.3$ Hz, SO_2–$\underline{Ph}$–CH_3), 7.79 (m, 2H, 2 × Ar–H_8), 7.87–8.01 (m, 4H, 2 × CO–$\underline{Ph}$–, 2H), 8.04–8.14 (m, 4H, 2 × CO–$\underline{Ph}$–, 2H), 8.33 (s, 1H, CO$\underline{NH}$–CH), 8.45 (s, 1H, CO$\underline{NH}$–CH), 10.50 (s, 2H, 2 × –CONH–. MS (FAB) = 651 (MH^+-free base). Anal. CHN $C_{31}H_{34}N_6O_8S \times CH_3COOH \times 2\ H_2O$.

*5.2.56. (2S)-2-{[(2,4-Dimethyl-7-{[4-(amino(imino)methyl)-benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)-carbonyl]amino}-3-{[(4-methylphenyl)sulphonyl]-amino}propanoic acid in the form of acetate (**57**)*

Prepared according to the general procedure as in Section 5.2.1.7 from **56** (0.29 g, 0.4 mmol). The compound was treated with 4 M HCl, washed with diethyl ether and dried in

vacuo. *m* = 0.20 g (73%). M.p. 204–216 °C. IR (KBr, cm^{-1}): 3333, 1676, 1515, 1390, 1316, 1157, 1092, 1030, 816, 661. $[\alpha]_D^{20}$= –3.1° (c 0.66; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.70 (s, 6H, 2 × 2-CH_3), 2.30 (s, 3H, Ph–CH_3), 2.38 (s, 3H, Ph–CH_3), 3.00 (m, 4H, 2 × NH–CH_2–CH), 3.30 (s, 6H, 2 × N–CH_3), 4.13 (m, 1H, CH), 4.22 (m, 1H, CH), 7.14 (m, 2H, 2 × Ar–H_5), 7.29 (d, 2H, *J* = 8.3 Hz, SO_2–Ph–CH_3), 7.38 (d, 2H, *J* = 7.9 Hz, SO_2–Ph–CH_3), 7.48–7.55 (m, 3H, 2 × Ar–H_6, $NHSO_2$), 7.58 (d, 2H, *J* = 8.3 Hz, SO_2–Ph–CH_3), 7.64 (d, 2H, *J* = 8.3 Hz, SO_2–Ph–CH_3), 7.70 (t, 1H, *J* = 6.0 Hz, $NHSO_2$), 7.77 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.82 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 8.00 (d, 4H, *J* = 7.9 Hz, 2 × CO–Ph–, 2H), 8.05 (d, 1H, *J* = 7.9 Hz, CONH–CH), 8.20 (m, 4H, 2 × CO–Ph–, 2H), 8.28 (d, 1H, *J* = 7.9 Hz, CONH–CH), 9.39 (s, 4H, 2 × NH_2), 9.59 (s, 4H, 2 × NH_2^+), 10.60 (s, 1H, –CONH–), 10.65 (s, 1H, –CONH–), 12.81 (s, 2H, 2 × CH–COOH). MS (FAB) = 623 (MH^+-free base). Anal. CHN $C_{29}H_{30}N_6O_8S \times CF_3COOH \times 3\ H_2O$.

*5.2.57. 2-Methyl-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-carboxylic acid (**60**) [10]*

Prepared according to the general procedure as in Section 5.2.1.6 from 4 (6.72 g, 24.0 mmol). *m* = 5.62 g (93%). M.p. 163–165 °C. IR (KBr, cm^{-1}): 3492, 3098, 2605, 1694, 1607, 1539, 1505, 1384, 1343, 1262, 1126, 975, 894, 745. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.73 (s, 3H, 2-CH_3), 7.10 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.81 (d, 1H, *J* = 2.5 Hz, Ar–H_8), 7.93 (dd, 1H, *J* = 8.7, 2.5, Ar–H_6). MS (FAB) = 253 (MH^+).

*5.2.58. Ethyl 2-{[(2-methyl-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}-3-phenylpropanoate (**61**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **60** (1.26 g, 5.0 mmol) and L-phenylalanine ethyl ester hydrochloride (1.27 g, 5.5 mmol). *m* = 1.85 g (86%). M.p. 165–167 °C. IR (KBr, cm^{-1}): 3346, 3248, 1732, 1662, 1608, 1532, 1324, 1125, 1019, 881, 744, 491. $[\alpha]_D^{20}$= –25.8° (*c* = 0.40; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.95 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.14 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.53 (s, 3H, 2-CH_3), 1.71 (s, 3H, 2-CH_3), 2.93 (m, 2H, Ph–CH_2–CH), 3.08 (m, 2H, Ph–CH_2–CH), 3.87 (m, 2H, O–CH_2–CH_3), 4.07 (m, 2H, O–CH_2–CH_3), 4.41 (m, 2H, 2 × 1H, CH_2–CH), 6.91–7.22 (m, 12H, 2 × Ar–H_5, 2 × 5 Ar–H), 7.85–7.96 (m, 4H, 2 × Ar–H_6, 2 × Ar–H_8), 8.62 (t, 1H, *J* = 8.3 Hz, CONH), 8.69 (t, 1H, *J* = 8.3 Hz, CONH), 11.32 (s, 2H, CONH). MS (FAB) = 428 (MH^+). Anal. CHN $C_{21}H_{21}N_3O_7$.

*5.2.59. Ethyl 2-{[(2-methyl-7-nitro-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}-2-{[(4-methylphenyl)sulphonyl]amino}propanoate (**62**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **60** (2.02 g, 8.0 mmol) and **71** (2.84 g, 8.8 mmol). *m* = 3.49 g (84%). M.p. 96–100 °C. IR (KBr, cm^{-1}): 3271, 2984, 1722, 1608, 1532, 1327, 1162, 1092, 816, 663, 550. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.94 (m, 3H, CH_2CH_3), 1.65 (d, 3H, *J* = 6.0 Hz, 2-CH_3), 2.37 (s, 3H, Ph–CH_3), 3.06 (m, 1H, NH–CH_2–CH), 3.30 (m, 1H, NH–CH_2–CH), 3.66 (m, 2H, O–CH_2–CH_3), 4.18 (m, 1H, NH–CH_2–CH), 7.03 (d, 1H, *J* = 8.7, Ar–H_5), 7.35–7.60 (dd, 4H, *J* = 8.7, 7.91 Hz, SO_2–Ph–CH_3), 7.89 (m, 1H, Ar–H_6), 7.94 (d, 1H, Ar–H_8), 8.19 (m, 1H, SO_2NH), 8.38 (s, 1H, –CONH–), 11.39 (dd, 1H, *J* = 11.30, 9.0 Hz, –CONH–). MS (FAB) = 521 (MH^+). Anal. CHN $C_{22}H_{24}N_4O_9S$.

*5.2.60. Ethyl 2-{[(7-amino-2-methyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}-3-phenylpropanoate (**63**)*

Prepared according to the general procedure as in Section 5.2.1.3 from **61** (1.49 g, 3.50 mmol). *m* = 1.37 g (99%). M.p. 69–71 °C. IR (KBr, cm^{-1}): 3364, 2980, 1699, 1636, 1520, 1375, 1180, 1027, 810, 702. $[\alpha]_D^{20}$ = –14.8° (*c* = 0.50; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.05 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 1.12 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 1.46 (s, 3H, 2-CH_3), 1.60 (s, 3H, 2-CH_3), 3.00 (m, 4H, Ph–CH_2–CH), 4.00 (m, 4H, O–CH_2–CH_3), 4.27 (m, 1H, CH_2–CH), 4.41 (m, 1H, CH_2–CH), 4.88 (s, 1H, 7-NH_2), 4.94 (s, 1H, 7-NH_2), 6.16–6.22 (m, 2H, 2 × Ar–H_6), 6.28–6.36 (2d, 2H, *J* = 2.3 Hz, 2 × Ar–H_8), 6.50–6.53 (2d, 2H, *J* = 6.0 Hz, 2 × Ar–H_5), 7.11–7.29 (m, 10H, 2 × 5 Ar–H), 8.10 (d, 1H, *J* = 7.9 Hz, CONH), 8.24 (d, 1H, *J* = 7.9 Hz, CONH), 11.24 (s, 1H, CONH), 11.27 (s, 1H, CONH). MS (FAB) = 398 (MH^+). Anal. CHN $C_{21}H_{23}N_3O_5$.

*5.2.61. Ethyl 2-{[(7-amino-2-methyl-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}-2-{[(4-methylphenyl)sulphonyl]amino}propanoate (**64**)*

Prepared according to the general procedure as in Section 5.2.1.3 from **62** (3.02 g, 5.8 mmol). *m* = 2.89 g (100%). M.p. 103–106 °C. IR (KBr, cm^{-1}): 3367, 2983, 1697, 1520, 1427, 1320, 1162, 1092, 1020, 814, 666, 551. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.00 (m, 3H, CH_2CH_3), 1.44 (d, 3H, *J* = 12.40 Hz, 2-CH_3), 2.37 (s, 3H, Ph–CH_3), 2.89 (m, 1H, NH–CH_2–CH), 3.41 (m, 1H, NH–CH_2–CH), 3.76 (m, 2H, CH_2–CH_3), 3.92 (m, 1H, NH–CH_2–CH), 4.90 (s, 2H, 7-NH_2), 6.16 (dd, 1H, *J* = 8.3, 2.3 Hz, Ar–H_6), 6.26 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 6.52 (d, 1H, *J* = 8.3 Hz, Ar–H_5), 7.37–7.64 (dd, 4H, *J* = 8.3, 7.5 Hz, SO_2–Ph–CH_3), 8.04 (m, 1H, SO_2NH), 8.23 (s, 1H, –CONH–), 11.26 (m, 1H, –CONH–). MS (FAB) = 491 (MH^+). Anal. CHN $C_{22}H_{26}N_4O_7S \times H_20$.

*5.2.62. Ethyl 2-{[(2-methyl-7-(4-cyanobenzoylamino)-2*H*-1,4-benzoxazine-3(4*H*)-one-2-yl)carbonyl]amino}-3-phenylpropanoate (**65**)*

Prepared according to the general procedure as in Section 5.2.1.4 from **63** (0.569 g, 1.50 mmol) and 0.27 g (1.58 mmol) of 4-cyanobenzoyl chloride. *m* = 0.655 g (83%). M.p. 225–228 °C. IR (KBr, cm^{-1}): 3344, 2230, 1736, 1687, 1655, 1516, 1394, 1247, 1186, 1117, 1016, 853, 756. $[\alpha]_D^{20}$ = –11.6° (*c* = 0.40; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.98 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 1.12 (t, 3H, *J* = 7.2 Hz,

$CH_2\underline{CH_3}$), 1.51 (s, 3H, 2-CH_3), 1.67 (s, 3H, 2-CH_3), 3.01 (m, 4H, 2 × 2H, Ph–$\underline{CH_2}$–CH), 3.91 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 4.04 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 4.29 (m, 1H, CH_2–$\underline{CH}$), 4.40 (m, 1H, CH_2–$\underline{CH}$), 6.80 (d, 1H, *J* = 8.3 Hz, Ar–H_5), 6.83 (d, 1H, *J* = 8.3 Hz, Ar–H_5), 6.99–7.31 (m, 12H, 2 × Ar–H_6, 2 × 5 Ar–H), 7.69 (m, 2H, 2 × Ar–H_8), 8.02–8.13 (m, 8H, Ar–4H), 8.39 (d, 1H, *J* = 8.3 Hz, –CONH–), 8.47 (d, 1H, *J* = 8.3 Hz, –CONH–), 10.46 (s, 1H, –CONH–), 10.47 (s, 1H, –CONH–), 10.69 (s, 1H, –CONH–), 10.71 (s, 1H, –CONH–). MS (FAB) = 527 (MH^+). Anal. CHN $C_{26}H_{29}N_4O_6 \times 0.5\ H_2O$.

*5.2.63. Ethyl 2-{[(2-methyl-7-(4-cyanobenzoylamino)-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]amino}-2-{[(4-methylphenyl)sulphonyl]amino}propanoate (**66**)*

Prepared according to the general procedure as in Section 5.2.1.4 from **64** (1.24 g, 2.5 mmol), 0.40 ml (2.6 mmol) Et_3N and 0.45 g (2.6 mmol) of 4-cyanobenzoyl chloride. *m* = 1.07 g (69%). M.p. 233–237 °C. IR (KBr, cm^{-1}): 3280, 2232, 1711, 1674, 1519, 1419, 1328, 1166, 1058, 855, 669, 562. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.90 (m, 3H, $CH_2\underline{CH_3}$), 1.58 (d, 3H, *J* = 10.9 Hz, 2-CH_3), 2.36 (s, 3H, Ph–$\underline{CH_3}$), 3.02 (m, 1H, NH–$\underline{CH_2}$–CH), 3.34 (m, 1H, NH–$\underline{CH_2}$–CH), 3.73 (m, 2H, $\underline{CH_2}$–CH_3), 3.94 (m, 1H, NH–CH_2–$\underline{CH}$), 6.83 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.28 (dd, 1H, *J* = 8.7, 1.9 Hz, Ar–H_6), 7.35 (d, 2H, *J* = 7.9 Hz, SO_2–$\underline{Ph}$–CH_3) 7.64 (m, 3H′, SO_2–$\underline{Ph}$–CH_3, Ar–H_8), 8.02–8.09 (dd, 4H, *J* = 8.7, 7.9 Hz, Ar–H), 8.21 (m, 2H, $SO_2\underline{NH}$,–CONH–), 10.47 (s, 1H, –CONH–), 10.74 (m, 1H, –CONH–). MS (FAB) = 620 (MH^+). Anal. CHN $C_{30}H_{29}N_5O_8S$.

*5.2.64. Ethyl 2-{[(2-methyl-7-{[4-(amino(imino)methyl)-benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)-carbonyl]amino}-3-phenylpropanoate in the form of acetate (**67**)*

Prepared according to the general procedure as in Section 5.2.1.5 from **65** (0.526 g, 1.00 mmol). *m* = 0.287 g (48%). M.p. >220 °C (decomposes without melting). IR (KBr, cm^{-1}): 3352, 1701, 1578, 1518, 1412, 1180, 1014, 864, 702. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.99 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.10 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.51 (s, 3H, 2-CH_3), 1.66 (s, 3H, 2-CH_3), 1.73 (s, 6H, $C\underline{H_3}COO^-$), 3.01 (m, 4H, 2 × 2H, Ph–$\underline{CH_2}$–CH), 3.91 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 4.04 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 4.29 (m, 1H, CH_2–$\underline{CH}$), 4.39 (m, 1H, CH_2–$\underline{CH}$), 6.81 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 6.85 (d, 1H, *J* = 8.3 Hz, Ar–H_5), 7.00–7.26 (m, 10H, 2 × 5 Ar–H), 7.32 (s, 1H, Ar–H_6), 7.36 (s, 1H, Ar–H_6), 7.73 (m, 2H, 2 × Ar–H_8), 7.95–8.11 (m, 8H, 2 × Ar–4H), 8.39 (s, 1H, –CONH–), 8.46 (s, 1H, –CONH–), 10.56 (s, 2H, 2 × –CONH–), signals for amidine protons and for the lactam proton were not observed. MS (FAB) = 544 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.65. Ethyl 2-{[(2-methyl-7-{[4-(amino(imino)methyl)-benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)-carbonyl]amino}-2-{[(4-methylphenyl)sulphonyl]amino}-propanoate in the form of acetate (**68**)*

Prepared according to the general procedure as in Section 5.2.1.5 from 0.75 g **66** (1.2 mmol). *m* = 0.17 g (20%). M.p. 232–235 °C. IR (KBr, cm^{-1}): 3425, 1700, 1558, 1520, 1417, 1338, 1161, 1020, 815, 644. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.95 (m, 3H, $CH_2\underline{CH_3}$), 1.58 (s, 3H, 2-CH_3), 1.80 (s, 6H, CH_3COO^-), 2.34 (s, 3H, Ph–$\underline{CH_3}$), 3.05 (m, 1H, NH–$\underline{CH_2}$–CH), 3.30 (m, 1H, NH–$\underline{CH_2}$–CH), 3.77 (m, 2H, $\underline{CH_2}$–CH_3), 3.97 (m, 1H, NH–CH_2–$\underline{CH}$), 6.84 (d, 1H, *J* = 8.6 Hz, Ar–H_5), 7.33 (m, 3H, Ar–H_6, SO_2–$\underline{Ph}$–CH_3) 7.63 (dd, 2H, *J* = 8.3 Hz, SO_2–$\underline{Ph}$–CH_3), 7.68 (d, 1H, *J* = 1.9 Hz, Ar–H_8), 7.93–8.11 (dd, 4H, *J* = 8.26, 8.26 Hz, Ar–H), 8.27 (m, 1H, –CONH–), 10.47 (s, 1H, –CONH–), signals for amidine protons and the lactam proton were not observed. MS (FAB) = 637 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.66. 2-{[(2-Methyl-7-{[4-(amino(imino)methyl)benzoyl]-amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]-amino}-3-phenylpropanoic acid in the form of acetate (**69**)*

Prepared according to the general procedure as in Section 5.2.1.7 from **67** (0.147 g, 0.24 mmol). *m* = 0.103 g (73%). M.p. >230 °C (decomposes without melting). IR (KBr, cm^{-1}): 3394, 1694, 1617, 1518, 1401, 1328, 1133, 864, 702. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.59 (s, 3H, 2-CH_3), 1.61 (s, 3H, 2-CH_3), 1.78 (s, 1.5 H, $C\underline{H_3}COO^-$), 2.97 (m, 4H, 2 × 2H, Ph–$\underline{CH_2}$–CH), 3.82 (m, 1H, CH_2–$\underline{CH}$), 3.93 (m, 1H, CH_2–$\underline{CH}$), 6.85 (m, 2H, Ar–H_5), 6.95–7.25 (m, 10H, 2 × 5 Ar–H), 7.42 (m, 1H, Ar–H_6), 7.48–7.53 (m, 2H, Ar–H_6, Ar–H_8), 7.63 (d, 1H, *J* = 1.9 Hz, Ar–H_8), 7.74–7.91 (dd, 4H, *J* = 8.3 Hz, Ar–H), 8.01–8.12 (dd, 4H, *J* = 8.3 Hz, Ar–H), 10.58 (s, 2H, 2 × CO$\underline{NH}$), signals for amidine protons, carboxylic proton and for the lactam proton were not observed; only half an equivalent of acetic acid was observed. MS (FAB) = 516 (MH^+-free base). Anal. CHN $C_{27}H_{25}N_5O_6 \times 0.5\ CH_3COOH \times 5\ H_2O$.

*5.2.67. 2-{[(2-Methyl-7-{[4-(amino(imino)methyl)benzoyl]-amino}-2H-1,4-benzoxazine-3(4H)-one-2-yl)carbonyl]-amino}-2-{[(4-methylphenyl)sulphonyl]amino}propanoic acid in the form of acetate (**70**)*

Prepared according to the general procedure as in Section 5.2.1.7 from **68** 0.11 g, (0.2 mmol). *m* = 0.04 g (35%). M.p. 245–248 °C. IR (KBr, cm^{-1}): 3425, 1700, 1558, 1520, 1417, 1338, 1161, 1020, 815, 644. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.68 (s, 3H, 2-CH_3), 1.78 (s, 3H, CH_3COO^-), 2.28 (s, 3H, Ph–$\underline{CH_3}$), 2.97 (m, 2H, NH–$\underline{CH_2}$–CH), 3.97 (m, 1H, NH–CH_2–$\underline{CH}$), 6.84 (d, 1H, *J* = 8.3 Hz, Ar–H_5), 7.30 (m, 3H, Ar–H_6, SO_2–$\underline{Ph}$–CH_3), 7.51 (m, 1H, Ar–H_8), 7.64 (dd, 2H, *J* = 8.3 Hz, SO_2–$\underline{Ph}$–CH_3), 7.82–8.03 (dd, 4H, *J* = 7.5 Hz, Ar–H), 8.20 (m, 1H, –CONH–), signals for amidine protons, carboxylic proton and the lactam proton

were not observed. MS (FAB) = 609 (MH^+-free base). Anal. CHN $C_{30}H_{32}N_6O_{10}S \times 2\ H_2O$.

*5.2.68. Ethyl 2-(7-nitro-2H-1,4-benzoxazine-3(4H)-one-4-il)acetate (**72**) [12]*

Prepared according to general procedure as in Section 5.2.1.1 from **7** (3.88 g, 20 mmol) and ethyl bromoacetate (2.28 ml, 20 mmol). *m* = 4.68 g (84%). M.p. 116–118 °C. IR (KBr, cm^{-1}): 3459, 3094, 2996, 1733, 1687, 1603, 1522, 1433, 1346, 1225, 1022, 890, 811, 744. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.31 (t, 3H, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 4.24 (m, 2H, $\underline{CH_2}CH_3$), 4.72 (s, 3H, N–CH_2), 4.79 (s, 2H, O–CH_2–CO), 6.86 (d, 1H, *J* = 8.9 Hz, Ar–H_5), 7.91 (d, 1H, *J* = 2.6 Hz, Ar–H_8), 7.96 (dd, 1H, *J* = 8.9, 2.6 Hz, Ar–H_6). MS (FAB) = 281 (MH^+). Anal. CHN $C_{12}H_{12}N_2O_6$.

*5.2.69. Ethyl (2S)-2-{[(4-methylphenyl)sulphonyl]amino}-3-[2-(7-nitro-2H-1,4-benzoxazine-3(4H)-one-4-yl)acetyl]-aminopropanoate (**73**)*

Prepared according to the general procedure as in Section 5.2.1.6 from **72** (4.49 g, 16 mmol). The crude product was used without further purification according to the general procedure as in Section 5.2.1.8 with **71** (1.48 g, 4.6 mmol) to yield **73**. *m* = 0.65 g (7.8%). M.p. 184–185 °C. IR (KBr, cm^{-1}): 3315, 3254, 2980, 1732, 1667, 1600, 1532, 1397, 1341, 1254, 1165, 1091, 1049, 925, 816, 742, 670. $[\alpha]_D^{20}$= –8.7° (*c* = 0.41; DMF). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 0.99 (t, 3H, *J* = 7.2 Hz, CH_2–$\underline{CH_3}$), 2.38 (s, 3H, Ph–$\underline{CH_3}$), 3.19 (m, 1H, N–$\underline{CH_2}$–CH), 3.38 (m, 1H, N–$\underline{CH_2}$–CH), 3.78 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$–CH_3), 3.93 (m, 1H, CH), 4.56 (s, 2H, N–$\underline{CH_2}$–CO), 4.86 (s, 2H, O–$\underline{CH_2}$–CO), 7.06 (d, 1H, *J* = 9.0 Hz, Ar–H_5), 7.37 (dd, 2H, *J* = 8.3 Hz, –SO_2–$\underline{Ph}$–), 7.65 (dd, 2H, *J* = 8.3 Hz, –SO_2–$\underline{Ph}$–), 7.82 (d, 1H, *J* = 2.6 Hz, Ar–H_8), 7.90 (dd, 1H, *J* = 9.0, 2.6 Hz, Ar–H_6), 8.29 (d, 1H, *J* = 9.1 Hz, Ph–$\underline{NH}SO_2$), 8.45 (t, 1H, *J* = 5.7 Hz, –CONH–). MS (FAB) = 521 (MH^+). Anal. CHN $C_{22}H_{24}N_4O_9S$.

*5.2.70. Ethyl (2S)-2-{[(4-methylphenyl)sulphonyl]amino}-3-[2-(7-amino-2H-1,4-benzoxazine-3(4H)-one-4-yl)acetyl]-aminopropanoate (**74**)*

Prepared according to the general procedure as in Section 5.2.1.3 from **73** (0.50 g, 0.96 mmol). *m* = 0.46 g (100%). M.p. 89–91 °C. IR (KBr, cm^{-1}): 3364, 2980, 1734, 1670, 1517, 1430, 1339, 1160, 1091, 1046, 814, 661. $[\alpha]_D^{20}$ = +18.2° (c 0.73; MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.01 (t, 3H, *J* = 7.2 Hz, CH_2–$\underline{CH_3}$), 2.38 (s, 3H, Ph–$\underline{CH_3}$), 3.17 (m, 1H, N–$\underline{CH_2}$–CH), 3.40 (m, 1H, N–$\underline{CH_2}$–CH), 3.79 (m, 2H, O–$\underline{CH_2}$–CH_3), 3.92 (m, 1H, CH), 4.31 (s, 2H, N–$\underline{CH_2}$–CO), 4.54 (s, 2H, O–$\underline{CH_2}$–CO), 5.24 (s, 2H, NH_2), 6.20–6.25 (m, 2H, Ar–H_6, Ar–H_8), 6.50 (d, 1H, *J* = 8.3 Hz, Ar–H_5), 7.38 (d, 2H, *J* = 8.1 Hz, –SO_2–$\underline{Ph}$–), 7.65 (d, 2H, *J* = 8.1 Hz, –SO_2–$\underline{Ph}$–), 8.22–8.34 (m, 2H, –NH–O_2–, –CONH–). MS (FAB) = 491 (MH^+). Anal. CHN $C_{22}H_{26}N_4O_7S \times 2\ H_2O$.

*5.2.71. Ethyl (2S)-2-{[(4-methylphenyl)sulphonyl]amino}-3-[2-(7-{[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl) benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-4-yl) acetyl]aminopropanoate (**75**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **74** (0.58 g, 1.2 mmol) and **59** (0.255 g, 1.2 mmol). *m* = 0.60 g (69%). M.p. 258–262 °C. IR (KBr, cm^{-1}): 3749, 3365, 1790, 1656, 1518, 1400, 1316, 1158, 1094, 941, 809, 660. $[\alpha]_D^{20}$ = –17.8° (c 0.69; 0.05 M NaOH). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.02 (t, 3H, *J* = 7.2 Hz, CH_2–$\underline{CH_3}$), 2.37 (s, 3H, Ph–$\underline{CH_3}$), 3.20 (m, 1H, N–$\underline{CH_2}$–CH), 3.39 (m, 1H, N–$\underline{CH_2}$–CH), 3.81 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$$CH_3$), 3.94 (m, 1H, CH), 4.45 (s, 2H, N–$\underline{CH_2}$–CO), 4.70 (s, 2H, O–$\underline{CH_2}$–CO), 6.82 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.40 (m, 3H, SO_2–$\underline{Ph}$–, 2H, Ar–H_6), 7.56 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.66 (d, 2H, *J* = 8.3 Hz, SO_2–$\underline{Ph}$–), 7.96 (d, 2H, *J* = 8.7 Hz, CO–$\underline{Ph}$–), 8.12 (d, 2H, *J* = 8.7 Hz, CO–$\underline{Ph}$–), 8.31 (d, 1H, *J* = 9.0 Hz, $NHSO_2$), 8.38 (t, 1H, *J* = 6.4 Hz, CO–$\underline{NH}$–CH_2), 10.41 (s, 1H, –CONH–). MS (FAB) = 679 (MH^+). Anal. CHN $C_{31}H_{30}N_6O_{10}S \times 1.5\ H_2O$.

*5.2.72. Ethyl (2S)-2-{[(4-methylphenyl)sulphonyl]amino}-3-[2-(7-{[4-(amino(imino)methyl)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-4-yl)acetyl]aminopropanoate in the form of acetate (**76**)*

Prepared according to the general procedure as in Section 5.2.1.9 from **75** (0.446 g, 0.65 mmol). *m* = 0.44 g (97%). M.p. 138–142 °C. IR (KBr, cm^{-1}): 3318, 1734, 1670, 1516, 1406, 1339, 1161, 1091, 1050, 814, 661. $[\alpha]_D^{20}$ = +4.2° (c 0.51; DMF). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.02 (t, 3H, *J* = 7.2 Hz, CH_2–$\underline{CH_3}$), 1.79 (s, 3H, $\underline{CH_3}$COOH), 2.38 (s, 3H, Ph–$\underline{CH_3}$), 3.20 (m, 1H, N–$\underline{CH_2}$–CH), 3.39 (m, 1H, N–$\underline{CH_2}$–CH), 3.81 (q, 2H, *J* = 7.2 Hz, O–$\underline{CH_2}$$CH_3$), 3.92 (m, 1H, CH), 4.44 (s, 2H, N–$\underline{CH_2}$–CO), 4.70 (s, 2H, O–$\underline{CH_2}$–CO), 6.82 (d, 1H, *J* = 9.0 Hz, Ar–H_5), 7.39 (m, 3H, SO_2–$\underline{Ph}$–, 2H, Ar–H_6), 7.57 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.67 (d, 2H, *J* = 8.3 Hz, SO_2–$\underline{Ph}$–), 7.94 (d, 2H, *J* = 8.7 Hz, CO–$\underline{Ph}$–), 8.11 (d, 2H, *J* = 8.7 Hz, CO–$\underline{Ph}$–), 8.5 (t, 1H, *J* = 6.0 Hz, CO–$\underline{NH}$–CH_2), 10.44 (s, 1H, –CONH–), signals for amidine protons and the sulphonamide proton were not observed. MS (FAB) = 637 (MH^+-free base). Anal. CHN $C_{31}H_{32}N_6O_8S \times CH_3COOH \times 2.25\ H_2O$.

*5.2.73. (2S)-2-{[(4-Methylphenyl)sulphonyl]amino}-3-[2-(7-{[4-(amino(imino)metil)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-4-yl)acetyl]aminopropanoic acid in the form of acetate (**77**)*

Prepared according to the general procedure as in Section 5.2.1.7 from **76** (0.26 g, 0.38 mmol). *m* = 0.18 g (71%). M.p. 230–233 °C. IR (KBr, cm^{-1}): 3337, 1665, 1610, 1518, 1407, 1324, 1153, 1091, 868, 812. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 2.34 (s, 3H, Ph–$\underline{CH_3}$), 3.81 (m, 1H, CH), 4.37–4.58 (m, 2H, N–$\underline{CH_2}$–CO), 4.70 (s, 2H, O–$\underline{CH_2}$–CO), 6.91 (d, 1H, *J* = 8.7 Hz, Ar–H_5), 7.37 (m, 4H, SO_2–$\underline{Ph}$–, 2H, Ar–H_6, Ar–H_8), 7.70 (d, 2H, *J* = 8.3 Hz, SO_2–$\underline{Ph}$–), 7.83–

7.94 (m, 4H, CO–Ph–), 8.13 (m, 1H, CO–NH–H_2), 10.29 (s, 1H, –CONH–), signals for amidine protons, sulphonamide and carboxylic protons were not observed. MS (FAB) = 609 (MH^+-free base). Compound was too hygroscopic for CHN analysis.

*5.2.74. Ethyl 5-(7-nitro-2H-1,4-benzoxazine-3(4H)-one-4-yl)pentanoate (**78**)*

To a stirred suspension of **7** (5.53 g, 28.5 mmol), K_2CO_3 (9.87 g, 71.3 mmol) and benzyltriethylammonium chloride (1.30 g, 5.7 mmol) in acetonitrile (150 ml), ethyl 5-bromopentanoate (5.94 ml, 37.05 mmol) was added dropwise. The reaction mixture was heated at 75 °C for 48 h. The precipitated K_2CO_3 was filtered off and the solvent evaporated. The oily residue was recrystallized from ethanol (60 ml) to give yellow crystals. *m* = 7.26 g (79%). M.p. 94–95 °C. IR (KBr, cm^{-1}): 3425, 3088, 1731, 1701, 1598, 1506, 1343, 1241, 1091, 900, 742. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.16 (t, 3H, *J* = 7.0 Hz, O–CH_2–CH_3), 1.58 (m, 4H, N–CH_2–CH_2–CH_2–CO), 2.34 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 3.98 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 4.03 (q, 2H, *J* = 7.0 Hz, O–CH_2–CH_3), 4.80 (s, 2H, O–CH_2–CO), 7.44 (d, 1H, *J* = 9.1 Hz, Ar–H_5), 7.81 (d, 1H, *J* = 2.6 Hz, Ar–H_8), 7.94 (dd, 2H, *J* = 9.1, 2.6 Hz, Ar–H_6). MS (EI) = 322 (M^+). Anal. CHN $C_{15}H_{18}N_2O_6$.

*5.2.75. Ethyl 5-(7-amino-2H-1,4-benzoxazine-3(4H)-one-4-yl)pentanoate (**79**)*

Prepared according to the general procedure as in Section 5.2.1.3 from **78** (7.00 g, 21.7 mmol). *m* = 6.20 g (98%). M.p. 73–75 °C. IR (KBr, cm^{-1}): 3325, 2938, 1728, 1658, 1515, 1423, 1313, 1196, 1046, 846, 802, 654, 587. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.26 (t, 3H, *J* = 7.2 Hz, O–CH_2–CH_3), 1.71 (m, 4H, N–CH_2–CH_2–CH_2–CO), 2.36 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 3.93 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 4.14 (q, 2H, *J* = 7.2 Hz, O–CH_2–CH_3), 4.55 (s, 2H, O–CH_2–CO), 6.50–6.53 (m, 2H, Ar–H_8, Ar–H_6), 6.81 (d, 1H, *J* = 9.1 Hz, Ar–H_5). MS (EI) = 292 (M^+). Anal. CHN $C_{15}H_{20}N_2O_4$.

*5.2.76. Ethyl 5-(7-{[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-4-yl)-pentanoate (**80**)*

Prepared according to the general procedure as in Section 5.2.1.8 from **79** (0.70 g, 2.39 mmol) and **59** (0.542 g, 2.64 mmol). The crude product was recrystallized from ethanol (40 ml) to give pale yellow crystals. *m* = 0.77 g (67%). M.p. 225–229 °C. IR (KBr, cm^{-1}): 3301, 2987, 1805, 1773, 1730, 1686, 1648, 1587, 1514, 1408, 1280, 1179, 1046, 940, 865, 815, 755, 665. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.17 (t, 3H, *J* = 7.0 Hz, O–CH_2–CH_3), 1.58 (m, 4H, N–CH_2–CH_2–CH_2–CO), 2.34 (t, 2H, *J* = 6.8 Hz, N–CH_2–CH_2–CH_2–CH_2–CO), 3.91 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 4.04 (q, 2H, *J* = 7.0 Hz, O–CH_2–CH_3), 4.65 (s, 2H, O–CH_2–CO), 7.21 (d, 1H, *J* = 9.1 Hz, Ar–H_5), 7.48 (dd, 1H, *J* = 9.1, 2.3 Hz, Ar–H_6), 7.55 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.95–8.14 (dd, 4H, *J* = 8.7 Hz, Ar–H), 8.01 (s, 1H, –CONH–), 10.27 (s, 1H, –CONH–). MS (FAB) = 481 (MH^+). Anal. CHN $C_{24}H_{24}N_4O_7 \times 1\ H_2O$.

*5.2.77. Ethyl 5-(7-{[4-(amino(imino)methyl)benzoyl]-amino}-2H-1,4-benzoxazine-3(4H)-one-4-yl)pentanoate in the form of acetate (**81**)*

Prepared according to the general procedure as in Section 5.2.1.9 from **80** (0.300 g, 0.624 mmol). *m* = 0.234 g (75%). M.p. 177–179 °C. IR (KBr, cm^{-1}): 2962, 1728, 1665, 1605, 1514, 1408, 1049, 861, 702. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.17 (t, 3H, *J* = 7.1 Hz, O–CH_2–CH_3), 1.57 (m, 4H, N–CH_2–CH_2–CH_2–CH_2–CO), 1.77 (s, 3H, CH_3COO^-), 2.34 (t, 2H, *J* = 6.4 Hz, N–CH_2–CH_2–CH_2–CH_2–CO), 3.91 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 4.04 (q, 2H, *J* = 7.1 Hz, O–CH_2–CH_3), 4.64 (s, 2H, O–CH_2–CO), 7.21 (d, 1H, *J* = 9.0 Hz, Ar–H_5), 7.48 (dd, 1H, *J* = 9.0, 2.3 Hz, Ar–H_6), 7.56 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.92–8.11 (dd, 4H, *J* = 8.3 Hz, Ar–H), 10.45 (s, 1H, –CONH–), signals for amidine protons were not observed. MS (FAB) = 439 (MH^+-free base). Anal. CHN $C_{25}H_{30}N_4O_7$.

*5.2.78. 5-(7-{[4-(Amino(imino)methyl)benzoyl]amino}-2H-1,4-benzoxazine-3(4H)-one-4-yl)pentanoic acid in the form of acetate (**82**)*

Prepared according to the general procedure as in Section 5.2.1.7 from **80** (0.250 g, 0.553 mmol). The crude product was used in the next step (general procedure as in Section 5.2.1.9) without further purification. *m* = 0.267 g (89%). M.p. 226–231 °C. IR (KBr, cm^{-1}): 2936, 1730, 1668, 1640, 1514, 1411, 1327, 1098, 1054, 861, 703. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.56 (m, 4H, N–CH_2–CH_2–CH_2–CH_2–CO), 1.83 (s, 3H, CH_3COO^-), 2.14 (t, 2H, *J* = 8.3 Hz, N–CH_2–CH_2–CH_2–CH_2–CO), 2.73 (s, 3H, CH_3–*N,N*-DMF*), 2.89 (s, 3H, CH_3–*N,N*-DMF*), 3.91 (m, 2H, N–CH_2–CH_2–CH_2–CH_2–CO), 4.64 (s, 2H, O–CH_2–CO), 7.22 (d, 1H, *J* = 9.0 Hz, Ar–H_5), 7.48 (dd, 1H, *J* = 9.0, 2.3 Hz, Ar–H_6), 7.52 (d, 1H, *J* = 2.3 Hz, Ar–H_8), 7.92–8.09 (dd, 4H, *J* = 8.3 Hz, Ar–H), 10.47 (s, 1H, –NHCO–), signals for amidine protons and the amide proton from *N,N*-DMF were not observed. Compound precipitated as pale yellowish crystals with one equivalent of *N,N*-DMF, which was characterized by NMR and CHN analysis. MS (FAB) = 411 (MH^+-free base). Anal. calcd. for $C_{23}H_{26}N_4O_7 \times 1.5\ H_2O \times$ *N,N*-DMF.

5.3. Inhibition of ADP-induced platelet aggregation assay [34]

Venous blood was collected from apparently healthy volunteers who were using no medication, and centrifuged at room temperature for 15 min at 1000 × *g* to give platelet-rich plasma (PRP). The remaining blood was further centrifuged for 10 min at 2000 × *g* to give platelet-poor plasma (PPP). In PRP the number of platelets was adjusted to 250 ± 25 × 10^6/ml by dilution with PPP. Platelet aggregation was mea-

sured at 37 °C by light transmittance in an automatic blood coagulation analyzer (Behring Coagulation Timer, Behring Diagnostics GmbH). A 15 μl of test compounds at various concentrations were added to 135 μl of PRP and after 5 min aggregation was initiated by adding 15 μl ADP at a final concentration of 20 μM. Results were expressed relative to platelet aggregation with no test compound added. The concentration of the test compound which reduced platelet aggregation by 50% (IC_{50}) was determined from the dose–response curve [35]. Final IC_{50} values were determined as the average of determinations conducted on plasma samples from at least two blood donors. The average standard error for determinations was ± 20%. RGDS and tirofiban (Aggrastat®, Merck & Co., Inc.) were included as controls, with IC_{50} 260 ± 52 μM and 0.0075 ± 0.0011 μM, respectively (the results are in accordance with those previously reported).

5.4. Inhibition of in vitro binding of fibrinogen to isolated $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrins assay [36]

The binding affinities of the synthesized compounds to integrins $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ were characterized by a solid-phase competitive displacement assay. Human fibrinogen (100 mg) was dissolved in aqueous NaCl (0.3 M, 5 ml) at 30 °C and then further diluted with 0.1 M $NaHCO_{3(aq)}$ to a final concentration of 1 mg/ml. Biotin *N*-hydroxysuccinimide ester (2 mg) was dissolved in *N,N*-dimethylformamide (2 ml) and added to 6 ml of fibrinogen solution. The reaction mixture was incubated for 90 min at 30 °C and dialyzed for 3 h at RT against buffer 1 (3 l, 20 mM Tris, 150 mM NaCl, pH 7.4). After dialysis, the solution was centrifuged for 5 min at 5400 rpm and Tween 20 (0.005%) was added (stock solution). Human integrins (10 μl of $\alpha_{IIb}\beta_3$ or 5 μl of $\alpha_V\beta_3$) were diluted in 10.2 ml of buffer 2 (20 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, pH 7.4) and adsorbed to 96-well (100 μl/well) high-binding microtiter plates (Greiner, Lumitrac 600) overnight at 4 °C. The remaining integrin solution was thrown away and non-specific receptor-binding sites were blocked with 1% BSA in buffer 2 (200 μl/well). Following incubation for 1 h at RT, the plates were washed twice with buffer 3 (buffer 2 containing 0.1% of Tween 20). The potential antagonists were serially diluted with buffer and solutions added (50 μl/well) at the same time as biotinylated fibrinogen (50 μl/well, 1:10 dilution of stock solution in buffer 2) to each well. The plates were incubated for 2 h at RT and then washed twice with buffer 3. In each well, peroxidase-conjugated antibiotin goat antibody (100 μl/well, 1:1000 dilution of purchased solution in buffer 3 + 0.1% of BSA) was added and incubated for another hour. The microtiter plates were washed with buffer 3 three times. Finally, chemiluminescence substrate (50 μl/well) was added and the luminescence detected with a GENios (Tecan Group AG) multimode research reader. Positive controls received no inhibitors while negative controls received no ligands. RGDS was used as the internal standard. The assays were performed at least in triplicate. The mean experimental data were fitted to the sigmoid model and IC_{50} values were calculated from the dose–response curve (OriginPro, OriginLab®, Version 7.5).

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