Original article

Towards dual antithrombotic compounds − Balancing thrombin inhibitory and fibrinogen GPIIb/IIIa binding inhibitory activities of 2,3-dihydro-1,4-benzodioxine derivatives through regio- and stereoisomerism

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ARTICLE INFO

ABSTRACT

Enantiomers of 2,3-dihydro-1,4-benzodioxine derivatives possessing both thrombin and fibrinogen GPIIb/IIIa binding inhibitory activities were prepared from (R)- and (S)-glycidol as potential dual antithrombotic compounds. The influence of chirality and substitution pattern on thrombin inhibition and on inhibition of fibrinogen binding to GPIIb/IIIa was analyzed. Docking studies were used in an attempt to rationalize the results. The (S)-isomers of both 2,3-dihydro-1,4-benzodioxine regioisomers at positions 6 and 7 were found to be better thrombin inhibitors than the corresponding (R)-enantiomers, whereas we observed that stereochemistry does not display a consistent influence on fibrinogen GPIIb/IIIa binding inhibitory activity. Compound **11b**, the (S)-isomer of the 6-substituted regioisomer, possessed the best balanced dual activity, with $K_{i(thrombin)} = 1.67 \pm 0.27$ μM and $IC_{50(GPIIb/IIIa)} = 0.665 \pm 0.26$ μM, raising the hope that merging anticoagulant and platelet antiaggregatory activities in the same molecule could lead to successful multitarget antithrombotic agents.

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1. Introduction

Cardiovascular diseases are the main cause of death in developed countries [1]. Existing anticoagulant and antiplatelet therapy, despite its importance and effectiveness in the treatment of cardiovascular diseases, has numerous limitations such as bleeding, metabolism that differs in different individuals due to genetic polymorphism, and interaction with other drugs and food [2,3].

During the last decade much effort have been made to design novel anticoagulant drugs that target the coagulation enzymes thrombin, factor Xa, factor VII and factor IX, with the aim of obtaining a simple and satisfactory, orally applicable replacement for the existing warfarin treatment [4–11]. Ximelagatran, a prodrug of melagatran, was the first oral direct thrombin inhibitor introduced to the market, however it was withdrawn from the market in 2006 due to expressed hepatotoxicity [12,13]. Dabigatran etexilate is a new oral prodrug for the direct thrombin inhibitor dabigatran (Fig. 1) that entered the market in 2008 for the prevention of blood clotting following hip or knee surgery, and for the prevention of stroke in patients with non-valvular atrial fibrillation [14]. Good progress has also been made in the field of direct and indirect factor Xa inhibitors. Rivaroxaban, a recently introduced, highly selective oral factor Xa inhibitor, promised effectiveness and safety in preventing venous thromboembolism [15]. Other novel factor Xa inhibitors include the recently approved apixaban, and idraparinux that is in the final stage of clinical investigation [16].

Platelets play an essential role in the maintenance of hemostasis; they are responsible for clot formation when damage of endothelium of blood vessels occurs [17]. The platelet glycoprotein IIb/IIIa antagonists abciximab, eptifibatide and tirofiban are potent antiplatelet agents, and are used to prevent pathological platelet
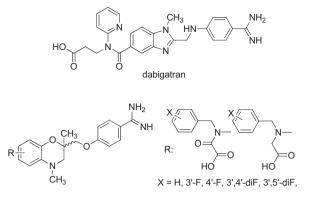
dabigatran

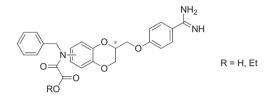previously reported racemic 6- and 7-substituted 1,4-benzoxazine derivatives

X = H, 3'-F, 4'-F, 3',4'-diF, 3',5'-diF,

R = H, Et

new enantiomers of 6- and 7-substituted 1,4-benzodioxines

Fig. 1. Structures of the clinically used thrombin inhibitor dabigatran, the racemic 1,4-benzoxazine derivatives [25,30] and the enantiomers of 1,4-benzodioxine derivatives reported in this paper.

aggregation in patients with acute coronary syndrome, heart attack and those undergoing invasive heart procedures [18].
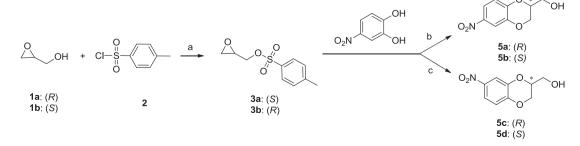
In clinical practice, an effective prophylaxis and treatment of thromboembolic diseases, such as deep vein thrombosis, thromboembolic stroke, pulmonary embolism and myocardial infarction, is achieved by using a combination of various antiaggregatory and anticoagulant drugs [19]. Since the combined use of thrombin inhibitors and glycoprotein IIb/IIIa antagonists in the prevention of cardiovascular diseases has shown additional benefits over treatment directed against thrombin or against platelets alone [20], we envisaged merging anticoagulant and platelet antiaggregatory activity in the same molecule as a promising approach towards novel multitarget antithrombotic agents [21–25]. Recently, other groups have reported the preparation of dual anticoagulant/antiplatelet agents featuring high molecular weight, polysulfated conjugates [26], in contrast to our dual acting compounds with strongly overlapping pharmacophores and low molecular weight.

In our previous work on potential dual antithrombotic compounds, combining in one molecule both thrombin inhibitory and fibrinogen receptor antagonistic activities [24,25], 3,4-dihydro-2*H*-1,4-benzoxazine [27] was selected as a suitable scaffold, on the basis of docking experiments, enabling convenient attachment of

substituents in positions 2, 4, 6 and 7 [25]. The benzamidine moiety, a typical P1 group of thrombin inhibitors and an arginine mimetic of the RGD motif [28], was attached at position 2 of the benzoxazine core, while P3 benzyl and carboxylic acid moieties, required for thrombin inhibition and GPIIb/IIIa binding [29], were bound at positions 6 or 7 of the 1,4-benzoxazine skeleton (Fig. 1). Molecular modeling studies predicted 7-isomers to be better thrombin inhibitors while 6-isomers were expected to possess better inhibition of fibrinogen GPIIb/IIIa binding, as was later confirmed in biological assays [25]. Focusing on optimization of the P3 part, with a combination of different aromatic and carboxylate group moieties, we succeeded in preparing well-balanced dual compounds with thrombin K_i values and IC_{50} values for inhibition of fibrinogen binding to platelet GPIIb/IIIa in the high nanomolar/low micromolar ranges [30]. Replacement of the highly basic benzamidine moiety with the less basic [1,2,4] triazolo[4,3-*b*]pyridazine-6-yl group resulted in loss of both thrombin inhibitory and fibrinogen receptor antagonistic activities [31], showing the importance of the benzamidine group for binding to both targets. The failure to achieve better affinities of the designed compounds for thrombin and fibrinogen receptor demonstrates the difficulty in reaching a compromise that would meet the constraints imposed by both binding sites.
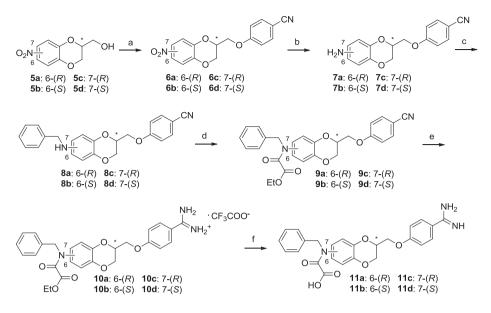
When designing multiple ligands with highly overlapping pharmacophores, balancing activities for both targets is demanding, so all structural information is of the utmost value [32–35]. In the present work we report a 1,4-benzodioxine series of enantiomeric compounds that combine, in the same molecule, highly overlapping pharmacophores of thrombin inhibitors and fibrinogen receptor antagonists (Fig. 1). They resulted from replacement of the 1,4-benzoxazine core by a 1,4-benzodioxine scaffold [36], a privileged heterocyclic skeleton applied in the design of selective α_1 adrenoceptor antagonists [37], antioxidants [38,39], radical scavenging compounds [40–42], hypolipidemic [43] and anti-inflammatory agents [44,45]. In order to explore the significance of chirality and regioisomerism in balancing the activity of the new 1,4-benzodioxine compounds at both targets, and thanks to the commercial availability of both (*R*)- and (*S*)-glycidol (required for enantioselective synthesis of the parent 2-substituted benzodioxine cores), we prepared both enantiomers of the benzodioxine 6- and 7-regioisomers and studied the effects of chirality and regioisomerism on binding the resulting potential dual antithrombotic compounds to thrombin and platelet fibrinogen receptor.

2. Chemistry

The synthesis of target 1,4-benzodioxine enantiomers **11a–d** from 4-nitrocatechol is presented in Schemes 1 and 2. The strongly electronegative nitro group in 4-nitrocatechol enabled effective control of the regioselectivity in construction of the 1,4-

1a: (*R*)
1b: (*S*)

2

3a: (*S*)
3b: (*R*)

5a: (*R*)
5b: (*S*)

5c: (*R*)
5d: (*S*)

Reagents and conditions: a) Et$_3$N, DMAP, DCM, 0 °C → rt, 1h; b) NaH, DMF, 80 °C, 2h; c) Na$_2$CO$_3$, DMF, 60 °C, 2h.

Scheme 1. Synthesis of compounds **5a–d**.

Reagents and conditions: a) 4-cyanophenol, PPh₃, DIAD, THF, reflux, 48h; b) H₂, Pd/C (10%), 25 bar, rt, 1h; c) benzaldehyde, MeOH, mol. sieves, rt, 12h, then NaBH₄, 1h; d) EtOCOCOCl, Et₃N, DCM, rt, 2h; e) HCl(g), EtOH, 0 °C (30 min) → rt, 24h, then CH₃COONH₄, 24h; f) 1M LiOH, THF/MeOH, rt, 1h.

Scheme 2. Synthesis of compounds **10a–d** and **11a–d**.

benzodioxine ring and, depending on the applied base, gave rise to 6- and 7-nitro isomers, respectively [46]. Thus, using a modified published procedure for the synthesis of the racemates [47], the reaction of 4-nitrocatechol with (R)- and (S)-(2-tosyloxymethyl) oxirane (**3b** and **3a**), in the presence of potassium carbonate in N,N-dimethylformamide afforded enantiomers of (7-nitro-2,3-dihydro-1,4-benzodioxin-2-yl)methanol (**5d** and **5c**), whereas the reaction of 4-nitrocatechol with **3a** or **3b**, using sodium hydride as a base, gave (R)- and (S)-6-nitro isomers **5a** and **5b** (Scheme 1). Both nitro isomers of the (R)- and (S)-series were reacted with 4-hydroxybenzonitrile under Mitsunobu conditions to give ethers **6a–d**, which were reduced in the next step to amines **7a–d**, using catalytic hydrogenation over palladium on

charcoal. These were benzylated, using benzaldehyde and sodium borohydride, and the resulting N-benzylamines **8a–d** acylated with ethyl oxalyl chloride to give compounds **9a–d**. Upon Pinner reaction they afforded esters **10a–d** which, after alkaline hydrolysis with lithium hydroxide in methanol/tetrahydrofuran, gave the enantiomeric carboxylic acids **11a–d** (Scheme 2). We tried to develop an HPLC method for determining the enantiomeric purity of products **5–11**, but succeeded only with compounds **8a–d** for which an enantiomeric excess >95% was obtained. The non-racemizing conditions in the last two reaction steps and consistent optical rotation values of the products allow the conclusion that the enantiomeric excess of compounds **10a–d** and **11a–d** remained higher than 95%.

Table 1
Biological activity of 1,4-benzodioxine derivatives **10a–d** and **11a–d**: the inhibition of serine proteases thrombin, factor Xa, trypsin, and the inhibition of fibrinogen binding to GPIIb/IIIa.

Compound	Substitution	R′	*	K_i (μM)			IC₅₀ (μM)
				Thrombin	Trypsin [selectivity][a]	FXa [selectivity][b]	GPIIb/IIIa[c]
10a	6	Et	R	1.65 ± 0.20	0.644 ± 0.089 [0.39]	2.15 ± 0.18 [1.30]	>200
10b	6	Et	S	0.436 ± 0.037	0.283 ± 0.044 [0.65]	1.51 ± 0.37 [3.46]	>200
10c	7	Et	R	0.675 ± 0.099	0.894 ± 0.097 [1.32]	0.637 ± 0.44 [0.95]	>200
10d	7	Et	S	0.0778 ± 0.005	0.210 ± 0.026 [2.71]	2.70 ± 0.15 [35.9]	>200
11a	6	H	R	5.18 ± 1.15	1.55 ± 0.22 [0.30]	5.19 ± 0.61 [1.30]	1.21 ± 0.32
11b	6	H	S	1.67 ± 0.27	0.463 ± 0.093 [0.28]	1.96 ± 0.17 [1.17]	0.665 ± 0.26
11c	7	H	R	2.69 ± 0.36	1.37 ± 0.029 [0.51]	1.27 ± 0.13 [0.47]	17.6 ± 3.0
11d	7	H	S	0.266 ± 0.03	0.267 ± 0.049 [1.00]	5.08 ± 0.57 [19.1]	42.6 ± 5.3

[a] Selectivity for thrombin *vs.* trypsin.
[b] Selectivity for thrombin *vs.* factor Xa.
[c] Compounds were also tested for inhibition of fibrinogen binding to $\alpha_V\beta_3$ receptor and none of them showed binding in the test system (IC₅₀($\alpha_V\beta_3$) > 200 μM).

3. Biological results

Compounds **10a–d** and **11a–d**, comprising enantiomers of the 6- and 7-isomers, were tested for inhibition of thrombin and, in order to assess their selectivity for related serine proteases, also for inhibition of factor Xa and trypsin. Additionally, they were tested for inhibition of fibrinogen binding to GPIIb/IIIa. The results are presented in Table 1. The general structure–activity relationship which was observed in the 1,4-benzoxazine series can also be seen in the case of the 1,4-benzodioxine series. Esters **10a–d** were found to be more potent thrombin inhibitors than the corresponding carboxylic acids **11a–d**, but they lacked fibrinogen GPIIb/IIIa binding activity (IC$_{50}$ > 200 μM). Compounds bearing a P3 moiety in position 6 (**11a–b**) displayed more pronounced fibrinogen GPIIb/IIIa binding inhibitory activity (IC$_{50}$ 0.66 and 1.21 μM), while compounds bearing a P3 moiety at position 7 exhibited more pronounced thrombin inhibitory activity (K_i = 0.078–2.69 μM). While compounds **10a–d** and **11a–d** were not selective towards two similar serine proteases, trypsin and factor Xa, the compound with the most potent thrombin inhibitory activity (**10d**) was found also to be the most selective. As demonstrated in Fig. 1, compound **11b**, the (S)-isomer of the 6-substituted regioisomer, possessed the best balanced dual activity ($K_{i(thr)}$ = 1.67 ± 0.27 μM, IC$_{50(GPIIb/IIIa)}$ = 0.665 ± 0.26 μM). Compound **10d**, the (S)-isomer of the 7-regioisomer, is the most potent thrombin inhibitor, with $K_{i(thr)}$ = 77.8 ± 5.0 nM, and compound **11b**, ((S)-isomer of the 6-regioisomer) has the most potent fibrinogen GPIIb/IIIa binding inhibitory activity, with IC$_{50(IIb/IIIa)}$ = 665 ± 260 nM. This demonstrates that, in the development of dual antithrombotic compounds based on the 1,4-benzodioxine scaffold, optimizing fibrinogen receptor activity is a more difficult task than optimizing thrombin inhibition.

The (S)-isomers of the 6- and 7-series were found to be better thrombin inhibitors than the (R)-isomers. Overall, these results are comparable with those for the 1,4-benzoxazine analogues. The racemic 1,4-benzoxazine derivative substituted at position 6 showed higher activity on both targets ($K_{i(thr)}$ = 0.62 ± 0.10 μM, IC$_{50(GPIIb/IIIa)}$ = 2.8 ± 1.5 μM), while the racemic 1,4-benzoxazine derivative substituted at position 7 had more pronounced thrombin inhibitory activity ($K_{i(thr)}$ = 0.19 ± 0.02 μM, IC$_{50(GPIIb/IIIa)}$ = 39 ± 18 μM) [26]. When considering eudismic ratios, the results in Table 1 demonstrate that, in the case of esters **10a–d** in the 6-regioisomer series, the (S)-isomer is 3.8 times more potent and, in the 7-regioisomer series, 8.7 times more potent thrombin inhibitor than corresponding (R)-isomer. Similar results are seen in the case of acids **10a–d** where, in 6-substituted compounds, the (S)-isomer is 3.1 times more potent and, in the 7-substituted compound, 10.1-fold more potent as a thrombin inhibitor. Stereochemistry does not appear to have such a consistent influence on fibrinogen GPIIb/IIIa binding inhibitory activity. In the case of the 6-substituted compound, the (S)-isomer **11b** is 1.8 times more potent than the (R)-isomer **11a**, while in case of 7-substituted compounds the (R)-isomer **11c** is 2.4 times more potent than the (S)-isomer **11d**. It can thus be concluded that, in the 1,4-benzodioxine series, the (S)-6-isomers offer the best opportunities for optimization towards balanced dual acting compounds.

4. Docking studies

In an attempt to rationalize the differences in the binding affinities of (R)- and (S)-enantiomers for thrombin and fibrinogen receptor, we performed docking of compounds **11a** and **11b** – both enantiomers of the more balanced 6-regioisomer docked to the thrombin active site and to the binding site of GPIIb/IIIa are depicted in Fig. 2. Given the similarity of our compounds to dabigatran, the crystal structure of the dabigatran–thrombin complex (PDB: **1KTS**) [48] was used for docking experiments. Compounds **11a** and **11b** bind to the thrombin active site in a similar manner to dabigatran but the configuration at chiral centers slightly influences the difference in their binding modes. The distance between the C-atoms of the Asp189 carboxylate and the amidine group is 3.9 Å and 4.0 Å for **11a** and **11b**, and two hydrogen bonds connect the amidine hydrogens and carboxylate oxygen atoms. Additional hydrogen bonds of 1.98 Å and 2.0 Å between the benzamidine and the Gly219 backbone for the two compounds are observed. The 2,3-dihydro-1,4-benzodioxine scaffold is located in the S2 binding pocket and the benzyl group reaches into the lipophilic S3 binding pocket, with the P3 carboxylate stretching
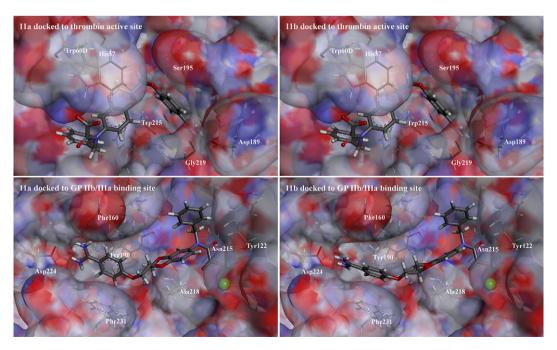

Fig. 2. Compounds **11a** (*R*-isomer, left) and **11b** (*S*-isomer, right) docked to thrombin active site (top) and GPIIb/IIIa binding site (bottom).

outwards from the thrombin surface. The methyleneoxy groups adjacent to the chiral carbon of 3,4-dihydro-1,4-benzodioxine are oriented differently in the two enantiomers. The distance between the oxygen of the methyleneoxy group and the Ser195 OH group (O−H•••O) is 3.1 Å in **11b** and 3.6 Å in **11a**. The carbon atom of the methylenoxy groups adjacent to the chiral center is the atom that differs most in its positioning in **11a** and **11b** – by 1.92 Å. In **11b**, the methylene group is closer to the protein surface while, in **11a**, it points outwards from the thrombin surface.

The analysis of the binding modes of **11a** and **11b** docked in the binding site of GPIIb/IIIa (using PDB: **2VDM** – the only published crystal structure of GPIIb/IIIa in a complex with a low molecular weight RGD mimetic) [49] shows that the compounds take an extended conformation. The carboxylic acid moiety occupies an identical location in **11a** and **11b**, making electrostatic interactions with the Mg^{2+} atom and hydrogen-bonding to the amide proton of Asn215 of the β_3 subunit of the GPIIb/IIIa. The phenyl rings of **11a** and **11b** also occupy identical positions, making hydrophobic interactions with Tyr122 of the β_3 subunit. The methylenoxy groups and the benzamidine moieties of **11a** and **11b** occupy slightly different positions. The Ala218 of the β_3 subunit makes, with its methyl group, hydrophobic interactions with the exocyclic methylenoxy group of the ligand (distances of 4.2 Å for **11b** and 5.5 Å for **11a**) and polar interactions of its C═O group with the exocyclic ether oxygen of the ligand (2.9 Å for **11b** and 3.3 Å for **11a**). Further, positioning of the benzamidine moiety, which forms hydrogen bonds with Asp224 and the Phe160 from the α_{IIb} subunit of the GPIIb/IIIa, and hydrophobic interactions with Tyr190, Phe231, and Phe191 of the α_{IIb} subunit, differs between the *R*- and *S*-isomers by 0.94 Å difference in the position of the carbon atom of the amidine moieties of **11a** and **11b**. The docking experiments thus show better binding interactions of **11b** than that of **11a** to platelet fibrinogen receptor (GPIIb/IIIa).

In summary, the measured activities and analysis of the docking positions of **11a** and **11b** at the two targets lead to the conclusion that the configuration at the chiral center, and the consequent different positioning of the methylenoxy moiety, accounts, at least partly, for the difference in potency of the two enantiomers. Whereas the methylenoxy moiety situated in close proximity to the chiral center is required for flexibility of the molecule (compounds **10a**−**11d** adopt a bent shape in the thrombin active site and extended conformations in the fibrinogen receptor-binding site), this could also explain the low eudismic ratios resulting from the − CH_2O− group imposed molecular flexibility that diminishes the difference between the binding conformations of two enantiomers at a particular target.

5. Conclusion

A novel 1,4-benzodioxine enantiomeric series of compounds have been prepared that act both as thrombin inhibitors and as fibrinogen GPIIb/IIIa binding inhibitors. The influence of chirality and regioisomerism on their thrombin inhibitory and fibrinogen GPIIb/IIIa binding inhibitory activities has been analyzed. The differences in potency of isomers and observed eudismic ratios can be explained in terms of the docking poses of the two stereoisomers. Unfortunately, we were not able to observe antithrombotic action of this series of compounds in *in vivo* model of venous thrombosis in rat, indicating that their potencies on both targets are still too weak to be measured *in vivo*. The attempt to balance thrombin inhibition and fibrinogen GPIIb/IIIa binding inhibitory activities on the one hand and to achieve lower nanomolar K_i (thrombin) and IC_{50} (GPIIb/IIIa) values on the other – which could result in *in vivo* antithrombotic activity – remains a viable, but highly demanding challenge in the search for effective dual antithrombotic drugs.

6. Experimental section

6.1. General

Chemicals were obtained from Acros, Aldrich Chemical Co. and Fluka and used without further purification. Analytical TLC was performed on silica gel Merck 60 F_{254} plates (0.25 mm), using visualization with ultraviolet light. 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. ^1H NMR and ^{13}C NMR spectra were recorded on a 400 MHz Bruker AVANCE III NMR spectrometer (^1H NMR at 400.13 MHz and ^{13}C NMR at 100.61 MHz) and on a Bruker Avance DPX300 spectrometer (^1H NMR at 300 MHz, ^{13}C NMR at 75 MHz) in DMSO-d_6 solution, with TMS as internal standard. ^{13}C NMR spectra were assigned using gradient COSY, HSQC and HMBC experiments. The following abbreviations were used to describe peak splitting patterns wherever appropriate: br = broad, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, and m = multiplet. IR spectra were recorded 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 were within ±0.4% of the theoretical values. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. Optical rotations were measured on a Perkin−Elmer 1241 MC polarimeter. Reverse phase HPLC was performed on an Agilent Technologies HP 1100 instrument with G1365B UV−vis detector (254 nm), using an Eclipse Plus C18 column 5 μm (4.6 × 150 mm) at flow rate of 1 mL/min. The eluent was a mixture of 0.1% TFA in water (A) and methanol (B) and the gradient was 40% B to 80% B in 15 min. Chiral HPLC analyses were performed on a JASCO ChromPass Software Version 1.8 with JASCO UV-2075 UV−vis detector (244 nm), using a Chiralcel OJ-H 5 μm 4.6 × 250 mm column at flow rate of 1 mL/min. Compounds were separated under isocratic conditions using as eluent a mixture of 60% *n*-hexane and 40% ethanol. All the compounds reported in this paper were >95% pure. Esters **10a**−**d** were purified by reverse phase column chromatography, using a Flash Purification System ISOLERA™. The eluent was a mixture of 0.1% TFA in water (A) and methanol (B) and the gradient was 40% B to 80% B in 30 column volumes.

6.2. Synthesis of (R)-glycidyl tosylate (**3b**)

To a solution of (*S*)-glycidol (5.50 g, 74.24 mmol), triethylamine (8.26 g, 81.66 mmol) and 4-(dimethylamino)pyridine (2%, 181 mg, 1.48 mmol) in 60 mL dichloromethane at 0 °C, tosyl chloride (14.15 g, 74.24 mmol) was added and the mixture was stirred at room temperature for 3 h with formation of a white precipitate. Dichloromethane (40 mL) was added and the mixture was subsequently washed with 100 mL 5% aq. K_2CO_3, 100 mL 1 M HCl, and 100 mL water. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The crude product (15.56 g colourless oil, 92% yield) was used in the next step without further purification; $[\alpha]_D = -16.7$ (*c* = 0.46, CHCl₃), lit.: [50] $[\alpha]_D = -17.0$ (*c* = 2.83, CHCl₃); ^1H NMR (300 MHz, CDCl₃): δ (ppm) 2.47 (s, 3H, CH₃), 2.61 (dd, *J* = 4.8, 2.5 Hz, 1H, CH₂O), 2.83 (t, *J* = .4 Hz, 1H, CH₂O), 3.16−3.24 (m, 1H, CH), 4.0 (dd, *J* = 11.4, 6.0 Hz, 1H, CH₂OS), 4.27 (dd, *J* = 11.4, 3.5 Hz, 1H, CH₂OS), 7.37 (d, *J* = 8.1 Hz, 2H, Ar−H³, Ar−H⁵), 7.82 (d, *J* = 8.1 Hz, 2H, Ar−H², Ar−H⁶).

6.3. Synthesis of (S)-glycidyl tosylate (**3a**)

To a solution of (*R*)-glycidol (4.00 g, 54.00 mmol), triethylamine (6.01 g, 59.50 mmol) and 4-(dimethylamino)pyridine (2%, 132 mg, 1.08 mmol) in 50 mL dichloromethane at 0 °C, tosyl chloride (10.30 g,

54.00 mmol) was added and the mixture was stirred at room temperature for 1 h. During stirring a white precipitate was formed. The reaction mixture was subsequently washed with 100 mL 5% aq. K_2CO_3, 100 mL 1 M HCl, and 100 mL water. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The crude product (11.63 g colourless oil, 95% yield) was used in the next step without further purification; $[\alpha]_D = +16.3$ ($c = 0.57$, CHCl$_3$), lit.: [51] $[\alpha]_D = +17.5$ ($c = 2.13$, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 2.47 (s, 3H, CH$_3$), 2.61 (dd, $J = 4.8$, 2.5 Hz, 1H, CH$_2$O), 2.81–2.85 (m, 1H, CH$_2$O), 3.17–3.24 (m, 1H, CH), 3.98 (dd, $J = 11.4$, 6.0 Hz, 1H, CH$_2$OS), 4.27 (dd, $J = 11.4$, 3.5 Hz, 1H, CH$_2$OS), 7.37 (d, $J = 8.1$ Hz, 2H, Ar–H^3, Ar–H^5), 7.83 (d, $J = 8.1$ Hz, 2H, Ar–H^2, Ar–H^6).

6.4. Synthesis of (R)-(6-nitro-2,3-dihydro-1,4-benzodioxin-2-yl) methanol (5a)

To a suspension of NaH (60% dispersion, 120 mg, 1.5 mmol) in 5 mL DMF at 0 °C, 4-nitrocatechol (310 mg, 2.00 mmol) in 1 mL DMF was added carefully. The mixture was stirred at 0 °C for 30 min (until gas formation ceased) and then a solution of (S)-glycidyl tosylate (502 mg, 2.20 mmol) in 1 mL DMF was added. The reaction mixture was stirred at room temperature for 30 min and then heated at 80 °C for 2 h. Upon cooling, the mixture was poured onto 15 mL water. The mixture was extracted with 3 × 20 mL diethyl ether. The combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness. The crude product obtained was purified by column cromatography (petrolether:ethyl acetate 1:1) to give 178 mg (42%) of pale yellow crystals; mp 112–114 °C; $[\alpha]_D = +87.61$ ($c = 0.57$, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 1.91 (t, $J = 6.20$ Hz, 1H, OH), 3.86–4.04 (m, 2H, CH$_2$OH), 4.09–4.28 (m, 1H, CH), 4.33–4.47 (m, 2H, CH$_2$O), 6.94–7.08 (m, 1H, Ar–H^8), 7.75–7.90 (m, 2H, Ar–H^5, Ar–H^7).

6.5. Synthesis of (S)-(6-nitro-2,3-dihydro-1,4-benzodioxin-2-yl) methanol (5b)

To a suspension of NaH (60% dispersion, 120 mg, 1.5 mmol) in 5 mL DMF at 0 °C, 4-nitrocatechol (310 mg, 2.00 mmol) in 1 mL DMF was added. The mixture was stirred at 0 °C for 30 min (until gas formation ceased) and then a solution of (R)-glycidyl tosylate (502 mg, 2.20 mmol) in 1 mL DMF was added. The reaction mixture was stirred at room temperature for 30 min and then heated at 80 °C for 2 h. Upon cooling, the mixture was poured onto 15 mL water. The mixture was extracted with 3 × 20 mL diethyl ether. The combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness. The crude product obtained was purified by column cromatography (petrolether:ethyl acetate = 1:1) to give 212 mg (50%) of pale yellow crystals; mp 112–114 °C; $[\alpha]_D = -86.6$ ($c = 0.51$, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 1.91 (t, $J = 6.20$ Hz, 1H, OH), 3.85–4.04 (m, 2H, CH$_2$OH), 4.10–4.25 (m, 1H, CH), 4.33–4.46 (m, 2H, CH$_2$O), 6.95–7.04 (m, 1H, Ar–H^8), 7.76–7.88 (m, 2H, Ar–H^5, Ar–H^7).

6.6. Synthesis of (R)-(7-nitro-2,3-dihydro-1,4-benzodioxin-2-yl) methanol (5c)

A suspension of 4-nitrocatechol (310 mg, 2.00 mmol), (S)-glycidyl tosylate (545 mg, 2.40 mmol) and K$_2$CO$_3$ (663 mg, 4.80 mmol) in 5 mL DMF was heated at 60 °C for 2 h. The mixture was poured onto 15 mL water and extracted with 3 × 20 mL diethyl ether. The combined organic phases were subsequently washed with 3 × 20 mL 10% aq. K$_2$CO$_3$, 20 mL water and 20 mL brine. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The crude product obtained was purified by column cromatography (petrolether:ethyl acetate = 1:1) to give 191 mg of pale yellow crystals (45%); mp 117–119 °C; $[\alpha]_D = +59.72$ ($c = 0.5$ in CHCl$_3$), lit.:

[52] +65.5 ($c = 0.58$, 96% EtOH); ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 1.94 (t, $J = 6.21$ Hz, 1H, OH), 3.84–4.08 (m, 2H, CH$_2$OH), 4.12–4.28 (m, 1H, CH), 4.28–4.49 (m, 2H, CH$_2$O), 6.94–7.02 (m, 1H, Ar–H^5), 7.74–7.87 (m, 2H, Ar–H^6, Ar–H^8).

6.7. Synthesis of (S)-(7-nitro-2,3-dihydro-1,4-benzodioxin-2-yl) methanol (5d)

A suspension of 4-nitrocatechol (310 mg, 2.00 mmol), (R)-glycidyl tosylate (545 mg, 2.40 mmol) and K$_2$CO$_3$ (663 mg, 4.80 mmol) in 5 mL DMF was heated at 60 °C for 2 h. The mixture was poured onto 15 mL water and extracted with 4 × 20 mL diethyl ether. The combined organic phases were subsequently washed with 3 × 20 mL 10% aq. K$_2$CO$_3$, 20 mL water and 20 mL brine. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The crude product obtained was purified by column cromatography (petrolether:ethyl acetate = 1:1) to give 180 mg of yellow crystals (43%); mp 117–119 °C; $[\alpha]_D = -61.6$ ($c = 0.5$ in CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm) 1.93 (t, $J = 6.19$ Hz, 1H, OH), 3.86–4.04 (m, 2H, CH$_2$OH), 4.13–4.27 (m, 1H, CH), 4.30–4.47 (m, 2H, CH$_2$O), 6.93–7.03 (m, 1H, Ar–H^5), 7.75–7.86 (m, 2H, Ar–H^6, Ar–H^8).

6.8. (R)-4-((6-nitro-2,3-dihydro-1,4-benzodioxin-2-yl)methoxy) benzonitrile (6a). General procedure for synthesis of compounds 6a–d

The benzodioxine alcohol 5a (2.80 g, 13.26 mmol) was dissolved in anhydrous THF (50 mL). Under argon flow and with ice/water cooling, 4-cyanophenol (1.74 g, 14.59 mmol) and triphenylphosphine (6.96 g, 26.52 mmol) were added. Diizopropyl azodicarboxylate (DIAD) (5.36 g, 26.52 mmol) dissolved in 15 mL anhydrous THF was added dropwise at 0 °C. The solution was stirred for 30 min at 0 °C, and then heated to reflux for 48 h. The mixture was evaporated to dryness and the crude product was purified by recrystallization from MeOH to give 2.15 g (52%) of 6a as pale yellow crystals; mp 159–162 °C; $[\alpha]_D = +83.4$ ($c = 0.5$ in CDCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.28 (dd, $J = 11.8$, 7.2 Hz, 1H, 3-CH$_2$), 4.39 (dd, $J = 11.1$, 5.7 Hz, 1H, CH$_2$O), 4.46 (dd, $J = 11.1$, 3.7 Hz, 1H, CH$_2$O), 4.58 (dd, $J = 11.8$, 2.5 Hz, 1H, 3-CH$_2$), 4.77–4.85 (m, 1H, 2-CH), 7.13–7.22 (m, 3H, Ar–H^8, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.77–7.84 (m, 4H, Ar–H^5, Ar–H^7, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 64.4 (C-3), 66.5 (CH$_2$O), 72.2 (C-2), 103.4 (C-4'), 112.7 (C-7), 115.6 (C-2', C-6'), 117.6, 117.7 (C-5, C-8), 119.0 (CN), 134.2 (C-3', C-5'), 141.1 (C-6), 142.7 (C-4a), 148.8 (C-8a), 161.4 (C-1'); HRMS (ESI) m/z calcd for C$_{16}$H$_{13}$N$_2$O$_5$ [M + H]$^+$ 313.0824, found 313.0830; IR (KBr, v, cm^{-1}): 2222, 1605, 1522, 1471, 1347, 1252, 1174, 839; HPLC: 100%, t_r 16.0 min; Anal. (C$_{16}$H$_{12}$N$_2$O$_5$) C, H, N.

6.9. (S)-4-((6-nitro-2,3-dihydro-1,4-benzodioxin-2-yl)methoxy) benzonitrile (6b)

Synthesized from 5b (1.17 g, 5.54 mmol) according to the general procedure for synthesis of compounds 6a–d; pale yellow crystals, yield 865 mg (50%); mp 172–175 °C; $[\alpha]_D = -80.4$ ($c = 0.5$, CDCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.33 (dd, $J = 11.7$, 7.3 Hz, 1H, 3-CH$_2$), 4.38 (dd, $J = 11.1$, 5.6 Hz, 1H, CH$_2$O), 4.45 (dd, $J = 11.1$, 3.7 Hz, 1H, CH$_2$O), 4.62 (dd, $J = 11.7$, 2.5 Hz, 1H, 3-CH$_2$), 4.72–4.78 (m, 1H, 2-CH), 7.11–7.23 (m, 3H, Ar–H^8, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.76–7.84 (m, 4H, Ar–H^5, Ar–H^7, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 65.0 (C-3), 66.5 (CH$_2$O), 71.4 (C-2), 103.4 (C-4'), 112.7 (C-7), 115.7 (C-2', C-6'), 117.5, 117.6 (C-5, C-8), 119.0 (CN), 134.2 (C-3', C-5'), 141.2 (C-6), 142.4, 149.0 (C-4a, C-8a), 161.4 (C-1'); HRMS (ESI) m/z calcd for C$_{16}$H$_{13}$N$_2$O$_5$ [M + H]$^+$ 313.0824, found 313.0813; IR (KBr, v, cm^{-1}): 2227, 1603, 1514, 1349, 1256, 1174, 839; HPLC: 100%, t_r 16.0 min; Anal. (C$_{16}$H$_{12}$N$_2$O$_5$) C, H, N.

6.10. (R)-4-((7-nitro-2,3-dihydro-1,4-benzodioxin-2-yl)methoxy) benzonitrile (**6c**)

Synthesized from **5c** (1.17 g, 5.54 mmol) according to the general procedure for synthesis of compounds **6a–d**; pale yellow crystals, yield 865 mg (50%); mp 159–162 °C; $[\alpha]_D = -29.7$ ($c = 0.5$, CDCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.33 (dd, $J = 11.7$, 7.3 Hz, 1H, 3-CH$_2$), 4.38 (dd, $J = 11.2$, 5.7 Hz, 1H, CH$_2$O), 4.45 (dd, $J = 11.2$, 3.7 Hz, 1H, CH$_2$O), 4.62 (dd, $J = 11.7$, 2.5 Hz, 1H, 3-CH$_2$), 4.71–4.79 (m, 1H, 2-CH), 7.14–7.21 (m, 3H, Ar–H^5, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.75–7.85 (m, 4H, Ar–H^6, Ar–H^8, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 65.0 (C-3), 66.5 (CH$_2$O), 71.4 (C-2), 103.4 (C-4'), 112.7 (C-6), 115.7 (C-2', C-6'), 117.5, 117.6 (C-5, C-8), 119.0 (CN), 134.2 (C-3', C-5'), 141.2 (C-7), 142.5, 149.0 (C-4a, C-8a), 161.4 (C-1'); HRMS (ESI) m/z calcd for C$_{16}$H$_{13}$N$_2$O$_5$ [M + H]$^+$ 313.0824, found 313.0827; IR (KBr, v, cm^{-1}): 2227, 1605, 1515, 1457, 1350, 1256, 1175, 839; HPLC: 100%, t_r 16.0 min; Anal. (C$_{16}$H$_{12}$N$_2$O$_5$) C, H, N.

6.11. (S)-4-((7-nitro-2,3-dihydro-1,4-benzodioxin-2-yl)methoxy) benzonitrile (**6d**)

Synthesized from **5d** (1.17 g, 5.54 mmol) according to the general procedure for synthesis of compounds **6a–d**; pale yellow crystals, yield 830 mg (48%); mp 168–171 °C; $[\alpha]_D = +30.2$ ($c = 0.5$, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.33 (dd, $J = 11.7$, 7.3 Hz, 1H, 3-CH$_2$), 4.38 (dd, $J = 11.1$, 5.7 Hz, 1H, CH$_2$O), 4.45 (dd, $J = 11.1$, 3.7 Hz, 1H, CH$_2$O), 4.62 (dd, $J = 11.7$, 2.5 Hz, 1H, 3-CH$_2$), 4.68–4.81 (m, 1H, 2-CH), 7.11–7.23 (m, 3H, Ar–H^5, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.74–7.85 (m, 4H, Ar–H^6, Ar–H^8, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 64.9 (C-3), 66.5 (CH$_2$O), 71.4 (C-2), 103.4 (C-4'), 112.7 (C-6), 115.7 (C-2', C-6'), 117.5, 117.6 (C-5, C-8), 119.0 (CN), 134.2 (C-3', C-5'), 141.2 (C-7), 142.5, 149.0 (C-4a, C-8a), 161.4 (C-1'); HRMS (ESI) m/z calcd for C$_{16}$H$_{13}$N$_2$O$_5$ [M + H]$^+$ 313.0824, found 313.0833; IR (KBr, v, cm^{-1}): 2225, 1600, 1504, 1349, 1251, 820; HPLC: 100%, t_r 16.0 min; Anal. (C$_{16}$H$_{12}$N$_2$O$_5$) C, H, N.

6.12. (R)-4-((6-(benzylamino)-2,3-dihydro-1,4-benzodioxin-2-yl) methoxy)benzonitrile (**8a**). General procedure for synthesis of compounds **8a–d**

To a solution of compound **6a** (800 mg, 2.56 mmol) in 100 mL MeOH, 10% Pd/C (80 mg) was added and the mixture was stirred in a hydrogenator at 25 bar for 1 h till the reaction was completed. The catalyst was filtered off and the solvent was evaporated *in vacuo* to yield 720 mg (100%) of **7a** which was used in the next step without purification. To a solution of the crude amine **7a** (722 mg, 2.56 mmol) in methanol (50 mL) containing molecular sieves under argon atmosphere, benzaldehyde (406 mg, 3.83 mmol) was added and the mixture was stirred at room temperature for 12 h, until the aldimine formation was completed. The reaction mixture containing aldimine in methanol was carefully treated with NaBH$_4$ (154 mg, 4.08 mmol) and stirred for additional 1 h, filtered, and the solvent was evaporated *in vacuo*. The crude residue was dissolved in dichloromethane (50 mL) and washed successively with saturated NaHCO$_3$ solution (3 × 50 mL) and brine (1 × 50 mL). The organic phase was dried over Na$_2$SO$_4$ and the solvent evaporated under reduced pressure. The oily product was purified by column chromatography using dichloromethane as eluant to obtain 439 mg (46%) of **8a** as yellow crystals; mp 144–147 °C; $[\alpha]_D = +32.4$ ($c = 0.5$, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.05 (dd, $J = 11.4$, 7.9 Hz, 1H, 3-CH$_2$), 4.18 (s, 2H, NCH$_2$), 4.22–4.36 (m, 3H, 3-CH$_2$, CH$_2$O), 4.38–4.47 (m, 1H, 2-CH), 5.92 (bs, 1H, NH), 6.09 (d, $J = 2.6$ Hz, 1H, Ar–H^5), 6.15 (dd, $J = 8.7$, 2.6 Hz, 1H, Ar–H^7), 6.62 (d, $J = 8.7$ Hz, 1H, Ar–H^8), 7.16 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.19–

7.25 (m, 1H, Ph), 7.27–7.39 (m, 4H, Ph), 7.78 (d, $J = 9.0$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 47.0 (Ph–CH$_2$), 64.7 (C-3), 66.9 (CH$_2$O), 70.7 (C-2), 100.4 (C-5), 103.2 (C-4'), 106.5 (C-7), 115.6 (C-2', C-6'), 117.2 (C-8), 119.0 (CN), 126.5 (C-4''), 127.1 (C-2'', C-6''), 128.2 (C-3'', C-5''), 133.3 (C-8a), 134.2 (C-3', C-5'), 140.4 (C-6), 143.0 (C-1''), 143.7 (C-4a), 161.6 (C-1'); HRMS (ESI) m/z calcd for C$_{23}$H$_{21}$N$_2$O$_3$ [M + H]$^+$ 373. 1552, found 373.1547; IR (KBr, v, cm^{-1}): 3378, 2221, 1508, 1250, 1176, 828; HPLC: 100%, t_r 11.7 min; Anal. (C$_{23}$H$_{20}$N$_2$O$_3$) C, H, N.

6.13. (S)-4-((6-(benzylamino)-2,3-dihydro-1,4-benzodioxin-2-yl) methoxy)benzonitrile (**8b**)

Synthesized from **6b** (800 mg, 2.56 mmol) according to the general procedure for synthesis of compounds **8a–d**; yellow crystals, yield 439 mg (46%); mp 88–91 °C; $[\alpha]_D = -32.8$ ($c = 0.5$ in CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.00 (dd, $J = 11.4$, 7.2 Hz, 1H, 3-CH$_2$), 4.18 (d, $J = 5.9$ Hz, 2H, NCH$_2$), 4.23–4.35 (m, 3H, 3-CH$_2$, CH$_2$O), 4.44–4.55 (m, 1H, 2-CH), 5.93 (bt, $J = 6.1$ Hz, 1H, NH), 6.07 (d, $J = 2.6$ Hz, 1H, Ar–H^5), 6.15 (dd, $J = 8.7$, 2.7 Hz, 1H, Ar–H^7), 6.62 (d, $J = 8.7$ Hz, 1H, Ar–H^8), 7.16 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.19–7.26 (m, 1H, Ph), 7.28–7.37 (m, 4H, Ph), 7.78 (d, $J = 9.0$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 47.0 (Ph–CH$_2$), 64.2 (C-3), 66.9 (CH$_2$O), 71.4 (C-2), 100.3 (C-5), 103.2 (C-4'), 106.3 (C-7), 115.6 (C-2', C-6'), 117.1 (C-8), 119.0 (CN), 126.4 (C-4''), 127.1 (C-2'', C-6''), 128.2 (C-3'', C-5''), 133.6 (C-8a), 134.2 (C-3', C-5'), 140.4 (C-6), 142.7 (C-1''), 143.9 (C-4a), 161.6 (C-1'); HRMS (ESI) m/z calcd for C$_{23}$H$_{21}$N$_2$O$_3$ [M + H]$^+$ 373.1552, found 373.1534; IR (KBr, v, cm^{-1}): 2219, 1606, 1504, 1260, 1174, 1045, 830; HPLC: 98.1%, t_r 11.7 min; Anal. (C$_{23}$H$_{20}$N$_2$O$_3$) C, H, N.

6.14. (R)-4-((7-(benzylamino)-2,3-dihydro-1,4-benzodioxin-2-yl) methoxy)benzonitrile (**8c**)

Synthesized from **6c** (800 mg, 2.56 mmol) according to the general procedure for synthesis of compounds **8a–d**; yellow crystals, yield 420 mg (44%); mp 111–114 °C; $[\alpha]_D = -42.5$ ($c = 0.8$, CDCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.00 (dd, $J = 11.5$, 7.2 Hz, 1H, 3-CH$_2$), 4.18 (d, $J = 4.8$ Hz, 2H, NCH$_2$), 4.22–4.35 (m, 3H, 3-CH$_2$, CH$_2$O), 4.43–4.57 (m, 1H, 2-CH), 5.93 (bt, $J = 5.6$ Hz, 1H, NH), 6.07 (d, $J = 2.6$ Hz, 1H, Ar–H^8), 6.15 (dd, $J = 8.7$, 2.6 Hz, 1H, Ar–H^6), 6.62 (d, $J = 8.7$ Hz, 1H, Ar–H^5), 7.16 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.19–7.25 (m, 1H, Ph), 7.27–7.38 (m, 4H, Ph), 7.78 (d, $J = 9.0$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 47.0 (Ph–CH$_2$) 64.2 (C-3), 66.7 (CH$_2$O), 71.4 (C-2), 100.3 (C-8), 103.2 (C-4'), 106.3 (C-6), 115.6 (C-2', C-6'), 117.1 (C-5), 119.0 (CN), 126.5 (C-4''), 127.1 (C-2'', C-6''), 128.2 (C-3'', C-5''), 133.6 (C-8a), 134.2 (C-3', C-5'), 140.4 (C-7), 142.7 (C-1''), 143.9 (C-4a), 161.6 (C-1'); HRMS (ESI) m/z calcd for C$_{23}$H$_{21}$N$_2$O$_3$ [M + H]$^+$ 373. 1552, found 373.1545; IR (KBr, v, cm^{-1}): 3387, 3050, 2220, 1508, 1262, 1174, 832; HPLC: 98.8%, t_r 11.7 min; Anal. (C$_{23}$H$_{20}$N$_2$O$_3$) C, H, N.

6.15. (S)-4-((7-(benzylamino)-2,3-dihydro-1,4-benzodioxin-2-yl) methoxy)benzonitrile (**8d**)

Synthesized from **7d** (800 mg, 2.56 mmol) according to the general procedure for synthesis of compounds **8a–d**; yellow crystals, yield 430 mg (45%); mp 86–89 °C; $[\alpha]_D = +44.4$ ($c = 0.8$, CDCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.00 (dd, $J = 11.4$, 7.2 Hz, 1H, 3-CH$_2$), 4.18 (d, $J = 5.7$ Hz, 2H, NCH$_2$), 4.24–4.34 (m, 3H, 3-CH$_2$, CH$_2$O), 4.46–4.53 (m, 1H, 2-CH), 5.93 (bt, $J = 5.9$ Hz, 1H, NH), 6.07 (d, $J = 2.6$ Hz, 1H, Ar–H^8), 6.15 (dd, $J = 8.7$, 2.6 Hz, 1H, Ar–H^6), 6.62 (d, $J = 8.7$ Hz, 1H, Ar–H^5), 7.16 (d, $J = 8.9$ Hz, 2H,

Ar–H$^{2'}$, Ar–H$^{6'}$), 7.18–7.26 (m, 1H, Ph), 7.28–7.37 (m, 4H, Ph), 7.78 (d, $J = 8.9$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 47.0 (Ph–$\underline{CH_2}$), 64.2 (C-3), 66.9 (CH$_2$O), 71.4 (C-2), 100.3 (C-8), 103.2 (C-4'), 106.3 (C-6), 115.6 (C-2', C-6'), 117.1 (C-5), 119.0 (CN), 126.4 (C-4''), 127.1 (C-2'', C-6''), 128.2 (C-3'', C-5''), 133.6 (C-8a), 134.2 (C-3', C-5'), 140.4 (C-7), 142.7 (C-1''), 143.9 (C-4a), 161.6 (C-1'); HRMS (ESI) m/z calcd for C$_{23}$H$_{21}$N$_2$O$_3$ [M + H]$^+$ 373.1552, found 373.1548; IR (KBr, v, cm^{-1}): 3386, 3059, 2220, 1606, 1509, 1262, 1174, 832; HPLC: 93.6%, t_r 11.8 min; Anal. (C$_{23}$H$_{20}$N$_2$O$_3$) C, H, N.

6.16. (R)-ethyl 2-(benzyl(2-((4-cyanophenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-6-yl)amino)-2-oxoacetate (9a). General procedure for the synthesis of compounds 9a–d

Ethyl oxalyl chloride (185 mg, 1.35 mmol) was added to a solution of **8a** (420 mg, 1.13 mmol) and triethylamine (136 mg, 1.35 mmol) in dichloromethane (50 mL) and the mixture was stirred for 2 h. The solvent was removed under reduced pressure, the residue dissolved in ethyl acetate (50 mL) and washed successively with a 10% citric acid solution (3 × 50 mL), saturated NaHCO$_3$ solution (3 × 50 mL) and brine (1 × 50 mL). The organic phase was dried over Na$_2$SO$_4$ and the solvent evaporated under reduced pressure. The oily product was purified by column chromatography on silica gel using dichloromethane as eluant to obtain 497 mg (93%) of **9a** as pale yellow solid; mp 42–45 °C; [α]$_D$ = +28.4 (c = 0.5, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 0.93 (t, $J = 7.1$ Hz, 3H, CH$_2$$\underline{CH_3}$), 4.02 (q, $J = 7.1$ Hz, 2H, $\underline{CH_2}$CH$_3$), 4.13 (dd, $J = 11.6$, 7.4 Hz, 1H, 3-CH$_2$), 4.30 (dd, $J = 11.0$, 5.7 Hz, 1H, CH$_2$O), 4.37 (dd, $J = 11.0$, 3.7 Hz, 1H, CH$_2$O), 4.44 (dd, $J = 11.6$, 2.4 Hz, 1H, 3-CH$_2$), 4.56–4.64 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.62 (dd, $J = 8.6$, 2.7 Hz, 1H, Ar–H^7), 6.78 (d, $J = 2.7$ Hz, 1H, Ar–H^5), 6.89 (d, $J = 8.6$ Hz, 1H, Ar–H^8), 7.15 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.18–7.22 (m, 2H, Ph), 7.27–7.37 (m, 3H, Ph), 7.79 (d, $J = 9.0$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 13.4 (CH$_2$–$\underline{CH_3}$), 50.8 (Ph–$\underline{CH_2}$), 61.3 ($\underline{CH_2}$–CH$_3$), 64.4 (C-3), 66.6 (CH$_2$O), 71.3 (C-2), 103.3 (C-4'), 115.6 (C-2', C-6'), 116.2 (C-7), 117.4 (C-5), 119.0 (CN), 120.9 (C-8), 127.5 (C-4''), 128.0 (C-2'', C-6''), 128.6 (C-3'', C-5''), 132.3 (C-6), 134.2 (C-3', C-5'), 136.1 (C-1''), 142.6, 142.7 (C-4a, C-8a), 161.4, 161.5, 162.4 ($\underline{CO}$–COO, CO–$\underline{COO}$, C-1'); HRMS (ESI) m/z calcd for C$_{27}$H$_{25}$N$_2$O$_6$ [M + H]$^+$ 473.1713, found 473.1706; IR (KBr, v, cm^{-1}): 2224, 1605, 1507, 1252, 1175, 1016, 834; HPLC: 100%, t_r 17.6 min; Anal. (C$_{27}$H$_{24}$N$_2$O$_6$) C, H, N.

6.17. (S)-ethyl 2-(benzyl(2-((4-cyanophenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-6-yl)amino)-2-oxoacetate (9b)

Synthesized from **8b** (420 mg, 1.13 mmol) according to the general procedure for the synthesis of compounds **9a–d**; pale yellow solid, yield 481 mg (90%); mp 41–44 °C; [α]$_D$ = −27.8 (c = 0.5, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 0.90 (t, $J = 7.1$ Hz, 3H, CH$_2$$\underline{CH_3}$), 4.00 (q, $J = 7.1$ Hz, 2H, $\underline{CH_2}$CH$_3$), 4.14 (dd, $J = 11.6$, 7.3 Hz, 1H, 3-CH$_2$), 4.29 (dd, $J = 11.0$, 5.9 Hz, 1H, CH$_2$O), 4.37 (dd, $J = 11.0$, 3.5 Hz, 1H, CH$_2$O), 4.44 (dd, $J = 11.6$, 2.4 Hz, 1H, 3-CH$_2$), 4.56–4.63 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.62 (dd, $J = 8.6$, 2.5 Hz, 1H, Ar–H^7), 6.80 (d, $J = 2.5$ Hz, 1H, Ar–H^5), 6.88 (d, $J = 8.6$ Hz, 1H, Ar–H^8), 7.16 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.17–7.21 (m, 2H, Ph), 7.23–7.36 (m, 3H, Ph), 7.79 (d, $J = 8.9$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 13.3 (CH$_2$–$\underline{CH_3}$), 50.8 (Ph–$\underline{CH_2}$), 61.3 ($\underline{CH_2}$–CH$_3$), 64.4 (C-3), 66.6 (CH$_2$O), 71.3 (C-2), 103.3 (C-4'), 115.6 (C-2', C-6'), 116.2 (C-7), 117.2 (C-5), 119.0 (CN), 120.7 (C-8), 127.5 (C-4''), 127.9 (C-2'', C-6''), 128.6 (C-3'', C-5''), 132.5 (C-6), 134.2 (C-3', C-5'), 136.1 (C-1''), 142.5, 142.8 (C-4a, C-8a), 161.4, 161.5, 162.4 ($\underline{CO}$–COO, CO–$\underline{COO}$, C-1'); HRMS (ESI) m/z calcd for C$_{27}$H$_{25}$N$_2$O$_6$ [M + H]$^+$ 473.1713, found 473.1732; IR (KBr, v, cm^{-1}): 2225, 1741,

6.18. (R)-ethyl 2-(benzyl(3-((4-cyanophenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-7-yl)amino)-2-oxoacetate (9c)

Synthesized from **8c** (420 mg, 1.13 mmol) according to the general procedure for the synthesis of compounds **9a–d**; pale yellow solid, yield 486 mg (91%); mp 43–46 °C; [α]$_D$ = −14.2 (c = 0.5, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 0.90 (t, $J = 7.1$ Hz, 3H, CH$_2$$\underline{CH_3}$), 4.00 (q, $J = 7.1$ Hz, 2H, $\underline{CH_2}$CH$_3$), 4.14 (dd, $J = 11.6$, 7.3 Hz, 1H, 3-CH$_2$), 4.29 (dd, $J = 11.0$, 5.8 Hz, 1H, CH$_2$O), 4.37 (dd, $J = 11.0$, 3.6 Hz, 1H, CH$_2$O), 4.44 (dd, $J = 11.6$, 2.4 Hz, 1H, 3-CH$_2$), 4.55–4.64 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.62 (dd, $J = 8.6$, 2.6 Hz, 1H, Ar–H^6), 6.80 (d, $J = 2.6$ Hz, 1H, Ar–H^8), 6.88 (d, $J = 8.6$ Hz, 1H, Ar–H^5), 7.16 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.17–7.21 (m, 2H, Ph), 7.25–7.35 (m, 3H, Ph), 7.80 (d, $J = 9.0$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 13.3 (CH$_2$–$\underline{CH_3}$), 50.8 (Ph–$\underline{CH_2}$), 61.3 ($\underline{CH_2}$–CH$_3$), 64.4 (C-3), 66.6 (CH$_2$O), 71.3 (C-2), 103.3 (C-4'), 115.6 (C-2', C-6'), 116.2 (C-6), 117.2 (C-5), 119.0 (CN), 120.7 (C-8), 127.5 (C-4''), 127.9 (C-2'', C-6''), 128.6 (C-3'', C-5''), 132.5 (C-7), 134.2 (C-3', C-5'), 136.1 (C-1''), 142.5, 142.8 (C-4a, C-8a), 161.4, 161.5, 162.4 ($\underline{CO}$–COO, CO–$\underline{COO}$, C-1'); HRMS (EI) m/z calcd for C$_{27}$H$_{25}$N$_2$O$_6$ [M + H]$^+$ 473.1713, found 473.1718; IR (KBr, v, cm^{-1}): 2224, 1741, 1669, 1605, 1508, 1257, 1173, 1028, 834; HPLC: 100%, t_r 17.5 min; Anal. (C$_{27}$H$_{24}$N$_2$O$_6$) C, H, N.

6.19. (S)-ethyl 2-(benzyl(3-((4-cyanophenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-7-yl)amino)-2-oxoacetate (9d)

Synthesized from **8d** (420 mg, 1.13 mmol) according to the general procedure for the synthesis of compounds **9a–d**; pale yellow solid, yield 470 mg (88%); mp 45–48 °C; [α]$_D$ = +12.8 (c = 0.5, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 0.93 (t, $J = 7.1$ Hz, 3H, CH$_2$$\underline{CH_3}$), 4.02 (q, $J = 7.1$ Hz, 2H, $\underline{CH_2}$CH$_3$), 4.13 (dd, $J = 11.6$, 7.4 Hz, 1H, 3-CH$_2$), 4.30 (dd, $J = 11.0$, 5.8 Hz, 1H, CH$_2$O), 4.37 (dd, $J = 11.0$, 3.7 Hz, 1H, CH$_2$O), 4.44 (dd, $J = 11.6$, 2.4 Hz, 1H, 3-CH$_2$), 4.56–4.64 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.62 (dd, $J = 8.6$, 2.5 Hz, 1H, Ar–H^6), 6.78 (d, $J = 2.5$ Hz, 1H, Ar–H^8), 6.89 (d, $J = 8.6$ Hz, 1H, Ar–H^5), 7.15 (d, $J = 9.0$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.17–7.22 (m, 2H, Ph), 7.25–7.38 (m, 3H, Ph), 7.79 (d, $J = 9.0$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 13.4 (CH$_2$–$\underline{CH_3}$), 50.8 (Ph–$\underline{CH_2}$), 61.3 ($\underline{CH_2}$–CH$_3$), 64.4 (C-3), 66.6 (CH$_2$O), 71.3 (C-2), 103.3 (C-4'), 115.6 (C-2', C-6'), 116.1 (C-6), 117.4 (C-5), 119.0 (CN), 120.9 (C-8), 127.5 (C-4''), 128.0 (C-2'', C-6''), 128.6 (C-3'', C-5''), 132.3 (C-7), 134.2 (C-3', C-5'), 136.1 (C-1''), 142.6, 142.7 (C-4a, C-8a), 161.4, 161.5, 162.4 ($\underline{CO}$–COO, CO–$\underline{COO}$, C-1'); HRMS (ESI) m/z calcd for C$_{27}$H$_{25}$N$_2$O$_6$ [M + H]$^+$ 473.1713, found 473.1704; IR (KBr, v, cm^{-1}): 2225, 1741, 1670, 1606, 1508, 1256, 1172, 1029, 835; HPLC: 100%, t_r 17.5 min; Anal. (C$_{27}$H$_{24}$N$_2$O$_6$ × 1/2H$_2$O) C, H, N.

6.20. (R)-ethyl 2-(benzyl(2-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-6-yl)amino)-2-oxoacetate trifluoroacetate (10a). General procedure for synthesis of amidines 10a–d

Gaseous HCl was slowly introduced over 30 min into a solution of the nitrile **9a** (480 mg, 1.02 mmol) in anhydrous ethanol (30 mL). The reaction mixture was closed tightly and stirred for 24 h at room temperature. The solvent was evaporated *in vacuo* and the residue washed 3 times with anhydrous diethyl ether. Obtained iminoether was dissolved in anhydrous EtOH (30 mL), ammonium acetate (3 eq, 237 mg, 3.06 mmol) was added and the reaction mixture stirred for 24 h at room temperature. The solvent was evaporated and crude product was purified by reverse phase column chromatography with gradient using methanol/trifluoroacetic acid (40–80%

in 30 min) as eluant. After evaporation of methanol white crystals were precipitated from trifluoroacetic acid, filtered off and dried to yield 350 mg (70%) of **10a** as a white powder; yield 350 mg (58%); mp 188–191 °C; $[\alpha]_D$ = +31.4 (c = 0.5, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.93 (t, J = 7.1 Hz, 3H, CH$_2$CH$_3$), 4.02 (q, J = 7.1 Hz, 2H, CH$_2$CH$_3$), 4.14 (dd, J = 11.6, 7.4 Hz, 1H, 3-CH$_2$), 4.32 (dd, J = 11.0, 5.8 Hz, 1H, CH$_2$O), 4.39 (dd, J = 11.0, 3.7 Hz, 1H, CH$_2$O), 4.46 (dd, J = 11.6, 2.4 Hz, 1H, 3-CH$_2$), 4.57–4.65 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.63 (dd, J = 8.6, 2.6 Hz, 1H, Ar–H[7]), 6.79 (d, J = 2.6 Hz, 1H, Ar–H[6]), 6.89 (d, J = 8.6 Hz, 1H, Ar–H[8]), 7.17–7.24 (m, 4H, Ph, Ar–H[2'], Ar–H[6']), 7.25–7.38 (m, 3H, Ph), 7.83 (d, J = 9.0 Hz, 2H, Ar–H[3'], Ar–H[5']), 8.92 (bs, 2H, NH$_2$), 9.16 (bs, 2H, NH$_2^+$); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 13.4 (CH$_2$–CH$_3$), 50.8 (Ph–CH$_2$), 61.3 (CH$_2$–CH$_3$), 64.4 (C-3), 66.7 (CH$_2$O), 71.3 (C-2), 114.8 (C-2', C-6'), 116.2 (C-7), 117.4 (C-5), 120.1 (C-4'), 120.9 (C-8), 127.6 (C-4''), 128.0 (C-2'', C-6''), 128.6 (C-3'', C-5''), 130.2 (C-3', C-5'), 132.3 (C-6), 136.1 (C-1''), 142.6, 142.7 (C-4a, C-8a), 161.5, 162.3, 162.4, 164.5 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$), 117,1 (CF$_3$–COOH, [1]$J_{C,F}$ = 299,3 Hz), 158,7 (CF$_3$–COOH, [2]$J_{C,F}$ = 31,5 Hz); HRMS (ESI) m/z calcd for C$_{27}$H$_{28}$N$_3$O$_6$ [M + H]$^+$ 490.1978, found 490.1967; IR (KBr, v, cm^{-1}): 3299, 3116, 1737, 1667, 1506, 1203, 844; HPLC: 99.0%, t_r 12.5 min; Anal. (C$_{27}$H$_{27}$N$_3$O$_6$ × CF$_3$COOH) C, H, N.

6.21. (S)-ethyl 2-(benzyl(2-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-6-yl)amino)-2-oxoacetate trifluoroacetate (10b)

Synthesized from **9b** (480 mg, 1.02 mmol) according to the general procedure for synthesis of amidines **10a–d**; white powder, yield 325 mg (53%); mp 190–193 °C; $[\alpha]_D$ = −32.0 (c = 0.5 in EtOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.93 (t, J = 7.1 Hz, 3H, CH$_2$CH$_3$), 4.02 (q, J = 7.1 Hz, 2H, CH$_2$CH$_3$), 4.14 (dd, J = 11.6, 7.4 Hz, 1H, 3-CH$_2$), 4.32 (dd, J = 11.0, 5.7 Hz, 1H, CH$_2$O), 4.39 (dd, J = 11.0, 3.6 Hz, 1H, CH$_2$O), 4.46 (dd, J = 11.6, 2.4 Hz, 1H, 3-CH$_2$), 4.57–4.66 (m, 1H, 2-CH), 4.91 (s, 2H, NCH$_2$), 6.63 (dd, J = 8.6, 2.7 Hz, 1H, Ar–H[7]), 6.79 (d, J = 2.7 Hz, 1H, Ar–H[5]), 6.89 (d, J = 8.6 Hz, 1H, Ar–H[8]), 7.16–7.25 (m, 4H, Ph, Ar–H[2'], Ar–H[6']), 7.27–7.37 (m, 3H, Ph), 7.83 (d, J = 9.0 Hz, 2H, Ar–H[3'], Ar–H[5']), 8.92 (bs, 2H, NH$_2$), 9.16 (bs, 2H, NH$_2^+$); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 13.4 (CH$_2$–CH$_3$), 50.8 (Ph–CH$_2$), 61.3 (CH$_2$–CH$_3$), 64.4 (C-3), 66.7 (CH$_2$O), 71.3 (C-2), 114.8 (C-2', C-6'), 116.2 (C-7), 117.4 (C-5), 120.1 (C-4'), 120.9 (C-8), 127.6 (C-4''), 128.0 (C-2'', C-6''), 128.6 (C-3'', C-5''), 130.2 (C-3', C-5'), 132.3 (C-6), 136.1 (C-1''), 142.6, 142.7 (C-4a, C-8a), 161.5, 162.3, 162.4, 164.5 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$), 117.1 (CF$_3$–COOH, [1]$J_{C,F}$ = 299.2 Hz), 158.8 (CF$_3$–COOH, [2]$J_{C,F}$ = 31.5 Hz); HRMS (ESI) m/z calcd for C$_{27}$H$_{28}$N$_3$O$_6$ [M + H]$^+$ 490.1978, found 490.1963; IR (KBr, v, cm^{-1}): 3298, 3122, 1737, 1665, 1506, 1203, 843; HPLC: 100%, t_r 12.4 min; Anal. (C$_{27}$H$_{27}$N$_3$O$_6$ × CF$_3$COOH × 1/9H$_2$O) C, H, N.

6.22. (R)-ethyl 2-(benzyl(3-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-7-yl)amino)-2-oxoacetate triluoroacetate (10c)

Synthesized from **9c** (480 mg, 1.02 mmol) according to the general procedure for synthesis of amidines **10a–d**; white powder, yield 335 mg (54%); mp 185–188 °C; $[\alpha]_D$ = +11.8 (c = 2.0, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.91 (t, J = 7.0 Hz, 3H, CH$_2$CH$_3$), 4.00 (q, J = 7.0 Hz, 2H, CH$_2$CH$_3$), 4.15 (dd, J = 11.6, 7.2 Hz, 1H, 3-CH$_2$), 4.31 (dd, J = 11.0, 6.0 Hz, 1H, CH$_2$O), 4.39 (dd, J = 11.0, 3.4 Hz, 1H, CH$_2$O), 4.46 (dd, J = 11.6, 2.2 Hz, 1H, 3-CH$_2$), 4.58–4.64 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.63 (dd, J = 8.7, 2.5 Hz, 1H, Ar–H[6]), 6.80 (d, J = 2.5 Hz, 1H, Ar–H[8]), 6.89 (d, J = 8.7 Hz, 1H, Ar–H[5]), 7.15–7.38 (m, 7H, Ph, Ar–H[2'], Ar–H[6']), 7.83 (d, J = 8.9 Hz, 2H, Ar–H[3'], Ar–H[5']), 8.91 (bs, 2H, NH$_2$), 9.17 (bs, 2H, NH$_2^+$); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 13.3 (CH$_2$–CH$_3$), 50.8 (Ph–CH$_2$), 61.3 (CH$_2$–CH$_3$), 64.4 (C-3), 66.7 (CH$_2$O), 71.3 (C-2), 114.8 (C-2', C-6'), 116.2 (C-6), 117.3 (C-5), 120.1 (C-4'), 120.8 (C-8), 127.5 (C-4''), 127.9 (C-2'', C-6''), 128.6 (C-3'', C-5''), 130.2 (C-3', C-5'), 132.5 (C-7), 136.1 (C-1''), 142.5, 142.8 (C-4a, C-8a), 161.5, 162.3, 162.4, 164.5 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$), 117.1 (CF$_3$–COOH, [1]$J_{C,F}$ = 299.2 Hz), 158.8 (CF$_3$–COOH, [2]$J_{C,F}$ = 31.5 Hz); HRMS (ESI) m/z calcd for C$_{27}$H$_{28}$N$_3$O$_6$ [M + H]$^+$ 490.1978, found 490.1996; IR (KBr, v, cm^{-1}): 3345, 3109, 1742, 1670, 1500, 1197, 843; HPLC: 98.4%, t_r 12.7 min; Anal. (C$_{27}$H$_{27}$N$_3$O$_6$ × CF$_3$COOH) C, H, N.

6.23. (S)-ethyl 2-(benzyl(3-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-7-yl)amino)-2-oxoacetate (10d)

Synthesized from **9d** (480 mg, 1.02 mmol) according to the general procedure for synthesis of amidines **10a–d**; white powder, yield 345 mg (56%); mp 188–191 °C; $[\alpha]_D$ = −12.8 (c = 2.0, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.91 (t, J = 7.1 Hz, 3H, CH$_2$CH$_3$), 4.00 (q, J = 7.1 Hz, 2H, CH$_2$CH$_3$), 4.15 (dd, J = 11.6, 7.3 Hz, 1H, 3-CH$_2$), 4.31 (dd, J = 11.0, 5.9 Hz, 1H, CH$_2$O), 4.39 (dd, J = 11.0, 3.5 Hz, 1H, CH$_2$O), 4.46 (dd, J = 11.6, 2.2 Hz, 1H, 3-CH$_2$), 4.53–4.68 (m, 1H, 2-CH), 4.90 (s, 2H, NCH$_2$), 6.63 (dd, J = 8.6, 2.5 Hz, 1H, Ar–H[6]), 6.80 (d, J = 2.5 Hz, 1H, Ar–H[8]), 6.89 (d, J = 8.6 Hz, 1H, Ar–H[5]), 7.17–7.24 (m, 4H, Ph, Ar–H[2'], Ar–H[6']), 7.26–7.37 (m, 3H, Ph), 7.83 (d, J = 8.9 Hz, 2H, Ar–H[3'], Ar–H[5']), 8.93 (bs, 2H, NH$_2$), 9.17 (bs, 2H, NH$_2^+$); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 13.3 (CH$_2$–CH$_3$), 50.8 (Ph–CH$_2$), 61.3 (CH$_2$–CH$_3$), 64.4 (C-3), 66.7 (CH$_2$O), 71.3 (C-2), 114.8 (C-2', C-6'), 116.2 (C-6), 117.3 (C-5), 120.1 (C-4'), 120.8 (C-8), 127.5 (C-4''), 127.9 (C-2'', C-6''), 128.6 (C-3'', C-5''), 130.2 (C-3', C-5'), 132.5 (C-7), 136.1 (C-1''), 142.5, 142.8 (C-4a, C-8a), 161.5, 162.3, 162.4, 164.5 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$); HRMS (ESI) m/z calcd for C$_{27}$H$_{28}$N$_3$O$_6$ [M + H]$^+$ 490.1978, found 490.1987; IR (KBr, v, cm^{-1}): 3342, 3110, 1742, 1670, 1499, 1196, 844; HPLC: 98.4%, t_r 12.7 min; Anal. (C$_{27}$H$_{27}$N$_3$O$_6$ × CF$_3$COOH) C, H, N.

6.24. (R)-2-(benzyl(2-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-6-yl)amino)-2-oxoacetic acid (11a). General procedure for synthesis of compounds 11a–d

To a solution of **10a** (150 mg, 0.31 mmol) in a mixture of tetrahydrofuran (3 mL) and methanol (1 mL), 1 M LiOH (1.50 mL, 1.50 mmol) was added and the reaction mixture stirred at room temperature for 1 h. The solvents was evaporated in vacuum and the resulting aqueous solution neutralized with 0.1% trifluoroacetic acid until the product started to precipitate (pH = 2). The product was filtered off and dried to obtain 79 mg (68%) of **11a** as a white powder; mp 290–293 °C; $[\alpha]_D$ = +59.6 (c = 2.0, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 4.14 (dd, J = 11.6, 7.6 Hz, 1H, 3-CH$_2$), 4.33 (dd, J = 11.0, 5.6 Hz, 1H, CH$_2$O), 4.40 (dd, J = 11.0, 3.7 Hz, 1H, CH$_2$O), 4.46 (dd, J = 11.6, 2.3 Hz, 1H, 3-CH$_2$), 4.57–4.66 (m, 1H, 2-CH), 4.88 (s, 2H, NCH$_2$), 6.67 (dd, J = 8.6, 2.5 Hz, 1H, Ar–H[7]), 6.80 (d, J = 2.5 Hz, 1H, Ar–H[5]), 6.89 (d, J = 8.6 Hz, 1H, Ar–H[8]), 7.18–7.24 (m, 4H, Ph, Ar–H[2'], Ar–H[6']), 7.27–7.35 (m, 3H, Ph), 7.83 (d, J = 8.9 Hz, 2H, Ar–H[3'], Ar–H[5']), 9.04 (bs, 2H, NH$_2$), 9.17 (bs, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 49.6 (Ph–CH$_2$), 64.4 (C-3), 66.7 (CH$_2$O), 71.0 (C-2), 114.7 (C-2', C-6'), 115.9 (C-7), 116.6 (C-5), 120.2 (C-4'), 120.6 (C-8), 127.0 (C-4''), 127.6 (C-2'', C-6''), 128.3 (C-3'', C-5''), 129.8 (C-3', C-5'), 132.3 (C-6), 137.7 (C-1''), 141.4, 142.0 (C-4a, C-8a), 162.0, 163.2, 164.0, 164.6 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$); HRMS (ESI) m/z calcd for C$_{25}$H$_{24}$N$_3$O$_6$ [M + H]$^+$ 462.1665, found 462.1674; IR (KBr, v, cm^{-1}): 3368, 1609, 1503, 1255, 844; HPLC: 96.1%, t_r 9.5 min; Anal. (C$_{25}$H$_{23}$N$_3$O$_6$ × 1/3CF$_3$COOH) C, H, N.

6.25. (S)-2-(benzyl(2-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-6-yl)amino)-2-oxoacetic acid (11b)

Synthesized from **10b** (150 mg, 0.25 mmol) according to the general procedure for synthesis of compounds **11a**–**d**; white powder, yield 69 mg (60%); mp 294–297 °C; $[\alpha]_D = -56.8$ ($c = 2.0$ in DMSO); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 3.91 (dd, $J = 11.2$, 7.1 Hz, 1H, 3-CH$_2$), 3.97 (dd, $J = 10.1$, 5.9 Hz, 1H, CH$_2$O), 4.08 (dd, $J = 10.1$, 5.5 Hz, 1H, CH$_2$O), 4.31 (dd, $J = 11.2$, 1.4 Hz, 1H, 3-CH$_2$), 4.36–4.44 (m, 1H, 2-CH), 4.83 (d, $J = 15.3$ Hz, 1H, NCH$_2$), 4.89 (d, $J = 15.3$ Hz, 1H, NCH$_2$), 6.73–6.85 (m, 3H, Ar–H^8, Ar–H^7, Ar–H^5), 7.03 (d, $J = 8.8$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.17–7.24 (m, 3H, Ph), 7.27–7.33 (m, 2H, Ph), 7.82 (d, $J = 8.8$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$), 9.01 (bs, 2H, NH$_2$), 9.96 (bs, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 50.6 (Ph–CH$_2$), 64.4 (C-3), 66.8 (CH$_2$O), 71.3 (2-C), 114.9 (C-2', C-6'), 116.1 (C-7), 117.3 (C-5), 120.1 (C-4'), 120.9 (C-8), 127.4 (C-4''), 127.8 (C-2'', C-6''), 128.5 (C-3'', C-5''), 130.2 (C-3', C-5'), 132.8 (C-6), 136.3 (C-1''), 142.5, 142.6 (C-4a, C-8a), 162.3, 163.0, 164.1, 164.5 (COCOO, CO–COO, C-1', C(=NH)NH$_2$); HRMS (ESI) m/z calcd for C$_{25}$H$_{24}$N$_3$O$_6$ [M + H]$^+$ 462.1665, found 462.1669; IR (KBr, v, cm^{-1}): 3311, 1610, 1500, 1267, 840; HPLC: 96.6%, t_r 9.4 min; Anal. (C$_{25}$H$_{23}$N$_3$O$_6$ × 1/15CF$_3$COOH) C, H, N.

6.26. (R)-2-(benzyl(3-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-7-yl)amino)-2-oxoacetic acid (11c)

Synthesized from **10c** (150 mg, 0.25 mmol) according to the general procedure for synthesis of compounds **11a**–**d**; white powder, yield 76 mg (66%); mp 291–294 °C; $[\alpha]_D = +16.8$ ($c = 2.0$ in DMSO); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 3.86–3.99 (m, 2H, 3-CH$_2$, CH$_2$O), 4.08 (dd, $J = 10.1$, 5.3 Hz, 1H, CH$_2$O), 4.30 (dd, $J = 11.3$, 1.8 Hz, 1H, 3-CH$_2$), 4.35–4.45 (m, 1H, 2-CH), 4.82 (d, $J = 15.3$ Hz, 1H, NCH$_2$), 4.90 (d, $J = 15.3$ Hz, 1H, NCH$_2$), 6.74–6.79 (m, 2H, Ar–H^8, Ar–H^6), 6.81 (d, $J = 8.6$ Hz, 1H, Ar–H^5), 7.01 (d, $J = 8.8$ Hz, 2H, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.16–7.26 (m, 3H, Ph), 7.27–7.33 (m, 2H, Ph), 7.83 (d, $J = 8.8$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$), 9.06 (bs, 2H, NH$_2$), 9.80 (bs, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 49.4 (Ph–CH$_2$), 64.5 (C-3), 65.9 (CH$_2$O), 71.0 (C-2), 114.7 (C-2', C-6'), 115.2 (C-8), 116.9 (C-6), 118.9 (C-4'), 120.1 (C-5), 126.9 (C-4''), 127.3 (C-2'', C-6''), 128.3 (C-3'', C-5''), 129.8 (C-3', C-5'), 135.5 (C-7), 137.8 (C-1''), 141.1, 141.6 (C-4a, C-8a), 161.9, 163.2, 164.0, 164.2 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$); HRMS (ESI) m/z calcd for C$_{25}$H$_{24}$N$_3$O$_6$ [M + H]$^+$ 462.1665, found 462.1668; IR (KBr, v, cm^{-1}): 3385, 1609, 1508, 1259, 845; HPLC: 94.0%, t_r 9.7 min; Anal. (C$_{25}$H$_{23}$N$_3$O$_6$ × 2/5CF$_3$COOH × 2/5H$_2$O) C, H, N.

6.27. (S)-2-(benzyl(3-((4-carbamimidoylphenoxy)methyl)-2,3-dihydro-1,4-benzodioxin-7-yl)amino)-2-oxoacetic acid (11d)

Synthesized from **10d** (150 mg, 0.25 mmol) according to the general procedure for synthesis of compounds **11a**–**d**; yield 72 mg (62%); mp 290–293 °C; $[\alpha]_D = -17.5$ ($c = 2.0$ in DMSO); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 4.13 (dd, $J = 11.6$, 7.9 Hz, 1H, 3-CH$_2$), 4.34 (dd, $J = 10.9$, 5.6 Hz, 1H, CH$_2$O), 4.40 (dd, $J = 10.9$, 3.6 Hz, 1H, CH$_2$O), 4.48 (dd, $J = 11.6$, 2.2 Hz, 1H, 3-CH$_2$), 4.55–4.65 (m, 1H, 2-CH), 4.88 (s, 2H, NCH$_2$), 6.68 (dd, $J = 8.6$, 2.5 Hz, 1H, Ar–H^6), 6.80 (d, $J = 2.5$ Hz, 1H, Ar–H^8), 6.89 (d, $J = 8.6$ Hz, 1H, Ar–H^5), 7.18–7.37 (m, 7H, Ph, Ar–H$^{2'}$, Ar–H$^{6'}$), 7.83 (d, $J = 8.9$ Hz, 2H, Ar–H$^{3'}$, Ar–H$^{5'}$), 8.91 (s, 2H, NH$_2$), 9.17 (s, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d$_6$): δ (ppm) 50.6 (Ph–CH$_2$), 64.4 (C-3), 66.8 (CH$_2$O), 71.4 (C-2), 114.9 (C-2', C-6'), 116.1 (C-8), 117.3 (C-6), 120.1 (C-4'), 120.7 (C-5), 127.4 (C-4''), 127.8 (C-2'', C-6''), 128.5 (C-3'', C-5''), 130.2 (C-3', C-5'), 133.0 (C-7), 136.3 (C-1''), 142.5, 142.6 (C-4a, C-8a), 162.3, 163.1, 164.2, 164.5 (CO–COO, CO–COO, C-1', C(=NH)NH$_2$); HRMS (ESI) m/z calcd for C$_{25}$H$_{24}$N$_3$O$_6$ [M + H]$^+$ 462.1665, found 462.1684; IR (KBr, v, cm^{-1}): 3309, 1609, 1492, 1262, 839; HPLC: 96.2%, t_r 9.7 min; Anal. (C$_{25}$H$_{23}$N$_3$O$_6$ × 1/11CF$_3$COOH) C, H, N.

6.28. Docking studies

The binding modes of the ligands to both targets were studied by Autodock 4.0 [53]. The energetically most favorable conformation was found for each stereoisomer with HyperChem, using the semiempirical method MNDO. All amide bonds had *trans* configuration and were marked as non-rotatable. Compounds were protonated on the basic center (amidino, or amino group) and were assigned with a negative charge on the acidic center (carboxylic group). The crystal structures of thrombin (PDB entry code: **1KTS**) [48] and GP IIb/IIIa (PDB entry code: **2VDM**) [49] were extracted from the Brookhaven Protein Database. The PDB crystal structures were prepared for docking by removing water molecules and ligands. In the case of **2VDM**, the magnesium ion in the active site was retained and manually assigned a charge of +2 and solvation parameters. The ligands were docked into a restricted box centered in the active site (22 Å × 22 Å × 22 Å). For the global search using the LGA (Lamarckian genetic algorithm), the size of the initial random population was 250 individuals, the maximal number of energy evaluations 1.25×10^6, the maximal number of generations 500, the number of top individuals that survived into the next generation (the elitism) 1, the probability that a gene would undergo a random change 0.02, and the crossover probability 0.80. The average of the worst energy was calculated over a window of 10 generations. For a purely local search, the pseudo Solis and Wets method was used, whereas the Solis and Wets method was used for the LGA part of the local search. The parameters used for local search in both cases were a maximum of 1000 iterations per local search, the probability of performing a local search on an individual was 1.0, the maximal number of consecutive successes or failures before doubling or halving the step size of the local search was 4, and the lower bound on the step size, 0.01, was the termination criterion for the local search. A total of 250 dockings were performed, and ligands with RMSD less than 1 were joined in clusters. The ligand with the lowest docked energy was chosen for interpreting the docking results.

6.29. Biochemical evaluation

6.29.1. Enzyme assay for inhibition of serine proteases

The enzymic amidolytic method for determining inhibition of thrombin, factor Xa and trypsin was based on the spectrophotometric determination of absorbance of the product formed after amide bond cleavage of a chromogenic substrate in the presence of the enzyme. K_i, which is a quantitative measure of inhibitor potency, was determined from the kinetics of substrate hydrolysis with and without the addition of the inhibitor. Measurements (spectrophotometer, BioTek Synergy H4) were performed in 96-well microtiter plates with a final volume of 200 μL. Thrombin was tested in a final concentration of 0.5 NIH E/mL with the substrate S-2238 (Chromogenix) at 20 and 40 μM final concentration, and factor Xa at the final concentration of 1 mBAEE E/mL with the substrate S-2222 (Chromogenix) at 100 and 200 μM final concentrations.

Trypsin was assayed at a final concentration of 0.5 nkat/mL using the substrate S-2222 (Chromogenix) at 50 and 100 μM final concentrations. Inhibitors were dissolved in DMSO (concentration of stock solutions, 10 mmol/L) and diluted with distilled water to concentrations from 0.5 to 100 μM. Reaction rates were measured in the presence and the absence of the inhibitor. Then 50 μM HBSA buffer, the 50 μM solution of each inhibitor concentration (or of HBSA buffer in case of measurement without inhibitor), and

50 μM of enzyme solution were pipetted into the microtiter wells. The plate was incubated for 15 min at 25 °C and 50 μL of chromogenic substrate then added. Absorbance at 405 nm was measured every 10 s at 25 °C. Measurements were carried out in triplicate with three concentrations of the inhibitor and two concentrations of the substrate. For every combination of concentrations, K_i was calculated from the change of absorbance in the initial, linear part of the curve according to the method of Cheng and Prusoff [54] and the final result was given as their average value. *Dabigatran* (thrombin, $K_i = 6.3 \pm 1.1$ nM) was used as control.

6.29.2. Inhibition of in vitro binding of fibrinogen to isolated GPIIb/IIIa and GPV/IIIa receptors

Binding affinities to GPIIb/IIIa and GPV/IIIa receptor were measured 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 it was diluted with 0.1 M NaHCO₃ in water to a final concentration of 1 mg/mL. Biotin *N*-hydroxysuccinimide ester (2 mg) was dissolved in DMF (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 room temperature 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%) added to give the stock solution. Human integrin (10 μL of GPIIb/IIIa receptor solution (Calbiochem), 1 μL of GPV/IIIa receptor solution (Calbiochem)) was diluted in 10.2 mL of *buffer 2* (50 mM Tris, 150 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM MnCl₂, pH 7.4) and adsorbed to 96-well (100 μL/well) high-binding microtiter plates (Greiner, Lumitrac 600) overnight at 4 °C. Nonspecific receptor-binding sites were blocked with 1% BSA in *buffer 2* (200 μL/well). Following incubation for 1 h at room temperature, the plates were washed twice with *buffer 3* (*buffer 2* containing 0.1% Tween-20). The potential antagonists were serially diluted with *buffer 2* containing 0.1% of BSA and test solutions added (50 μL/well) together with biotinylated fibrinogen (50 μL/well, 1:42 dilution of stock solution in *buffer 2* containing 0.1% of BSA) to each well. The plates were incubated for 2 h at room temperature, and then washed twice with *buffer 3*. Peroxidase-conjugated antibiotin goat antibody (100 μL/well of a 1:1000 dilution of purchased solution (Calbiochem) in *buffer 2* containing 0.1% of BSA) was added to each well and incubated for another hour. The microtiter plates were washed three times with *buffer 3*. Finally, chemiluminescent substrate (POD, Roche Diagnostics, Boehringer Mannheim) (100 μL/well) was added and the luminescence measured with a BioTek Synergy H4 multimode research reader three times over 10 min. Positive controls received no inhibitors, while negative controls received no receptor. *Tirofiban* (IC$_{50(\text{GPIIb/IIIa})}$ = 0.37 ± 0.11 nM) was used as the internal standard. Assays were performed in duplicate and repeated at least three times on various days. The mean experimental data were fitted to the sigmoid model and IC$_{50}$ values calculated from the dose–response curve.

Acknowledgments

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