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Original article

Novel pyrazinone and pyridinone thrombin inhibitors incorporating weakly basic heterobicyclic P_1-arginine mimetics

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Abstract

The design, synthesis and biological activity of new thrombin inhibitors with a pyridinone or pyrazinone core and different heterobicyclic P_1 arginine side-chain mimetics are described. The arginine side-chain mimetics used in this study are (±)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine and both enantiomers thereof, (±)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine and the corresponding *R* enantiomer. Compound **25**, the most potent in the series of pyrazinone inhibitors, exhibited a K_i of 41 nM *in vitro* and high selectivity against trypsin and factor Xa.

Keywords: Thrombin inhibitors; Arginine side chain mimetics; Enantiomers; Stereoselective action

1. Introduction

The pathophysiological role of an excessively active coagulation cascade, with subsequent inappropriate thrombus formation in blood vessels is well recognized. Venous thrombosis, which often leads to pulmonary embolism, is frequently triggered by vascular injury at the time of surgery or trauma [1]. In contrast, arterial thrombosis may lead to acute coronary syndromes, often as a result of plaque rupture. The current paradigm is that thrombosis is the major reason for complications of atherosclerosis, such as myocardial infarction and stroke, and the major factor responsible for atherosclerosis-related mortality [2]. Due to the central role played by thrombin in the pathogenesis of thromboembolic disorders, by triggering fibrin formation and platelet activation, antithrombin agents are an important component in the treatment of patients with acute coronary syndromes and venous thromboembolism [3]. The main limitation of the clinically used indirect thrombin inhibitors heparin and low molecular weight heparins, as well as of the natural occuring thrombin inhibitor hirudin (K_i = 22 fM), its recombinant analogs lepirudin and desirudin, synthetic hirulog bivalirudin and the non-peptide low molecular weight inhibitor argatroban, is their requirement for parenteral application. Consequently they are used for acute and not for chronic therapy. Enormous efforts have been dedicated over the last decades towards the design of potent and specific small molecule thrombin inhibitors having a wide safety margin and appropriate physicochemical and pharmacokinetic properties permitting oral dosing [4–12]. Ximelagatran, a very recently approved prodrug of the thrombin inhibitor melagatran, fulfils, to a certain extent, the listed goals and could replace warfarin, a vitamin K antagonist used for almost 50 years for chronic treatment of some thrombotic indications [13].

While the prodrug approach has been successfully employed to produce P_1 benzamidine-based thrombin inhibitors with acceptable oral bioavailability, the majority of recent approaches in the development of oral thrombin inhibitors

Abbreviations: DMF, *N*-*N*-dimethylformamide; EDC, *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide; HOBT, 1-hydroxybenzotriazole.

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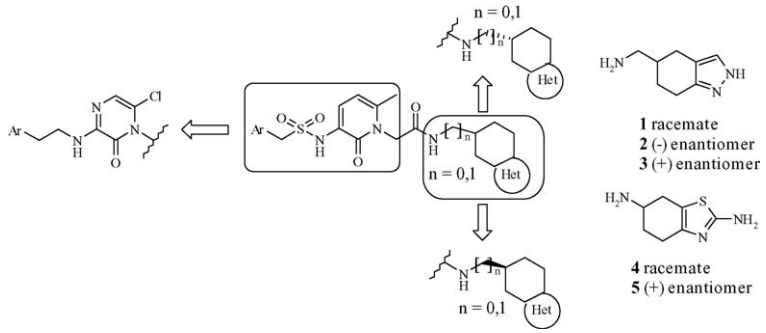


Fig. 1. Optimization strategy of 3-amino-6-methyl-2-pyridinone acetamide thrombin inhibitors.

have focused on compounds that incorporate less basic P_1 moieties [14–18]. Thus, we have focused on the design and synthesis of thrombin inhibitors that incorporate weakly basic, partially saturated heterobicyclic P_1-arginine side chain mimetics [19–23] and have reported on a series of proline-based thrombin inhibitors and 3-amino-2-pyridinone acetamide thrombin inhibitors incorporating different lipophilic residues in the P_3 part of the molecule [24–29]. The pyridinone template was previously shown by other groups, in the design of human leukocyte elastase inhibitors, thrombin inhibitors, and tissue factor/factor VIIa inhibitors, to be an appropriate peptidomimetic template that mimics the hydrogen bond array of the backbone of peptide inhibitors and provides a good fit of the inhibitor in the enzyme active site [15,30–33]. As a replacement for the pyridinone core, the pyrazinone scaffold has been frequently used [9,32,34] and it has been observed by Merck scientists that this modification improves the pharmacokinetic properties of thrombin inhibitors. Further improvement of the pharmacokinetics of pyrazinone thrombin inhibitors was observed also on substitution of the 6-methyl group by chlorine [9,32].

In this article we report the optimization of our previously described 3-amino-6-methyl-2-pyridinone acetamide thrombin inhibitors [27,28]. The optimization strategy included the synthesis of single enantiomers and replacement of the pyridinone core by the pyrazinone scaffold (Fig. 1). The arginine side-chain mimetics of low basicity used in this study comprise racemic 4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine (**1**), its two enantiomers **2** and **3**, and 4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine in racemic form (**4**) and in the form of the (+)-enantiomer **5** (Fig. 1).

2. Chemistry

We have recently reported a convenient synthetic approach to the conformationally restricted arginine side chain mimetics **1** and **4** [19–22,24]. In the synthesis of the enantiomers (-)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine (**2**) and (+)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine (**3**), optical resolution of (±)-**1** with L- and D-tartaric acid, respectively, was employed (Fig. 2). The resolution of (±)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole (**4**) has already been described [35].

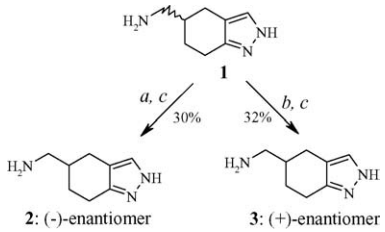


Fig. 2. Reagents and conditions: (a) L-(+)-tartaric acid, H_20/EtOH (2:1), 75 °C, then 5 °C, 3 days; (b) D-(-)-tartaric acid, H_20/EtOH (2:1), 75 °C, then 5 °C, 3 days; (c) 85% aq KOH, 10 °C.

Figs. 3,4 outline the elaboration of P_3-P_2 pyridinone and P_3-P_2 pyrazinone synthons **6** [32] and **10** [9] and the ultimate coupling reactions with P_1-arginine mimetics which led to target inhibitors **15**-**25**. The coupling reactions were performed using *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBT), both in dry *N*,*N*-dimethylformamide, as condensing reagents.

3. Pharmacology

The ability of the new thrombin inhibitors to inhibit the enzymatic activities of thrombin, trypsin and factor Xa was measured as described previously [25] by amidolytic enzyme assay using chromogenic substrates and is expressed as inhibition constants, K_i [37]. Values for K_i were calculated according to Cheng and Prusoff [38] based on IC_{50} values, or from a relation between reaction velocity equations in the absence and presence of inhibitor, using the relevant K_m [39]. The

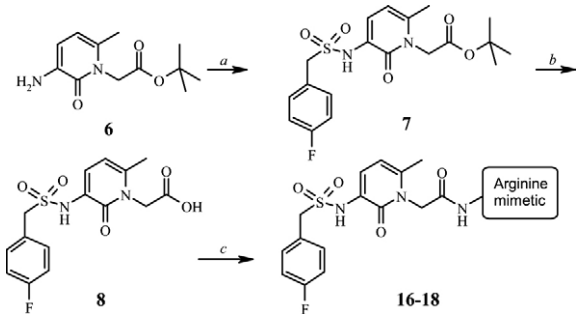


Fig. 3. Reagents and conditions: (a) *p*-F-C_6H_4-CH_2SO_2Cl [36], CH_2Cl_2, Et_3N, 0 °C to rt, 2 h; (b) HCl_g, EtOAc, 0 °C, 20 min; (c) arginine mimetic **1**, **2** or **3**, EDC, HOBt, *N*-methyl-morpholine, DMF, rt, 12 h.

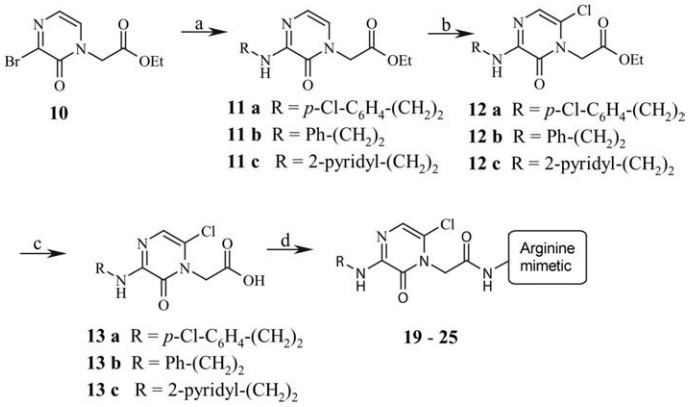


Fig. 4. Reagents and conditions : (a) $RCH_2CH_2NH_2$, Et_3N, toluene/EtOH (3:1), 120 °C, 15 h; (b) *N*-chlorosuccinimide, 1,2-dichloroethane, reflux, 2,5 h; (c) 1 M aq KOH, dioxane, 3 h; (d) arginine mimetic **1**, **2**, **3**, **4** or **5**, EDC, HOBt, *N*-methylmorpholine, DMF, rt, 12 h.

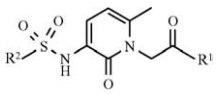
selectivity for thrombin over trypsin was expressed as the ratio $K_{i(trypsin)}/K_{i(thrombin)}$.

4. Results and discussion

The in vitro inhibitory potencies of inhibitors **15-25** are listed in Tables 1,2. Based on the crystal structure of thrombin inhibitor **15** complexed to human thrombin [27], which indicated preferential binding of the *R* enantiomer to the active site, we prepared the single enantiomers of its *p*-fluoro analogue **16,** which showed improved inhibitory potencies compared to **15**. The (+) enantiomer **18** (K_i = 47 nM) binded with almost 2-fold higher affinity to the thrombin active site than the (-) enantiomer **17** (K_i = 76 nM). Although the (-) enantiomer also showed good affinity for thrombin, that for the (+) enantiomer was higher and showed 1815-fold selectivity against trypsin and 1772-fold selectivity against factor Xa. Preferential binding of the *R* enantiomer was also observed in the series of pyridinone inhibitors incorporating 4,5,6,7-tetrahydro-1,3-benzothiazol-2-amine as P_1 arginine mimetic, where the *R* enantiomer binds with 16-fold higher affinity than the *S* enantiomer [28]. Comparison of these two series of thrombin inhibitors leads to the conclusion that the absolute configuration at C6 of a P_1 partially saturated heterobicyclic ring plays a less important role in the 4,5,6,7-tetrahydro-2*H*-indazol-6-ylmethanamine series than in the 4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine series.

In the pyrazinone series of thrombin inhibitors, we synthesized analogues with 2-phenylethyl-amino-, 2-(4-chlorophenyl)ethylamino- and 2-(2-pyridyl)ethylamino residues in the P_3 part and two different P_1 arginine side chain mimetics 4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine and 4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine. In this series, the 4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine and 4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine arginine side chain mimetics in the P_1 part of molecule contribute

Table 1
Inhibitory potencies of compounds **15-18**



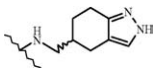
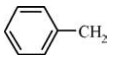
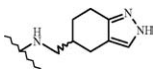
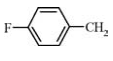
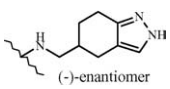
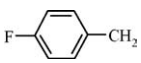
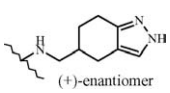
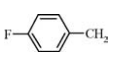
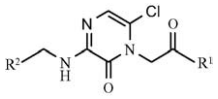
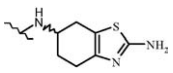
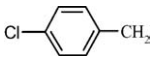
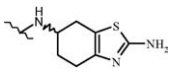
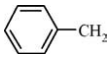
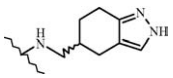
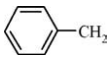
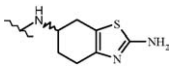
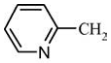
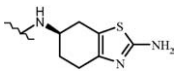
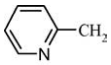
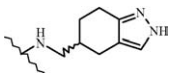
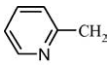
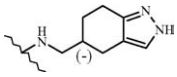
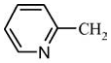
Compound	R^1	R^2	K_i (μM)			*Selectivity*
			Thrombin	Trypsin	FXa	*Thrombin/Trypsin*
15			0.17	>500	*103*	*>2941*
16			0.057	113.2	*87.9*	*1985*
17	 (-)-enantiomer		0.076	133.9	*58.2*	*1761*
18	 (+)-enantiomer		0.047	85.3	*83.3*	*1815*

Table 2
Inhibitory potencies of compounds **19-25**



Compound	R^1	R^2	K_i (μM)			*Selectivity*
			Thrombin	Trypsin	FXa	*Thrombin/Trypsin*
19			0,493	15,4	138,5	31
20			0,745	46,5	>100	62
21			1,33	>100	>100	>75
22			0,060	>100	*59,0*	*>1665*
23			0,053	>100	*>100*	*>1887*
24			0,085	>100	*121,5*	*>1176*
25			0,041	>100	*82,5*	*>2440*

Microanalyses;
18 Calcd.: C, 54.64; H, 5.58; N, 13.85 Found: C, 54.78; H, 5.79; N, 13.90;
19 HRMS (EI): Calcd.: 492.09125 Found: 492.09020;
20 Calcd.: C, 53.69; H, 5.19; N, 17.89 Found: C, 54.08; H, 5.18; N, 17.56;
21 Calcd.: C, 58.73; H, 5.82; N, 18.68 Found: C, 59.07; H, 5.94; N, 18.44;
22 Calcd.: C, 52.23; H, 4.82; N, 21.32 Found: C, 52.25; H, 5.05; N, 20.99;
23 Calcd.: C, 52.23; H, 4.82; N, 21.32 Found: C, 52.22; H, 5.05; N, 20.99;
24 Calcd.: C, 57.08; H, 5.47; N, 22.19 Found: C, 56.84; H, 5.65; N, 21.84;
25 Calcd.: C, 54.84; H, 5.70; N, 21.32 Found: C, 55.16; H, 5.75; N, 20.98.

similarly to the overall binding affinity of the inhibitors (compounds **20**, **21** and **22**, **24** respectively)

Modifications in the P_3 part of the pyrazinone series of thrombin inhibitors demonstrated that, irrespective of the P_1 moiety, replacement of the P_3 phenyl ring with a 2-pyridyl ring improved the potency of inhibitors (cf. **20** (K_i = 745 nM) and **22** (K_i = 60 nM); **21** (K_i = 1.33 μM) and **24** (K_i = 85 nM); From the crystal structure of a related thrombin inhibitor in a complex with human thrombin [9], it is evident that an electron-deficient P_3 pyridine reinforces the edge-to-face δ-π interactions in the S_3 pocket between the P_3 pyridine group and the π-rich Trp 215, providing a greater overall binding affinity versus its phenyl counterpart. Introduction of the *p*-chloro substituent (**19;** K_i = 493 nM) led to a slightly greater binding affinity over the phenyl derivative **20** (K_i = 745 nM), but the K_i value of **19** was still 8-fold lower than that of inhibitor **22** (K_i = 60 nM) which incorporates pyridine as a P_3 group. Since the pyridinone inhibitor **15** (K_i = 170 nM; Table 1), with a benzylsulfonyl group in the P_3 part, exhibits an 8-fold higher affinity for thrombin than the pyrazinone analogue **21** (K_i = 1.33 μM) incorporating the identical P_1 moiety, it can be concluded that the central heterocyclic P_2 core and the sulfonyl group have only weak influence on binding of the inhibitors to the thrombin active site.

To determine the influence of chirality in the pyrazinone series of thrombin inhibitors, we prepared single enantiomers **23** and **25** of racemic inhibitors **22** and **24** that both incorporate a P_3-pyridine moiety. The inhibitory potencies of compound **22** and its *R*-enantiomer **23,** possessing a 4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine arginine mimetic in the P_1 part, are practically the same (**22**: K_i = 60 nM; **23**: K_i = 53 nM), suggesting that, in contrast to the similar pyridinone series of inhibitors [28], the binding affinities of the pyrazinone series are not strongly dependent on the absolute

configuration at C6 of the heterobicyclic ring. A similar minimal influence of chirality was also observed in the pyrazinone inhibitor **24** with a P_3 2-(2-pyridyl)ethylamino moiety and 4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine in the P_1 part of the molecule. Thus, the (-) enantiomer **25** (K_i = 41 nM) showed a 2-fold better binding affinity than the corresponding racemic inhibitor **24** (K_i = 85 nM). Comparison of thrombin inhibitory potency and selectivity of our most potent compound **25** (K_i = 41 nM; > 2400-fold selectivity versus trypsin) and melagatran (K_i = 2 nM; 2-fold selectivity versus trypsin) [40], an active form of a recently approved prodrug ximelagatran [41], shows that while possessing excellent selectivity the thrombin inhibitory constant of compound **25** is of one order of magnitude lower than that of melagatran. This is probably due to the presence of a bicyclic ring which hinders **25** from achieving optimal interaction with the enzyme active site. On the other side, the bicyclic ring which enters the S_1 pocket could be responsible for the observed high selectivity versus trypsin. In the pyridazinone series the selectivity of the inhibitors versus trypsin was generally high in compounds with P_3 2-(2-pyridyl)ethylamino moiety. All thrombin inhibitors listed in Tables 1,2 exhibited good (58- to 138-fold) selectivity for inhibition of thrombin versus inhibition of factor Xa. Thus, regarding inhibition of thrombin and factor Xa, they could be regarded as pure anti-thrombin type of coagulation factors' inhibitors.

5. Conclusion

Optimization of our previously described 3-amino-6-methyl-2-pyridinone acetamide thrombin inhibitors resulted in potent and selective inhibitors **18** and **25**. This study revealed that the (+) enantiomer **18** of P_1 4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine compound **16**, with K_i of 47 nM and 1815-fold selectivity versus trypsin, binds to the thrombin active site with only 2-fold higher affinity than the (-) enantiomer **17**. In the pyrazinone series of inhibitors possessing tetrahydroindazole in the P_1 part, better binding affinity was achieved with (-) enantiomer **25** than with the racemic inhibitor **24**. Compound **25** is a potent thrombin inhibitor, with an in vitro K_i of 41 nM and over 2440-fold selectivity against trypsin. As in the pyrazinone series the affinity for thrombin improved markedly on replacement of the P_3 benzlysulfonyl group by the 2-(2-pyridyl)ethyl moiety, it can be concluded that the 2-(2-pyridyl)ethyl group contributes importantly to the inhibitory potency of compounds **22**, **23**, **24** and **25**.

6. Experimental protocols

6.1. Chemistry

6.1.1. Materials and methods

Chemicals were obtained from Aldrich Chemical Co., Fluka and Synthetech and used without further purification. THF was kept over sodium and distilled immediately prior to use. Analytical TLC was performed on Merck silica gel (60 F 254) plates (0.25 mm), with visualization with ultraviolet light and ninhydrin. Column chromatography was carried out on Florisil® (particle size 100-200 mesh) and 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 a Bruker AVANCE DPX_{300} spectrometer in $CDCl_3$ or DMSO 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. Elemental analyses were within ± 0.4% of the theoretical values. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. HPLC analyses were performed on Agilent Technologies HP 1100 instrument with G1365B UV-VIS detector, using a Eurospher C_{18} column (4.6 x 250 mm). The eluant was a mixture of acetonitrile (60%) and 0.1 M ammonium acetate buffer pH 4.15 (40%). Chemical names were generated using ACD/Name software.

*6.1.2. Resolution of (±)-4,5,6,7-tetrahydro-2H-indazol-5-ylmethanamine dihydrochloride (**1**×2HCl).*

To an aqueous solution of the dihydrochloride of **1** (1.24 g, 5.53 mmol), 5 M solution of NaOH (1.12 ml) was added. The resulting solution was extracted with dichloromethane (4×40 ml), the organic layers collected and dried (Na_2SO_4) and the solvent removed in vacuo, giving 0.65 g (78%) of **1** as a white solid. D-(-)-tartaric acid (0.65 g, 4.30 mmol) was added to a suspension of **1** (0.65 g, 4.30 mmol) in 5 ml of mixture of water/ethanol (2:1) and the mixture was heated to reflux for 10 minutes. After cooling, the mixture was stood for 3 days in a refrigerator. A precipitate formed and was collected and recrystallized two times from a mixture of water/ethanol (2:1). The crystalline D-(-)-tartrate was suspended in 20 ml of water and concentrated aqueous HCl was added dropwise until a clear solution resulted. After addition of 85% aqueous KOH (0.57 ml) at 10 °C, the free base was extracted with dichloromethane, the organic layers collected and dried (Na_2SO_4), and the solvent removed in vacuo to yield 120 mg (18%) of (-)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethan-amine **2** as a white solid; mp 129-132 °C; $[\alpha]_D^{20}$ = -16.69° (c = 0.29, H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.36-1.50 (m, 1H, H-5), 1.87-2.10 (m, 2H, 2H-6), 2.12-2.23 and 2.62-2.72 (2×m, 4H, 2H-4, 2H-7), 2.80-2.87 (m, 2H, $\underline{CH_2}NH_2$), 7.23 (s, 1H, H-3).

(+)-4,5,6,7-Tetrahydro-2*H*-indazol-5-ylmethanamine (**3**) was obtained from **1** (0.38 g, 1.70 mmol) using L-(+) tartaric acid (0.26 g, 1.70 mmol) and following the same procedure as described above; yield 70 mg (28%), $[\alpha]_D^{20}$ = +15.36° (c = 0.25, H_2O); The product was in al other respects identical to **2**.

*6.1.2.1. tert-Butyl 2-[3-{[(4-fluorobenzyl)sulfonyl]amino}-6-methyl-2-oxo-1(2H)-pyridinyl]acetate (**7**).* (4-Fluorophenyl) methanesulfonyl chloride (1.59 g, 7.62 mmol) was added in

portions to a stirred solution of *tert*-butyl (3-amino-6-methyl-2-oxo-1(2*H*)-pyridinyl)acetate (**6**) [32] (1.30 g, 5.45 mmol) and triethylamine (1.5 ml, 10.78 mmol) in dichloromethane (15 ml) cooled to 0 °C. After two hours 10% $KHSO_4$ solution (15 ml) was added and the aqueous phase was extracted twice with dichloromethane. The pooled organic phases were dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 40:1) to give 750 mg (34%) of a white solid; mp 149-151 °C; IR (KBr): ν 3142, 2982, 1742, 1652, 1595, 1511, 1449, 1353, 1225, 1155, 896, 772, 577, 496 cm^{-1}. MS (FAB): 411 (MH^+, 73%), 154 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.54 (s, 9H, *t*-Bu), 2.29 (s, 3H, 6-CH_3), 4.30 (s, 2H, *p*-F-C_6H_4- $\underline{CH_2}$), 4.76 (s, 2H, NCH_2), 6.04 (d, 1H, *J* = 7.54 Hz, H-5), 6.98-7.05 (m, 2H, 2×CH), 7.16-7.21 (s br, 1H, $NHSO_2$), 7.23-7.29 (m, 2H, 2×CH) 7.38 (d, 1H, *J* = 7.54 Hz, H-4).

*6.1.2.2. 2-[3-{[(4-Fluorobenzyl)sulfonyl]amino}-6-methyl-2-oxo-1(2H)-pyridinyl]acetic acid (**8**).* HCl gas was bubbled through a stirred suspension of *tert*-butyl 2-[3-{[(4-fluorobenzyl)-sulfonyl]amino}-6-methyl-2-oxo-1(*2H*)-pyridinyl]-acetate (**7**) (745 mg, 1.82 mmol) in ethylacetate cooled to 0 °C, until a clear solution was obtained. The HCl-saturated clear solution was stirred for one hour at room temperature and evaporated to give a brown solid; yield: 606 mg (94%), mp 117-120 °C; IR (KBr): ν 3142, 1714, 1654, 1601, 1510, 1458, 1370, 1227, 1152, 1032, 879, 584 cm^{-1}. MS (FAB): 355 (MH^+, 72%), 154 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.27 (s, 3H, CH_3), 4.53 (s, 2H, *p*-F-C_6H_4- $\underline{CH_2}$), 4.78 (s, 2H, NCH_2), 6.11 (d, 1H, *J* = 7.54 Hz, H-5), 7.11-7.20 (m, 3H, 2×CH, H-4), 7.37-7.44 (m, 2H, 2×CH), 8.66 (s, 1H, $NHSO_2$), 13.13 (s, 1H, COOH).

*6.1.2.3. (+)-2-[3-{[(4-Fluorobenzyl)sulfonyl]amino}-6-methyl-2-oxo-1(2H)-pyridinyl]-N-(4,5,6,7-tetrahydro-2H-indazol-5-ylmethyl)acetamide (**18**). General procedure for the synthesis of compounds **16-18** by coupling of P_3-P_2 fragment **8** with (±)-4,5,6,7-tetrahydro-2H-indazol-5-ylmethanamine (**1**), (-)-4,5,6,7-tetrahydro-2H-indazol-5-ylmethanamine (**2**) and (+)-4,5,6,7-tetrahydro-2H-indazol-5-ylmethanamine (**3**).* 2-[3-{[(4-Fluorobenzyl)sulfonyl]-amino}-6-methyl-2-oxo-1(2*H*)-pyridinyl]acetic acid (**8**) (160 mg, 0.45 mmol) and (+)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine (**3**) (68 mg, 0.45 mmol) were dissolved in *N,N*-dimethylformamide (1.0 ml). HOBT (61 mg, 0.45 mmol) and, after adjusting the pH to 8 with *N*-methylmorpholine, EDC (86 mg, 0.45 mmol) were added. The reaction mixture was stirred overnight at room temperature. It was then partitioned between ethyl acetate and saturated aqueous $NaHCO_3$, and the organic layer washed with brine, dried over Na_2SO_4 and filtered and the solvent removed under vacuum. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) to give 57 mg (26%) of a faintly brown powder; mp 116-119 °C; $[\alpha]_D^{20}$ = +12.50° (c = 0.095, CH_3OH); IR (KBr): ν 3295, 2924, 1652, 1601, 1569, 1508, 1443, 1355, 1223, 1157, 771, 571 cm^{-1}. MS (FAB): 488 (MH^+, 11%), 73 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.50-1.59 (m, 1H, CH-5), 1.90-2.23 (m, 2H, 6-CH_2), 2.18-2.26, 2.62-2.69, 2.71-2.78 (3×m, 4H, 4-CH_2, 7-CH_2), 2.48 (s, 3H, CH_3), 3.23-3.38 (m, 2H, $\underline{CH_2}$NH), 4.29 (s, 2H, *p*-F-$C_6H_4\underline{CH_2}$), 4.65 (s, 2H, NCH_2), 6.09 (d, 1H, *J* = 7.54 Hz, Py-H-5), 6.80-6.89 (m, 2H, CH, 2-NH), 6.91-6.99 (m, 2H, 2×CH), 7.21-7.29 (m, 3H, 3-CH, NHCO, CH), 7.38 (d, 1H, *J* = 7.54 Hz, Py-H-4) 8.05 (s, 1H, $NHSO_2$); HPLC: purity 96.5%; Anal. $C_{23}H_{26}FN_5O_4S \times H_2O$ (C, H, N).

*6.1.2.4. (-)-2-[3-{[(4-Fluorobenzyl)sulfonyl]amino}-6-methyl-2-oxo-1(2H)-pyridinyl]-N-(4,5,6,7-tetrahydro-2H-indazol-5-ylmethyl)acetamide (**17**).* Using the general procedure described above, **17** was prepared from 2-[3-{[(4-fluorobenzyl)-sulfonyl]amino}-6-methyl-2-oxo-1(2*H*)-pyridinyl]-acetic acid (**8**) (117 mg, 0.33 mmol) and (-)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine (**2**) (50 mg, 0.33 mmol); yield 40 mg (25%), white powder; HPLC: purity 89.3%; $[\alpha]_D^{20}$ = -10.00° (c = 0.11, CH_3OH); The product was in all other respects (mp, IR, MS, NMR) identical to compound **18**;

*6.1.2.5. (±)-2-[3-{[(4-Fluorobenzyl)sulfonyl]amino}-6-methyl-2-oxo-1(2H)-pyridinyl]-N-(4,5,6,7-tetrahydro-2H-indazol-5-ylmethyl)acetamide (**16**).* Using the general procedure described above, **16** was prepared from 2-[3-{[(4-fluorobenzyl)-sulfonyl]amino}-6-methyl-2-oxo-1(2*H*)-pyridinyl]acetic acid (**8**) (106 mg, 0.30 mmol) and (±)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine dihydrochloride (**1**·HCl) (67 mg, 0.30 mmol); yield 40 mg (27%), white powder; HPLC: purity 89.8%; The product was in all respects (mp, IR, MS, NMR) identical to compound **18**.

*6.1.2.6. Ethyl 2-[3-[(4-chlorophenethyl)amino]-2-oxo-1(2H)-pyrazinyl]acetate (**11a**). General procedure for the synthesis of compounds **11a-c** by reaction of ethyl 2-[3-bromo-2-oxo-1(2H)-pyrazinyl]acetate (**10**) with 2-(4-chlorophenyl)-1-ethanamine, 2-phenyl-1-ethanamine and 2-(2-pyridinyl)-1-ethanamine.* A solution of 2-(4-chlorophenyl)-1-ethanamine (417 mg, 2.68 mmol), triethylamine (0.37 ml, 2.68 mmol) and ethyl 2-[3-bromo-2-oxo-1(2*H*)-pyrazinyl]acetate (**10**) (700 mg, 2.68 mmol) in a mixture of 2 ml toluene and 0.35 ml ethanol was heated under reflux overnight. The mixture was concentrated and the residue partitioned between dichloromethane (11 ml) and saturated aqueous $NaHCO_3$ (11 ml). The aqueous layer was washed with dichloromethane (4x10 ml) and the combined organic layers dried over Na_2SO_4. The solvent was removed at reduced pressure to give a yellow solid that was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 20:1) to give 372 mg (41%) of **11a** as a white solid which was used without further purification for the synthesis of **12a**. IR (KBr): ν 3322, 2989, 1742, 1649, 1608, 1563, 1495, 1374, 1217, 1016, 757 cm^{-1}. MS (FAB): 336 (MH^+, 32%), 75 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, 3H, *J* = 7.16 Hz, CH_3), 2.93 (t, 2H, *J* = 7.16 Hz,

p-$ClC_6H_4\underline{CH_2}$), 3.67 (dt, 2H, J_1 = 7.16 Hz, J_2 = 6.02 Hz, $C\underline{H_2}NH$), 4.27 (q, 2H, *J* = 7.16 Hz, CH_2O), 4.56 (s, 2H, CH_2CO), 6.16 (s br, 1H, NH), 6.40 (d, 1H, *J* = 4.52 Hz, Pz-H), 6.89 (d, 1H, *J* = 4.52 Hz, Pz-H), 7.18 (d, 2H, *J* = 8.53 Hz, 2×CH), 7.29 (d, 2H, *J* = 8.53 Hz, 2×CH).

*6.1.2.7. Ethyl 2-[2-oxo-3-(phenethylamino)-1(2H)-pyrazinyl]-acetate (**11b**).* Using the general procedure described above, ethyl 2-[2-oxo-3-(phenethylamino)-1(2*H*)-pyrazinyl]acetate (**11b**) was prepared from 2-phenyl-1-ethanamine (279 mg, 2.30 mmol), triethylamine (0,32 ml, 2,30 mmol) and ethyl 2-[3-bromo-2-oxo-1(2*H*)-pyrazinyl]acetate (**15**) (600 mg, 2,30 mmol). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) to give 517 mg (75%) of **11b** as a pink solid which was used without further purification for the synthesis of **12b**. IR (KBr): ν 3330, 2988, 1746, 1648, 1558, 1373, 1206, 1033, 703 cm^{-1}. MS (EI): 301 (M^+, 37%), 210 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, 3H, *J* = 7.13 Hz, CH_3), 2.96 (t, 2H, *J* = 7.12 Hz, Ph-CH_2), 3.70 (q, 2H, *J* = 6.96 Hz, $C\underline{H_2}NH$), 4.27 (q, 2H, *J* = 7.13 Hz, CH_2O), 4.56 (s, 2H, CH_2CO), 6.19 (s br, 1H, NH), 6.39 (d, 1H, *J* = 4.62 Hz, Pz-H), 6.90 (d, 1H, *J* = 4.62 Hz, Pz-H), 7.22-7.36 (m, 5H, Ph-H).

*6.1.2.8. Ethyl 2-[2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2H)-pyrazinyl]acetate (**11c**).* Using the general procedure described above, ethyl 2-[2-oxo-3-{[2-(2-pyridinyl)ethyl]-amino}-1(2*H*)-pyrazinyl]acetate (**11c**) was prepared from 2-(2-pyridinyl)-1-ethanamine (715 mg, 5.85 mmol), triethylamine (0.81 ml, 5.85 mmol) and ethyl 2-[3-bromo-2-oxo-1(2*H*)-pyrazinyl]acetate (**10**) (1.53 g, 5.85 mmol); yield 1.56 g (88%), brown solid. The crude product was used immediately without purification for the synthesis of **12c**; IR (KBr): ν 3323, 2993, 2938, 1746, 1657, 1603, 1557, 1433, 1371, 1224, 1112, 990, 891, 780, 738 cm^{-1}. MS (FAB): 303 (MH^+, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, 3H, *J* = 7.16 Hz, CH_3), 3.18 (t, 2H, *J* = 6.78 Hz, Py-$\underline{CH_2}$), 3.85 (dt, 2H, J_1 = 6.78 Hz, J_2 = 6.41 Hz, $C\underline{H_2}NH$), 4.25 (q, 2H, *J* = 7.16 Hz, CH_2O), 4.57 (s, 2H, CH_2CO), 6.39 (d, 1H, *J* = 4.71 Hz, Pz-H), 6.62 (t br, 1H, NH), 6.70 (d, 1H, *J* = 4.71 Hz, Pz-H), 7.19 (m, 2H, 2×Py-H), 7.62 (m, 1H, Py-H), 8.60 (d, 1H, *J* = 7.54 Hz, Py-H).

*6.1.2.9. Ethyl 2-[6-chloro-3-[(4-chlorophenethyl)amino]-2-oxo-1(2H)-pyrazinyl]acetate (**12a**). General procedure for the synthesis of compounds **12a-c**.* *N*-chlorosuccinimide (137 mg, 1.03 mmol) was added to a stirred solution of ethyl 2-[3-[(4-chlorophenethyl)amino]-2-oxo-1(2*H*)-pyrazinyl]-acetate (**11a**) (372 mg, 1.11 mmol) in 1,2-dichloroethane (6 ml) and the reaction mixture heated to reflux for 2.5 h. During this time additional *N*-chlorosuccinimide was added (13 mg, (0,097 mmol) after 1 h and 4 mg (0.030 mmol) after 1.5 h). The solution was then cooled to room temperature and partitioned between dichloromethane (9 ml) and saturated aqueous $NaHCO_3$ (11 ml). The layers were separated and the aqueous phase backwashed with CH_2Cl_2 (2 ×11 ml). The combined organic layers were dried over Na_2SO_4, and the solution concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 50:1) to give 178 mg (43%) of **12a** as a white solid which was used without further purification for the synthesis of **13a;** IR (KBr): ν 3351, 2938, 1753, 1639, 1572, 1485, 1210, 1090, 1014, 808, 660 cm^{-1}. MS (EI): 369 (M^+, 21%), 244 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, 3H, *J* = 7.16 Hz, CH_3), 2.92 (t, 2H, *J* = 7.16 Hz, *p*-$ClC_6H_4\underline{CH_2}$), 3.66 (dt, 2H, J_1 = 7.15 Hz, J_2 = 6.03 Hz, $C\underline{H_2}NH$), 4.28 (q, 2H, *J* = 7.16 Hz, CH_2O), 4.90 (s, 2H, CH_2CO), 6.07 (t, 1H, *J* = 5.61 Hz, NH), 6.98 (s, 1H, Pz-H), 7.18 (d, 2H, *J* = 8.52 Hz, 2×CH), 7.29 (d, 2H, *J* = 8.52 Hz, 2×CH).

*6.1.2.10. Ethyl 2-[6-chloro-2-oxo-3-(phenethylamino)-1(2H)-pyrazinyl]acetate (**12b**).* Using the general procedure described above **12b** was prepared from ethyl 2-[2-oxo-3-(phenethylamino)-1(2*H*)-pyrazinyl]acetate (**11b**) (326 mg, 1.08 mmol) and *N*-chlorosuccin-imide (147 mg, 1.10 mmol); yield 363 mg (100%), red solid. The product was used without further purification in the next step for the synthesis of **13b**. IR (KBr): ν 3351, 2985, 1756, 1648, 1573, 1486, 1210, 1033, 700 cm^{-1}. MS (EI): 335 (M^+, 33%), 244 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, 3H, *J* = 7.16 Hz, CH_3), 2.95 (t, 2H, *J* = 7.17 Hz, Ph-$\underline{CH_2}$), 3.71 (dt, 2H, J_1 = 6.98 Hz, J_2 = 6.0 Hz, $C\underline{H_2}NH$), 4.28 (q, 2H, *J* = 7.16 Hz, CH_2O), 4.90 (s, 2H, CH_2CO), 6.10 (t, 1H, *J* = 6.0 Hz, NH), 6.98 (s, 1H, Pz-H), 7.21-7.36 (2×m, 5H, Ph-H).

*6.1.2.11. Ethyl 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]-amino}-1(2H)-pyrazinyl]acetate (**12c**).* Using the general procedure described above, **12c** was prepared from ethyl 2-[2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2*H*)-pyrazinyl]-acetate (**11c**) (1.08 g, 3.57 mmol) and *N*-chlorosuccinimide (489 mg, 3.66 mmol); yield 1.19 g (98%), brown solid. The product was used without further purification in the next step for the synthesis of **13c**. IR (KBr): ν 3390, 3339, 2972, 2755, 1744, 1645, 1575, 1485, 1415, 1223, 1026, 777, 608 cm^{-1}. MS (FAB): 337 (MH^+, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, 3H, *J* = 7.16 Hz, CH_3), 3.15 (t, 2H, *J* = 6.75 Hz, Py-CH_2), 3.85 (dt, 2H, J_1 = 6.78 Hz, J_2 = 6.40 Hz, $C\underline{H_2}NH$), 4.25 (q, 2H, *J* = 7.16 Hz, CH_2O), 4.90 (s, 2H, CH_2CO), 6.60 (t br, 1H, NH), 6.98 (s, 1H, Pz-H), 7.19 (m, 2H, 2×Py-H), 7.63 (m, 1H, Py-H), 8.60 (m, 1H, Py-H).

*6.1.2.12. 2-[6-Chloro-3-[(4-chlorophenethyl)amino]-2-oxo-1(2H)-pyrazinyl]acetic acid (**13a**). General procedure for the synthesis of compounds **13a-13c**.* 1 M aqueous KOH (0.96 ml, 0.96 mmol) was added to a stirred solution of ethyl 2-[3-[(4-chlorophenethyl)amino]-2-oxo-1(2*H*)-pyrazinyl]acetate (**12a**) (178 mg, 0.48 mmol) in dioxane (5 ml). After 3 h the solution was neutralized to pH 7 with concentrated hydrochloric acid, and the mixture evaporated under reduced pressure (toluene was added 2-3 times to form azeotrope) to give 203 mg of a yellow solid containing **13a**

(164 mg, yield: 100%) and KCl. IR (KBr): ν 3400, 1610, 1484, 1385, 1229, 1090, 804, 703 cm^{-1}. MS (EI): 341 (M^+, 7%), 216 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.85 (t, 2H, *J* = 7.16 Hz, *p*-$ClC_6H_4\underline{CH_2}$), 3.49 (dt, 2H, J_1 = 7.16 Hz, J_2 = 6.03 Hz, $C\underline{H_2}NH$), 4.29 (s, 2H, CH_2CO), 6.84 (s, 1H, Pz-H), 7.12 (t, 1H, *J* = 6.03 Hz, NH), 7.25 (d, 2H, *J* = 8.66 Hz, 2×CH), 7.34 (d, 2H, *J* = 8.66 Hz, 2×CH).

*6.1.2.13. 2-[6-Chloro-2-oxo-3-(phenethylamino)-1(2H)-pyrazinyl]acetic acid (**13b**).* Using the general procedure described above, 2-[6-chloro-2-oxo-3-(phenethylamino)-1(2*H*)-pyrazinyl]acetic acid (**13b**) was prepared from ethyl 2-[6-chloro-2-oxo-3-(phenethylamino)-1(2*H*)-pyrazinyl]-acetate (**12b**) (340 mg, 1.01 mmol) and 1 M aqueous KOH (2.02 ml, 2.02 mmol). yield: 444 mg of a yellow solid containing **13b** (311 mg, yield: 100%) and KCl. IR (KBr): ν 3366, 1617, 1574, 1483, 1373, 1314, 1234, 1110, 870, 699, 554 cm^{-1}. MS (EI): 307 (M^+, 29%), 216 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.86 (t, 2H, *J* = 7.07 Hz, $Ph\underline{CH_2}$), 3.50 (dt, 2H, J_1 = 7.07 Hz, J_2 = 6.05 Hz, $C\underline{H_2}NH$), 4.29 (s, 2H, CH_2CO), 6.84 (s, 1H, Pz-H), 7.08 (t, 1H, *J* = 6.05 Hz, NH), 7.16-7.34 (m, 5H, Ph-H).

*6.1.2.14. 2-[6-Chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2H)-pyrazinyl]acetic acid (**13c**).* Using the general procedure described above, 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)-ethyl]-amino}-1(2*H*)-pyrazinyl]acetic acid (**13c**) was prepared from ethyl 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]-amino}-1(2*H*)-pyrazinyl]acetate (**12c**) (1.16 g, 3.45 mmol) and 1 M aqueous KOH (6.90 ml, 6.90 mmol). yield 1.01 g of a yellow solid containing **13c** (1065 mg, yield: 100%) and KCl. IR (KBr): ν 3339, 1709, 1654, 1581, 1484, 1379, 1392, 1114, 987, 772, 760 cm^{-1}. MS (FAB): 309 (MH^+, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.02 (t, 2H, *J* = 7.53 Hz, Py-$\underline{CH_2}$), 3.63 (dt, 2H, J_1 = 7.53 Hz, J_2 = 6.78 Hz, $C\underline{H_2}NH$), 4.38 (s, 2H, CH_2CO), 6.85 (s, 1H, Pz-H), 7.21 (m, 2H, 2×Py-H), 7.71 (m, 1H, Py-H), 8.50 (d, 1H, Py-H).

*6.1.2.15. (±)-N-(2-Amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl)-2-[6-chloro-3-[(4-chlorophenethyl)amino]-2-oxo-1(2H)-pyrazinyl]acetamide (**19**). General procedure for the synthesis of compounds **19**, **20**, **22** and **23** by coupling P_3-P_2 fragments **13** with (±)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-amine dihydrobromide (**4**×2HBr) and (-)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-amine (**5**).* 2-[6-Chloro-3-[(4-chlorophenethyl)amino]-2-oxo-1(2*H*)-pyrazinyl]acetic acid (**13a**) (81 mg, 0.236 mmol), (±)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-amine dihydrobromide (**4**×2HBr) (71 mg, 0.215 mmol) and 1-hydroxybenzotriazole (32 mg, 0.236 mmol) were dissolved in *N,N*-dimethylformamide (1.0 ml), the pH adjusted to pH 8 with *N*-methylmorpholine and 1-(3-dimethylaminopropyl)-3'-ethyl-carbodiimide hydrochloride (45 mg, 0.236 mmol) then added. The reaction mixture was stirred overnight at room temperature, then diluted with saturated $NaHCO_3$ (4.4 ml) and water (6.6 ml) and the resulting precipitate filtered to yield a white solid. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) to give 37 mg (yield: 35%) of **19** as a white powder; mp 234-237 °C. IR (KBr): ν 3346, 3274, 2936, 1644, 1586, 1530, 1490, 1231, 1091, 1014, 802 cm^{-1}. MS (FAB): 493 (MH^+, 24%), 154 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.66-1.91 (m, 2H, CH_2), 2.23-2.50 (m, 3H, 2×CH_2), 2.72-2.82 (m, 1H, CH_2), 2.86 (t, 2H, *J* = 7.16 Hz, *p*-ClC_6H_4-$\underline{CH_2}$), 3.51 (dt, 2H, J_1 = 7.16 Hz, J_2 = 6.40 Hz, $C\underline{H_2}NH$), 3.96-4.08 (m, 1H, CH-6), 4.70 (s, 2H, CH_2CO), 6.65 (s, 2H, NH_2), 6.94 (s, 1H, Pz-H), 7.25 (d, 2H, *J* = 8.47 Hz, 2×CH), 7.32-7.41 (m, 1H, Pz-NH), 7.34 (d, 2H, *J* = 8.47 Hz, 2×CH), 8.36 (d, 1H, *J* = 7.53 Hz, NHCO). HRMS (EI) $C_{21}H_{22}Cl_2N_6O_2S$: calcd.: 492.09125; found: 492.09020.

*6.1.2.16. (±)-N-(2-Amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl)-2-[6-chloro-2-oxo-3-(phenethylamino)-1(2H)-pyrazinyl]acetamide (**20**).* Using the general procedure described above, **20** was prepared from 2-[6-chloro-2-oxo-3-(phenethylamino)-1(2*H*)-pyrazinyl]acetic acid (**13b**) (110 mg, 0.356 mmol) and (±)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-amine dihydrobromide (**4**×2HBr) (107 mg, 0.324 mmol). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) to give 40 mg (27%) of white solid; mp 242-246 °C. IR (KBr): ν 3335, 3286, 2933, 1660, 1581, 1488, 1232, 695 cm^{-1}. MS (FAB): 459 (MH^+, 89%), 57 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.69-1.90 (m, 2H, CH_2), 2.34-2.56 (m, 3H, 2×CH_2), 2.71-2.81 (m, 1H, CH_2), 2.87 (t, 2H, *J* = 7.53 Hz, Ph-CH_2), 3.52 (dt, 2H, J_1 = 7.53 Hz, J_2 = 6.40 Hz, $C\underline{H_2}NH$), 3.97-4.08 (m, 1H, CH-6), 4.70 (s, 2H, CH_2CO), 6.65 (s, 2H, NH_2), 6.94 (s, 1H, Pz-H), 7.17-7.37 (m, 6H, Ph, Pz-NH), 8.36 (d, 1H, *J* = 7.91 Hz, NHCO) Anal. $C_{21}H_{23}ClN_6O_2S$×0,6 H_2O (C, H, N).

*6.1.2.17. (±)-N-(2-Amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl)-2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2H)-pyrazinyl]acetamide (**22**).* Using the general procedure described above, **22** was prepared from 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2*H*)-pyrazinyl]acetic acid (**13c**) (95 mg, 0.308 mmol) and (±)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-amine dihydrobromide (**4**×2HBr) (93 mg, 0.280 mmol). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 7:1) to give 54 mg (42%) of tan solid; mp 246-249 °C. IR (KBr): ν 3276, 2922, 1646, 1588, 1476, 1229, 1108, 747 cm^{-1}. MS (FAB): 460 (MH^+, 18%), 55 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.66-1.91 (m, 2H, CH_2), 2.34-2.58 (m, 3H, 2×CH_2), 2.71-2.82 (m, 1H, CH_2), 3.02 (t, 2H, *J* = 7.53 Hz, Py-CH_2), 3.65 (dt, 2H, J_1 = 7.53 Hz, J_2 = 6.03 Hz, $C\underline{H_2}NH$), 3.95-4.04 (m, 1H, CH-6), 4.70 (s, 2H, CH_2CO), 6.65 (s, 2H, NH_2), 6.93 (s, 1H, Pz-H), 7.22 (ddd, 1H, J_1 = 7.54 Hz, J_2 = 4.90 Hz, J_3 = 1.13 Hz, Py-H-3), 7.27 (d, 1H, *J* = 7.54 Hz, Py-H-4), 7.41 (t, 1H, *J* = 6.03 Hz, Pz-NH), 7.71 (m, 1H, Py-H-5), 8.36 (d, 1H, *J* = 7.54 Hz, NHCO), 8.50 (m, 1H, Py-H-6). Anal. $C_{20}H_{22}ClN_7O_2S$ (C, H, N).

*6.1.2.18. N-[(6R)2-Amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl]-2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2H)-pyrazinyl]acetamide (**23**).* Using the general procedure described above, **23** was prepared from 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2*H*)-pyrazinyl]acetic acid (**13c**) (92 mg, 0.297 mmol) and (+)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine (**5**) (46 mg, 0.270 mmol). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 7:1) to give 30 mg (24%) of tan solid; mp 207-212 °C. IR (KBr): ν 3360, 2933, 1652, 1586, 1229, 1109, 746 cm^{-1}. MS (FAB): 460 (MH^+, 9%), 55 (100%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.66-1.91 (m, 2H, CH_2), 2.34-2.58 (m, 3H, 2×CH_2), 2.72-2.83 (m, 1H, CH_2), 3.03 (t, 2H, *J* = 7.16 Hz, Py-$\underline{CH_2}$), 3.65 (dt, 2H, J_1 = 7.16 Hz, J_2 = 6.03 Hz, $C\underline{H_2}NH$), 3.96-4.08 (m, 1H, CH-6), 4.70 (s, 2H, CH_2CO), 6.65 (s, 2H, NH_2), 6.94 (s, 1H, Pz-H), 7.22 (ddd, 1H, J_1 = 7.54 Hz, J_2 = 4.90 Hz, J_3 = 1.13 Hz, Py-H-3), 7.27 (d, 1H, *J* = 7.54 Hz, Py-H-4), 7.41 (t, 1H, *J* = 6.03 Hz, Pz-NH), 7.71 (m, 1H, Py-H-5), 8.36 (d, 1H, *J* = 7.53 Hz, NHCO), 8.50 (m, 1H, Py-H-6). Anal. $C_{20}H_{22}ClN_7O_2S$ (C, H, N).

*6.1.2.19. (±) 2-[6-Chloro-2-oxo-3-(phenethylamino)-1(2H)-pyrazinyl]-N-(4,5,6,7-tetrahydro-2H-indazol-5-ylmethyl)acetamide (**21**). General procedure for the synthesis of compounds **21**, **24** and **25** by coupling P_3-P_2 fragments **13b** and **13c** with (±)-4,5,6,7-tetrahydro-2H-indazol-5-ylmethanamine dihydrochloride (**1**×2HCl) and (-)-4,5,6,7-tetrahydro-2H-indazol-5-ylmethanamine (**2**).* 2-[6-Chloro-2-oxo-3-(phenethylamino)-1(2*H*)-pyrazinyl]acetic acid (**13b**) (200 mg, 0.65 mmol) and (±)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine dihydrochloride (**1**×2HCl) (146 mg, 0.65 mmol) were dissolved in *N,N*-dimethylformamide (1.0 ml). 1-hydroxybenzo-triazole (90 mg, 0.67 mmol) and, after adjusting the pH of the resulting solution to 8 with *N*-methylmorpholine, 1-(3-dimethylaminopropyl)-3'-ethyl-carbodiimide hydrochloride (125 mg, 0.65 mmol) were added. The reaction mixture was stirred overnight at room temperature and partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, filtered and the solvent removed in vacuo. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 20:1) to give 60 mg (21%) of white powder; mp 120-123 °C. IR (KBr): ν 3344, 2924, 1655, 1648, 1583, 1487, 1240, 780, 694 cm^{-1}. MS (FAB): 441 (MH^+, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.83-0.98 (m, 1H, CH-5), 1.47-1.59 (m, 2H, CH_2-6), 2.64-2.69, 2.71-2.80, 2.83-2.88 (3×m, 4H, CH_2-4, CH_2-7), 2.96 (t, 2H, *J* = 7.16 Hz, Ph-$\underline{CH_2}$), 3.27-3.40 (m, 2H, $\underline{CH_2}NHCO$), 3.68 (m, 2H, $CH_2\underline{CH_2}NH$), 4.83 (s, 2H, CH_2CO), 6.00 (t br, 1H, Pz-NH), 6.11 (t br, 1H, NHCO), 7.03 (s, 1H, Pz-H), 7.22-7.37 (m, 6H, Ph-H, CH-3) Anal. $C_{22}H_{25}N_6O_2Cl \times 0.5\ H_2O$ (C, H, N).

*6.1.2.20. (±) 2-[6-Chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2H)-pyrazinyl]-N-(4,5,6,7-tetrahydro-2H-indazol-5-ylmethyl)acetamide (**24**).* Using the general procedure described above, **24** was prepared from 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2*H*)-pyrazinyl]acetic acid (**13c**) (200 mg, 0.65 mmol) and (±)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine dihydrochloride (**1**×2HCl) (146 mg, 0.65 mmol). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) to give 80 mg (28%) of white powder; mp 103-105 °C. IR (KBr): ν 3331, 2924, 1652, 1581, 1479, 1443, 1434, 1226, 957, 748 cm^{-1}. MS (FAB): 442 (MH^+, 91%), 149 (100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.85-0.96 (m, 1H, CH-5), 1.46-1.58 (m, 2H, CH_2-6), 2.15-2.26, 2.57-2.68, 2.71-2.80 (3×m, 4H, CH_2-4, CH_2-7), 3.12 (t, 2H, *J* = 6.78 Hz, Py-$\underline{CH_2}$), 3.25-3.38 (m, 2H, $\underline{CH_2}NHCO$), 3.85 (dt, 2H, J_1 = J_2 = 6.40 Hz, $CH_2\underline{CH_2}NH$), 4.83 (s, 2H, $\underline{CH_2}CO$), 6.15 (t br, 1H, PzNH), 6.68 (t br, 1H, NHCO), 7.01 (s, 1H, Pz-H), 7.16-7.21 (m, 2H, 2×Py-H), 7.30 (s, 1H, CH-3), 7.62 (m, Py-H), 8.58 (d, 1H, *J* = 1.88 Hz, Py-H). Anal. $C_{21}H_{24}N_7O_2Cl$ (C, H, N).

*6.1.2.21. (-) 2-[6-Chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2H)-pyrazinyl]-N-(4,5,6,7-tetrahydro-2H-indazol-5-ylmethyl)acetamide (**25**).* Using the general procedure described above, **25** was prepared from 2-[6-chloro-2-oxo-3-{[2-(2-pyridinyl)ethyl]amino}-1(2*H*)-pyrazinyl]acetic acid (**13c**) (93 mg, 0.30 mmol) and (-)-4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethanamine (**2**) (45 mg, 0.30 mmol). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) to give 50 mg (37%) of white powder; mp 101-104 °C; $[\alpha]_D^{20}$ = -11.54° (c = 0.13, MeOH). The product was in all other respects (IR, MS, ^{1}H-NMR) identical with **24**. Anal. $C_{21}H_{24}N_7O_2Cl \times H_2O$ (C, H, N).

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