

Design, Synthesis and Evaluation of Serine Protease Inhibitor Analogues

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Recent efforts in the field of thrombin inhibitor research have focused on the identification of pyrazinone-containing compounds. In this manuscript we describe the synthesis of the new pyrazinones **12–36**. All the targets were fully characterised and screened for serine protease inhibition.

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Introduction

Serine proteases are involved in many critical physiological processes, including digestion, hemostasis, apoptosis, signal transduction, reproduction, and immune response.^[1]

The synthesis of novel compounds that can inhibit the action of these proteases has important medicinal applications. One of the more important ones is in the field of the treatment of cardiovascular diseases due to excessive blood coagulation. These remain the most common cause of mortality in the western world.^[2] The more prominent conditions that occur are deep vein thrombosis (DVT), pulmo-

nary embolism (PE), and thromboembolic stroke. The use of standard therapies such as warfarin or heparin^[3] is severely limited due to slow onset of action and lack of selectivity leading to bleeding side effects that require close patient monitoring. Drug development has now been expanded to include a number of inhibiting agents having as target either earlier or later factors of the coagulation cascade. These include inhibitors of the factor VIIa/tissue factor complex,^[4] inhibitors of factor IXa,^[5] selective direct or indirect inhibitors of factor Xa,^[6] and direct thrombin inhibitors.^[7]

Recent papers published by the Merck laboratories describe the design and synthesis of compounds that inhibit thrombin and the factor VIIa/tissue factor complex.^[7] These inhibitors are built around a central pyrazinone template (Figure 1).^[7c–8] This pyrazinone core is assumed to orient the substituents R^1 , R^2 and R^3 on the scaffold in the

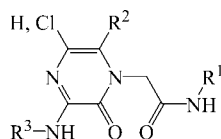


Figure 1. Pyrazinone core structure.

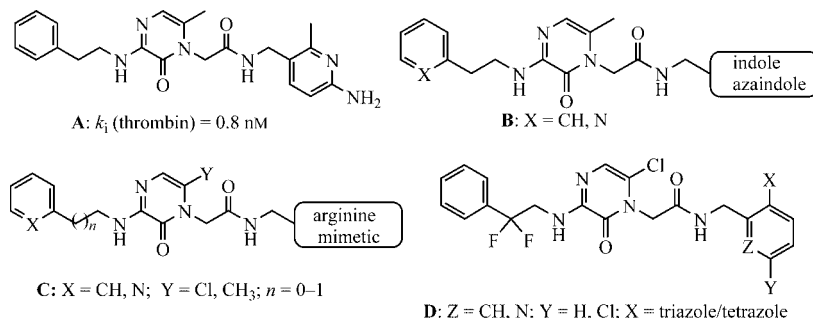


Figure 2. Protease inhibitors.

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correct spatial arrangement to interact with the S^1 , S^2 and S^3 pockets of the enzyme.

The same pyrazinone core was a building block in other library synthesis projects (compounds of type **A–D**, Figure 2).^[7,8] For example, the 2-amino-6-methylpyridine-deriv-

atized pyrazinone **A**, is developed as a selective, efficacious and orally active thrombin inhibitor (Figure 2).^[9]

In an effort to bring more diversity into the libraries of compounds that can be synthesized based on this pyrazinone core, we modified a number of the Merck inhibitors (Figure 1). In these compounds, we inverted the substituents at the 1- and the 3-position of the pyrazinone scaffold because simple computational models showed that this structure more or less represented the side chains in the same fashion as the original compounds (Figure 3). Our goal was to check the ability of the targets to inhibit the enzymatic action of serine proteases.

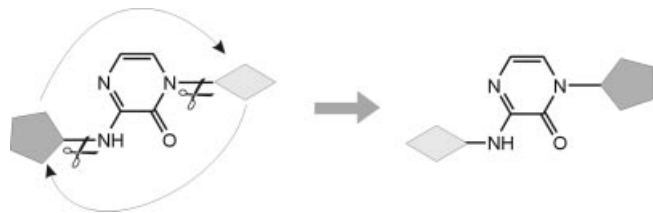


Figure 3. Design of the target pyrazinones.

From a synthetic point of view, we for the first time synthesized pyrazinones substituted with (β)-amino acids at the 3-position. Hence, these completely new scaffolds are also interesting systems for broader screening.

Design of the Targets

The targets were designed keeping in mind our experience with the functionalisation of dichloropyrazinone derivatives. The aim of this work was to selectively functionalise the 3-position of dichloropyrazinones by the amino function of a carboxy-protected amino acid. The deblocked acid function of this amino acid could then serve as an easy attachment point for combinatorial variation. At the 1-po-

sition of the pyrazinone core, benzyl and phenethyl groups were planned to be attached, as these functionalities were also present in the original Merck compounds (Figure 2). For synthetic reasons, a chlorine atom is present at the 5-position of our pyrazinones.

The idea was to check if the new compounds (Figure 4) retained their serine protease inhibiting activity.

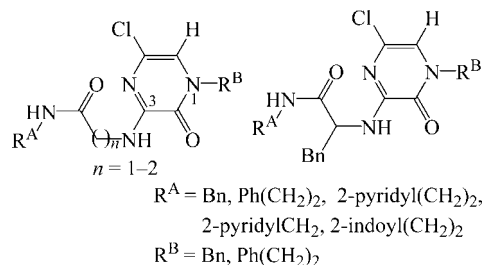


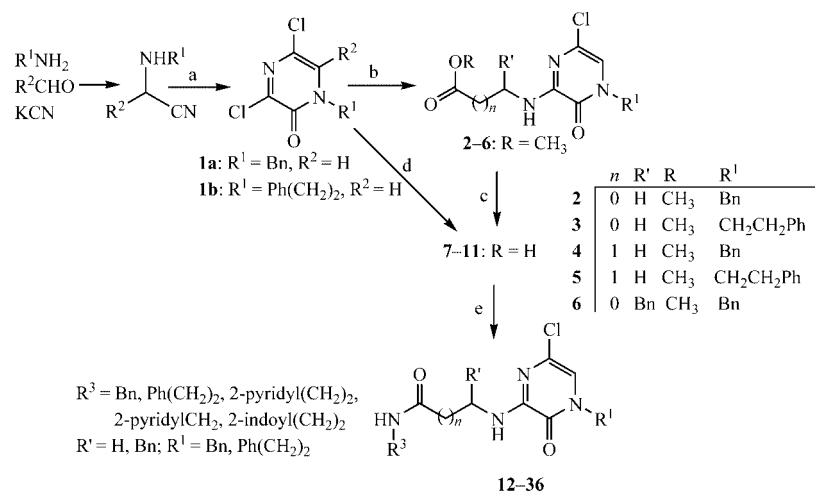
Figure 4. Target compounds.

Chemistry

Scheme 1 outlines the elaboration of the 1- and the 3-position of pyrazinone **1** and the ultimate coupling reactions with amines which lead to the desired target compounds. The proposed strategy is general and both the R^3 and R^1 substituents are easily varied.

Our approach towards the synthesis of the target compounds started from 3,5-dichloropyrazinones **1**. These have been developed in our laboratory starting from amino nitriles and oxalyl chloride.^[10] The R^1 and R^2 moieties were contained in the starting amine and aldehyde, respectively, and thus are hardwired in place at the beginning of the synthesis.

The subsequent step of the synthetic sequence was to perform a nucleophilic displacement of the 3-chlorine atom



Scheme 1. Synthesis of the target compounds. Reagents and conditions: a) $(\text{COCl})_2$, chlorobenzene, $\text{NEt}_3 \cdot \text{HCl}$, room temp., 24 h; b) **1** (1 equiv.), methyl ester (glycine, β-alanine, L-phenylalanine) (2 equiv.), NEt_3 (4 equiv.), DMF, 80 °C, 16 h (51–95%); c) LiOH (5–8 equiv.), THF/MeOH/ H_2O (3:2:1), room temp., 16 h; d) Na_2CO_3 (6 equiv.), EtOH/ H_2O , methyl ester (glycine, β-alanine, L-phenylalanine) (2 equiv.), 80 °C to reflux; e) **7–11** (1 equiv.), amine (1 equiv.), HOBT (1 equiv.), DIC (1.5 equiv.), NMM (1.2 equiv.), DMF/MeCN (2:1), room temp., 4–12 h (30–80%).

of pyrazinone **1** with the desired amino acid methyl ester (glycine, β -alanine and L-phenylalanine). As usual with 3,5-dichloro-2(1*H*)-pyrazinones, the 5-chloro position does not react at all under these conditions. In the initial optimisation studies, different methods were examined. Treatment of pyrazinone **1** with methyl glycinate hydrochloride (1.5 equiv.) and NEt_3 (3 equiv.) in MeOH as solvent was attempted. The desired reaction was not observed in this case neither with heating nor at room temperature, instead 3-methoxy-substituted pyrazinone was formed. The use of freshly distilled DMF as the solvent and NEt_3 as the base at 80 °C for 16 h seem to be the best conditions if the methyl esters **2–6** are to be obtained (51–95%). Alternatively, the compounds **7–11** could also be prepared directly from the dichloropyrazinone by using conditions as described in Scheme 1.

The second step of the synthesis was the conversion of the methyl esters **2–6** into the acid derivatives **7–11** under standard conditions with lithium hydroxide in THF/MeOH/ H_2O (3:2:1) at room temperature. Upon completion of the hydrolysis, the reaction mixture was acidified with dilute HCl. After extraction with ethyl acetate, the combined organic layers were dried and then concentrated to dryness. Chromatographic separation of the residue on a silica gel column eluting with EtOAc/ CH_2Cl_2 (70:30) as an eluent provided the acid derivatives **7–11** in 62–90% yield.

The last step of the synthesis incorporated the R^3 unit on the pyrazinone by amide coupling. Coupling reactions generally employed HOBt and *N,N'*-diisopropylcarbodiimide (DIC) as coupling reagents with 1.2 equiv. of *N*-methylmorpholine (NMM) as a base at room temperature in DMF/MeCN (2:1) solvent mixtures. Chromatographic pu-

rification afforded pure products **12–36** in moderate to good yield as outlined in Table 1.

Table 1. Novel substituted pyrazinone series **12–36**.

Compound	R^3	R^1	n	R'	Yield (%)
12	CH_2Ph	Bn	0	Bn	50
13		Bn	0	H	50
14		Bn	1	H	50
15		$\text{CH}_2\text{CH}_2\text{Ph}$	0	H	40
16		$\text{CH}_2\text{CH}_2\text{Ph}$	1	H	34
17	$\text{CH}_2\text{CH}_2\text{Ph}$	Bn	0	Bn	45
18		Bn	0	H	30
19		Bn	1	H	32
20		$\text{CH}_2\text{CH}_2\text{Ph}$	0	H	80
21		$\text{CH}_2\text{CH}_2\text{Ph}$	1	H	62
22	CH_2Pyr	Bn	0	Bn	50
23		Bn	0	H	40
24		Bn	1	H	42
25		$\text{CH}_2\text{CH}_2\text{Ph}$	0	H	40
26		$\text{CH}_2\text{CH}_2\text{Ph}$	1	H	30
27	$\text{CH}_2\text{CH}_2\text{Pyr}$	Bn	0	Bn	33
28		Bn	0	H	40
29		Bn	1	H	30
30		$\text{CH}_2\text{CH}_2\text{Ph}$	0	H	30
31		$\text{CH}_2\text{CH}_2\text{Ph}$	1	H	54
32	$\text{CH}_2\text{CH}_2\text{Ind}$	Bn	0	Bn	30
33		Bn	0	H	88
34		Bn	1	H	50
35		$\text{CH}_2\text{CH}_2\text{Ph}$	0	H	80
36		$\text{CH}_2\text{CH}_2\text{Ph}$	1	H	40

The structure of all synthesized compounds **12–36** was evidenced by ^1H and ^{13}C NMR spectroscopy (in CDCl_3 or CD_3OD) and mass spectrometry. This library of 25 compounds was tested for inhibition of serine proteases.

The X-ray crystal structure of compound **33** was determined as shown in Figure 5.^[11]

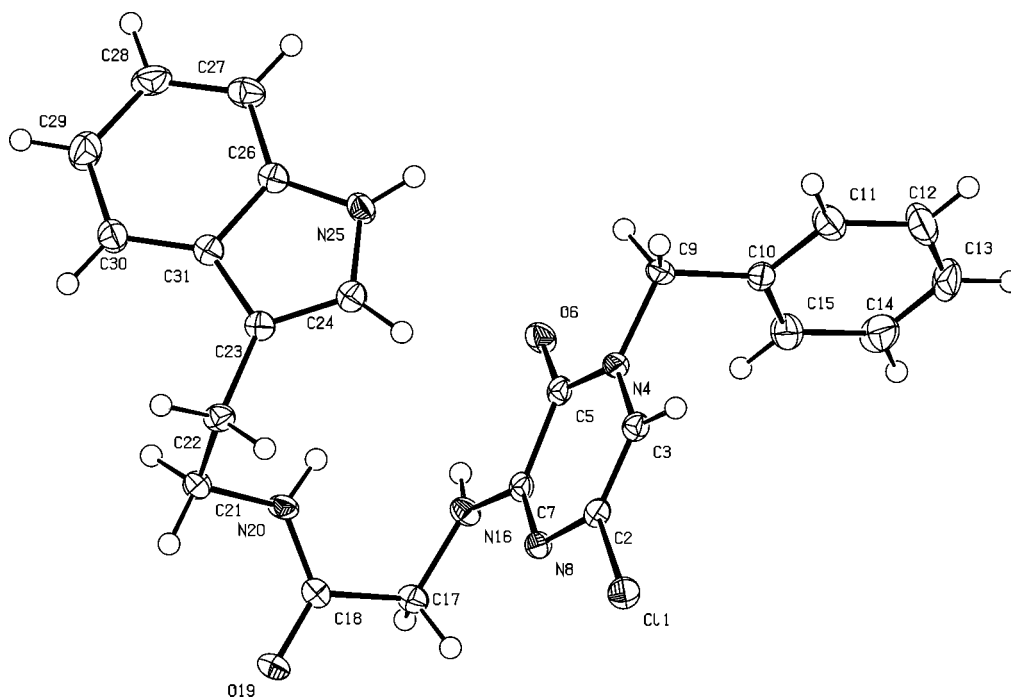


Figure 5. X-ray crystal structure of compound **33**, ORTEP representation.

Enzyme Kinetic Experiments

The ability of the new ligands **12–36** to inhibit the enzymatic activities of α -thrombin, trypsin and α -chymotrypsin was measured spectrophotometrically by amidolytic enzyme assays using chromogenic substrates and is expressed as inhibition constants, K_i .^[12] Values for K_i were calculated from the equation for competitive inhibition relating the reaction velocity in the presence of inhibitor to the substrate concentration, using the relevant K_m .^[13] The assays were carried out by optimizing the methods described in the literature.^[15]

Results and Discussion

The ligands **12–36** were screened for activity against thrombin, trypsin and chymotrypsin. With trypsin none of the compounds was found to show inhibitory activity. However, compounds containing the R³ pyridinyl moiety exhibit a moderate effect on thrombin (**22** and **27**, $K_i = 30 \mu\text{M}$). For the rest of the investigated compounds in Table 1, no inhibitory effect on thrombin or only a negligible one (e.g. **23**, $K_i = 160 \mu\text{M}$ and **21**, $K_i = 250 \mu\text{M}$) was observed. Furthermore, the pyridine series maintained the low micromolar activity against chymotrypsin as compared with thrombin (**22**, $K_i = 10 \mu\text{M}$ and **27**, $K_i = 70 \mu\text{M}$), this was not seen in the remaining compounds of the pyrazinone series.

The tolerance of the pockets of the enzyme (thrombin and chymotrypsin) to the pyridine ring would prove to be a valuable finding, as it seems to be an important requirement to obtain inhibitors that show activity against serine proteases.

Modeling

In order to rationalise the improved binding of the pyridine-containing compounds,^[16] molecular modeling was used to dock the ligands in the active sites of thrombin and chymotrypsin.

The ligands used in this study were modeled using the Sybyl 6.97 software.^[17] All models were sketched and optimised using the Tripos forcefield, and Gasteiger–Marsili charges. Minimalisation methods were Simplex as an initial minimalisation, followed by Conjugated Gradient, with a maximum of 1000000 iterations. Docking of the molecules was done using the FlexX2 docking software^[18] loaded with the standard FlexX-score scoring function. As receptors the crystal structure for thrombin (pdb 1SL3)^[19] and α -chymotrypsin (pdb 4CHA)^[20] were taken and docking was restricted to the active site. All docked results were compared and the following observation was made (Figure 6): The putative binding of inhibitor **22** in the thrombin (light gray) and chymotrypsin (dark grey) pocket is marked by the formation of a hydrogen bonding between the lone pair of the nitrogen atom in the pyridine ring and the cysteine hydrogen atom of thrombin/chymotrypsin (Cys-42). This hydrogen bond can stabilise the complex formed and is not present in the other compounds. This is a feature which can be taken into consideration in future development of inhibitors of these serine proteases.

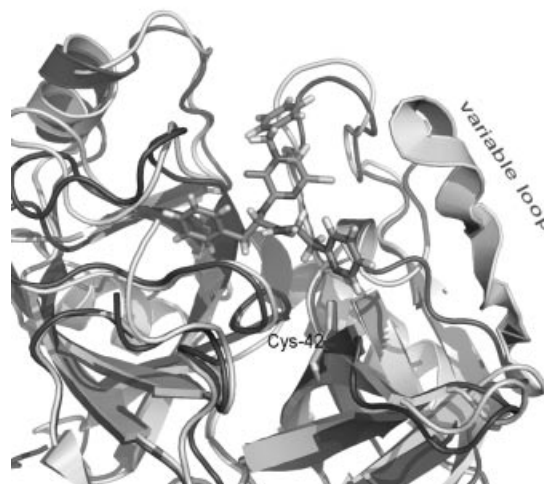


Figure 6. Putative binding of the inhibitors.

Conclusions

We have described the design, the preparation and the evaluation of a library of analogues of serine protease inhibitors **12–36**. They are accessible through the substituted pyrazinone **1** which has been developed in our laboratory.

Experimental Section

General Remarks: Analytical and preparative thin layer chromatography were performed on TLC plates coated with Alugram Sil G/UV₂₅₄. Column chromatography was carried out by using 70–230 mesh silica gel 60 (E. M. Merck) as the stationary phase. ¹H and ¹³C NMR spectra were recorded with Bruker AMX 400 and Bruker Avance 300 spectrometers, and chemical shifts (δ) are given in ppm relative to tetramethylsilane as an internal reference. Mass spectra were recorded with a Hewlett Packard MS-Engine 5989A apparatus for EI and CI spectra and a Kratos MS 50TC instrument using a DS90 data system for exact mass measurements performed in the EI mode at a resolution of 10,000. APCI and ESI spectra were recorded with a Thermo Finnigan LCQ Advantage mass spectrometer. Infrared spectra were recorded with a Perkin–Elmer 1720 Fourier transform spectrometer. All melting points are uncorrected, and were measured with an Electrothermal IA 9000 digital melting point apparatus.

Enzymes and Substrates for Kinetic Assays: Human-derived α -thrombin (Biochemika, Fluka, 0.89 NIH units·mg⁻¹) with tosyl-GPR-pNA (Chromozym TH, Sigma); bovine trypsin (Sigma) with benzyl-DL-arginine-pNA (BAPA, Sigma) and bovine α -chymotrypsin (Sigma) with succinyl-AAPF-pNA (Biochemika, Fluka) (where suc is succinyl, pNA is *p*-nitroaniline, bzl is benzoyl). Stock solutions of the enzymes were prepared in milliQ water (thrombin at 1.0 mg/mL) or in 1 mM HCl (trypsin and chymotrypsin at 0.335 mg/mL). For the assays these solutions were diluted in 50 mM Tris/HCl buffer pH = 8.2, containing 0.1 M NaCl and 0.1 % (w/v) polyethyl-

ene glycol (PEG) 6000 to a concentration of 38 $\mu\text{g/mL}$ (1.1 μM), 9.3 $\mu\text{g/mL}$ (0.40 μM) and 0.46 $\mu\text{g/mL}$ (19 nM) for thrombin, trypsin and chymotrypsin, respectively. PEG 6000 was included in the Tris buffer to avoid loss of enzyme or inhibitor due to adsorption to the plastic walls of the cuvettes.^[14] The stock solutions of the substrates were: 1.9 mM tos-GPR-*p*-NA in water, 100 mM bzI-DL-R-*p*-NA in DMSO and 40 mM suc-AAPF-*p*-NA in DMSO. Measurements were performed at 25 °C with a Shimadzu UV-1601 spectrophotometer, equipped with a temperature-controlled cuvette holder and PC for data storage, using 1-cm polystyrene cuvettes. The reference cuvette was filled with the Tris buffer. In the test cuvette, the enzyme solution (700 μL) was mixed with DMSO (50 μL , control experiment) or with 50 μL of a solution of the ligand dissolved in DMSO at 1 mg/mL ($\approx$ 1 mM; final concentration 50 μM). After incubation at 25 °C for 1–2 min, 2–30 μL of a solution of the appropriate substrate was added so as to reach a final substrate concentration in the range 5–500 μM , and the total reaction volume was brought to 800 μL with water. The contents of the cuvette were well mixed, and the absorbance at 405 nm, indicative of the release of *p*-nitroaniline, was measured for 240 s against the Tris buffer (reference cuvette). Each kinetic analysis was performed at 3–4 substrate concentrations.

X-ray Structure Analysis: Crystals of compound **33** were grown from ethanol. Crystal dimensions 0.15 $\times$ 0.36 $\times$ 0.42 mm, triclinic $P\bar{1}$, $a = 8.9974(9)$ Å, $b = 9.5444(9)$ Å, $c = 13.9283(13)$ Å, $\alpha = 79.104(6)^\circ$, $\beta = 79.104(6)^\circ$, $\gamma = 68.284(6)^\circ$, $V = 1054.64(18)$ Å³; $Z = 2$, $\rho_{\text{calcd.}} = 1.373$ g cm⁻³, $2\theta_{\text{max}} = 142^\circ$, $\mu(\text{Cu-K}\alpha) = 1.858$ cm⁻¹, Bruker SMART 6000 detector, Cu-K α ($\lambda = 1.54178$ Å), crossed Göbel mirrors, $T = 100$ K, 10780 measured reflections, 3847 independent reflections. The data were corrected for Lorentz, absorption, and polarization effects. The structure was solved by direct methods. Full matrix least-squares refinement based on $|F^2|$, 280 parameters, hydrogen atoms placed at calculated positions with temperature factors 20% higher than those of the parent atoms, $R_1 = 0.0412$ [for 3187 with $I > 2\sigma(I)$], $\omega R_2 = 0.1093$, max./min. residual electron density 0.25/–0.35 e⁻ Å⁻³. Crystallographic data (excluding structure factors) for compound **33** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-616659. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033 or E-mail: deposit@ccdc.cam.ac.uk) or via www.ccdc.cam.ac.uk/data_request/cif.

I. Synthesis of Pyrazinones

I.1. Synthesis of 1-Benzyl-3,5-dichloro-1H-pyrazin-2-one: See ref.^[10]

I.2. Synthesis of 3,5-Dichloro-1-phenethyl-1H-pyrazin-2-one (1b):^[21] Yield 4.92 g (65%). M.p. 190 °C. IR (KBr, assignment): $\tilde{\nu} = 1660$ (CO), 1589 (C=N) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.33$ – 7.12 (m, 5 H, Ph-H), 6.82 (s, 1 H, 6-H), 4.14 (t, $J = 8$ Hz, 2 H, NCH₂), 3.06 (t, $J = 8$ Hz, 2 H, CH₂Ph) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 163.9$ (C-3), 157.5 (CO), 136.7 (C-*ipso* Ph), 129.4, 129.1, 127.8 (Ph-C), 127.3 (C-6), 123.8 (C-5), 53.6 (NCH₂), 34.6 (CH₂) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 268 (5) [M⁺], 91 (26) [PhCH₂⁺]. HRMS (EI): calcd. for C₁₃H₁₂Cl₂N₂O 268.0170; found 268.0174.

II. Synthesis of 3-Substituted Pyrazinones

General Method: A solution of pyrazinone (1 equiv.), glycine methyl ester hydrochloride (or L-phenylalanine methyl ester hydrochloride or β -alanine methyl ester hydrochloride) (2 equiv.) and NEt₃ (4 equiv.) in DMF (10 mL) was stirred at 80 °C overnight. Then the mixture was concentrated to dryness and redissolved in

EtOAc (10 mL) and 0.5 N HCl (10 mL). After separation of the organic layer and further extraction of the aqueous phase with EtOAc (3 $\times$ 50 mL), the combined organic layers were washed with brine and dried with Na₂SO₄. Finally, purification by column chromatography (silica gel, eluent EtOAc/CH₂Cl₂, 10:90) yielded the desired 3-substituted pyrazinones.

II.1. Synthesis of Methyl [(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]acetate (3): Yield 0.36 g (40%). M.p. 172 °C. IR (KBr, assignment): $\tilde{\nu} = 3320$ (NH), 1670 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.31$ – 7.17 (m, 5 H, Ph-H), 6.71 (br. s, 1 H, C2-NH), 6.35 (s, 1 H, 5-H), 4.20 (d, $J = 5$ Hz, 2 H, CH₂CO), 4.03 (t, $J = 8$ Hz, 2 H, NCH₂), 3.79 (s, 3 H, OCH₃), 3.02 (t, $J = 8$ Hz, 2 H, CH₂Ph) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 169.8$ (CO), 150.0 (C-3), 149.7 (C-2), 137.1 (C-*ipso* Ph), 128.9, 128.7, 127.0 (Ph-C), 126.1 (C-6), 113.7 (C-5), 52.3 (CH₃), 50.9 (NCH₂), 42.6 (NHCH₂CO), 34.6 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 321 (100) [M⁺], 262 (19) [M⁺ – CO-OCH₃]. HRMS (EI): calcd. for C₁₅H₁₆ClN₃O₃ 321.0880; found 321.0880.

II.2. Synthesis of Methyl [(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]acetate (2): Yield 0.82 g (42%). M.p. 134 °C. IR (KBr, assignment): $\tilde{\nu} = 3323$ (NH), 1744 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.35$ – 7.28 (m, 5 H, Ph-H), 6.85 (t, $J = 6$ Hz, 1 H, C2-NH), 6.53 (s, 1 H, 5-H), 5.00 (s, 2 H, NCH₂), 4.18 (d, $J = 6$ Hz, 2 H, NH CH₂CO), 3.76 (s, 3 H, OCH₃) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 169.8$ (CO), 150.2 (C-3), 150.0 (C-2), 135.2 (C-*ipso* Ph), 129.4, 128.9, 128.7 (Ph-C), 126.9 (C-6), 113.1 (C-5), 52.8 (CH₃), 52.2 (NCH₂), 43.0 (NHCH₂CO) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 307 (35) [M⁺], 248 (6) [M⁺ – COOCH₃], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₁₄H₁₄ClN₃O₃ 307.0722; found 307.0723.

II.3. Synthesis of Methyl 3-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]propionate (4): Yield 1.86 g (73%). M.p. 87 °C. IR (KBr, assignment): $\tilde{\nu} = 3322$ (NH), 1734 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.20$ (br. s, 5 H, Ph-H), 6.95 (t, $J = 5$ Hz, 1 H, C2-NH), 6.46 (s, 1 H, 5-H), 4.89 (s, 2 H, NCH₂), 4.01 (q, $J = 8$ Hz, 1 H, CH_ANH), 3.61 (q, $J = 8$ Hz, 1 H, CH_BNH), 3.55 (s, 3 H, OCH₃), 2.55 (t, $J = 8$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 172.5$ (CO), 150.6 (C-3), 150.5 (C-2), 135.4 (C-*ipso* Ph), 129.2, 128.6, 128.5 (Ph-C), 127.1 (C-6), 112.5 (C-5), 52.1 (CH₃), 52.0 (NCH₂), 36.9 (NHCH₂), 34.4 (CH₂CO) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 321 (63) [M⁺], 290 (6) [M⁺ – OCH₃], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₁₅H₁₆ClN₃O₃ 321.0872; found 321.0880.

II.4. Synthesis of Methyl 3-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]propionate (5): Yield 1.35 g (51%). M.p. 114 °C. IR (KBr, assignment): $\tilde{\nu} = 3332$ (NH), 1653 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.20$ – 7.05 (m, 5 H, Ph-H), 6.82 (br. t, $J = 7$ Hz, 1 H, C2-NH), 6.28 (s, 1 H, 5-H), 3.90 (t, $J = 8$ Hz, 2 H, NCH₂), 3.63– 3.56 (m, 5 H, C2NHCH₂ + OCH₃), 2.88 (t, $J = 8$ Hz, 2 H, CH₂CO), 2.56 (t, $J = 8$ Hz, 2 H, CH₂Ph) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 172.6$ (CO), 150.4 (C-3), 150.3 (C-2), 137.6 (C-*ipso* Ph), 129.1, 129.0, 127.2 (Ph-C), 126.7 (C-6), 113.0 (C-5), 52.1 (CH₃), 51.0 (NCH₂), 36.8 (NHCH₂), 34.8 (CH₂CO), 33.5 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 335 (90) [M⁺], 105 (100) [PhCH₂CH₂⁺]. HRMS (EI): calcd. for C₁₆H₁₈ClN₃O₃ 335.1036; found 335.1037.

II.5. Synthesis of Methyl 2-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]-3-phenylpropionate (6): Yield 1.56 g (95%). Oil. IR (KBr, assignment): $\tilde{\nu} = 3566$ (NH), 1734 (CO), 1669 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.30$ – 7.13 (m, 10 H, Ph-H), 6.78 (d, $J = 8$ Hz, 1 H, C2-NH), 6.48 (s, 1 H, 5-H), 4.94– 4.86 (m, 3 H,

$NCH_2 + CHCO$), 3.65 (s, 3 H, OCH_3), 3.20 (dd, $J = 14$ Hz, 6 Hz, 1 H, CH_APh), 3.15 (dd, $J = 14$ Hz, 6 Hz, 1 H, CH_BPh) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.2$ (CO), 149.8 (C-3), 149.0 (C-2), 135.6, 134.6 (C-*ipso* Ph), 128.9, 128.6, 128.3, 128.1, 127.8, 126.8 (Ph-C), 126.1 (C-6), 112.9 (C-5), 54.5 (NHCH), 51.9 (OCH_3), 51.5 (NCH_2), 37.4 (CH_2Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 397 (30) [M^+], 338 (11) [$M^+ - COOCH_3$], 91 (100) [$PhCH_2^+$]. HRMS (EI): calcd. for $C_{21}H_{20}ClN_3O_3$ calculated: 397.1193; found 397.1198.

III. Hydrolysis of the Methyl Ester Derivatives and Synthesis of Linear Analogues of Serine Protease Inhibitors

General Method: To a solution of the methyl ester (1 equiv.) in THF/MeOH/ H_2O (3:2:1, 10 mL), lithium hydroxide was added (5–8 equiv.). After stirring at room temperature for 4 h, the residue was concentrated and then acidified with dilute HCl. The residue was then extracted with EtOAc (3×50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, eluent EtOAc/ CH_2Cl_2 , 70:30) to provide the acid derivatives **7–11**. The latter were dissolved in a solvent mixture of DMF/ CH_3CN (2:1, 10 mL), then the amine (1 equiv.) followed by DIC (1.5 equiv.) was added. After stirring at 0 °C for 15 min, NMM (1.2 equiv.) and HOBt (1 equiv.) were added. The reaction mixture was stirred at room temperature for 4 h. After extraction with EtOAc (3×50 mL), the residue was subjected to column chromatography (silica gel, eluent EtOAc/ CH_2Cl_2 , 50:50).

III.1. Synthesis of *N*-(Benzyl)-2-[(6-chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]acetamide (15): Yield 0.12 g (40%). M.p. 115 °C. IR (KBr, assignment): $\tilde{\nu} = 3391$ (NH), 1646 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.30$ –7.13 (m, 10 H, Ph-H), 6.89 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 6.48 (t, $J = 5$ Hz, 1 H, CONH or C2-NH), 6.35 (s, 1 H, 5-H), 4.45 (d, $J = 6$ Hz, 2 H, CH_2CO), 4.10 (d, $J = 6$ Hz, 2 H, $NHCH_2Ph$), 4.00 (t, $J = 8$ Hz, 2 H, NCH_2), 2.98 (t, $J = 8$ Hz, 2 H, CH_2Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.4$ (CO), 150.0 (C-3 + C-2), 138.6, 137.8 (C-*ipso* Ph), 128.8, 128.7, 127.5 (Ph-C), 126.0 (C-6), 114.1 (C-5), 50.9 (NCH_2), 45.0 (CH_2CO), 43.4 ($NHCH_2Ph$), 34.6 (CH_2Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 396 (64) [M^+], 105 (100) [$PhCH_2CH_2^+$]. HRMS (EI): calcd. for $C_{21}H_{21}ClN_4O_2$ 396.1353; found 396.1358.

Synthesis of 2-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-*N*-(phenethyl)acetamide (20): Yield 0.20 g (80%). M.p. 140 °C. IR (KBr, assignment): $\tilde{\nu} = 3300$ (NH), 1699 (CO), 1652 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.32$ –7.13 (m, 10 H, Ph-H), 6.83 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 6.37 (s, 1 H, 5-H), 6.11 (t, $J = 5$ Hz, 1 H, CONH or C2-NH), 4.03 (t, $J = 8$ Hz, 2 H, NCH_2), 4.01 (d, $J = 6$ Hz, 2 H, CH_2CO), 3.53 (qd, $J = 7$ Hz, 2 H, $NHCH_2CH_2Ph$), 3.00 (t, $J = 7$ Hz, 2 H, CH_2Ph), 2.80 (t, $J = 8$ Hz, 2 H, CH_2Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.3$ (CO), 150.0 (C-3), 149.9 (C-2), 137.0 (C-*ipso* Ph), 128.7, 127.0, 126.4 (Ph-C), 126.0 (C-6), 113.9 (C-5), 50.9 (NCH_2), 44.9 (CH_2CO), 40.6 ($NHCH_2CH_2Ph$), 35.5 ($NHCH_2CH_2Ph$), 34.6 (CH_2Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 410 (65) [M^+], 290 (16) [$M^+ - NHCH_2CH_2Ph$], 105 (100) [$PhCH_2CH_2^+$]. HRMS (EI): calcd. for $C_{22}H_{23}ClN_4O_2$ 410.1507; found 410.1509.

Synthesis of 2-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-*N*-(2-pyridin-2-ylethyl)acetamide (30): Yield 0.10 g (30%). M.p. 162 °C. IR (KBr, assignment): $\tilde{\nu} = 3321$ (NH), 1683 (CO), 1652 (CO) cm^{-1} . 1H NMR (400 MHz, CD_3OD): $\delta = 8.44$ (d, $J = 6$ Hz, 1 H, 6'-H), 7.65 (td, $J = 7$ Hz, 2 Hz, 1 H, 4'-H), 7.33–7.16 (m, 7 H, Ph-H + 3'-H + 5'-H), 6.39 (s, 1 H, 5-H), 4.06 (d, $J =$

6 Hz, 2 H, CH_2CO), 3.87 (t, $J = 8$ Hz, 2 H, NCH_2), 3.61 (t, $J = 8$ Hz, 2 H, $NHCH_2CH_2pyr$), 3.02 (t, $J = 8$ Hz, 2 H, CH_2pyr), 2.96 (t, $J = 8$ Hz, 2 H, CH_2Ph) ppm. ^{13}C NMR (100 MHz, CD_3OD): $\delta = 169.5$ (CO), 159.1 (C-*ipso* pyr), 150.4 (C-3), 150.0 (C-2), 148.8, 137.7 (pyr-C), 137.4 (C-*ipso* Ph), 129.1, 129.0, 127.3 (Ph-C), 126.5 (C-6), 124.3, 122.2 (pyr-C), 114.5 (C-5), 51.3 (NCH_2), 44.5 (CH_2CO), 39.3 ($NHCH_2CH_2pyr$), 37.1 (CH_2pyr), 34.8 (CH_2Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 411 (100) [M^+], 262 (11) [$M^+ - CONHCH_2CH_2pyr$]. HRMS (EI): calcd. for $C_{22}H_{23}ClN_4O_2$ 411.1456; found 411.1462.

Synthesis of 2-[(6-Chloro-3-oxo-4-phenethyl-3,4-dihydropyrazin-2-yl)amino]-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide (35): Yield 0.41 g (80%). M.p. 120 °C. IR (KBr, assignment): $\tilde{\nu} = 3317$ (NH), 1647 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): $\delta = 8.20$ (s, 1 H, NH-ind), 7.56 (d, $J = 8$ Hz, 1 H, 4i-H), 7.32 (d, $J = 8$ Hz, 1 H, 7i-H), 7.29–7.21 (m, 3 H, Ph-H), 7.18 (t, $J = 8$ Hz, 1 H, 6i-H), 7.16–7.15 (m, 2 H, Ph-H), 7.10 (t, $J = 8$ Hz, 1 H, 5i-H), 7.00 (d, $J = 2$ Hz, 1 H, 2i-H), 6.85 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 6.30 (s, 1 H, 5-H), 6.12 (t, $J = 5$ Hz, 1 H, CONH or C2-NH), 4.00 (d, $J = 6$ Hz, 2 H, CH_2CO), 3.96 (t, $J = 8$ Hz, 2 H, NCH_2), 3.64 (qd, $J = 6$ Hz, 2 H, $NHCH_2CH_2ind$), 2.97 (t, $J = 6$ Hz, 2 H, CH_2ind), 2.96 (t, $J = 8$ Hz, 2 H, CH_2Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.3$ (CO), 149.9 (C-3), 149.8 (C-2), 137.0 (C-*ipso* Ph), 134.7 (C-7ai), 128.8, 128.7, 127.0 (Ph-C), 127.2 (C-3ai), 125.9 (C-6), 122.2 (C-2i), 122.0 (C-6i), 119.4 (C-5i), 118.5 (C-4i), 113.8 (C-5), 112.5 (C-3i), 111.2 (C-7i), 50.8 (NCH_2), 44.8 (CH_2CO), 39.6 ($NHCH_2CH_2ind$), 34.5 (CH_2Ph), 25.0 (CH_2ind) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 449 (14) [M^+], 306 (35) [$M^+ - CH_2=CHind$], 143 (100) [$indCH=CH_2^+$]. HRMS (EI): calcd. for $C_{24}H_{24}ClN_5O_2$ 449.1618; found 449.1618.

Synthesis of 2-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-*N*-(pyridin-2-ylmethyl)acetamide (25): Yield 0.14 g (40%). M.p. 151 °C. IR (KBr, assignment): $\tilde{\nu} = 3320$ (NH), 1683 (CO), 1652 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): $\delta = 8.44$ (d, $J = 6$ Hz, 1 H, 6'-H), 7.65 (td, $J = 8$ Hz, 2 Hz, 1 H, 4'-H), 7.29–7.15 (m, 8 H, 5'-H + Ph-H + 3'-H + CONH or C2-NH), 7.02 (t, $J = 5$ Hz, C2-NH or CONH), 6.35 (s, 1 H, 5-H), 4.57 (d, $J = 6$ Hz, 2 H, CH_2CO), 4.18 (d, $J = 6$ Hz, 2 H, CH_2pyr), 4.02 (t, $J = 8$ Hz, 2 H, NCH_2), 3.35 (t, $J = 8$ Hz, 2 H, CH_2Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.5$ (CO), 158.5 (C-*ipso* pyr), 151.3 (C-3), 151.2 (C-2), 149.2, 138.6 (pyr-C), 138.3 (C-*ipso* Ph), 129.5, 129.4, 127.6 (Ph-C), 127.1 (C-6), 123.3, 122.5 (pyr-C), 115.1 (C-5), 51.6 (NCH_2), 45.1 (CH_2CO), 45.0 (CH_2pyr), 35.1 (CH_2Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 398 (37) [M^+], 105 (100) [$PhCH_2CH_2^+$]. HRMS (EI): calcd. for $C_{20}H_{20}ClN_5O_2$ 397.1305; found 397.1306.

Synthesis of *N*-Benzyl-2-[(4-benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]acetamide (13): Yield 0.31 g (50%). M.p. 135 °C. IR (KBr, assignment): $\tilde{\nu} = 3306$ (NH), 1648 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.33$ –7.21 (m, 12 H, Ph-H + C2-NH + CONH), 6.55 (s, 1 H, 5-H), 4.95 (s, 2 H, NCH_2), 4.42 (d, $J = 5$ Hz, 2 H, CH_2Ph), 4.06 (d, $J = 5$ Hz, 2 H, CH_2CO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.7$ (CO), 150.1 (C-3), 149.9 (C-2), 137.4 (C-*ipso* Ph), 134.4 [C-*ipso* Ph (CH_2N)], 128.8, 128.3, 128.1, 127.2, 127.1 (Ph-C), 126.4 (C-6), 113.0 (C-5), 51.7 (NCH_2), 44.4 (CH_2CO), 43.1 (CH_2Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 382 (5) [M^+], 91 (100) [$PhCH_2^+$]. HRMS (EI): calcd. for $C_{20}H_{19}ClN_4O_2$ 382.1196; found 382.1196.

Synthesis of 2-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]-*N*-(phenethyl)acetamide (18): Yield 0.20 g (30%). M.p. 137 °C. IR (KBr, assignment): $\tilde{\nu} = 3312$ (NH), 1646 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.36$ –7.11 (m, 10 H, Ph-H), 6.89 (t,

$J = 5$ Hz, 1 H, C2-NH or CONH), 6.54 (s, 1 H, 5-H), 6.15 (t, $J = 5$ Hz, 1 H, CONH or C2-NH), 4.99 (s, 2 H, NCH₂), 4.01 (d, $J = 6$ Hz, 2 H, CH₂CO), 3.52 (q, $J = 7$ Hz, 2 H, NHCH₂CH₂Ph), 2.79 (t, $J = 7$ Hz, 2 H, CH₂Ph) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 168.3$ (CO), 150.2 (C-3), 150.0 (C-2), 138.6 (C-*ipso* Ph), 134.4 [C-*ipso* Ph (CH₂N)], 129.0, 128.7, 128.6 (Ph-C), 126.4 (C-6), 113.3 (C-5), 51.9 (NCH₂), 44.9 (CH₂CO), 40.5 (NHCH₂CH₂Ph), 35.5 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 396 (21) [M⁺], 248 (18) [M⁺ – CONHCH₂CH₂Ph]. HRMS (EI): calcd. for C₂₀H₁₉ClN₄O₂ 396.1352; found 396.1353.

Synthesis of 2-[(4-Benzyl-6-chloro-3-oxo-3,4-dihydropyrazin-2-yl)-amino]-N-(2-pyridin-2-ylethyl)acetamide (28): Yield 0.23 g (40%). M.p. 150 °C. IR (KBr, assignment): $\tilde{\nu} = 3382$ (NH), 1650 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 8.36$ (d, $J = 5$ Hz, 1 H, 6'-H), 7.54 (td, $J = 7$ Hz, 2 H, 1 H, 4'-H), 7.54–7.27 (m, 5 H, Ph-H), 7.26 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 7.12 (d, $J = 7$ Hz, 1 H, 3'-H), 7.04 (t, $J = 7$ Hz, 2 H, 5'-H), 7.02 (t, $J = 5$ Hz, 1 H, CONH or C2-NH), 6.51 (s, 1 H, 5-H), 4.99 (s, 2 H, NCH₂), 4.05 (d, $J = 6$ Hz, 2 H, CH₂CO), 3.67 (q, $J = 6$ Hz, 2 H, NHCH₂CH₂pyr), 2.98 (t, $J = 6$ Hz, 2 H, CH₂pyr) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 168.1$ (CO), 159.3 (C-*ipso* pyr), 150.2 (C-3), 149.9 (C-2), 148.8, 136.6 (pyr-C), 134.8 (C-*ipso* Ph), 129.0, 128.5, 128.3 (Ph-C), 126.5 (C-6), 123.5, 121.1 (pyr-C), 113.0 (C-5), 51.8 (NCH₂), 44.7 (CH₂CO), 38.5 (NHCH₂CH₂pyr), 35.5 (CH₂pyr) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 397 (47) [M⁺], 249 (7) [M⁺ – CONHCH₂CH₂pyr]. HRMS (EI): calcd. for C₂₀H₂₀ClN₅O₂ 397.1298; found 397.1305.

Synthesis of 2-[(4-Benzyl-6-chloro-3-oxo-3,4-dihydropyrazin-2-yl)-amino]-N-(pyridin-2-ylmethyl)acetamide (23): Yield 0.13 g (40%). M.p. 190 °C. IR (KBr, assignment): $\tilde{\nu} = 3260$ (NH), 1647 (CO) cm⁻¹. ¹H NMR (400 MHz, [D₆]MeOH): $\delta = 8.44$ (d, $J = 6$ Hz, 1 H, 6'-H), 7.75 (td, $J = 7$ Hz, 2 H, 1 H, 4'-H), 7.42 (d, $J = 7$ Hz, 1 H, 3'-H), 7.37–7.30 (m, 5 H, Ph-H), 7.24 (t, $J = 7$ Hz, 1 H, 5'-H), 6.69 (s, 1 H, 5-H), 5.03 (s, 2 H, NCH₂), 4.13 (s, 2 H, CH₂CO), 3.35 (s, 2 H, CH₂pyr) ppm. ¹³C NMR (100 MHz, CD₃OD): $\delta = 169.5$ (CO), 156.6 (C-*ipso* pyr), 149.7 (C-3), 149.4 (C-2), 147.7, 137.1 (pyr-C), 134.2 (C-*ipso* Ph), 128.3, 127.8, 127.6 (Ph-C), 126.0 (C-6), 122.0, 121.2 (pyr-C), 112.9 (C-5), 51.2 (NCH₂), 43.5 (CH₂CO), 35.9 (CH₂pyr) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 383 (36) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₁₉H₁₈ClN₅O₂ 383.1149; found 383.1149.

Synthesis of 2-[(4-Benzyl-6-chloro-3-oxo-3,4-dihydropyrazin-2-yl)-amino]-N-[2-(1H-indol-3-yl)ethyl]acetamide (33): Yield 0.20 g (40%). M.p. 188 °C. IR (KBr, assignment): $\tilde{\nu} = 3325$ (NH), 1669 (CO), 1650 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 10.8$ (s, 1 H, NH-ind), 8.10 (t, $J = 5$ Hz, 1 H, CONH), 7.75 (t, $J = 5$ Hz, 1 H, C2-NH), 7.58 (d, $J = 8$ Hz, 1 H, 4i-H), 7.39 (d, $J = 8$ Hz, 1 H, 7i-H), 7.35–7.17 (m, 5 H, Ph-H), 7.20 (s, 1 H, 2i-H), 7.14 (s, 1 H, 5-H), 7.10 (t, $J = 8$ Hz, 1 H, 5i-H), 7.01 (t, $J = 8$ Hz, 1 H, 6i-H), 5.05 (s, 2 H, NCH₂), 3.96 (d, $J = 5$ Hz, 2 H, CH₂CO), 3.45 (q, $J = 6$ Hz, 2 H, NHCH₂CH₂ind), 2.90 (t, $J = 6$ Hz, 2 H, CH₂ind) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 168.1$ (CO), 150.2 (C-3), 149.9 (C-2), 136.3 (C-7ai), 136.0 (C-*ipso* Ph), 128.6, 128.0, 127.8 (Ph-C), 127.2 (C-3ai), 125.1 (C-6), 122.6 (C-2i), 120.9 (C-6i), 118.2 (C-5i), 118.2 (C-4i), 113.4 (C-5), 111.7 (C-3i), 111.3 (C-7i), 51.0 (NCH₂), 43.8 (CH₂CO), 39.7 (NHCH₂CH₂ind), 25.1 (CH₂ind) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 435 (6) [M⁺], 292 (24) [M⁺ – CH₂=CHind], 143 (100) [indCH=CH₂⁺]. HRMS (EI): calcd. for C₂₃H₂₂ClN₅O₂ 435.1462; found 435.1464.

Synthesis of 3-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)-amino]-N-(pyridin-2-ylmethyl)propionamide (24): Yield 0.27 g (42%). M.p. 149 °C. IR (KBr, assignment): $\tilde{\nu} = 3316$ (NH), 1652

(s, CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 8.49$ (d, $J = 5$ Hz, 1 H, 6'-H), 7.67 (dt, $J = 7$ Hz, 2 H, 1 H, 4'-H), 7.36–7.27 (m, 6 H, Ph-H + 3'-H), 7.21 (t, $J = 7$ Hz, 1 H, 5'-H), 7.01 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 6.90 (t, $J = 5$ Hz, CONH or C2-NH), 6.45 (s, 1 H, 5-H), 4.97 (s, 2 H, NCH₂), 4.57 (d, $J = 5$ Hz, 2 H, CH₂pyr), 3.78 (qd, $J = 8$ Hz, 2 H, NHCH₂CH₂), 2.62 (t, $J = 8$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 171.8$ (CO), 156.9 (C-*ipso* pyr), 150.2 (C-3), 150.1 (C-2), 148.3, 137.3 (pyr-C), 134.7 (C-*ipso* Ph), 128.8, 128.2, 128.0 (Ph-C), 126.7 (C-6), 122.4, 122.1 (pyr-C), 111.9 (C-5), 51.6 (NCH₂), 44.2 (CH₂pyr), 37.1 (C2NHCH₂), 34.8 (CH₂CO) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 397 (85) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₂₀H₂₀ClN₅O₂ 397; 1305; found 397.1318.

Synthesis of 3-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)-amino]-N-[2-(pyridin-2-yl)ethyl]propionamide (29): Yield 0.20 g (30%). M.p. 157 °C. IR (KBr, assignment): $\tilde{\nu} = 3303$ (NH), 1699 (CO), 1646 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 8.47$ (d, $J = 5$ Hz, 1 H, 6'-H), 7.56 (td, $J = 7$ Hz, 2 H, 1 H, 3'-H), 7.35–7.27 (m, 5 H, Ph-H), 7.15–7.09 (m, 2 H, 3'-H + 5'-H), 6.84 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 6.68 (t, $J = 5$ Hz, 1 H, C2-NH or CONH), 6.45 (s, 1 H, 5-H), 4.96 (s, 2 H, NCH₂), 3.71 (q, $J = 6$ Hz, 2 H, C2NHCH₂), 3.66 (qd, $J = 6$ Hz, 2 H, NHCH₂CH₂pyr), 2.98 (t, $J = 6$ Hz, 2 H, CH₂pyr), 2.48 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 168.1$ (CO), 159.3 (C-*ipso* pyr), 150.2 (C-3), 149.9 (C-2), 148.8, 136.6 (pyr-C), 134.8 (C-*ipso* Ph), 129.0, 128.5, 128.3 (Ph-C), 126.5 (C-6), 123.5, 121.1 (pyr-C), 113.0 (C-5), 51.8 (NCH₂), 44.7 (NHCH₂CH₂pyr), 38.5 (C2NHCH₂), 37.2 (CH₂pyr), 35.5 (CH₂CO) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 411 (58) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₂₁H₂₂ClN₅O₂ 411.1462; found 411.1463.

Synthesis of N-Benzyl-3-[(4-benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]propionamide (14): Yield 0.30 g (50%). M.p. 191 °C. IR (KBr, assignment): $\tilde{\nu} = 3278$ (NH), 1699 (CO), 1636 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃ + CD₃OD): $\delta = 7.36$ –7.23 (m, 10 H, Ph-H), 6.55 (s, 1 H, 5-H), 5.00 (s, 2 H, NCH₂), 4.38 (br. s, 2 H, CH₂Ph), 3.71 (t, $J = 6$ Hz, 2 H, C2NHCH₂), 2.57 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃ + CD₃OD): $\delta = 171.6$ (CO), 150.2 (C-3), 150.0 (C-2), 137.9 (C-*ipso* Ph), 134.6 [C-*ipso* Ph (N-1)], 128.7, 128.3, 128.2, 128.0, 127.3, 127.0 (Ph-C), 126.6 (C-6), 111.9 (C-5), 51.6 (NCH₂), 43.1 (CH₂Ph), 37.2 (C2NHCH₂), 34.9 (CH₂CO) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 396 (66) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₂₁H₂₁ClN₄O₂ 396.1353; found 396.1356.

Synthesis of 3-[(4-Benzyl-6-chloro-3-oxo-3,4-dihydropyrazin-2-yl)-amino]-N-(phenethyl)propionamide (19): Yield 0.14 g (32%). M.p. 162 °C. IR (KBr, assignment): $\tilde{\nu} = 3628$ (NH), 1699 (CO), 1645 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.36$ –7.15 (m, 10 H, Ph-H), 6.83 (t, $J = 5$ Hz, 1 H, C2NH or CONH), 6.47 (s, 1 H, 5-H), 5.81 (t, $J = 5$ Hz, 1 H, CONH or C2NH), 4.97 (s, 2 H, NCH₂), 3.71 (qd, $J = 6$ Hz, 2 H, C2NHCH₂), 3.52 (qd, $J = 7$ Hz, 2 H, NHCH₂CH₂Ph), 2.79 (t, $J = 7$ Hz, 2 H, CH₂Ph), 2.45 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 170.7$ (CO), 150.4 (C-3), 150.3 (C-2), 138.8 (C-*ipso* Ph), 134.9 (C-*ipso* Ph CH₂N), 129.0, 128.7, 128.6, 128.5, 128.3 (Ph-C), 126.4 (C-6), 112.0 (C-5), 51.8 (NCH₂), 40.6 (NHCH₂CH₂Ph), 37.2 (C2NHCH₂), 35.6 (CH₂CO), 35.4 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 410 (66) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₂₂H₂₃ClN₄O₂ 410.1508; found 410.1509.

Synthesis of 3-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)-amino]-N-[2-(1H-indol-3-yl)ethyl]propionamide (34): Yield 0.35 g (50%). M.p. 141 °C. IR (KBr, assignment): $\tilde{\nu} = 3396$ (s, NH), 1652 (s, CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 8.68$ (s, 1 H, NH-

ind), 7.54 (d, $J = 7$ Hz, 1 H, 4i-H), 7.39 (d, $J = 7$ Hz, 1 H, 7i-H), 7.32–7.23 (m, 5 H, Ph-H), 7.23–7.22 (m, 1 H, 2i-H), 7.14 (t, $J = 7$ Hz, 1 H, 5i-H), 7.05 (t, $J = 7$ Hz, 1 H, 6i-H), 6.91 (t, $J = 5$ Hz, 1 H, C2NH or CONH), 6.40 (s, 1 H, 5-H), 6.13 (t, $J = 5$ Hz, 1 H, CONH or C2NH), 4.89 (s, 2 H, NCH₂), 3.65 (qd, $J = 6$ Hz, 2 H, C2NHCH₂), 3.54 (qd, $J = 6$ Hz, 2 H, NHCH₂CH₂ind), 2.90 (t, $J = 6$ Hz, 2 H, CH₂ind), 2.37 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 171.1$ (CO), 150.0 (C-3), 149.9 (C-2), 136.3 (C-7ai), 134.7 (C-*ipso* Ph), 128.6, 128.1, 127.9 (Ph-C), 127.1 (C-3ai), 126.6 (C-6), 122.1 (C-2i), 121.2 (C-6i), 118.5 (C-5i), 118.0 (C-4i), 112.0 (C-5), 111.8 (C-3i), 111.0 (C-7i), 51.5 (NCH₂), 39.8 (NHCH₂CH₂ind), 37.1 (NHCH₂), 34.8 (CH₂CO), 24.8 (CH₂ind) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 449 (29) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₂₄H₂₄ClN₅O₂ 449.1618; found 449.1620.

Synthesis of 3-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-N-(pyridin-2-ylmethyl)propionamide (26): Yield 0.14 g (30%). M.p. 145 °C. IR (KBr, assignment): $\tilde{\nu} = 3323$ (NH), 1652 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃ + CD₃OD): $\delta = 8.05$ (d, $J = 6$ Hz, 1 H, 6'-H), 7.32 (td, $J = 7$ Hz, 2 H, 4'-H), 6.93 (d, $J = 7$ Hz, 1 H, 3'-H), 6.88–6.76 (m, 6 H, Ph-H + 5'-H), 6.09 (s, 1 H, 5-H), 4.11 (s, 2 H, CH₂pyr), 3.62 (t, $J = 8$ Hz, 2 H, NCH₂), 3.31 (t, $J = 6$ Hz, 2 H, C2NHCH₂), 2.57 (t, $J = 8$ Hz, 2 H, CH₂Ph), 2.24 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃ + CD₃OD): $\delta = 172.1$ (CO), 157.1 (C-*ipso* pyr), 149.8 (C-3), 149.7 (C-2), 148.1, 137.1 (pyr-C), 136.9 (C-*ipso* Ph), 128.2, 128.1, 126.3 (Ph-C), 126.1 (C-6), 122.0, 121.4 (pyr-C), 112.4 (C-5), 50.2 (NCH₂), 43.9 (CH₂pyr), 36.8 (NHCH₂), 34.4 (CH₂CO), 33.9 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 411 (91) [M⁺], 105 (100) [PhCH₂CH₂⁺]. HRMS (EI): calcd. for C₂₁H₂₂ClN₅O₂ 411.1159; found 411.1462.

Synthesis of N-Benzyl-3-[(6-chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]propionamide (16): Yield 0.30 g (34%). M.p. 132 °C. IR (KBr, assignment): $\tilde{\nu} = 3395$ (NH), 1734 (CO), 1652 (CO). ¹H NMR (400 MHz, CDCl₃): $\delta = 7.30$ –7.13 (m, 10 H, Ph-H), 6.89 (t, $J = 5$ Hz, 1 H, C2NH), 6.68 (t, $J = 5$ Hz, 1 H, CONH), 6.28 (s, 1 H, 5-H), 4.37 (d, $J = 6$ Hz, 2 H, NHCH₂Ph), 3.95 (t, $J = 8$ Hz, 2 H, NCH₂), 3.68 (qd, $J = 6$ Hz, 2 H, C2NHCH₂), 2.95 (t, $J = 8$ Hz, 2 H, CH₂Ph), 2.52 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 171.0$ (CO), 150.1 (C-3), 150.0 (C-2), 138.2, 137.2 (C-*ipso* Ph), 128.8, 128.7, 128.6, 127.7, 127.4, 127.3, 126.9 (Ph-C), 126.4 (C-6), 112.5 (C-5), 50.7 (NCH₂), 43.6 (C2NHCH₂), 37.3 (CH₂CO), 35.3 (CH₂pyr), 34.6 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 410 (100) [M⁺]. HRMS (EI): calcd. for C₂₂H₂₃ClN₄O₂ 410.1505; found 410.1509.

Synthesis of 3-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-N-(phenethyl)propionamide (21): Yield 0.41 g (62%). M.p. 138 °C. IR (KBr, assignment): $\tilde{\nu} = 3319$ (NH), 1699 (CO), 1652 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.29$ –7.14 (m, 10 H, Ph-H), 6.85 (t, $J = 6$ Hz, 1 H, C2NH), 6.29 (s, 1 H, 5-H), 6.08 (t, $J = 5$ Hz, 1 H, CONH), 3.95 (t, $J = 8$ Hz, 2 H, NCH₂), 3.68 (qd, $J = 6$ Hz, 2 H, C2NHCH₂), 3.49 (qd, $J = 7$ Hz, 2 H, CONHCH₂), 2.96 (t, $J = 7$ Hz, 2 H, CH₂Ph), 2.79 (t, $J = 7$ Hz, 2 H, NHCH₂CH₂Ph), 2.52 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 170.9$ (CO), 150.2 (C-3), 150.1 (C-2), 138.9, 137.2 (C-*ipso* Ph), 128.8, 128.7, 128.5, 126.9, 126.4 (Ph-C), 126.3 (C-6), 112.6 (C-5), 50.8 (NCH₂), 40.6 (CONHCH₂), 37.3 (C2NHCH₂), 35.6 (NHCH₂CH₂Ph), 35.4 (CH₂CO), 34.6 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 424 (100) [M⁺]. HRMS (EI): calcd. for C₂₃H₂₅ClN₄O₂ 424.1663; found 424.1665.

Synthesis of 3-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-N-[2-(pyridin-2-yl)ethyl]propionamide (31): Yield 0.25 g (54%). M.p. 130 °C. IR (KBr, assignment): $\tilde{\nu} = 3320$ (NH), 1683 (CO), 1635 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 8.43$ (d, $J = 6$ Hz, 1 H, 6'-H), 7.55 (td, $J = 7$ Hz, 2 H, 4'-H), 7.27–6.90 (m, 9 H, Ph-H + 5'-H + 3'-H + 2NH), 6.27 (s, 1 H, 5-H), 3.94 (t, $J = 8$ Hz, 2 H, NCH₂), 3.68 (q, $J = 8$ Hz, 2 H, C2NHCH₂), 3.60 (qd, $J = 8$ Hz, 2 H, CONHCH₂), 2.96–2.76 (m, 4 H, CH₂Ph + CH₂pyr), 2.04 (t, $J = 8$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 170.9$ (CO), 159.4 (C-*ipso* pyr), 150.2 (C-3), 150.1 (C-2), 148.9, 137.2 (pyr-C), 136.7 (C-*ipso* Ph), 128.7, 128.6, 126.8 (Ph-C), 126.4 (C-6), 123.4, 121.5 (pyr-C), 112.4 (C-5), 50.7 (NCH₂), 38.8 (CONHCH₂), 37.3 (C2NHCH₂), 36.9 (CH₂pyr), 35.2 (CH₂CO), 34.5 (CH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 425 (95) [M⁺], 105 (100) [PhCH₂CH₂⁺]. HRMS (EI): calcd. for C₂₂H₂₄ClN₅O₂ 425.1601; found 425.1618.

Synthesis of 3-[(6-Chloro-3,4-dihydro-3-oxo-4-phenethylpyrazin-2-yl)amino]-N-[2-(1H-indol-3-yl)ethyl]propionamide (36): Yield 0.20 g (40%). Oil. IR (KBr, assignment): $\tilde{\nu} = 3648$ (NH), 1684 (CO), 1652 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 8.95$ (br. s, 1 H, NH-ind), 7.49 (d, $J = 7$ Hz, 1 H, 4i-H), 7.27–7.11 (m, 6 H, Ph-H + 7i-H), 7.10 (t, $J = 7$ Hz, 1 H, 5i-H), 7.00 (t, $J = 7$ Hz, 1 H, 6i-H), 6.94 (t, $J = 7$ Hz, 1 H, C2NH), 6.90 (s, 1 H, 2i-H), 6.24 (t, $J = 5$ Hz, 1 H, CONH), 6.18 (s, 1 H, 5-H), 3.84 (t, $J = 8$ Hz, 2 H, NCH₂), 3.60 (q, $J = 8$ Hz, 2 H, C2NHCH₂), 3.56 (qd, $J = 6$ Hz, 2 H, CONHCH₂), 2.85 (m, 4 H, CH₂ind + CH₂Ph), 2.34 (t, $J = 6$ Hz, 2 H, CH₂CO) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 171.0$ (CO), 149.8 (C-2 and C-3), 136.9 (C-*ipso* Ph), 136.1 (C-7ai), 128.5, 128.4, 126.7 (Ph-C), 127.0 (C-3ai), 126.1 (C-6), 122.1 (C-2i), 121.5 (C-6i), 118.2 (C-4i), 118.8 (C-5i), 112.0 (C-5), 111.2 (C-7i), 50.4 (NCH₂), 39.5 (CONHCH₂), 37.0 (C2NHCH₂), 34.8 (CH₂CO), 34.2 (CH₂Ph), 24.8 (CH₂ind) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 463 (23) [M⁺], 143 (100) [indCH=CH₂⁺]. HRMS (EI): calcd. for C₂₅H₂₆ClN₅O₂ 463.1775; found 463.1779.

Synthesis of N-Benzyl-2-[(4-benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]-3-phenylpropionamide (12): Yield 0.35 g (50%). Oil. IR (KBr, assignment): $\tilde{\nu} = 3384$ (NH), 1683 (CO), 1635 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.28$ –7.04 (m, 15 H, Ph-H), 6.94 (d, $J = 7$ Hz, 1 H, C2NH), 6.66 (t, $J = 6$ Hz, 1 H, CONH), 6.44 (s, 1 H, 5-H), 4.83 (s, 2 H, NCH₂), 4.69 (q, $J = 7$ Hz, 1 H, CHCO), 4.30 (dd, $J = 14$ Hz, 6 Hz, 1 H, NHCH_A Ph), 4.26 (dd, $J = 14$ Hz, 6 Hz, 1 H, NHCH_B Ph), 3.15 (d, $J = 7$ Hz, 2 H, CHCH₂ Ph) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 170.3$ (CO), 149.9 (C-3), 149.4 (C-2), 137.6, 136.4, 134.4 (C-*ipso* Ph), 129.0, 128.7, 128.3, 128.2, 128.0, 127.1, 126.9, 126.6 (Ph-C), 126.1 (C-6), 113.0 (C-5), 56.3 (CHCO), 51.5 (NCH₂), 43.0 (NHCH₂Ph), 37.4 (CHCH₂Ph) ppm. EIMS {(relative intensity) [assignment]}: m/z (%) = 472 (24) [M⁺], 91 (100) [PhCH₂⁺]. HRMS (EI): calcd. for C₂₇H₂₅ClN₄O 472.1666; found 472.1668.

Synthesis of 2-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)amino]-3-phenyl-N-(phenethyl)propionamide (17): Yield 0.30 g (45%). Oil. IR (KBr, assignment): $\tilde{\nu} = 3365$ (NH), 1652 (CO) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.34$ –6.99 (m, 15 H, Ph-H), 6.76 (d, $J = 7$ Hz, 1 H, C2NH), 6.49 (s, 1 H, 5-H), 6.06 (t, $J = 6$ Hz, 1 H, CONH), 4.97 (d, $J = 14$ Hz, 1 H, NCH_APh), 4.88 (d, $J = 14$ Hz, 1 H, NCH_BPh), 4.59 (qd, $J = 7$ Hz, 1 H, CHCO), 3.49–3.35 (m, 2 H, NHCH₂), 2.71–2.62 (m, 2 H, NHCH₂CH₂), 3.15 (d, $J = 7$ Hz, 2 H, CHCH₂ Ph) ppm. ¹³C NMR (100 MHz, CDCl₃): $\delta = 170.1$ (CO), 149.9 (C-3), 149.3 (C-2), 138.5, 136.4, 134.5 (C-*ipso* Ph), 129.1, 128.8, 128.5, 128.4, 128.2, 126.8, 126.3 (Ph-C), 126.2 (C-6), 113.0 (C-5), 56.2 (CHCO), 51.8 (NCH₂), 40.5 (NHCH₂), 37.7 (CHCH₂Ph), 35.2 (NHCH₂CH₂) ppm. EIMS {(relative intensity)

[assignment]}; m/z (%) = 486 (20) [M^+], 91 (100) [$PhCH_2^+$]. HRMS (EI): calcd. for $C_{28}H_{27}ClN_4O_2$ 486.1822; found 486.1830.

Synthesis of 2-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)-amino]-3-phenyl-N-[2-(pyridin-2-yl)ethyl]propionamide (27): Yield 0.21 g (33%). Oil. IR (KBr, assignment): $\tilde{\nu}$ = 3300 (NH), 1653 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): δ = 8.20 (d, J = 6 Hz, 1 H, 6'-H), 7.35 (td, J = 7 Hz, 2 Hz, 1 H, 4'-H), 7.24–7.04 (m, 12 H, Ph-H + 2NH), 6.96 (d, J = 9 Hz, 1 H, 3'-H), 6.91–6.85 (m, 1 H, 5'-H), 6.43 (s, 1 H, 5-H), 4.97 (d, J = 14 Hz, 1 H, NCH_APh), 4.88 (d, J = 14 Hz, 1 H, NCH_BPh), 4.59 (qd, J = 9 Hz, 1 H, $CHCO$), 3.54–3.48 (m, 2 H, $NHCH_2$), 3.09 (d, J = 8 Hz, 2 H, $CHCH_2Ph$), 2.78–2.72 (m, 2 H, CH_2pyr) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9 (CO), 158.6 (C-*ipso* pyr), 149.5 (C-3), 149.0 (C-2), 148.3, 136.4 (Pyr-C), 135.9, 134.5 (C-*ipso* Ph), 128.7, 128.4, 127.9, 127.8, 126.2 (Ph-C), 125.8 (C-6), 122.8, 120.8 (pyr-C), 112.6 (C-5), 55.8 ($CHCO$), 51.3 (NCH_2), 38.2 ($NHCH_2$), 37.5 ($CHCH_2Ph$), 36.1 (CH_2pyr) ppm. EIMS {(relative intensity) [assignment]}; m/z (%) = 487 (45) [M^+], 91 (100) [$PhCH_2^+$]. HRMS (EI): calcd. for $C_{27}H_{26}ClN_5O_2$ 487.1775; found 487.1775.

Synthesis of 2-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)-amino]-3-phenyl-N-(pyridin-2-ylmethyl)propionamide (22): Yield 0.36 g (50%). Oil. IR (KBr, assignment): $\tilde{\nu}$ = 3345 (NH), 1683 (CO), 1635 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): δ = 8.35 (d, J = 6 Hz, 1 H, 6'-H), 7.56 (td, J = 7 Hz, 2 Hz, 1 H, 4'-H), 7.50 (t, J = 6 Hz, 1 H, CONH), 7.29–7.19 (m, 10 H, Ph-H), 7.15 (d, J = 7 Hz, 1 H, 3'-H), 6.99 (d, J = 7 Hz, 1 H, C2NH), 6.94 (dt, J = 7 Hz, 2 Hz, 1 H, 5'-H), 6.48 (s, 1 H, 5-H), 4.89 (s, 2 H, NCH_2), 4.78 (qd, J = 7 Hz, 1 H, $CHCO$), 4.44 (d, J = 6 Hz, 2 H, CH_2pyr), 3.19 (d, J = 7 Hz, 2 H, $CHCH_2Ph$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5 (CO), 156.4 (C-*ipso* pyr), 149.8 (C-3), 149.4 (C-2), 148.3, 136.7 (pyr-C), 136.4, 134.5 (C-*ipso* Ph), 129.0, 128.6, 128.2, 128.1, 128.0, 126.5 (Ph-C), 126.1 (C-6), 121.9, 121.6 (pyr-C), 112.9 (C-5), 56.1 ($CHCO$), 51.5 (NCH_2), 44.2 (CH_2pyr), 37.7 ($CHCH_2Ph$) ppm. EIMS {(relative intensity) [assignment]}; m/z (%) = 473 (33) [M^+], 91 (100) [$PhCH_2^+$]. HRMS (EI): calcd. for $C_{26}H_{24}ClN_5O_2$ 473.1618; found 473.1623.

Synthesis of 2-[(4-Benzyl-6-chloro-3,4-dihydro-3-oxopyrazin-2-yl)-amino]-N-[2-(1H-indol-3-yl)ethyl]-3-phenylpropionamide (32): Yield 0.30 g (30%). Oil. IR (KBr, assignment): $\tilde{\nu}$ = 3445 (NH), 1652 (CO) cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): δ = 8.50 (s, 1 H, NH-ind), 7.49 (d, J = 8 Hz, 1 H, 4i-H), 7.30–7.13 (m, 12 H, Ph-H + 7i-H + C2NH), 7.12–7.02 (m, 2 H, 5i-H + 6i-H), 6.72 (d, J = 2 Hz, 1 H, 2i-H), 6.34 (s, 1 H, 5-H), 6.19 (t, J = 6 Hz, 1 H, CONH), 4.88 (d, J = 14 Hz, 1 H, NCH_APh), 4.74 (d, J = 14 Hz, 1 H, NCH_BPh), 4.58 (qd, J = 7 Hz, 1 H, $CHCO$), 3.50 (qd, J = 7 Hz, 2 H, $NHCH_2$), 3.11 (d, J = 7 Hz, 2 H, $CHCH_2Ph$), 2.86–2.78 (m, 2 H, CH_2ind) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6 (CO), 149.7 (C-3), 149.2 (C-2), 136.6 (C-7ai), 136.2, 134.7 (C-*ipso* Ph), 128.9, 128.7, 128.2, 128.1, 127.8, 126.5 (Ph-C), 126.9 (C-3ai), 126.1 (C-6), 122.1 (C-2i), 121.5 (C-6i), 118.8 (C-5i), 118.1 (C-4i), 113.0 (C-5), 111.7 (C-3i), 111.1 (C-7i), 56.4 ($CHCO$), 51.5 (NCH_2), 37.6 ($CHCH_2Ph$), 39.2 ($NHCH_2$), 24.8 (CH_2ind) ppm. EIMS {(relative intensity) [assignment]}; m/z (%) = 525 (19) [M^+], 91 (100) [$PhCH_2^+$]. HRMS (EI): calcd. for $C_{30}H_{28}ClN_5O_2$ 525.1931; found 525.1937.

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- [1] L. Hedstrom, *Chem. Rev.* **2002**, *102*, 4501–4523.
- [2] a) R. N. Anderson, B. L. Smith, *Natl. Vital Stat. Rep.* **2005**, *53*, 1–89; b) M. Nordstrom, B. Lindblad, D. Bergqvist, T. Kjellstrom, *J. Intern. Med.* **1992**, *232*, 155–160; c) V. Fuster, M. Verstraete, *Thrombosis in Cardiovascular Disorders*, W. B. Saunders Co., Philadelphia, **1992**.
- [3] M. B. Young, J. C. Barrow, L. Kristen, G. F. Lundell, C. L. Newton, J. M. Pellicore, K. E. Rittle, H. G. Selnick, K. J. Stauffer, J. P. Vacca, P. D. Williams, D. Bohn, F. C. Clayton, J. J. Cook, J. A. Krueger, L. C. Kuo, S. D. Lewis, B. J. Lucas, D. R. McMasters, C. Miller-Stein, B. L. Pietrak, A. A. Wallace, R. B. White, B. Wong, Y. Yan, P. G. Nantermet, *J. Med. Chem.* **2004**, *47*, 2995–3008.
- [4] a) G. Leone, E. Rossi, A. M. Leone, V. De Stefano, *Curr. Med. Chem. Cardiovasc. Hematol. Agents* **2004**, *2*, 311–326; b) B. A. Schweitzer, W. L. Neumann, H. K. Rahman, C. L. Kusturin, K. R. Sample, G. I. Poda, R. G. Kurumbail, A. M. Stevens, R. A. Stegeman, W. C. Stallings, M. S. South, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3006–3011.
- [5] a) J. Pieters, G. Willems, H. C. Hemker, T. Lindhout, *J. Biol. Chem.* **1988**, *263*, 5313–5318; b) J. S. Rosenberg, P. W. McKenna, R. D. Rosenberg, *J. Biol. Chem.* **1975**, *250*, 8883–8888.
- [6] T. Su, H. Yang, D. Volkots, J. Woolfrey, S. Dam, P. Wong, U. Sinha, R. M. Scarborough, B. Zhu, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 729–732.
- [7] a) L. P. Mašič, A. Kranjc, P. Marinko, G. Mlinsek, T. Solmajer, M. Stegnar, D. Kikelj, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3171–3176; b) C. S. Burgey, K. A. Robinson, T. A. Lyle, P. E. J. Sanderson, S. D. Lewis, B. J. Lucas, J. A. Krueger, R. Singh, C. Miller-Stein, R. B. White, B. Wong, E. A. Lyle, P. D. Williams, C. A. Coburn, B. D. Dorsey, J. C. Barrow, M. T. Stranieri, M. A. Holahan, G. R. Sitko, J. J. Cook, D. R. McMasters, C. M. McDonough, W. M. Sanders, A. A. Wallace, F. C. Clayton, D. Bohn, Y. M. Leonard, T. J. Detwiler, J. J. Lynch, Y. Yan, Z. Chen, L. Kuo, S. J. Gardell, J. A. Shafer, J. P. Vacca, *J. Med. Chem.* **2003**, *46*, 461–473; c) K. E. Rittle, J. C. Barrow, K. J. Curotona, K. L. Glass, J. A. Krueger, L. C. Kuo, S. D. Lewis, B. J. Lucas, D. R. McMasters, M. M. Morrisette, P. G. Nantermet, C. L. Newton, W. M. Sanders, Y. Yan, J. P. Vacca, H. G. Selnick, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3477–3482; d) P. E. J. Sanderson, M. G. Stanton, B. D. Dorsey, T. A. Lyle, C. McDonough, W. M. Sanders, K. L. Savage, A. M. Naylor-Olsen, J. A. Krueger, S. D. Lewis, B. J. Lucas, J. J. Lynch, Y. Yan, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 795–798.
- [8] a) J. J. Parlow, B. L. Case, T. A. Dice, R. L. Fenton, M. J. Hayes, D. E. Jones, W. L. Neumann, R. S. Wood, R. M. Lachance, T. J. Girard, N. S. Nicholson, M. Clare, R. A. Stegeman, A. M. Stevens, W. C. Stallings, R. G. Kurumbail, M. S. South, *J. Med. Chem.* **2003**, *46*, 4050–4062; b) S. Maignan, J. P. Guillo-teau, S. Pouzieux, Y. M. Choi-Sledeski, M. R. Becker, S. I. Klein, W. R. Ewing, H. W. Pauls, A. P. Spada, V. Mikol, *J. Med. Chem.* **2000**, *43*, 3226–3232; c) K. Kumata, H. Kawamoto, T. Honma, T. Iwama, S. H. Kim, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6630–6635; d) H. Brandstetter, A. Kühne, W. Bode, R. Huber, W. von der Saal, K. Wirthensohn, R. A. Engh, *J. Biol. Chem.* **1996**, *271*, 29988–29992.
- [9] a) P. E. J. Sanderson, T. A. Lyle, K. J. Cutrona, D. L. Dyer, B. D. Dorsey, C. M. McDonough, A. M. Naylor-Olsen, I. W. Chen, Z. Chen, J. J. Cook, C. M. Cooper, S. J. Gardell, T. R. Hare, J. A. Krueger, S. D. Lewis, J. H. Lin, B. J. Lucas, E. A. Lyle, J. J. Lynch, M. T. Stranieri, K. Vastag, Y. Yan, J. A. Shafer, J. P. Vacca, *J. Med. Chem.* **1998**, *41*, 4466–4474; b) A. Kranjc, L. P. Mašič, S. Reven, K. Mikic, A. Preželj, M. Stegnar, D. Kikelj, *Eur. J. Med. Chem.* **2005**, *40*, 782–791.
- [10] For a review on the synthesis and functionalisation of these compounds, see: V. G. Pawar, W. M. De Borggraeve, *Synthesis* **2006**, *17*, 2799–2814.
- [11] See X-ray structure analysis in the Exp. Sect.

- [12] Y. C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
- [13] R. B. Brandt, J. E. Laux, S. W. Yates, *Biochem. Med. Metab. Biol.* **1987**, *37*, 344–349.
- [14] a) R. Lottenberg, J. A. Hall, M. Blinder, E. P. Binder, C. M. Jackson, *Biochim. Biophys. Acta* **1983**, *742*, 539–557; b) T. Steinmetzer, M. Renatus, S. Künzel, A. Eichinger, W. Bode, P. Wikström, J. Hauptmann, J. Stürzebecher, *Eur. J. Biochem.* **1999**, *265*, 598–605; c) S. D. Lewis, A. S. Ng, J. J. Baldwin, N. Fusetani, A. M. Naylor, J. A. Shafer, *Throm. Res.* **1993**, *70*, 173–190.
- [15] K. Hilpert, J. Ackermann, D. W. Banner, A. Gast, K. Gubernator, P. Hadvary, L. Labler, K. Mueller, G. Schmid, *J. Med. Chem.* **1994**, *37*, 3889–3901.
- [16] Initial molecular modeling performed showed the compounds could be of interest. After biological evaluation, however, this work was revised and reinterpreted in terms of the biological results.
- [17] N. Vanopdenbosch, R. Cramer, F. F. Giarrusso, *J. Mol. Graph.* **1985**, *3*, 110–111.
- [18] M. Rarey, B. Kramer, T. Lengauer, G. Klebe, *J. Mol. Biol.* **1996**, *261*, 470–489.
- [19] M. B. Young, J. C. Barrow, K. L. Glass, G. F. Lundell, C. L. Newton, J. M. Pellicore, K. E. Rittle, H. G. Selnick, K. J. Stauffer, J. P. Vacca, P. D. Williams, D. Bohn, F. C. Clayton, J. J. Cook, J. A. Krueger, L. C. Kuo, S. D. Lewis, B. J. Lucas, D. R. McMasters, C. Miller-Stein, B. L. Pietrak, A. A. Wallace, R. B. White, B. Wong, Y. Yan, P. G. Nantermet, *J. Med. Chem.* **2004**, *47*, 2995–3008.
- [20] H. Tsukada, D. M. Blow, *J. Mol. Biol.* **1985**, *184*, 703–711.
- [21] P. K. Loosen, M. F. Khorasani, S. M. Toppet, G. J. Hoornaert, *Tetrahedron* **1991**, *47*, 9269–9278.

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