

Novel Inhibitors of B-RAF Based on a Disubstituted Pyrazine Scaffold. Generation of a Nanomolar Lead

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B-RAF, a serine/threonine kinase, plays an important role in the development of certain classes of cancer, especially melanoma. As a result of high-throughput screening of a 23,000 compound library, 2-(3,4,5-trimethoxyphenylamino)-6-(3-acetamidophenyl)pyrazine, **1**, was identified as a low micromolar ($IC_{50} = 3.5 \mu M$) B-RAF inhibitor. This compound was chosen as the starting point of a program aimed at producing potent inhibitors of B-RAF. We have synthesized a series of 40 novel compounds, which involved extensive modifications to the 2-(3,4,5-trimethoxyphenylamino) moiety (ring A) of **1**. Their biological profiles against isolated B-RAF and mutated B-RAF in a cellular assay have been determined. These efforts led to the identification of two compounds exhibiting activities lower than 800 nM against B-RAF.

Introduction

The protein kinase B-RAF forms part of a conserved signal transduction pathway that regulates cellular responses to extracellular signals.^{1,2} Under normal circumstances, B-RAF is activated downstream of receptors in the cell membrane in a RAS small G-protein dependent manner. It then phosphorylates and activates the protein kinase MEK, which in turn activates a third protein kinase called ERK. ERK phosphorylates transcription factors such as ELK-1, regulating gene expression and controlling how cells respond to extracellular signals.

B-RAF is mutated in approximately 7% of human cancers,^{3,4} especially melanoma (50–70%), ovarian (~35%), thyroid (~30%) and colorectal (~10%) cancers. The most common mutation (~90%) is a glutamic acid for valine substitution at position 600 (V600E). The kinase activity of ^{V600E}B-RAF is elevated 500-fold, providing cancer cells with both proliferation and survival signals and allowing them to grow as tumors in model systems.⁵ These data demonstrate that B-RAF is important both in tumor induction and maintenance and that it is an important and exciting new therapeutic target for several human cancers. Novel anticancer drugs that inhibit the activity of this kinase are therefore urgently sought.

High-throughput screening of a collection of potential inhibitors resulted in the identification of a novel lead compound, pyrazine **1**. This compound exhibited low micromolar activity against B-RAF and in a cellular assay *in vitro*. For this reason, along with its synthetic tractability, compound **1** was chosen as the initial point for structure optimization.

Rationale of the Design. RAF family proteins (A, B and C) are serine-threonine kinases.⁶ A number of inhibitors, mainly targeting C-RAF (Raf-1) such as benzylidene oxindoles,⁷ ZM 336372⁸ and sorafenib,^{9–12} have been developed. Recently isoquinolines were reported as B-RAF inhibitors.^{13,14} The most intensely studied, sorafenib, currently in phase III clinical trials, proved to be active against B-RAF in cellular assays. However, sorafenib, as a single agent, failed to show therapeutic activity in the treatment of malignant melanoma, despite positive results in renal carcinoma.¹⁵ This is probably because it also inhibits a

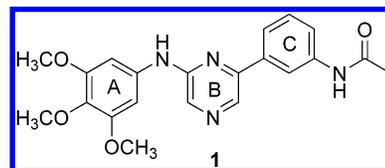


Figure 1. Structure of the hit **1**.

number of other kinases (such as VEGFR, PDGFR),⁹ which are also overexpressed in renal carcinoma. The reason for the failure in malignant melanoma may be attributed to its inability to reach a concentration in melanoma cells sufficient to inhibit B-RAF. Therefore, more potent and more selective inhibitors of B-RAF, able to produce clinical results in melanoma, are needed.

The pyrazine hit described in this paper has a chemical structure different from sorafenib and the other known RAF inhibitors listed above. Therefore, it represents a unique starting point for the development of B-RAF inhibitors.

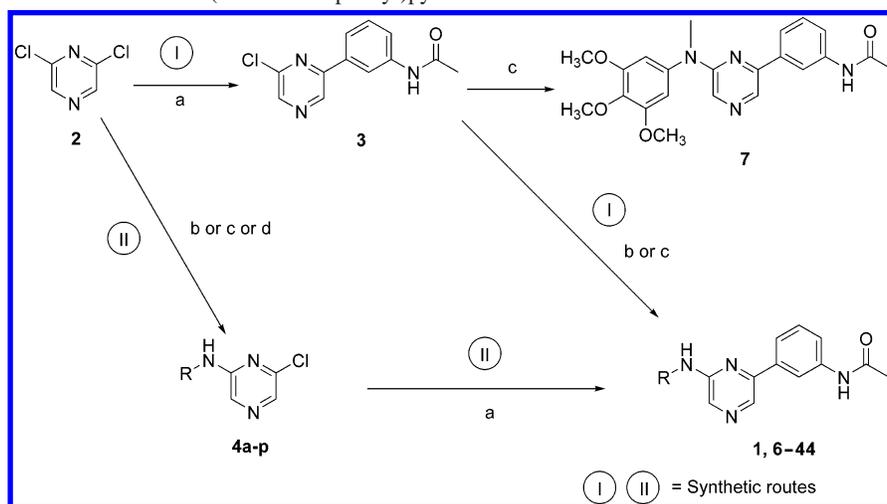
Chemistry. Three domains can be described in compound **1** (Figure 1): a pyrazine central core containing an amino functionality (denoted ring B), a trimethoxyphenyl moiety (ring A) and an acetamidophenyl group (ring C). Our first objective was the replacement of the trimethoxyphenyl group in order to evaluate its importance in binding to B-RAF and to improve the overall potency against the protein.

Compound **1** and 39 analogues (**1**, **6–44**) modified on ring A were synthesized in two steps starting from 2,6-dichloropyrazine **2** (Scheme 1). A Suzuki coupling^{16,17} with 3-acetamidophenyl boronic acid in the presence of PdCl₂-dppf:DCM complex as catalyst and sodium carbonate as base gave intermediate **3**.

Intermediate **3** was treated with a number of commercially available aromatic and heterocyclic amines (using liquid phase parallel synthesis), via palladium amination conditions,^{18,19} to generate a collection of analogues of the hit (compounds **6–30**) (Scheme 1). For the amination reactions, inert atmospheres (e.g., argon or nitrogen) and anhydrous degassed aprotic solvents (toluene, dioxane) were used. The reactions were typically performed at a temperature range of 80–110 °C.

Potassium *tert*-butoxide, sodium *tert*-butoxide or cesium carbonate gave good results as bases, with reaction times within

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Scheme 1. Synthesis of 2-N-Substituted-6-(3-acetamidophenyl)pyrazines^a

^a Reagents and conditions: (a) 3-Acetamidophenyl boronic acid, PdCl₂-dppf:DCM complex 1:1, 1 M Na₂CO₃, DME, 90 °C; (b) suitable arylamine, Pd₂dba₃, imidazolium ligands, KOtBu, dioxane, conventional or microwave heating, 100 °C; (c) methylphenylamine, Pd₂dba₃, BINAP, NaOtBu, toluene, 90 °C; (d) suitable benzylamine, Et₃N, DMF, 80 °C.

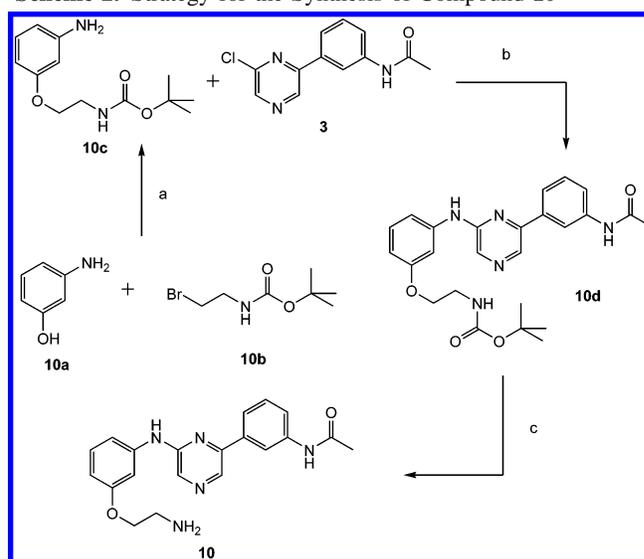
the range of 3–24 h under conventional heating. Alternatively, microwave heating was employed to reduce reaction times (15–30 min).

Tris(dibenzylideneacetone) dipalladium(0) (Pd₂dba₃) and palladacycles were used as palladium catalysts. Several ligands were used, such as 1,3-bis(diphenylphosphino)propane (dppp), rac-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP), 2-(dicyclohexyl)phosphino-2'-(dimethylamino) biphenyl, 1,3-bis(2,6-diisopropylphenyl)-4,5-dihydroimidazolium tetrafluoroborate, 1,3-bis(2,4,6-trimethyl phenyl)-4,5-dihydroimidazolium tetrafluoroborate, and also 2-dicyclohexylphosphino-2'-(*N,N*-dimethylamino)biphenyl.

For the synthesis of the benzylic amines, a different strategy was used. Common intermediate **3** failed to react with benzylic amines, probably because the chloropyrazine ring is rendered less reactive upon introduction of the acetamido-phenyl group. In contrast, the reaction of 2,6-dichloropyrazine with benzylic amines in the presence of triethylamine proceeds with good yield to afford intermediates **4a–p**. These were subsequently treated with 3-acetamidoboronic acid under Suzuki reaction conditions to give the final targets **31–44** (route II, Scheme 1). The Suzuki coupling was optimized in order to use liquid phase parallel synthesis. We found this strategy to be more convenient for the benzylic amines because of the ease of purification of the intermediates, since the reactions did not involve the use of a palladium catalyst or ligands and the yields were relatively high (70–80%).

Compound **10** was synthesized via a protection–deprotection strategy (Scheme 2). 3-Aminophenol **10a** was reacted with commercially available bromide **10b** in the presence of potassium *tert*-butoxide to afford amine **10c**. Potassium carbonate was added to decrease the reaction time. The oxy-ethylamino chain was introduced as the protected *t*-Boc version by using this method in order to prevent interference in the next step. Amine **10c** was reacted under the amination conditions described previously and subsequent cleavage of the *t*-Boc group with trifluoroacetic acid (TFA) gave the target product **10**.

Structure–Activity Relationships. The biological activities of the compounds were determined using two assays: the determination of the IC₅₀ against the V_{600E}B-RAF enzyme in vitro (IC₅₀B-RAF) and the determination of the growth inhibition

Scheme 2. Strategy for the Synthesis of Compound **10**^a

^a Reagents and conditions: (a) KOtBu, K₂CO₃, DMF, 90 °C; (b) Pd₂dba₃, KOtBu, imidazolium ligand, dioxane, 100 °C, microwave heating; (c) TFA, r.t.

in WM266.4 human melanoma cells expressing mutant B-RAF with sulforhodamine-B (IC₅₀SRB). The data are summarized in Table 1.

Our goal was to improve the potency of **1** by exploring the SAR of ring A. First, we focused on investigating the role of the methoxy groups (see Table 1). The removal of all of the methoxy groups led to only a 2-fold decrease in potency (compound **6**) for B-RAF.

Of the three possible mono-methoxy isomers (**8**, **9**, **11**) only the 3-position appears particularly interesting since the corresponding isomer retains activity whereas the 4- and 2-methoxy isomers are less active. Keeping two methoxy groups (in positions 3,4 and 3,5) (compounds **12** and **13**) led to at least equipotent compounds against B-RAF compared to **1**. Other functional groups were also introduced into the 3-position. Neither the Cl nor the OH substituents (compounds **15**, **16**) led to an improvement against B-RAF. However, bulky substituents including solubilizing side chain (oxyethylamino as in compound **10**) or isoxazole (compound **19**) were well tolerated by

Table 1. Analogues of Hit **1** by Modification of the Substituents^a

Compound number	R	IC ₅₀ B-RAF (μM)	95% CI	IC ₅₀ SRB (μM)	95% CI
Sorafenib	--	0.046	0.034-0.062	6.1	4.2-8.8
1		3.5	1.9-5.6	3.6	3.0-4.2
6		7.9	1.5-42.6	12	11.5-13.6
7^b		>100	--	49.6	44.1-55.6
8		74	29.6-183.9	ND ^c	--
9		4.8	2.0-11.8	4.0	3.6-4.5
10		3.8	2.2-6.7	5.2	3.8-7.0
11		26	10.6-65.4	ND	--
12		2.7	1.8-4.2	3.0	2.8-3.3
13		1.3	0.8-2.4	6.4	6.0-6.9
14		21	5.5-80.4	8.8	8.2-9.6
15		8.8	3.4-22.9	1.4	1.35-1.53
16		14	6.8-30.3	ND	--
17		84	21.1-331.6	5.2	4.3-6.2
18		>100	--	ND	--
19		0.79	0.4-1.8	45	37.3-54.0
20		8.8	3.7-21.0	8.3	7.5-9.2
21		10	7.7-13.1	2.3	1.5-3.6
22		10	6.0-18.1	0.032	0.012-0.067
23		5.4	1.7-16.3	3.0	2.7-3.2
24		4.5	2.3-8.7	3.3	3.0-3.6
25		39	8.4-186.9	ND	--
26		37	10.5-125.6	ND	--
27		>100	--	>100	--
28		0.74	0.4-1.2	0.49	0.44-0.55
29		0.95	0.4-2.3	ND	--
30		20	7.4-52.2	ND	--
31		34	8.5-222.3	ND	--
32		3.8	0.7-19.0	ND	--
33		4.2	2.6-6.9	38	35.5-42.5
34		14	0.2-991.5	18	10.0-33.5
35		>100	--	>100	--
36		18	4.7-43.1	ND	--
37		45	12.9-93.2	ND	--
38		>100	--	33	30.5-37.3
39		65	1.1-3928	33	25.0-42.3
40		58	1.5-2204	38	34.3-44.2
41		>100	--	37	13.9-124.5
42		8.0	2.3-27.8	ND	--
43		33	13.0-84.3	ND	--
44		99	35.6-276.4	27	16.9-40.7

^a See Experimental Section for a description of the assay conditions.

^b The H was replaced by a methyl group, which generated a tertiary amine.

^c ND = Not determined.

the protein. The 3-oxazole-phenyl-substituted analogue **19** was the most potent compound against B-RAF in this series (IC₅₀ = 0.79 μM). The 4-substitution resulted in inactive compounds (compounds **17** and **18**) against B-RAF. However, when bulky

substituents designed at improving solubility were introduced into the 4-position (compounds **20**, **21**), the potency was maintained in the B-RAF assay, indicating that these modifications were tolerated.

Since the naked phenyl seemed a promising scaffold (compound **6**), several fused rings were also synthesized (compounds **22–28**). Fluorine substitution of the fused ring proved detrimental (compounds **25–27**) whereas the unsubstituted 5, 6, 7-membered rings (compounds **22–24**) maintained the potency but did not improve it. However, introduction of a fused lactone (compound **28**) produced a derivative with improved activity against B-RAF (IC₅₀ = 0.74 nM). Replacement of the phenyl ring (ring A) by different heterocycles resulted in compounds **29** and **30**, the pyridine derivative **29**, exhibited good potency against the enzyme (IC₅₀ = 0.95 μM).

The role of the –NH– linker between rings A and B was also explored. Introduction of an extra methyl onto the nitrogen led to a tertiary amine, which turned out to be completely inactive (compound **7**). This finding suggests the –NH– linker is involved in a crucial interaction with the protein.

Extra methylene groups [(CH₂)_n, n = 1–3] between the amino group and ring A were also introduced (compounds **31–44**), as well as some heterocycles. One extra methylene group is well tolerated in the presence of different heterocycles (**32**, **33**). In general, however, introduction of extra methylenes is detrimental, and the drop in potency parallels the number of methylenes added (see **36–38**).

The activity in the SRB test shows figures comparable to sorafenib activity. However, no direct relationship was found between the potency of B-RAF inhibition and the efficacy in the SRB assay. The outstanding value reported for compound **22** (32 nM) in this test is believed to be an off-target effect.

Conclusions

High-throughput screening of a library of compounds resulted in the identification of hit **1** with a novel structure, which served as starting point for the design of new B-RAF inhibitors. This has resulted in two compounds (**19**, **28**) that both show an IC₅₀ below 800 nM for B-RAF. Our lead compound **28** has a similar IC₅₀ for the SRB assay. The secondary amino group between rings A and B seems to be essential for biological activity. Additional methylene groups proved detrimental for the activity except in some cases where different heterocycles were introduced. Additionally, we found that the potency against B-RAF was essentially maintained upon replacement of the trimethoxy-phenyl unit by appropriate solubilizing groups. These findings will be combined with efforts aimed at further optimization of the rest of the molecule.

Experimental Section

All starting materials, reagents and solvents for reactions were reagent grade and used as purchased. Chromatography solvents were HPLC grade and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) analysis using Merck silica gel 60 F-254 thin layer plates. Flash column chromatography was carried out on Merck silica gel 60 (0.015–0.040 mm) or in disposable Isolute Flash Si and Si II silica gel columns. Preparative TLC was performed on either Macherey-Nagel [809 023] precoated TLC plates SIL G-25 UV₂₅₄ or Analtech [2015] precoated preparative TLC plates, 2000 microns with UV₂₅₄. LC-MS analyses were performed on a Micromass LCT/Water's Alliance 2795 HPLC system with a Discovery 5 μm, C18, 50 mm × 4.6 mm i.d. column from Supelco at a temperature of 22 °C using the following solvent system: Solvent A: methanol; Solvent B: 0.1% formic acid in water, at a flow rate of 1 mL/min. Gradient starting with 10% A/90% B from 0 to 0.5 min then 10% A/90% B to 90%

A/10% B from 0.5 min to 6.5 min and continuing at 90% A/10% B up to 10 min. From 10 to 10.5 min the gradient reverted back to 10% A/90% B and was held until 12 min. UV detection was at 254 nm and ionization was positive or negative ion electrospray. The molecular weight scan range was 50–1000. Samples were supplied as 1 mg/mL in DMSO or methanol with 3 μ L injected on a partial loop fill. NMR spectra were recorded in DMSO- d_6 on a Bruker DPX 250 operating at 250.13 MHz or on a Bruker advance 500 operating at 500.26 MHz. The signal of the deuterated solvent was used as internal reference; the chemical shifts are expressed in ppm (δ). Preparative HPLC purifications were performed on an Axial Chromatospac Prep 10 (Jobin-Yvon) using Merck Kieselgel 60 (0.015–0.040). Microwave reactions were run on a CM discovery unit operating at 200 W with simultaneous stirring. The parallel synthesis in liquid phase were run on a Carousel (12 place reaction station) from Radley Discovery Technology.

2-(3-Acetamidophenyl)-6-chloropyrazine (3). Method A. Amounts of 1.00 g (6.70 mmol) 2,6-dichloropyrazine, 450 mg (0.54 mmol) PdCl₂.dppf:DCM 1:1, and 1.44 g (8.04 mmol) 3-acetamidophenyl boronic acid were dissolved in 100 mL dimethyl ethylene glycol (DME). To this solution 14 mL aq. 1 M Na₂CO₃ solution was added under stirring and the reaction mixture was heated at 90 °C (bath temperature) for 5 h. The reaction mixture was evaporated to dryness, the residue taken up in 50 mL AcOEt and filtered and the organic solution washed (2 \times 30 mL brine), dried (MgSO₄) and evaporated to 20 mL. A pale yellow solid, 160 mg was obtained. After filtration, the filtrate was submitted to preparative HPLC (AcOEt) and another fraction (749 mg) of **3** was obtained. Total yield: 909 mg (54.7%). ¹H NMR (DMSO- d_6) δ 2.07 (s, 3H, CH₃CO), 7.47 (t, 1H, J = 7.9 Hz), 7.79–7.84 (2d, 2H), 8.28 (s, 1H), 8.28 (s, 1H), 8.76 (s, 1H), 10.17 (s, 1H); ¹³C NMR (DMSO- d_6) δ : 23.99, 117.26, 121.07, 121.53, 129.58, 134.67, 140.08, 140.19, 142.78, 147.81, 151.32, 168.55. LC-MS: t_R = 6.27 min, m/z 248.0 (M + H)⁺, >98% purity; HRMS m/z (M + H)⁺ calcd for C₁₂H₁₀ClN₃O 248.0591, found 248.0592.

2-(3,4,5-Trimethoxyphenylamino)-6-chloropyrazine (4). Method B. To 5.00 g (33.5 mmol) 2,6-dichloropyrazine dissolved in 200 mL dry toluene in an oven dried flask were added under stirring 0.42 g (0.46 mmol) Pd(O)₂dba₃, 0.83 g (1.34 mmol) 2,2'-bis-(diphenylphosphino)-1,1'-binaphthyl (BINAP), 7.36 g (40.2 mmol) 3,4,5-trimethoxyaniline and 4.41 g (46.9 mmol) sodium *tert*-butoxide. The reaction mixture was heated at 90 °C (bath temperature) for 4 h under N₂. After cooling, the toluene solution was filtered. The solid was taken up in 100 mL acetone and filtered through a 5 cm column of Kieselgel 60, and the resulting solution was evaporated under vacuum. A portion of 2.53 g of **4** was obtained. The toluene filtrate was evaporated to dryness and then retaken in 70–80 mL boiling AcOEt. The solution was kept at 4 °C overnight, and 2.57 g of the solid title compound was collected by filtration. The filtrate was reduced to 18–20 mL and submitted to HPLC (AcOEt:cyclohexane 2:1). A third fraction of 0.94 g of **4** was collected. The total yield was 6.04 g (61.1%). ¹H NMR (DMSO- d_6) δ 3.63 (s, 3H), 3.77 (s, 6H), 7.02 (s, 2H), 7.96 (s, 1H), 8.14 (s, 1H), 9.79 (s, 1H); ¹³C NMR (DMSO- d_6) δ : 56.04, 60.03, 128.16, 128.86, 132.73, 135.61, 136.80, 144.87, 149.89, 149.89, 151.54, 152.84; LC-MS, t_R = 7.43 min; m/z : 296 (M + H)⁺ (C₁₃H₁₃ClN₃O₃); HRMS: (M + H)⁺ calcd for C₁₃H₁₄ClN₃O₃ 296.0802, found 296.0801.

2-(3,4,5-Trimethoxyphenylamino)-6-(3-acetamidophenyl)pyrazine (1). The catalyst was prepared in an oven dried flask by adding with stirring under an Ar atmosphere 40 mg (0.09 mmol) dppb and 30 mg (0.077 mmol) PdCl₂(benzotrile)₂ to 10 mL dry, degassed toluene. After 30 min, 225 mg (0.76 mmol) 2-(3,4,5-trimethoxyphenylamino)-6-chloropyrazine (**4**) 161 mg (0.9 mmol) 3-acetamido-boronic acid, 1.60 mL EtOH and 1.5 mL aq. 1 M Na₂CO₃ solution in 10 mL toluene were added, and the reaction mixture was heated at 90 °C (bath temperature) for 8 h. After cooling, AcOEt (50 mL) was added to the reaction, and the mixture was washed with brine (2 \times 100 mL), dried (MgSO₄), and evaporated to dryness. An amount of 165 mg of a solid was obtained and purified by preparative HPLC (AcOEt:EtOH 9:1). Finally, 55 mg

pure **1** (18.4%) was obtained. ¹H NMR (DMSO- d_6) δ 2.07 (s, 3H), 3.63 (s, 3H), 3.80 (s, 6H), 7.25 (s, 2H), 7.43 (t, 1H, J = 7.9 Hz), 7.65 (d, 1H, J = 8.1 Hz), 7.65 (d, 1H, J = 7.8 Hz), 8.16 (s, 1H), 8.27 (s, 1H), 8.38 (s, 1H), 9.57 (s, 1H), 10.06 (s, 1H); ¹³C NMR (DMSO- d_6) δ : 23.91, 55.42, 60.09, 117.40, 121.34, 129.18, 129.83, 131.90, 133.64, 136.87, 137.30, 139.78, 147.70, 151.81, 152.81, 169.70; LC-MS, t_R = 7.17 min; m/z : 395 (M + H)⁺ (C₂₁H₂₂N₄O₄); HRMS: (M + H)⁺ calcd for C₂₁H₂₂N₄O₄ 395.1719, found 395.1720.

2-(Phenylamino)-6-(3-acetamidophenyl)pyrazine (6). Method C. 2-(3-Acetamidophenyl)-6-chloropyrazine **3** (100 mg, 0.404 mmol), aniline (54 mg, 0.581 mmol), potassium *tert*-butoxide (68 mg, 0.605 mmol), 1,3-bis(2,6-di-*i*-propylphenyl)-4,5-dihydroimidazolium tetrafluoroborate (19 mg, 0.040 mmol) and Pd(O)₂dba₃ (18.5 mg, 0.020 mmol) in dry dioxane (2.5 mL) were added to a round-bottomed flask in an Ar atmosphere were placed and stirred at 95 °C and for 3.5 h. The solvent was evaporated under vacuum to yield a solid that was submitted to column chromatography (AcOEt) to produce 32 mg (26%) of **6**. ¹H NMR (DMSO- d_6) δ 2.11 (s, 3 H), 6.99 (t, 1H, J = 7.4 Hz), 7.33–7.49 (m, 3 H), 7.62 (d, 1 H, J = 7.9 Hz), 7.73 (d, 1 H, J = 7.9 Hz), 7.87 (d, 1 H, J = 7.6 Hz), 8.19 (s, 1 H), 8.44 (s, 1 H), 8.46 (bs, 1 H), 9.60 (s, 1 H), 10.10 (s, 1 H); ¹³C NMR (DMSO- d_6) δ : 24.11, 117.12, 118.21, 119.93, 121.04, 121.30, 128.91, 129.20, 129.96, 133.54, 137.05, 139.97, 140.66, 147.87, 151.46, 168.46; LC-MS: t_R = 7.11 min, m/z 305.1 (M + H)⁺, >90% purity; HRMS m/z (M + H)⁺ calcd for C₁₈H₁₇N₄O: 305.1402, found 305.1401.

2-(Phenyl-*N*-methylamino)-6-(3-acetamidophenyl)pyrazine (7). Method D. **3** (150 mg, 0.61 mmol), *N*-methylaniline (93 mg, 0.87 mmol), tris(dibenzylidene acetone)dipalladium (0) (Pd(O)₂dba₃) (28 mg, 0.030 mmol), 1,3-bis(2,6-di-*i*-propyl phenyl)-4,5-dihydroimidazolium tetrafluoro-borate (29 mg, 0.061 mmol), potassium *tert*-butoxide (102 mg, 0.91 mmol) and dioxane (3.5 mL) were added to a vessel suitable for microwave irradiation provided with stirring and Ar atmosphere. The vessel was irradiated for 30 min at 100 °C. The crude reaction mixture was passed through a very short silica gel column (2–3 g silica) and washed with AcOEt. After evaporation of the solvent, 117 mg (61%) of **7** was produced. ¹H NMR (DMSO- d_6) δ 2.08 (s, 3 H), 3.52 (s, 3 H), 7.32 (t, 1 H, J = 7.0 Hz), 7.38–7.54 (m, 5 H), 7.73 (d, 2 H, J = 8.6 Hz), 7.83 (s, 1 H), 8.31 (bs, 1 H), 8.38 (s, 1 H), 10.09 (s, 1 H); ¹³C NMR (DMSO- d_6) δ : 24.03, 37.69, 117.13, 120.06, 121.13, 126.04, 126.24, 128.99, 129.14, 129.95, 130.46, 136.93, 139.85, 144.69, 147.98, 153.29, 168.46; LCMS: t_R = 6.87 min, m/z 319.0, (M + H)⁺, >95% purity; HRMS: (M + H)⁺ calcd for C₁₉H₁₉N₄O 319.1543, found 319.1559.

2-(2-Methoxyphenylamino)-6-(3-acetamidophenyl)pyrazine (8). Using method D with *o*-anisidine (107 mg, 0.87 mmol), 103 mg (51%) of **8** was produced. ¹H NMR (DMSO- d_6) δ 2.09 (s, 3H), 3.89 (s, 3H), 7.01–7.08 (m, 3H), 7.42 (t, 1H, J = 7.9 Hz), 7.61 (d, 1H, J = 7.6 Hz), 7.70 (d, 1H, J = 7.9 Hz), 8.40 (s, 1H), 8.41 (s, 1H), 8.42 (s, 1H), 8.46–8.48 (m, 1H), 8.76 (s, 1H), 10.11 (s, 1H); ¹³C NMR (DMSO- d_6) δ : 24.14, 55.72, 110.93, 117.10, 119.60, 119.92, 120.70, 121.09, 122.29, 129.03, 129.23, 130.02, 134.15, 137.13, 139.95, 147.72, 149.09, 151.59, 168.49; LC-MS: t_R = 7.21 min, m/z 335.1 (M + H)⁺, (C₁₉H₁₈N₄O₂), >98% purity. HRMS m/z (M + H)⁺ calcd for C₁₉H₁₉N₄O₂ 335.1508, found 335.1516.

2-(3-Methoxyphenylamino)-6-(3-acetamidophenyl)pyrazine (9). Method D with *m*-anisidine (107 mg, 0.87 mmol) gave 24 mg (11.8%) of **9**. ¹H NMR (DMSO- d_6) δ 2.08 (s, 3 H), 3.77 (s, 3 H), 6.56 (d, 1 H, J = 7.4 Hz), 7.25 (t, 1 H, J = 8.1 Hz), 7.38–7.47 (m, 2 H), 7.56 (bs, 1 H), 7.65 (d, 1 H, J = 7.8 Hz), 7.73 (d, 1 H, J = 9.5 Hz), 8.20 (s, 1 H), 8.33 (bs, 1 H), 8.42 (s, 1 H), 9.61 (s, 1 H), 10.09 (s, 1 H); ¹³C NMR (DMSO- d_6) δ 24.02, 54.78, 103.29, 107.48, 110.59, 117.26, 120.13, 121.21, 129.22, 129.65, 130.09, 133.66, 137.15, 139.88, 141.91, 147.98, 151.46, 159.67, 168.43; LC-MS: t_R = 7.17 min, m/z 335.1 (M + H)⁺, >98% purity; Anal. (C₁₉H₁₈N₄O₂); C, H, N. (calcd C, 68.25; H, 5.43; N, 16.76. Found: C, 67.81; H, 5.49; N, 16.47). HRMS m/z (M + H)⁺ calcd for C₁₉H₁₉N₄O₂ 335.1508, found 335.1498.

tert-Butyl 2-(3-Aminophenoxy)ethylcarbamate (10c). 3-Aminophenol **10a** (600 mg, 5.48 mmol), K_2CO_3 (380 mg, 2.76 mmol), potassium *tert*-butoxide (648 mg, 5.76 mmol) and dry DMF (8 mL) were added to a round-bottomed flask provided with Ar atmosphere. The reaction mixture was stirred for 10 min, and a solution of 2-(Boc-amino) ethyl bromide (1.23 g, 5.50 mmol) in 6 mL of dry DMF was added dropwise and the temperature increased to 90 °C for 3 h. To this mixture was added a solution of sodium hydroxide (3.5 g) in water (100 mL). This mixture was extracted with AcOEt (3 × 40 mL). The organic layers were combined, dried ($MgSO_4$), filtered and evaporated under vacuum to produce a crude mixture that was chromatographed using a mixture of AcOEt: DCM (15:85) to furnish 713 mg (49%) of **10c**. 1H NMR (DMSO- d_6) δ 1.38 (s, 9H), 3.24 (c, 2 H, $J = 5.8$ Hz), 3.33 (s, 1 H), 3.82 (t, 2 H, $J = 5.9$ Hz), 5.02 (s, 2 H), 6.06 (d, 1 H, $J = 9.1$ Hz), 6.12–6.16 (m, 2 H), 6.88 (t, 1 H, $J = 7.7$ Hz), 6.95 (bt, 1 H, $J = 5.2$ Hz). ^{13}C NMR (DMSO- d_6) δ : 28.19, 65.81, 77.70, 99.98, 101.90, 106.91, 129.49, 149.93, 155.64, 159.37. LC-MS: $t_R = 4.16$ min, >77% purity. HRMS m/z (M + Na) $^+$ calcd for $C_{13}H_{20}N_2O_3Na$ 275.1372, found 275.1378.

2-[3-(tert-Butylcarbamoyl-N-ethyloxy)phenylamino]-6-(3-acetamidophenyl)pyrazine (10d). Method D with *tert*-butyl 2-(3-aminophenoxy)ethylcarbamate **10c** (190 mg, 0.753 mmol) was applied with heating for 20 min. The reaction mixture was filtered and washed with AcOEt (45 mL). The liquid was dried under vacuum and the residue chromatographed using a mixture of AcOEt: DCM (1:1) to furnish 120 mg (43%) of **10d**. 1H NMR (DMSO- d_6) δ 1.37 (s, 9 H), 2.09 (s, 3 H), 3.32 (m, 2 H), 3.98 (m, 2 H), 6.56 (dd, 1 H, $J_a = 8.0$ Hz, $J_b = 2.4$ Hz), 7.01 (bt, 1 H), 7.25 (t, 1 H, $J = 8.1$ Hz), 7.36 (bd, 1 H, $J = 8.4$ Hz), 7.45 (t, 1 H, $J = 7.9$ Hz), 7.59 (bs, 1 H), 7.66 (bd, 1 H, $J = 9.2$ Hz), 7.74 (bd, 1 H, $J = 8.0$ Hz), 8.19 (s, 1 H), 8.34 (bs, 1 H), 8.42 (s, 1 H), 9.60 (s, 1 H), 10.09 (s, 1 H). ^{13}C NMR (DMSO- d_6) δ 23.99, 28.18, 66.23, 77.77, 103.95, 107.82, 110.74, 117.11, 120.07, 121.24, 129.24, 129.66, 130.08, 133.66, 137.10, 139.90, 141.88, 147.96, 151.44, 155.65, 158.87, 168.53. LC-MS: $t_R = 7.67$ min, m/z 364.2 (M - Boc + H) $^+$ ($C_{20}H_{21}N_5O_2$), >98% purity. HRMS m/z (M + H) $^+$ calcd for $C_{25}H_{30}N_5O_4$ 464.2298, found 464.2284.

2-[3-(2-Aminoethoxy)phenylamino]-6-(3-acetamidophenyl)pyrazine (10). Compound **10b** (60 mg, 0.129 mmol) was deprotected by stirring with trifluoroacetic acid (TFA) (2.5 mL) for 3 h at room temperature. The TFA was evaporated under vacuum, and 340 μ L triethylamine was added. The residue was chromatographed using a gradient of AcOEt:EtOH 85:15 to 50:50 to give 15 mg of **10**. Yield: 32%. 1H NMR (250 MHz, DMSO- d_6) δ 2.09 (s, 3 H), 2.90 (t, 2 H, $J = 5.5$ Hz), 3.96 (t, 2 H, $J = 5.7$ Hz), 6.56 (d, 1 H, $J = 8.0$ Hz), 7.24 (t, 1 H, $J = 8.0$ Hz), 7.34 (d, 1 H, $J = 6.8$ Hz), 7.44 (t, 1 H, $J = 7.9$ Hz), 7.64 (bs, 1 H), 7.66 (d, 1 H, $J = 9.0$ Hz), 7.73 (d, 1 H, $J = 8.0$ Hz), 8.20 (s, 1 H), 8.34 (bs, 1 H), 8.42 (s, 1 H), 9.64 (s, 1 H), 10.16 (s, 1 H). ^{13}C NMR (DMSO- d_6) δ 23.98, 40.82, 69.56, 103.92, 107.96, 110.61, 117.24, 120.19, 121.26, 129.22, 129.61, 130.08, 133.68, 137.15, 139.90, 141.89, 147.98, 151.47, 159.08, 168.51. LC-MS: $t_R = 4.46$ min, m/z 364.2 (M + H) $^+$ ($C_{21}H_{20}N_5O_2$).

2-(3,5-Dimethoxyphenylamino)-6-(3-acetamidophenyl)pyrazine (12). Using method B, 180 mg (0.73 mmol) 2-(3-acetamidophenyl)-6-chloropyrazine (**3**), dissolved in 5 mL dry toluene in an oven dried flask, was reacted under N_2 with 24 mg (0.03 mmol) $Pd(O)_2dba_3$, 75 mg (0.12 mmol) BINAP, 134 mg (0.88 mmol) 3,4,5-trimethoxyaniline and 98 mg (1.02 mmol) sodium *tert*-butoxide at 90 °C (bath temperature) for 5 h. The toluene solution was filtered and evaporated under vacuum. The solid was taken up in 40 mL AcOEt, washed (2 × 40 mL water) and dried ($MgSO_4$). The catalyst was filtered and the mixture extracted with 10 mL of acetone. The organic solutions were pooled, evaporated to 10 mL volume and left at 4 °C overnight to produce 160 mg of **12**. The filtrate, submitted to HPLC (AcOEt) gave an additional 40 mg of **12**. The total yield was 170 mg (63.9%). 1H NMR (DMSO- d_6) δ 2.07 (s, 3H), 3.75 (s, 6H), 6.15 (s, 1H), 7.12 (s, 2H), 7.44 (t, 1H, $J = 7.8$ Hz), 7.67 (d, 1H, $J = 7.9$ Hz), 7.73 (d, 1H, $J = 7.6$ Hz), 8.19 (s, 1H), 8.24 (s, 1H), 8.39 (s, 1H), 9.64 (s, 1H), 10.03 (s, 1H); ^{13}C

NMR (DMSO- d_6) δ 23.91, 54.92, 94.33, 96.21, 117.21, 117.42, 118.10, 120.32, 121.28, 121.35, 129.20, 130.22, 133.71, 137.24, 139.80, 140.70, 142.43, 148.07, 151.47, 160.60, 168.38; LC-MS, $t_R = 7.35$ min; m/z : 365.3 (M + H) $^+$ ($C_{20}H_{20}N_4O_3$); HRMS: (M + H) $^+$ calcd for $C_{20}H_{21}N_4O_3$, 365.1637, found 365.1614.

2-(3,4-Dimethoxyphenylamino)-6-(3-acetamidophenyl)pyrazine (13). Using the same method B and the workup described for compound **12**, from 180 mg (0.73 mmol) **3** and 134 mg (0.88 mmol) 3,4-dimethoxyaniline (reaction time: 6 h), 72 mg (27.1%) of pure **13** was obtained after preparative HPLC (AcOEt) purification of the whole batch. 1H NMR (DMSO- d_6) δ 2.07 (s, 3H), 3.73 (s, 3H), 3.77 (s, 3H), 6.95 (d, 1H, $J = 8.7$ Hz), 7.26 (q, 1H, $J_a = 8.6$ Hz, $J_b = 2.3$ Hz), 7.42 (t, 1H, $J = 7.9$ Hz), 7.62 (s, 1H), 7.64 (d, 1H), 7.72 (d, 1H, $J = 7.6$ Hz), 8.13 (s, 1H), 8.30 (s, 1H), 8.35 (s, 1H), 9.45 (s, 1H), 10.07 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.00, 55.07, 55.84, 103.51, 110.03, 112.63, 117.62, 120.09, 121.20, 129.16, 129.37, 133.38, 134.39, 137.28, 139.82, 143.58, 147.87, 148.71, 151.60, 168.44; LC-MS: $t_R = 5.85$ min; m/z : 365.3 (M + H) $^+$ ($C_{20}H_{20}N_4O_3$); HRMS: (M + H) $^+$ calcd for $C_{20}H_{21}N_4O_3$, 365.1614, found 365.1602.

2-(2,4-Dimethoxyphenylamino)-6-(3-acetamidophenyl)pyrazine (14). Using method B, **3** (60 mg, 0.24 mmol), 1,3-dimethoxyaniline (54 mg, 0.35 mmol), $Pd(O)_2dba_3$ (9 mg, 0.01 mmol), BINAP (50 mg, 0.04 mmol) and sodium *tert*-butoxide (33 mg, 0.34 mmol) in degassed toluene (5 mL) were reacted at 85 °C for 16 h. The solvent was evaporated under vacuum and the resulting solid was washed with 20 mL hot AcOEt and filtered. The volume was reduced to 2 mL and chromatographed on silica gel (AcOEt) to furnish 61 mg of a mixture of two products. Preparative TLC (AcOEt) and crystallization in chloroform afforded 12 mg (14%) of **14**. 1H NMR (DMSO- d_6) δ 2.08 (s, 3 H), 3.77 (s, 3 H), 3.85 (s, 3 H), 6.59 (dd, 1 H, $J_a = 8.9$ Hz, $J_b = 2.7$ Hz), 6.68 (d, 1 H, $J = 2.6$ Hz), 7.40 (t, 1 H, $J = 7.9$ Hz), 7.65 (t, 2 H, $J = 9.3$ Hz), 8.08 (d, 1 H, $J = 8.8$ Hz), 8.19 (s, 1 H), 8.29 (bs, 1 H), 8.32 (s, 1 H), 8.55 (s, 1 H), 10.07 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.07, 55.29, 55.73, 99.03, 104.39, 117.11, 119.88, 121.09, 121.83, 122.25, 129.11, 129.26, 133.01, 137.23, 139.82, 147.89, 151.45, 152.12, 155.83, 168.40; LC-MS: $t_R = 7.10$ min, m/z 365.1 (M + H) $^+$, >99% purity; HRMS: (M + H) $^+$ calcd for $C_{20}H_{21}N_4O_3$ 365.1605, found 365.1614.

2-(3-Chlorophenylamino)-6-(3-acetamidophenyl)pyrazine (15). Using method A with 300 mg (1.25 mmol) 2-(3-chlorophenylamino)-6-chloropyrazine, **4b**, and 268 mg (1.5 mmol) 3-acetamidoboronic acid (reaction time: 16 h), 78 mg (28.1%) pure title **15** were obtained after purification by preparative HPLC (AcOEt). 1H NMR (DMSO- d_6) δ 2.09 (s, 3H), 7.03 (d, 1H, $J = 7.12$ Hz), 7.39 (t, 1H, $J = 6.4$ Hz), 7.45 (t, 1H, $J = 7.9$ Hz), 7.63 (d, 1H, $J = 7.9$ Hz), 7.73 (d, 1H, $J = 7.7$ Hz), 7.89 (m, 2H), 8.20 (s, 1H), 8.48 (s, 1H), 9.81 (s, 1H), 10.09 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 20.73, 24.02, 55.29, 110.87, 116.92, 119.82, 120.94, 123.11, 123.62, 127.67, 129.02, 130.26, 132.04, 137.30, 139.76, 140.32, 143.31, 147.87, 153.84, 168.42; LC-MS: $t_R = 6.43$ min, m/z 309.1 (M + H) $^+$ $C_{17}H_{16}N_4O_2$; HRMS: (M + H) $^+$ calcd for $C_{17}H_{16}N_4O_2$ 309.1352, found 309.1376.

2-(3-Hydroxyphenylamino)-6-(3-acetamidophenyl)pyrazine (16). Method B with 3-hydroxyaniline (95 mg, 0.87 mmol) afforded 158 mg (80%) of **16**. 1H NMR (DMSO- d_6) δ 2.11 (s, 3H), 6.41 (d, 1H, $J = 7.9$ Hz), 7.13 (t, 1H, $J = 7.9$ Hz), 7.27 (d, 1H, $J = 7.9$ Hz), 7.40 (s, 1H), 7.44 (t, 1H, $J = 7.9$ Hz), 7.59 (d, 1H, $J = 7.6$ Hz), 7.76 (d, 1H, $J = 7.2$ Hz), 8.18 (s, 1H), 8.42 (s, 1H), 8.51 (s, 1H), 9.29 (s, 1H), 9.45 (s, 1H), 10.15 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.08, 105.15, 108.73, 109.33, 117.36, 119.97, 121.22, 129.21, 129.59, 129.77, 133.60, 137.02, 139.74, 141.71, 147.80, 151.52, 157.66, 168.84; LC-MS: $t_R = 6.58$ min; m/z 321.0 (M + H) $^+$, >94% purity; HRMS: (M + H) $^+$ calcd for $C_{18}H_{17}N_4O_2$: 321.1330, found 321.1352.

2-[4-(Trifluoromethoxy)phenylamino]-6-(3-acetamidophenyl)pyrazine (17). Method B was followed starting from 4-(trifluoromethoxy)aniline (51 mg, 0.29 mmol). Purification by preparative TLC (AcOEt) furnished 19 mg (21%) of **17**. 1H NMR (DMSO- d_6) δ 2.10 (s, 3 H), 7.34–7.47 (m, 3 H), 7.60 (d, 1 H, $J = 7.9$ Hz),

7.74 (d, 1 H, $J = 7.8$ Hz), 8.00 (d, 2 H, $J = 9.1$ Hz), 8.21 (s, 1 H), 8.51 (bs, 2 H), 9.82 (s, 1 H), 10.10 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.09, 117.01, 119.09, 119.87, 120.94, 121.68, 129.15, 130.40, 133.52, 136.73, 139.92, 141.96, 141.99, 147.66, 151.00, 168.45; LCMS: $t_R = 8.06$ min; m/z 389.3 (M + H) $^+$, (C₁₉H₁₅F₃N₄O₂), >96% purity; HRMS: (M + H) $^+$ calcd for C₁₈H₁₆N₄O₂: 361.1295, found 361.1301.

2-(3-Chloro-4-fluorophenylamino)-6-(3-acetamidophenyl)pyrazine (18). Method B was followed starting from 3-chloro-4-fluoroaniline (42 mg, 0.29 mmol). Purification by preparative TLC (AcOEt) produced 4 mg (5%) of **18**. ^1H NMR (DMSO- d_6) δ 2.09 (s, 3 H), 7.38 (d, 1 H, $J = 9.5$ Hz), 7.46 (d, 1 H, $J = 7.9$ Hz), 7.61 (d, 1 H, $J = 7.9$ Hz), 7.72 (d, 1 H, $J = 7.5$ Hz), 7.86–7.91 (m, 1 H), 8.00–8.04 (m, 1 H), 8.18 (s, 1 H), 8.40 (bs, 1 H), 8.48 (s, 1 H), 9.80 (s, 1 H), 10.08 (s, 1 H); LCMS: $t_R = 6.79$ min; m/z 357.2 (M + H) $^+$, (C₁₈H₁₄ClFN₄O), >55% purity.

2-[3-(Oxazol-5-yl)phenylamino]-6-(3-acetamidophenyl)pyrazine (19). Method B was followed starting from 3-(1,3-oxazol-5-yl)aniline (56 mg, 0.35 mmol). Preparative TLC (AcOEt) afforded 23 mg (26%) of **19**. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3 H), 7.36 (d, 1 H, $J = 8.0$ Hz), 7.48 (t, 2 H, $J = 8.3$ Hz), 7.61 (s, 1 H), 7.65 (d, 1 H, $J = 8.4$ Hz), 7.82 (d, 2 H, $J = 7.3$ Hz), 8.23 (s, 1 H), 8.39 (bs, 2 H), 8.46 (s, 1 H), 8.47 (s, 1 H), 9.82 (s, 1 H), 10.09 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.0, 113.4, 117.1, 117.3, 118.3, 120.3, 121.3, 121.8, 127.9, 129.3, 129.8, 130.3, 133.7, 137.0, 139.9, 141.4, 147.9, 150.8, 151.3, 151.7, 168.5; LC-MS: $t_R = 7.23$ min, m/z 372.1 (M + H) $^+$, >96% purity (C₂₁H₁₇N₅O₂); HRMS: (M + H) $^+$ calcd for C₂₁H₁₈N₅O₂: 372.1461, found 372.1436.

2-[4-(Morpholinofonyl)phenylamino]-6-(3-acetamidophenyl)pyrazine (20). Method B was followed starting from 4-(morpholinofonyl)aniline (85 mg, 0.35 mmol). Column chromatography (AcOEt) afforded 34 mg (31%) of **20**. ^1H NMR (DMSO- d_6) δ 2.11 (s, 3 H), 2.86–2.89 (m, 4 H), 3.62–3.65 (m, 4 H), 7.45 (t, 1 H, $J = 7.8$ Hz), 7.54 (d, 1 H, $J = 8.1$ Hz), 7.73–7.80 (m, 3 H), 8.14 (d, 2 H, $J = 8.9$ Hz), 8.28 (s, 1 H), 8.57 (bs, 1 H), 8.61 (s, 1 H), 10.13 (s, 1 H), 10.18 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.19, 45.90, 65.28, 117.09, 117.50, 120.06, 121.09, 125.43, 129.22, 129.33, 131.54, 134.02, 136.56, 140.07; LC-MS: $t_R = 6.74$ min; m/z 454.2 (M + H) $^+$, >98% purity; HRMS: (M + H) $^+$ calcd for C₂₂H₂₄N₅O₄S: 454.1549, found 454.1524.

2-(4-Morpholinophenylamino)-6-(3-acetamidophenyl)pyrazine (21). Method B was followed starting from 4-morpholinoaniline (155 mg, 0.87 mmol). Crystallization from AcOEt produced 70 mg (30%) of **21**. ^1H NMR (DMSO- d_6) δ 2.09 (s, 3 H), 3.03–3.07 (bt, 4 H), 3.72–3.76 (bt, 4 H), 6.97 (d, 2 H, $J = 9.0$ Hz), 7.42 (t, 1 H, $J = 7.9$ Hz), 7.65–7.68 (m, 2 H), 7.70 (d, 2 H, $J = 8.9$ Hz), 8.11 (s, 1 H), 8.33 (bs, 2 H), 9.36 (s, 1 H), 10.09 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.11, 49.24, 66.16, 116.01, 117.08, 119.61, 119.89, 121.08, 129.16, 133.03, 133.15, 137.23, 139.87, 146.10, 147.95, 151.70, 168.45; LC-MS: $t_R = 6.42$ min; m/z 390.2 (M + H) $^+$, >88% purity; HRMS: (M + H) $^+$ calcd for C₂₂H₂₄N₅O₂: 390.1923, found 390.1930.

2-(3,4-Methylenedioxyphenylamino)-6-(3-acetamidophenyl)pyrazine (22). Using method B starting from 200 mg (0.33 mmol) **3** and 138 mg (1.0 mmol) 3,4-methylenedioxyaniline, 84 mg (29.0%) pure **22** was obtained after preparative HPLC (AcOEt) purification of the whole batch. Reaction time: 18 h. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3H), 5.99 (s, 2H), 6.91 (d, 1H, $J = 8.4$ Hz), 7.32 (q, 1H, $J_a = 8.4$ Hz, $J_b = 2.0$ Hz), 7.40 (d, 1H, $J = 1.8$ Hz), 7.43 (t, 1H, $J = 7.9$ Hz), 7.65 (d, 1H, $J = 8.9$ Hz), 7.68 (d, 1H, $J = 7.8$ Hz), 8.12 (s, 1H), 8.30 (s, 1H), 8.35 (s, 1H), 9.42 (s, 1H), 10.09 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.07, 100.62, 100.74, 108.27, 111.15, 117.07, 119.92, 121.09, 129.22, 129.61, 133.29, 135.25, 137.14, 139.87, 141.75, 147.19, 147.95, 151.53, 168.44; LC-MS: $t_R = 7.07$ min; m/z : 349.1 (M + H) $^+$ (C₁₉H₁₆N₄O₃); HRMS: (M + H) $^+$ calcd for C₁₉H₁₇N₄O₃: 349.1301, found 349.1308.

2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-ylamino)-6-(3-acetamidophenyl)pyrazine (23). Method B was followed starting from 6-amino-3,4-benzodioxane (45 mg, 0.29 mmol). Column chromatography (AcOEt) furnished 40 mg (46%) of **23**. ^1H NMR (DMSO- d_6) δ 2.09 (s, 3 H), 4.20–4.24 (m, 4 H), 6.85 (d, 1 H, $J = 8.7$ Hz),

7.25–7.38 (m, 1 H), 7.43 (t, 1 H, $J = 8.0$ Hz), 7.68 (t, 1 H, $J = 7.8$ Hz), 8.11 (s, 1 H), 8.28 (s, 1 H), 8.34 (s, 1 H), 9.38 (s, 1 H), 10.08 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.64, 64.29, 64.51, 110.82, 115.00, 117.69, 118.20, 121.10, 122.61, 129.56, 130.37, 131.03, 132.75, 137.34, 138.58, 140.38, 143.82, 149.32, 121.10, 122.61, 129.56, 130.37, 131.03, 132.75, 137.34, 138.58, 140.38, 143.82, 149.32, 151.99, 168.59; LC-MS: $t_R = 6.96$ min; m/z 363.1 (M + H) $^+$ (C₂₀H₁₈N₄O₃) >95% purity; HRMS: (M + H) $^+$ calcd for C₂₀H₁₉N₄O₃: 363.1457, found 363.1466.

2-(3,4-Dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-ylamino)-6-(3-acetamidophenyl)pyrazine (24). Method B was followed starting from 3,4-dihydro-7-amino-2*H*-1,5-benzo[*b*][1,4]dioxepin (58 mg, 0.35 mmol). Purification by preparative TLC (AcOEt) produced 33 mg (37%) of **24**. ^1H NMR (DMSO- d_6) δ 2.09 (bs, 5 H), 4.00–4.13 (m, 4 H), 6.98 (d, 1 H, $J = 8.7$ Hz), 7.33 (d, 1 H, $J = 2.7$ Hz), 7.43 (t, 1 H, $J = 7.9$ Hz), 7.54 (dd, 1 H, $J_a = 8.7$ Hz, $J_b = 2.6$ Hz), 7.62 (bd, 1 H, $J = 6.8$ Hz), 7.70 (bd, 1 H, $J = 7.9$ Hz), 8.12 (s, 1 H), 8.35 (bs, 1 H), 8.38 (s, 1 H), 9.47 (s, 1 H), 10.08 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.06, 31.99, 70.61, 111.53, 113.48, 117.21, 120.01, 121.08, 121.69, 129.21, 129.75, 133.29, 136.24, 137.10, 139.89, 145.73, 147.92, 151.17, 151.40, 168.42; LC-MS: $t_R = 7.02$ min; m/z 377.2 (M + H) $^+$, (C₂₁H₂₀N₄O₃), >98% purity; HRMS: (M + H) $^+$ calcd for C₂₁H₂₁N₄O₃: 377.1601, found 377.1614.

2-(2,2,3,3-Tetrafluoro-2,3-dihydrobenzo[*b*][1,4]dioxin-6-ylamino)-6-(3-acetamidophenyl)pyrazine (25). Method B was followed starting from 2,2,3,3-tetrafluoro-6-amino-1,4-benzodioxine (78 mg, 0.35 mmol). Crystallization from AcOEt gave 11 mg (10%) of **25**. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3 H), 7.44 (d, 1 H, $J = 9.1$ Hz), 7.47 (d, 1 H, $J = 7.8$ Hz), 7.59 (bd, 1 H, $J = 7.6$ Hz), 7.704–7.759 (m, 2 H), 7.96 (d, 1 H, $J = 2.4$ Hz), 8.21 (s, 1 H), 8.45 (bs, 1 H), 8.51 (s, 1 H), 9.95 (s, 1 H), 10.10 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.00, 106.13, 115.92, 117.34, 118.01, 120.13, 121.08, 129.31, 130.04, 130.98, 133.62, 136.16, 136.79, 139.03, 139.99, 147.90, 150.84, 168.40; LC-MS: $t_R = 8.38$ min; m/z 435.1 (M + H) $^+$, (C₂₀H₁₄F₄N₄O₃), >99% purity; HRMS: (M + H) $^+$ calcd for C₂₀H₁₅F₄N₄O₃: 435.1080, found 435.1027.

2-(2,2-Difluorobenzo[*d*][1,3]dioxol-5-ylamino)-6-(3-acetamidophenyl)pyrazine (26). Method B was followed starting from 5-amino-2,2-difluoro-1,3-benzodioxine (61 mg, 0.35 mmol). Crystallization from DCM and cyclohexane (4:1) provided 6 mg (6%) of **26**. ^1H NMR (DMSO- d_6) δ 2.09 (s, 3 H), 7.37 (d, 1 H, $J = 8.8$ Hz), 7.44 (t, 7.9 Hz), 7.57–7.65 (m, 2 H), 7.70 (d, 1 H, $J = 7.7$ Hz), 7.94 (d, 1 H, $J = 2.1$ Hz), 8.20 (s, 1 H), 8.40 (bs, 1 H), 8.45 (s, 1 H), 9.88 (s, 1 H), 10.12 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.00, 110.06, 113.48, 117.09, 119.91, 121.00, 129.20, 130.44, 133.49, 136.83, 136.95, 137.51, 139.91, 142.76, 147.81, 151.05, 168.38; LC-MS: $t_R = 8.03$ min; m/z 385.1 (M + H) $^+$ (C₁₉H₁₄F₂N₄O₃), >98% purity; HRMS: (M + H) $^+$ calcd for C₁₉H₁₅F₂N₄O₃: 385.1112, found 385.1073.

2-(2,2,3,3-Tetrafluoro-2,3-dihydrobenzo[*b*][1,4]dioxin-5-ylamino)pyrazin-2-yl)phenylacetamide (27). Method B was followed starting from 2,2,3,3-tetrafluoro-5-amino-1,4-benzodioxin (78 mg, 0.35 mmol). The solvent was evaporated in vacuo, the resulting crude was washed with 30 mL DCM and filtered. The insoluble solid was washed with 30 mL of hot AcOEt and filtered. The AcOEt was evaporated under vacuum to produce 39 mg (37%) of **27**. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3 H), 7.11 (d, 1 H, $J = 7.8$ Hz), 7.34–7.44 (m, 2 H), 7.60 (d, 1 H, $J = 7.2$ Hz), 7.70 (d, 1 H, $J = 7.3$ Hz), 8.34 (d, 1 H, $J = 7.4$ Hz), 8.42 (bs, 1 H), 8.45 (s, 1 H), 8.57 (s, 1 H), 9.41 (s, 1 H), 10.07 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ 24.09, 110.20, 117.01, 117.41, 120.01, 120.98, 125.83, 127.29, 129.13, 129.79, 131.57, 133.89, 139.49, 136.72, 139.88, 147.68, 150.70, 168.39; LC-MS: $t_R = 8.17$ min; m/z 435.1 (M + H) $^+$, (C₂₀H₁₃F₄N₄O₃), >95% purity; HRMS: (M + H) $^+$ calcd for C₂₀H₁₄F₄N₄O₃: 435.1013, found 435.1013.

2-(3-Oxo-1,3-dihydroisobenzofuran-5-ylamino)-6-(3-acetamidophenyl)pyrazine (28). Method B was followed starting from 6-amino-1,3-dihydroisobenzofuran-1-one (52 mg, 0.35 mmol). The solvent was evaporated in vacuo and the resulting solid washed with 30 mL DCM and filtered. The insoluble solid was washed

with acetone (60 mL), the acetone evaporated and the resulting solid crystallized from DMF:DCM; 1:10 to give 23 mg (26%) of **28**. ¹H NMR (DMSO-*d*₆) δ 2.09 (s, 3 H), 5.39 (s, 2 H), 7.45 (t, 1 H, *J* = 8.0 Hz), 7.62–7.69 (m, 2 H), 7.76 (d, 1 H, *J* = 7.9 Hz), 8.23 (s, 1 H), 8.24–8.29 (m, 2 H), 8.39 (bs, 1 H), 8.50 (s, 1 H), 9.99 (s, 1 H), 10.06 (s, 1 H); ¹³C NMR (DMSO-*d*₆) δ 24.05, 69.78, 112.78, 117.31, 120.16, 121.13, 123.36, 124.58, 125.59, 129.29, 130.73, 133.63, 136.83, 139.71, 139.94, 141.47, 147.88, 151.05, 168.47, 170.75; LC-MS: *t*_R = 6.73 min; *m/z* 361.1 (M + H)⁺ (C₁₈H₁₅N₄O₂), 100% purity; HRMS: (M + H)⁺ calcd for C₁₈H₁₆N₄O₂: 361.1295, found 361.1301.

2-(2,6-Dichloropyridin-4-ylamino)-6-(3-acetamidophenyl)pyrazine (29). Method B was followed starting from 4-amino-2,6-dichloropyridine (57 mg, 0.35 mmol). Preparative TLC (AcOEt) afforded 7 mg (8%) of **29**. ¹H NMR (DMSO-*d*₆) δ 2.08 (s, 3 H), 7.48 (t, 1 H, *J* = 7.9 Hz), 7.65 (d, 1 H, *J* = 8.0 Hz), 7.71 (d, 1 H, *J* = 7.6 Hz), 7.85 (s, 2 H), 8.30 (s, 1 H), 8.32 (bs, 1 H), 8.66 (s, 1 H), 10.10 (s, 1 H), 10.55 (s, 1 H); ¹³C NMR (DMSO-*d*₆) δ 23.9, 110.3, 117.47, 120.6, 121.2, 129.4, 133.4, 134.2, 136.3, 140.0, 148.3, 149.5, 149.7, 151.4, 168.3; LC-MS: *t*_R = 6.47 min; *m/z* 374.1 (M + H)⁺, (C₁₇H₁₃Cl₂N₅O), >80% purity.

2-(4-tert-Butylthiazol-2-ylamino)-6-(3-acetamidophenyl)pyrazine (30). Method B was followed starting from 4-(*tert*-butyl)-1,3-thiazolyl-2-amine (55 mg, 0.35 mmol). Column chromatography on silica gel (AcOEt: DCM, 2:1) furnished 19 mg (21%) of **30**. ¹H NMR (DMSO-*d*₆) δ 1.30 (s, 9 H), 2.10 (s, 3 H), 6.69 (s, 1 H), 7.47 (t, 1 H, *J* = 7.9 Hz), 7.69 (d, 1 H, *J* = 8.0 Hz), 7.89 (d, 1 H, *J* = 7.8 Hz), 8.38 (s, 1 H), 8.45 (bs, 1 H), 8.58 (s, 1 H), 10.11 (s, 1 H), 11.78 (bs, 1 H); ¹³C NMR (DMSO-*d*₆) δ 24.05, 29.80, 34.12, 103.13, 117.42, 120.48, 121.54, 129.30, 131.54, 133.28, 136.40, 139.92, 147.95, 158.12, 160.43, 168.46; LC-MS: *t*_R = 7.10 min; *m/z* 368.2 (M + H)⁺, (C₁₉H₂₁N₅O), >50% purity.

2-(3-Chlorophenylamino)-6-chloropyrazine (4b). The title compound was prepared by method B using 3-chloroaniline (520 mg, 3.5 mmol). The workup was different: to the reaction mixture 50 mL was added Et₂O, and the mixture was extracted with 100 mL H₂O. The aqueous layer was extracted again with 50 mL Et₂O and the organic solution pooled, dried (MgSO₄) and evaporated to dryness. A solid (0.852 g) was obtained and purified by preparative HPLC (AcOEt:cyclohexane 1:2). 0.419 g (50.0%) of **4b** was collected. ¹H NMR (DMSO-*d*₆) δ 7.02 (m, 1H, *J* = 7.9 Hz), 7.36 (t, 1H, *J* = 8.1 Hz), 7.51 (m, 1H, *J* = 8.3 Hz), 7.83 (t, 1H, *J* = 2.0 Hz), 8.03 (s, 1H), 8.18 (s, 1H), 10.03 (s, 1H); LC-MS: *t*_R = 8.41 min; *m/z*: 296 (M - H)⁻, (C₁₀H₇Cl₂N₃).

2-(4-Methoxyanilin)-6-chloropyrazine (4c). Using method B with 4-methoxyaniline (500 mg, 3.35 mmol) (reaction time: 5 h), **4c** was obtained (265 mg, 33.7%), after purification by preparative HPLC (cyclohexane:AcOEt 1:1). ¹H NMR (DMSO-*d*₆) δ 3.73 (s, 3H), 6.94 (d, 2H, *J* = 9.0 Hz), 7.51 (d, 2H), 7.89 (s, 1H), 8.08 (s, 1H), 9.65 (s, 1H).

2-(2,3-Dehydro-1,4-benzodioxin-6-methylamino)-6-chloropyrazine (4d). Method E. Amounts of 100 mg (0.61 mmol) 2,3-dehydro-1,4-benzodioxin-6-methylamine, 96 mg (0.15 mmol) 2,6-dichloropyrazine and 170 mL triethylamine were stirred in 10 mL DMF for 18 h at 80 °C. After cooling, the reaction mixture was diluted with 25 mL H₂O and extracted with 25 mL AcOEt. The organic layer was washed (2 × 25 mL brine), dried (MgSO₄) and evaporated to 2–3 mL. The resulting solution was purified on an Isolute flash Si-II column (10 g Si) (AcOEt:cyclohexane 2:1), from which 133 mg (78.6%) of **4d** were obtained, as a thick oil. ¹H NMR (DMSO-*d*₆) δ: 4.22 (s, 2H), 4.33 (d, 2H, *J* = 5.3 Hz), 6.81 (s, 1H), 6.83 (d, 2H), 7.71 (s, 1H), 7.91 (s, 1H), 7.96 (t, 1H); ¹³C NMR (DMSO-*d*₆) δ 43.19, 63.95, 116.20, 116.87, 120.37, 128.14, 131.73, 142.40, 143.13, 145.87, 154.44; LC-MS: *t*_R = 7.15 min; *m/z*: 278.05 (M + H)⁺, (C₁₃H₁₂ClN₃O₂); HRMS: (M + H)⁺ calcd for C₁₃H₁₂ClN₃O₂: 319.0962, found 319.0965.

2-(1,3-Benzodioxol-5-ylmethylamino)-6-chloropyrazine (4e). Using method E from 140 mg (0.92 mmol) 1,3-benzodioxol-5-ylmethylamine and 148 mg (1.01 mmol) 2,6-dichloropyrazine, 248 mg (72.6%) of **4e** was obtained after purification on an Isolute column (AcOEt:cyclohexane 2:1). ¹H NMR (DMSO-*d*₆) δ 4.37 (d,

2H, *J* = 5.7 Hz), 5.99 (s, 2H), 6.81–6.93 (m, 3H), 7.71 (s, 1H), 7.91 (s, 1H), 7.96 (t, 1H); ¹³C NMR (DMSO-*d*₆) δ 43.61, 100.83, 108.07, 108.08, 120.89, 128.38, 132.38, 143.08, 145.87, 146.23, 154.43; LC-MS: *t*_R = 7.24 min, *m/z*: 278.05 (M + H)⁺, (C₁₂H₁₂ClN₃O₂); HRMS: (M + H)⁺ calcd for C₁₄H₁₄ClN₄O₂ 305.0805, found 305.0812.

2-[(5-Methyl-3-isoxazolyl)methylamino]-6-chloropyrazine (4f). Using method E from 140 mg (1.25 mmol) 5-methyl-3-isoxazolyl-methylamine and 202 mg (1.38 mmol) 2,6-dichloropyrazine, 164 mg (58.4%) of **4f** was obtained after purification on an Isolute column (AcOEt:cyclohexane 2:1). ¹H NMR (DMSO-*d*₆) δ 2.38 (s, 3H), 4.48 (d, 2H, *J* = 5.9 Hz), 6.17 (s, 1H), 7.78 (s, 1H), 7.96 (s, 1H), 8.07 (t, 1H); ¹³C NMR (DMSO-*d*₆) δ 11.73, 30.64, 101.15, 128.86, 131.26, 145.79, 154.28, 161.67, 169.55; LC-MS, *t*_R = 5.59 min, *m/z*: 224.9 (M + H)⁺, (C₉H₉ClN₄O); HRMS: (M + H)⁺ calcd for C₉H₁₀ClN₄O 225.0543, found 225.0556.

2-[(1,5-Dimethyl-1H-pyrazol-3-yl)methylamino]-6-chloropyrazine (4g). Using method E from 140 mg (1.12 mmol) (1,5-dimethyl-1H-pyrazol-3-yl)methylamine and 180 mg (1.23 mmol) 2,6-dichloropyrazine, 105 mg (61.2%) of **4g** was obtained after purification on an Isolute column (AcOEt). Mp 122–3 °C; ¹H NMR (DMSO-*d*₆) δ 2.21 (s, 3H), 3.67 (s, 3H), 4.29 (d, 2H, *J* = 5.6 Hz), 5.96 (s, 1H), 7.70 (s, 1H), 7.84 (t, 1H, *J* = 5.2 Hz), 7.92 (s, 1H); LC-MS, *t*_R = 5.72 min, *m/z*: 237.9 (M + H)⁺, (C₁₀H₁₃ClN₅).

2-(2-Pyridylmethylamino)-6-chloropyrazine (4h). Using method E from 140 mg (1.30 mmol) 2-pyridylmethylamine and 210 mg (1.43 mmol) 2,6-dichloropyrazine, 53 mg (48.7%) of **4h** was obtained after purification on an Isolute column (AcOEt:cyclohexane 2:1). Mp 107–8.5 °C; ¹H NMR (DMSO-*d*₆) δ 4.57 (d, 2H, *J* = 5.89), 7.26–7.38 (m, 2H), 7.74 (s, 1H), 7.73–7.80 (m, 1H), 8.02 (s, 1H), 8.13 (s, 1H), 8.54 (d, 1H, *J* = 4.51); ¹³C NMR (DMSO-*d*₆) δ 45.56, 121.29, 122.25, 128.43, 128.40, 131.37, 136.76, 145.84, 154.57, 157.97; LC-MS, *t*_R = 2.69 min, *m/z*: 220.9 (M + H)⁺ (C₁₀H₁₀ClN₄). HRMS: (M + H)⁺ calcd for C₁₀H₁₀ClN₄ 221.0594, found 221.0600.

2-(Benzylamino)-6-chloropyrazine (4i). Using method E with 100 mg (0.93 mmol) benzylamine and 146 mg (1.00 mmol) 2,6-dichloropyrazine, 144 mg (70.5%) of **4i** was obtained after purification on an Isolute Si-II column (AcOEt:cyclohexane 2:1). ¹H NMR (DMSO-*d*₆) δ 4.47 (d, 2H, *J* = 5.9 Hz), 7.23–7.37 (m, 5H), 7.72 (s, 1H), 7.94 (s, 1H), 8.05 (t, 1H, *J* = 5.6 Hz); ¹³C NMR (DMSO-*d*₆) δ 43.78, 126.88, 127.95, 128.36, 128.40, 131.16, 138.80, 145.89, 154.55; LC-MS: *t*_R = 7.38 min, *m/z*: 219.95 (M + H)⁺, (C₁₁H₁₁ClN₃). HRMS: (M + H)⁺ calcd for C₁₁H₁₁ClN₃ 220.0642, found 220.0661.

2-(2-Phenylethylamino)-6-chloropyrazine (4j). Using method E with 400 mg (3.30 mmol) 2-phenylethylamine and 533 mg (3.63 mmol) 2,6-dichloropyrazine, 586 mg (53.4%) of **4j** was obtained after purification on an Isolute Si-II column, 20 g (AcOEt:cyclohexane 2:1). ¹H NMR (DMSO-*d*₆) δ 2.85 (t, 2H, *J* = 7.6 Hz), 3.48 (q, 2H), 7.18–7.34 (m, 5H), 7.64 (m, 1H), 7.68 (s, 1H), 7.88 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 34.55 41.88, 126.28, 127.25, 128.30, 128.67, 131.14, 143.09, 145.97, 154.65; LC-MS: *t*_R = 7.64 min, *m/z*: 233.1 (M + H)⁺, (C₁₂H₁₁ClN₃); HRMS: (M + H)⁺ calcd for C₁₂H₁₂ClN₃ 234.0798, found 234.0791.

2-(3-Phenylpropylamino)-6-chloropyrazine (4k). Using method E with 100 mg (0.74 mmol) 3-phenylpropylamine and 117 mg (0.80 mmol) 2,6-dichloropyrazine, 136 mg (74.3%) of **4k** was obtained after purification on an Isolute Si-II column, 10 g (AcOEt:cyclohexane 2:1). ¹H NMR (DMSO-*d*₆) δ: 1.78–1.91 (m, 2H), 2.67 (t, 2H, *J* = 7.3 Hz), 3.24 (q, 2H), 7.14–7.33 (m, 5H), 7.61 (t, 1H, *J* = 5.2 Hz), 7.65 (s, 1H), 7.88 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 30.07, 30.67, 32.45, 125.74, 127.95, 128.26, 128.40, 131.11, 141.56, 145.97, 154.78; LC-MS: *t*_R = 7.96 min, *m/z*: 247.97 (M + H)⁺, (C₁₃H₁₄ClN₃). HRMS: (M + H)⁺ calcd for C₁₃H₁₅ClN₃ 248.0955, found 248.0941.

2-(2-Phenoxyethylamino)-6-chloropyrazine (4l). Using method E with 400 mg (2.90 mmol) 2-phenoxyethylamine and 468 mg (3.19 mmol) 2,6-dichloropyrazine, 467 mg (64.7%) of **4l** was obtained after purification by chromatography on an Isolute Si-II column 10 g (AcOEt:cyclohexane 2:1). ¹H NMR (DMSO-*d*₆) δ 3.64 (q,

2H), 4.12 (t, 2H, $J = 5.6$ Hz), 6.94–7.00 (m, 3H), 7.26–7.33 (m, 2H), 7.72 (s, 1H), 7.82 (t, 1H, $J = 5.5$ Hz), 7.88 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 65.65, 114.47, 120.68, 128.12, 129.47, 131.43, 143.09, 145.85, 154.69, 158.31; LC-MS: $t_{\text{R}} = 7.52$ min; m/z : 247.97 (M + H) $^+$, (C₁₂H₁₂ClN₃O). HRMS: (M + H) $^+$ calcd for C₁₂H₁₃ClN₃O 250.0747, found 250.0742.

2-[2-(4-Methoxyphenoxy)ethylamino]-6-chloropyrazine (4m). Using method E with 140 mg (0.84 mmol) 2-(4-methoxyphenoxy)ethylamine and 135 mg (0.92 mmol) 2,6-dichloropyrazine, 176 mg (75.2%) of **4m** was obtained after purification on an Isolute Si-II column 10 g (AcOEt:cyclohexane 2:1). ^1H NMR (DMSO- d_6) δ 3.60 (q, 2H, $J = 5.5$ Hz), 3.70 (s, 3H), 4.06 (t, 2H, $J = 5.6$ Hz), 6.86 (d, 2H), 6.92 (m, 2H), 7.72 (s, 1H), 7.80 (t, 1H, $J = 5.3$ Hz), 7.96 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 55.33, 65.65, 114.60, 115.67, 128.08, 131.44, 143.09, 145.85, 151.80, 154.69; LC-MS: $t_{\text{R}} = 6.80$ min; m/z : 279.9 (M + H) $^+$, (C₁₃H₁₄ClN₃O₂); HRMS: (M + H) $^+$ calcd for C₁₅H₁₈ClN₄O₂ 321.1118, found 321.1122.

2-(3,4,5-Trimethoxybenzylamino)-6-chloropyrazine (4n). Using method E with 400 mg (2.03 mmol) 2-phenylethylamine and 327 mg (2.23 mmol) 2,6-dichloropyrazine, 434 mg (69.2%) of **4n** was obtained after purification on an Isolute Si-II column, 20 g (AcOEt:cyclohexane 2:1). ^1H NMR (DMSO- d_6) δ 3.64 (s, 6H), 3.77 (s, 3H), 4.38 (d, 2H, $J = 5.7$ Hz), 6.71 (s, 2H), 7.73 (s, 1H), 7.73 (m, 2H); ^{13}C NMR (DMSO- d_6) δ 44.22, 55.82, 59.95, 105.15, 128.26, 131.28, 134.26, 136.57, 145.84, 152.83, 154.48; LC-MS: $t_{\text{R}} = 6.83$ min; m/z : 309.93 (M + H) $^+$, (C₁₆H₁₆ClN₃O₃); HRMS: (M + H) $^+$ calcd for C₁₆H₁₇ClN₃O₃ 310.0958, found 310.0948.

3-(3-Fluorobenzylamino)-6-chloropyrazine (4o). In an oven dried flask, 50 mg (0.34 mmol) 3,6-dichloropyrazine, 50 mg (0.40 mmol) 3-fluoro-benzylamine, 48 mg (0.50 mmol) sodium *tert*-butoxide and 3.20 mg (0.007 mmol) palladacycle **20** were suspended in 2 mL dry toluene under stirring and N₂. The reaction mixture was heated for 2 h at 90 °C (bath temperature). The solvent was evaporated under vacuum, the residue taken up in 10 mL AcOEt, washed (2 × 10 mL brine), dried (MgSO₄) and evaporated to dryness and 40 mg of **4o**, as a brown oil, was obtained.

Using method D with 50 mg (0.34 mmol) of 2,6-dichloropyrazine and 50 mg (0.40 mmol) 3-fluorobenzylamine and, after purification on an Isolute flash Si column, 10 g (AcOEt) 80 mg (62.2%) of **4o**, as a thick oil, was obtained. ^1H NMR (DMSO- d_6) δ 4.49 (d, 2H, $J = 5.9$ Hz), 7.05–7.19 (m, 3H), 7.35–7.44 (m, 1H), 7.74 (s, 1H), 7.96 (s, 1H), 8.09 (t, 1H, $J = 5.5$ Hz); ^{13}C NMR (DMSO- d_6) δ 43.25, 113.79, 123.36, 128.53, 130.34, 131.21, 141.92, 145.84, 152.43, 161.26, 163.19; LC-MS: $t_{\text{R}} = 7.36$ min; m/z : 238 (M + H) $^+$, (C₁₁H₉ClFN₃). HRMS: (M + H) $^+$ calcd for C₁₁H₁₀ClFN₃ 238.0547, found 238.0533.

2-(3-Furylmethylamino)-6-chloropyrazine (4p). Using method B, with 100 mg (0.67 mmol) 2,6-dichloropyrazine and 80 mg (0.82 mmol) 3-furylmethylamine and after purification by preparative HPLC (AcOEt:cyclohexane 2:1), **4p** was obtained as thick oil crystallizing on storage: 128 mg (50.5%). ^1H NMR (DMSO- d_6) δ 4.28 (d, 2H, $J = 5.6$ Hz), 7.31 (s, 1H), 7.62–7.67 (m, 2H), 7.73 (s, 1H), 7.83 (t, 1H, $J = 5.2$ Hz), 7.92 (s, 1H); LC-MS, $t_{\text{R}} = 6.45$ min; m/z : 210 (M + H) $^+$, (C₉H₈ClFN₃O).

2-(3-Chloro-4-fluorobenzylamino)-6-chloropyrazine (4q). Using method B with 100 mg (0.67 mmol) 2,6-dichloropyrazine and 159 mg (1.00 mmol) 3-chloro-4-fluorobenzylamine and purification on Isolute column, 10 g (AcOEt) **4q** was obtained as a thick oil, 138 mg (76%). ^1H NMR (DMSO- d_6) δ 4.49 (d, 2H, $J = 5.9$ Hz), 7.05–7.19 (m, 3H), 7.35–7.44 (m, 1H), 7.74 (s, 1H), 7.96 (s, 1H), 8.09 (t, 1H, $J = 5.5$ Hz); ^{13}C NMR (DMSO- d_6) δ 42.66, 116.85, 128.09, 128.63, 129.47, 131.25, 136.88, 145.81, 154.30, 155.27, 157.22; LC-MS: $t_{\text{R}} = 7.94$ min; m/z : 238 (M + H) $^+$, (C₁₁H₈ClFN₃). HRMS: (M + H) $^+$ calcd for C₁₁H₉Cl₂FN₃ 272.0158, found 272.0155.

2-(2,3-Dehydro-1,4-benzodioxin-6-methylamino)-6-(3'-acetamidophenyl)pyrazine (31). Using method A, 100 mg (0.36 mmol) 2-(2,3-dehydro-1,4-benzodioxin-6-methylamino)-6-chloropyrazine, **4d**, and 97 mg (0.54 mmol, 1.5 equiv) 3-acetamidophenyl boronic acid were reacted at 80 °C (bath temperature) for 20 h in

a twelve-tube carousel. After purification on an Isolute column, Flash Si-II, 10 g (AcOEt), 89 mg (65.7%) of **31** was obtained. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3H), 4.22 (s, 2H), 4.86 (d, 2H, $J = 5.8$ Hz), 6.80 (d, 2H, $J = 7.7$ Hz), 6.89 (d, 2H, $J = 7.6$ Hz), 6.91 (s, 1H), 7.39 (t, 1H), 7.59 (s, 1H), 7.66–7.68 (m, 2H), 7.91 (s, 1H), 8.19 (s, 1H), 8.24 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.02, 43.03, 59.72, 63.95, 116.31, 116.79, 120.60, 127.66, 129.00, 131.86, 132.83, 137.33, 139.72, 142.28, 143.12, 147.97, 153.89, 168.38; LC-MS: $t_{\text{R}} = 6.84$ min; m/z : 377.1 (M + H) $^+$, (C₂₁H₂₀N₄O₃). HRMS: (M + H) $^+$ calcd for C₂₁H₂₁N₄O₃ 377.1614, found 377.1586.

2-(3,4-Methylenedioxyphenylaminomethyl)-6-(3-acetamidophenyl)pyrazine (30). Using method A, 100 mg (0.38 mmol) 2-(3,4-methylenedioxyphenylamino methyl)-6-chloropyrazine (**4e**) were reacted with 102 mg (0.58 mmol) 3-acetamido phenyl boronic acid (reaction time: 4 h), and after purification on an Isolute column, Flash SiII, 10 g (AcOEt), 70.0 mg (50.8%) of **30** was obtained. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3H), 4.76 (d, 2H, $J = 5.8$ Hz), 5.97 (s, 2H), 6.86 (d, 1H, $J = 7.9$ Hz), 6.93 (d, 1H, $J = 8.0$ Hz), 7.93 (t, 1H, $J = 7.9$ Hz), 7.61 (t, 1H, $J = 5.8$ Hz), 7.78 (d, 2H, $J = 7.8$ Hz), 7.92 (s, 1H), 8.20 (s, 1H), 8.26 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.02, 43.41, 100.74, 108.03, 108.25, 117.01, 119.81, 120.92, 127.71, 129.01, 131.88, 133.68, 137.30, 139.72, 146.06, 147.20, 147.94, 153.83, 168.39; LC-MS: $t_{\text{R}} = 6.92$ min; m/z : 363.1 (M + H) $^+$, (C₂₀H₁₈N₄O₃); HRMS: (M + H) $^+$ calcd for C₂₀H₁₉N₄O₃ 363.1457, found 363.1464.

2-[(5-Methyl-3-isoxazolyl)methylamino]-6-(3-acetamidophenyl)pyrazine (33). Using method A, 100 mg (0.44 mmol) 2-[(5-methyl-3-isoxazolyl)methylamino]-6-chloropyrazine (**4f**) and 120.0 mg (0.67 mmol) 3-acetamidophenyl boronic acid, (reaction time: 4 h) were reacted, and after purification on an Isolute column, Flash SiII, 10 g (AcOEt), 54.0 mg (34%) of **33** resulted. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3H), 2.36 (s, 3H), 4.62 (d, 2H, $J = 5.9$ Hz), 6.20 (s, 1H), 7.40 (t, 1H, $J = 7.9$ Hz), 7.61 (t, 1H, $J = 5.8$ Hz), 7.67–7.70 (m, 3H), 7.96 (s, 1H), 8.23 (s, 1H), 8.25 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 11.73, 24.02, 36.99, 101.32, 117.00, 119.90, 121.10, 128.25, 129.06, 131.97, 137.17, 139.73, 147.99, 153.63, 162.40, 168.40, 169.40; LC-MS: $t_{\text{R}} = 5.98$ min; m/z : 323.1 M + , (C₁₇H₁₇N₅O₂); HRMS: (M + H) $^+$, calcd for C₁₇H₁₈N₅O₂ 324.1461, found 324.1486.

2-[(1,5-Dimethyl-1H-pyrazol-3-yl)methylamino]-6-(3-acetamidophenyl)pyrazine (34). Using method A, 100 mg (0.42 mmol) 2-[(1,5-dimethyl-1H-pyrazol-3-yl)methylamino]-6-chloropyrazine (**4g**) and 113.0 mg (0.63 mmol) 3-acetamido phenyl boronic acid (reaction time: 4 h) gave, after purification on an Isolute column, Flash SiII, 10 g (AcOEt), 47.0 mg (34.6%) of **34**. ^1H NMR (DMSO- d_6) δ : 2.08 (s, 3H), 2.20 (s, 3H), 3.68 (s, 1), 4.46 (d, 2H, $J = 5.6$ Hz), 6.00 (s, 1H), 7.36–7.42 (m, 2H), 7.68–7.73 (m, 2H), 7.93 (s, 1H), 8.17 (s, 1H), 8.23 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 10.63, 24.01, 35.06, 37.78, 103.78, 117.03, 119.79, 121.03, 127.56, 129.00, 131.94, 137.40, 138.87, 139.71, 147.90, 153.96, 168.39; LC-MS: $t_{\text{R}} = 6.01$ min; m/z : 337.1 (M + H) $^+$, (C₁₈H₂₀N₆O); HRMS: (M + H) $^+$, calcd for C₁₈H₂₀N₆O 337.1777, found 337.1763.

2-(2-Pyridylmethylamino)-6-(3-acetamidophenyl)pyrazine (35). Using method A, 100 mg (0.42 mmol) 2-(2-pyridylmethylamino)-6-chloropyrazine (**4h**) and 122.0 mg (0.68 mmol) 3-acetamido phenyl boronic acid (reaction time: 4 h) gave after purification on an Isolute column, Flash SiII, 10 g (AcOEt), 117.0 mg (77.4%) of **35**. ^1H NMR (DMSO- d_6) δ 2.07 (s, 3H), 4.46 (d, 2H, $J = 5.9$ Hz), 7.24–7.29 (m, 1H), 7.37 (t, 1H, $J = 7.9$ Hz), 7.45 (d, 1H), 7.59–7.79 (m, 4H), 8.02 (s, 1H), 8.19 (s, 1H), 8.20 (s, 1H), 8.55 (d, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.01, 57.72, 119.83, 121.05, 121.45, 122.10, 127.92, 128.99, 132.00, 136.68, 137.26, 139.69, 148.00, 153.91, 158.96, 168.38; LC-MS: $t_{\text{R}} = 3.61$ min; m/z : 320.1 (M + H) $^+$, (C₁₈H₁₇N₅O); HRMS: (M + H) $^+$, calcd for C₁₈H₁₈N₅O 320.1511, found 320.1520.

2-(Benzylamino)-6-(3-acetamidophenyl)pyrazine (36). Using method A with 100 mg (0.46 mmol) 2-benzylamino-6-chloropyrazine (**4i**) and 123 mg (0.68 mmol) 3-acetamidophenyl boronic acid, 116 mg (79.2%) of **36** were obtained after purification on an Isolute Si-II column, 10 g (AcOEt). ^1H NMR (DMSO- d_6) δ 2.08

(s, 3H), 4.62 (d, 2H, $J = 5.8$ Hz), 7.21–7.46 (m, 7H), 7.64–7.72 (m, 2H), 7.94 (s, 1H), 8.20 (s, 1H), 8.25 (s, 1H), 10.04 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.02, 43.61, 117.01, 119.81, 121.08, 126.80, 127.70, 128.30, 131.89, 137.32, 139.72, 148.00, 153.95, 168.38; LC-MS: $t_{\text{R}} = 7.00$ min; m/z 319.1 (M + H) $^+$, (C₁₉H₁₈N₄O); HRMS: (M + H) $^+$, calcd for C₁₉H₁₈N₄O 319.1559, found 319.1582.

2-(Phenylethylamino)-6-(3-acetamidophenyl)pyrazine (37). Using method A with 100 mg (0.43 mmol) 2-(phenylethylamino)-6-chloropyrazine (**4j**) and 115 mg (0.64 mmol) 3-acetamidophenyl boronic acid, 96 mg (67.6%) of **37** was obtained, after purification on an Isolute Si-II column, 10 g (AcOEt). ^1H NMR (DMSO- d_6) δ : 2.08 (s, 3H), 2.93 (t, 2H, $J = 7.6$ Hz) 3.52 (q, 2H), 7.17–7.44 (m, 7H), 7.69 (d, 2H), 7.89 (s, 1H), 8.17 (s, 1H), 8.25 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 14.06, 34.87, 41.94, 117.12, 119.82, 121.05, 126.03, 127.45, 128.30, 128.80, 128.99, 131.95, 137.53, 139.69, 139.71, 148.15, 154.13, 168.37; LC-MS: $t_{\text{R}} = 7.38$ min; m/z 332.1 M + H $^+$, (C₂₀H₂₀N₄O); HRMS: (M + H) $^+$, calcd for C₂₀H₂₁N₄O 333.1715, found 333.1736.

2-(3-Phenylpropylamino)-6-(3-acetamidophenyl)pyrazine (38). Using method A with 100 mg (0.40 mmol) 2-(3-phenylpropylamino)-6-chloropyrazine (**4k**) and 107 mg (0.60 mmol) 3-acetamidophenyl boronic acid, 124 mg (89.5%) of **38** was obtained after purification on an Isolute Si-II column (AcOEt). ^1H NMR (DMSO- d_6) δ 1.85–1.98 (m, 2H), 2.72 (t, 2H, $J = 7.3$ Hz), 3.40 (q, 2H), 7.14–7.42 (m, 7H), 7.66 (dd, 2H), 7.89 (s, 1H), 8.15 (s, 1H), 8.22 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.02, 30.57, 32.66, 117.03, 119.78, 121.04, 125.86, 127.25, 128.24, 128.31, 128.97, 131.86, 137.48, 139.68, 141.82, 148.08, 154.08, 168.37; LC-MS: $t_{\text{R}} = 7.67$ min; m/z 347.1 (M + H) $^+$, (C₂₁H₂₂N₄O); HRMS: (M + H) $^+$, calcd for C₂₁H₂₃N₄O 347.1845, found 347.1847.

2-Phenoxyethylamino-6-(3-acetamidophenyl)pyrazine (39). Using method A, 100 mg (0.40 mmol) 2-phenoxyethylamino-6-chloropyrazine (**4l**) and 107.0 mg (0.60 mmol) 3-acetamidophenyl boronic acid were reacted, and after purification on an Isolute column, Flash Si-II (AcOEt), 112.0 mg (80.3%) of **39** was obtained. ^1H NMR (DMSO- d_6) δ 3.79 (dd, 2H, $J = 5.5$ Hz), 4.20 (t, 2H), 3.40 (q, 2H), 6.90–7.01 (m, 3H), 7.25–7.43 (m, 4H), 7.69 (t, 2H, $J = 7.5$ Hz), 7.97 (s, 1H), 8.19 (s, 1H), 8.22 (s, 1H), 10.06 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.03, 65.98, 114.48, 117.08, 119.79, 120.60, 121.08, 127.73, 129.48, 132.15, 137.43, 139.73, 148.09, 154.10, 158.42, 168.40; LC-MS: $t_{\text{R}} = 7.29$ min; m/z 349.1 (M + H) $^+$, (C₂₀H₂₀N₄O₂); HRMS: (M + H) $^+$, calcd for C₂₀H₂₁N₄O₂ 349.1665, found 349.1673.

2-(4-Methoxyphenoxyethylamino)-6-(3-acetamidophenyl)pyrazine (40). Using method A with 228 mg (0.97 mmol) 2-(4-methoxyphenoxyethylamino)-6-chloropyrazine (**4m**) and 147 mg (0.82 mmol) 3-acetamidophenyl boronic acid, 33 mg (10.2%) pure **40** was obtained, after purification on an Isolute SiII column, 10 g (AcOEt). Reaction time: 24 h. ^1H NMR (DMSO- d_6) δ : 2.09 (s, 3H), 3.75 (s, 3H), 7.27 (s, 2H), 6.96 (d, 2H, $J = 9.0$ Hz), 7.42 (t, 1H, $J = 7.9$ Hz), 7.63 (d, 2H, $J = 7.7$ Hz), 7.71 (d, 2H), 7.75 (d, 2H), 8.11 (s, 1H), 8.23 (d, 2H), 8.36 (s, 1H), 9.41 (s, 1H), 10.09 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.03, 55.30, 66.63, 114.58, 115.46, 117.06, 119.77, 121.08, 127.69, 129.01, 132.16, 137.43, 139.73, 148.08, 152.42, 153.40, 154.11, 161.33, 168.40; LC-MS: $t_{\text{R}} = 7.13$ min; m/z 379.1 (M + H) $^+$, (C₂₁H₂₂N₄O₃); HRMS: (M + H) $^+$, calcd for C₂₁H₂₃N₄O₃ 379.1770, found 379.1757.

2-(3,4,5-Trimethoxybenzylamino)-6-(3-acetamidophenyl)pyrazine (41). Using method A with 100 mg (0.32 mmol) 2-(3,4,5-trimethoxybenzylamino)-6-chloropyrazine (**4n**) and 87 mg (0.49 mmol) 3-acetamidophenyl boronic acid, 114 mg (87.2%) of **41** was obtained, after purification on an Isolute Si-II column, 10 g (AcOEt). ^1H NMR (DMSO- d_6) δ 2.07 (s, 3H), 3.62 (s, 6H), 3.74 (s, 3H), 4.53 (d, 2H, $J = 4.9$ Hz), 6.79 (s, 2H), 7.39 (t, 1H, $J = 7.9$ Hz), 7.62–7.72 (m, 3H), 7.94 (s, 1H), 8.23 (s, 1H), 8.33 (s, 1H), 10.06 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 23.99, 55.71, 59.91, 105.29, 116.95, 119.80, 120.94, 127.65, 128.99, 132.01, 135.44, 136.33, 137.24, 139.74, 147.77, 152.74, 153.85, 168.39; LC-MS: $t_{\text{R}} = 6.51$ min; m/z 408.1 (M + H) $^+$, (C₂₂H₂₄N₄O₄); HRMS: (M + H) $^+$, calcd for C₂₂H₂₅N₄O₄ 409.1876, found 409.1874.

2-(3-Fluorophenylmethylamino)-6-(3'-acetamidophenyl)pyrazine (42). Using method A and starting from 80 mg (0.33 mmol) 2-(3-fluorophenylmethyl amino)-6-chloropyrazine (**4o**) and 62 mg (0.38 mmol) 3-fluorobenzylamine, 26 mg (23.6%) pure **42** was obtained after purification by preparative HPLC (AcOEt:EtOH 95:5). Reaction time: 18 h. ^1H NMR (DMSO- d_6) δ 2.07 (s, 3H), 4.63 (d, 2H, $J = 5.9$ Hz), 7.06–7.50 (m, 5H), 7.60–7.70 (m, 2H), 7.74 (t, 1H, $J = 5.7$ Hz), 7.95 (s, 1H), 8.22 (s, 1H), 8.25 (s, 1H), 10.04 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.01, 43.14, 113.44, 114.14, 117.03, 119.86, 121.00, 123.63, 127.95, 129.00, 130.25, 131.90, 137.22, 143.06, 147.96, 153.79, 161.26, 168.37; LC-MS: $t_{\text{R}} = 7.07$ min; m/z 337.1 (M + H) $^+$, (C₁₉H₁₇FN₄O); HRMS: (M + H) $^+$, calcd for C₁₉H₁₈FN₄O 337.1465, found 337.1456.

2-(3-Furylmethylamino)-6-(3-acetamidophenyl)pyrazine (43). Using method A with 160 mg (0.76 mmol) 2-(3-furylmethylamino)-6-chloropyrazine (**4p**) and 168 mg (0.87 mmol) 3-acetamidophenyl boronic acid, 114 mg (48.7%) pure **43** was obtained after purification by preparative HPLC (AcOEt:EtOH 95:5). Reaction time: 18 h. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3H), 4.43 (d, 2H, $J = 5.8$ Hz), 6.53 (s, 1H), 7.30–7.50 (m, 2H), 7.52–7.75 (d, 4H), 7.95 (s, 1H), 8.22 (s, 1H), 8.30 (s, 1H), 10.04 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 20.73, 24.02, 110.87, 116.92, 119.82, 120.94, 123.11, 127.67, 129.02, 130.26, 132.04, 137.30, 139.76, 140.32, 143.31, 147.87, 153.84, 168.42; LC-MS: $t_{\text{R}} = 6.43$ min; m/z 309.1 (M + H) $^+$, (C₁₇H₁₆N₄O₂); HRMS: (M + H) $^+$, calcd for C₁₇H₁₇N₄O₂ 309.1352, found 309.1376.

2-(3-Chloro-4-fluorobenzylamino)-6-(3-acetamidophenyl)pyrazine (44). Using method A with 100 mg (0.37 mmol) 2-(3-chloro-4-fluorobenzylamino)-6-chloropyrazine (**4q**) and 76 mg (0.42 mmol) 3-acetamidophenyl boronic acid, 40 mg (29.2%) pure **44** was obtained after purification by preparative HPLC (AcOEt:EtOH 95:5). Reaction time: 18 h. ^1H NMR (DMSO- d_6) δ 2.08 (s, 3H), 4.58 (d, 2H, $J = 5.8$ Hz), 7.30–7.55 (m, 3H), 7.70 (d, 3H), 7.78 (s, 1H), 7.95 (s, 1H), 8.22 (s, 1H), 8.28 (s, 1H), 10.04 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 24.08, 100.62, 100.74, 111.15, 117.08, 119.92, 121.09, 129.22, 129.61, 133.28, 135.06, 137.15, 139.88, 141.76, 147.20, 147.96, 151.93, 168.45; LC-MS: $t_{\text{R}} = 7.56$ min; m/z 371.1 (M + H) $^+$, (C₁₉H₁₆ClFN₄O); HRMS: (M + H) $^+$, calcd for C₁₉H₁₇-ClFN₄O 371.1075, found 371.1061.

^{600}EB -RAF Kinase Assay. Full-length rabbit MEK1 protein was expressed with a GST tag at the N-terminus and a C-terminal histidine tag in *Escherichia coli* JM109 bacteria and purified by nickel-agarose affinity chromatography. ^{600}EB -RAF was generated by infection of SF9 insect cells cultured in SF-900 II medium (Invitrogen, Paisley, Scotland) with a baculovirus containing full-length human B-RAF with an N-terminal histidine tag. Assay buffer was 20 mM MOPS, pH 7.2, containing 5 mM EGTA, 10 mM MgCl₂, 0.1% β -ME and 25 mM β -glycerophosphate. All incubations were at room temperature with shaking. Amounts of 1 μg GST-MEK1, 0.07 μL insect cell ^{600}EB -RAF lysate and 0.5 μL inhibitor at the required concentrations (0.001 to 100 μM final concentration) were added to the wells of a glutathione-coated plate, and the plate was preincubated for 10 min. ATP in assay buffer (10 μL), to give a final concentration of 100 μM , was added to each well, and the plates were incubated for 45 min. The plates were then washed 3 \times with 200 μL 0.1% tween20/water. Primary antibody (rabbit anti-phospho MEK1/2 diluted 1/2000, Cell Signaling Technologies, Hitchin, UK) and Eu-labeled anti-rabbit secondary antibody (diluted 1/1000, Perkin-Elmer, Turku, Finland) were preincubated for 30 min and 50 μL added to the washed plates, which were incubated for a further hour. The plates were washed as before, and 100 μL DELFIA enhancement solution (Perkin-Elmer, Turku, Finland) was added. The plates were sealed and incubated for 30 min and europium counts measured on a Victor 2 reader (Perkin-Elmer, Turku, Finland).

SRB IC₅₀ for B-RAF Inhibitors. WM266.4 melanoma cells were cultured in DMEM/10% fetal bovine serum, at 37 $^{\circ}\text{C}$, in 5% CO₂ water saturated atmosphere. Cell suspensions (10 000/mL) were prepared and 100 μL /well dispensed into 96-well plates giving 1000 cells/well. The plates were returned to the incubator for 24 h to allow the cells to reattach. The compounds were initially prepared

at 20 mM in DMSO. Aliquots (200 μ L) were diluted into 20 mL culture medium giving 200 μ M, and 10 serial dilutions of 3 \times prepared. Aliquots (100 μ L) of each dilution were added to the wells, giving doses ranging from 100 μ M to 0.005 μ M. After a further 6 days growth, the cells are fixed in trichloroacetic acid (10%) and stained with sulforhodamine-B (0.1%). After rinsing, the bound stain was taken into solution and the absorbance at 540 nm determined. The percent control growth was plotted against the logarithm of the drug concentration and analyzed by nonlinear regression to a four-parameter logistic equation (Graphpad Prism, Graphpad Software Inc., San Diego, CA). The IC₅₀ generated by this procedure is the concentration of the drug that produces a percentage control A₅₄₀ midway between the saturation and zero-effect plateaus.

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