

Synthesis of Novel Piperazine-linked Anthranilic Acids as Potential Small Molecule Kinase Inhibitors

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ABSTRACT

Substituted anthranilic acid and piperazines were used as building blocks to prepare two libraries of compounds, with the aim being that they would exhibit biochemical activity as small molecule kinase inhibitors. The synthesized anthranilamide-piperazine compounds were subsequently tested against a panel of kinases including EGFR, Abl, Akt and Aurora B.

KEYWORDS

Small molecule kinase inhibitors, anthranilic acid, piperazines, EGFR.

1. Introduction

Anthranilic acids and their derivatives represent a structural motif that has seen much application in medicinal chemistry. In the field of oncology, a wide variety of substituted anthranilic acids have been tested and found to have cytotoxic activity against cancerous cells.^{1–5} In particular, it has been noted that this scaffold is a privileged structure when dealing with kinase inhibition.^{6,7} Examples include PD184352 **1** (aka CI-1040) from Parke-Davis – a MEK1 and MEK2 inhibitor, tranilast **2**⁸ and two anthranilic acid amides, AAL993 **3**⁹ and **4**, prepared during a cooperation between Novartis Pharma and Schering AG (Fig. 1). The last three compounds were all found to inhibit vascular endothelial growth factor (VEGF) receptor tyrosine kinase, an important therapeutic target in the field of oncology.¹⁰

In a 2004 set of papers, anthranilic acid **5** was identified as a possible scaffold for the inhibition of the tyrosine kinases Src and epidermal growth factor receptor (EGFR), resulting in the development of a potent set of benzamides and benzamidines (see for example structures **6** in Fig. 2).^{11,12} EGFR is a transmembrane protein classified as a ‘receptor tyrosine kinase’. This protein is typically activated by the extracellular binding of a number of ligands, including epidermal growth factor, and has been implicated in a number of important cellular processes. Importantly, its abnormal functioning has also been implicated in numerous human malignancies.¹³ The same authors extended the study of this class of compounds to demonstrate that vascular endothelial growth factor receptors (VEGFRs) were also selectively inhibited by further inhibitors based on the same anthranilic acid scaffold.¹⁴ Importantly, the authors of this work proposed that molecules like **6a** and **6b** most likely possessed a ‘slightly different’ binding conformation when compared to erlotinib (TarcevaTM) **7**, although this was not confirmed by an X-ray study.¹² Structures **6a** and **6b** (Fig. 2) have been drawn to show how the presence of an intramolecular hydrogen bond preorganizes **6** to mimic the quinazoline-portion of known EGFR inhibitors such as PD153035 **8** and erlotinib (TarcevaTM) **7**,

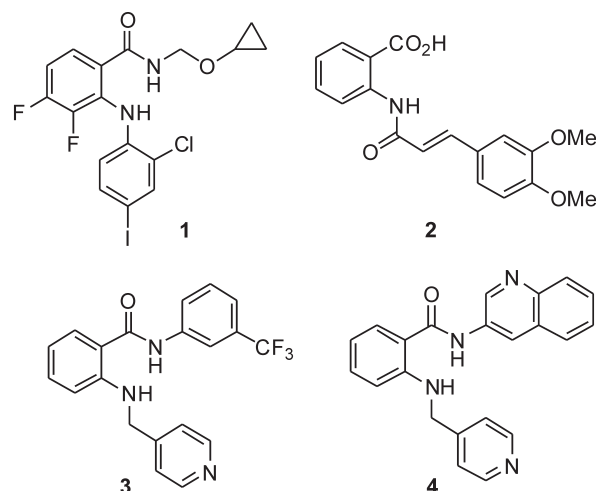


Figure 1 Examples of anthranilic acid-based kinase inhibitors.

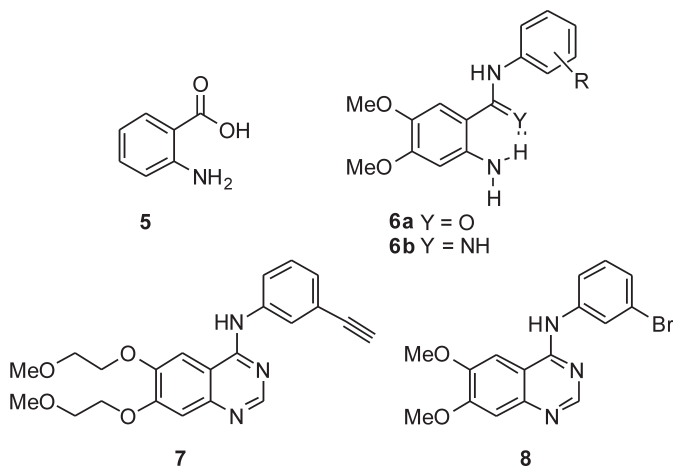


Figure 2 Design of anthranilic acid-based EGFR and VEGFR kinase inhibitors based on the ‘internal hydrogen bond’ concept.^{11,12,14}

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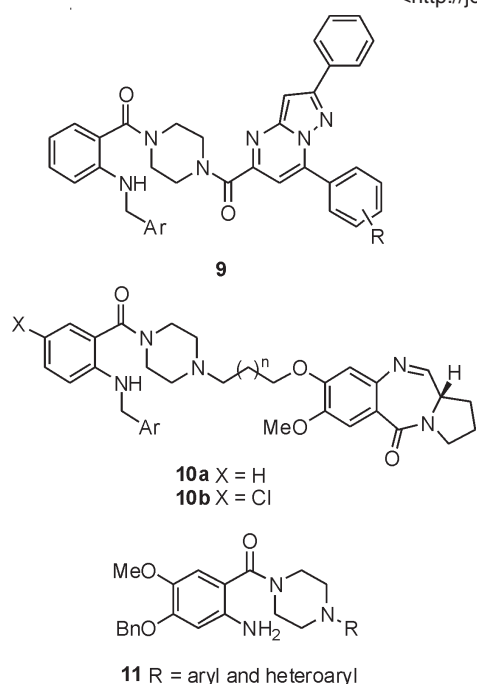


Figure 3 Example of generalized anthranilamide-piperazine conjugates with anti-cancer properties, as produced by Kamal *et al.*^{18–20}

although it should be realized that the aniline NH_2 is unlikely to act as an intermolecular hydrogen bond acceptor as is often proposed for the comparative quinazoline kinase inhibitors. It should be noted that other researchers have also designed potent kinase inhibitors on this particular basis,^{15,16} and that this topic has become an important concept in medicinal chemistry.¹⁷

In recent years, the piperazine moiety has seen increasing use as a structural component of compounds with relevance in oncology. As an example, and of particular relevance to this paper, Kamal, Pal-Bhadra and co-workers have recently found that the use of anthranilamide-piperazine conjugates, such as **9** and **10**, have demonstrated interesting activity in terms of

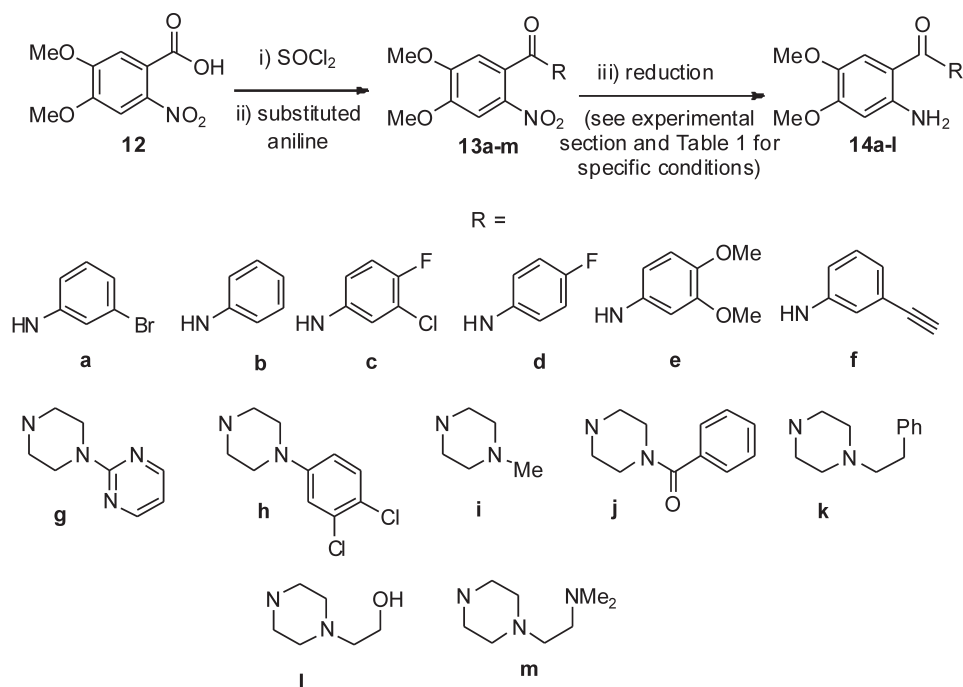
apoptosis induction in cancer cells (Fig. 3).^{18,19} These same researchers patented a series of simpler anthranilic acid derivatives with the generic structure **11**, as 'potential anticancer agents'.²⁰

Over the past years, our respective research groups have been interested in the design, synthesis and evaluation of small molecules for the selective inhibition of topical kinases,^{21,22} as well as the associated biochemical evaluations.^{23–25} The literature concerning the use of intramolecular hydrogen bonds, as well as the innovation of using the piperazine fragment as part of the scaffold,^{26,27} prompted us to combine these two structural features, resulting in the synthesis of two compound libraries. In the present work, twelve compounds based on the anthranilic acid skeleton and incorporating a piperazine within their structure were synthesized following a three-step protocol, to afford potential kinase-inhibiting anilines which were biochemically evaluated. Secondly, a focused library based on the piperazin-1-yl{2-[(pyridin-4-ylmethyl)amino]phenyl}methanone scaffold was also generated.

2. Synthesis

For the first set of potential inhibitors, 4,5-dimethoxy-2-nitrobenzoic acid **12** was converted into the corresponding acid chloride derivative, after which treatment with a number of substituted anilines and piperazines afforded the nitroamides **13a–l** in good to excellent yields over two steps (see Scheme 1 and Table 1 for details). Subsequent reduction of the nitro functional group led to the desired anthranilic acid derivatives **14a–l**. These compounds were all thoroughly characterized by spectroscopic techniques which included ^1H and ^{13}C NMR, as well as high resolution mass spectroscopy (HRMS).

At this point, it should also be stressed that compounds **14a** and **14c** were synthesized as potential reference compounds, as they were studied in the key Nakamura study mentioned earlier.¹² It should also be mentioned here that the palladium-mediated reduction of substrates **13a** and **13c** did not result in the expected anilines **14a** and **14c**, but gave compounds **14b** and **14d** instead, in which the bromine and chlorine atoms had been reductively



Scheme 1

Synthesis of 'Library 1'. For yields see Table 1.

Table 1 Yields for reactions in Scheme 1.

	Yield of reaction 12 → 13 /%, over two steps	Yield of reaction 13 → 14 /%
	13	14
a	73	23 ^{a,b}
b	–	100 ^c
c	56	35 ^b
d	–	86 ^d
e	58	43
f	68	43
g	94	40
h	100	69
i	96	100
j	76	93
k	68	75
l	71	96
m	55	– ^e

^a Based on recovered starting material; ^b reduction by Fe/HCl in EtOH; ^c Synthesized from **13a**; ^d synthesized from **13c**; ^e no reduced product obtained after work-up.

removed respectively. Application of an alternative reduction method making use of iron in an acidic media, did however afford the desired compounds **14a** and **14c**, albeit in less than satisfactory yields.

Furthermore, a second set of anthranilic acid analogues, this time containing pyridyl appendages, inspired by compounds **3** and **4**, was also synthesized. These compounds were produced by an initial reductive amination between the anthranilic ester **15** and 4-pyridine carboxaldehyde to afford the alkylated anilino scaffolds **16** and **17**, respectively (Scheme 2 and Table 2). Further-

Table 2 Yields for amidation reactions in Scheme 2.

Scaffold	Yields of benzamide formation/%					
	a	b	c	d	e	f
18	61	51	83	22	83	45
19	54	45	30	34	nd	nd

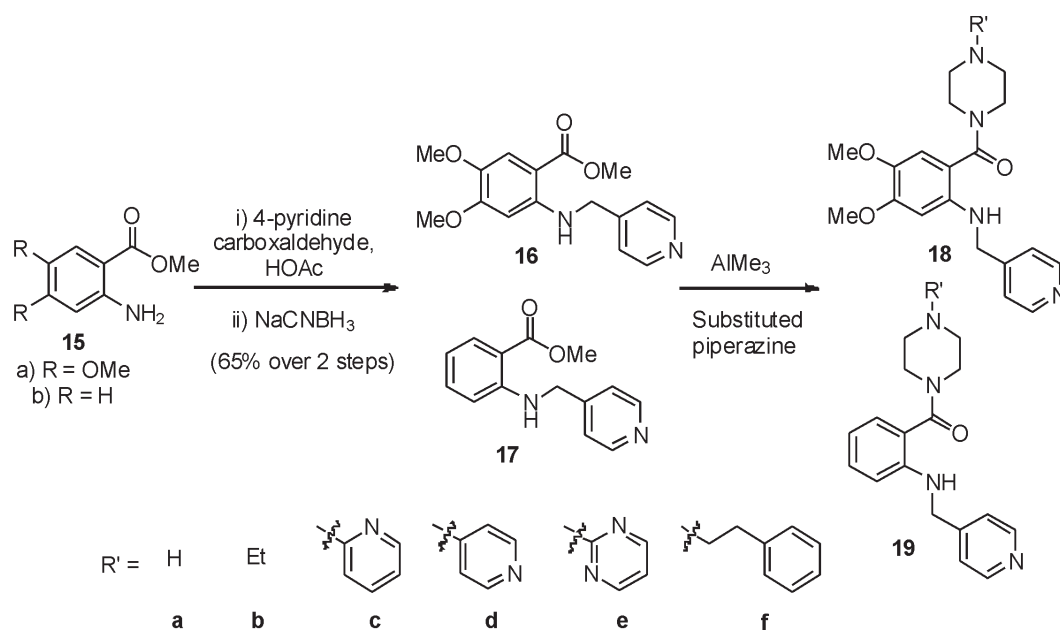
nd = not done.

more, conversion of the ester functional groups into the corresponding amides, facilitated by the Lewis acid trimethyl aluminium, then afforded the desired anthranilic acid derivatives **18** and **19**. These compounds thus featured a pyridine moiety and were decorated with a range of substituted piperazines, as shown in Scheme 2 and Table 2.

3. Biochemical Testing

As an initial screening, 12 compounds from 'Library 1' (**14a–l**) were evaluated for their ability to inhibit the kinase EGFR, which is commonly involved in carcinogenicity.²⁸ Mutations in EGFR account for 10–17 % of all non-small cell lung cancers (NSCLCs), with L858R and short in-frame deletions of exon 19 representing the most common activating mutations.²⁹ In contrast to other EGFR mutations involved in cancer, L858R does not cause resistance to TKI-based therapy, but instead improves the response to first line tyrosine kinase inhibitors such as gefitinib. Secondary mutations providing resistance to small molecule therapy often appear later, as in the case of T790M.³⁰ EGFR is a prime target in cancer therapy, which is emphasized by the large number of small molecules that have been developed to target this receptor tyrosine kinase.³¹ Furthermore, it is closely related to VEGFR on which the anthranilic scaffold originally was tested. For these reasons, EGFR wt as well as its L858R and T790M/L858R mutated species were used for testing in a biochemical assay setup.²² The results displayed in Table 3 were unfortunately disappointing, with only compounds **14a** (~10 μM) and **14c** (~30 μM), from the Nakamura study,¹² having any interesting activity on wt and L858R mutated EGFR. The synthesized novel compounds **14f** and **14h** also displayed slight inhibitory activity >200 μM in this assay. EGFR harboring both activating (L858R) and gatekeeper (T790M) mutation was not affected at all, up to compound concentrations of 200 μM.

In a further screen, it was decided to test the inhibition of the compounds from both libraries/sets against the Abl T315I kinase at a concentration of 10 μM. This particular kinase has been of interest due to the lack of inhibitors available to target this specific resistance mutation in chronic myelogenous leukemia (CML). Ponatinib (Iclusig), which was recently approved by the FDA, represents the only therapeutic to effectively target Abl

**Scheme 2**

Synthesis of 'Library 2'. For yields see Table 2.

Table 3 *In vitro* determined IC₅₀s on different EGFR species.

Compound	IC ₅₀ /μM		
	EGFR wt	EGFR L858R	EGFR T790M/L858R
14a	11.4 ± 2.9	6.1 ± 1.1	ni
14b	ni	ni	ni
14c	31.4 ± 8.1	29.9 ± 13.7	ni
14d	ni	ni	ni
14e	ni	ni	ni
14f	>200	>200	ni
14g	ni	ni	ni
14h	≥200	>200	ni
14i	ni	ni	ni
14j	ni	ni	ni
14k	ni	ni	ni
14l	ni	ni	ni

ni: no inhibitory effect at 200 μM compound concentration.

T315I, but comes along with severe side effects, indicating a rather complex pharmacology.³² The results from this assay were not encouraging, with none of the compounds demonstrating inhibition at this concentration, although the reference compound, staurosporine, displayed the expected inhibitory effect (See supplementary information, Fig. S1).

It was then decided to send two representative compounds from the first set, namely compounds **14a** and **14h**, which had shown some activity in the EGFR screen, for testing against the Dundee kinase library, which comprises 95 different kinases (see supplementary information, Figs S2 and S3).³³ Of interest was that **14a** showed good inhibition of AKT2 (aka PKBβ) (17 % remaining activity at 10 μM concentration), as well as moderate inhibition of HER4 and Aurora B (43% resp. 51% remaining activity at 10 μM). Compound **14h** also inhibited AKT2 (PKBβ) with moderate effect (64 %), as well as Aurora B (61 %) and CAMK1 (60 %). The two libraries, comprising compounds **14a–l**, **18a–f** and **19a–d**, were then evaluated for their ability to inhibit Akt2 (PKBβ) and its close isoform Akt1 (PKBα), with the enzymes at full length (Akt1 and Akt2) or kinase domain only (Akt1). Unfortunately, the results showed that the compounds did not effectively inhibit the kinases either (see supplementary information, S4).³⁴ Finally, as the Dundee screen had also shown **14a** and **14h** to inhibit Aurora B, both compounds were sent for testing against this particular kinase to Reaction Biology Corp.³⁵ Compounds **14a** and **14h** were subsequently tested in 10-dose IC₅₀ mode with threefold serial dilution starting at 300 μM and in the presence of 20 μM ATP. Unfortunately, both compounds showed little activity (IC₅₀ >300 μM)³⁶ in comparison to the positive control staurosporine, which had an IC₅₀ of 5 nM.

4. Conclusion

The present paper presents the synthesis of anthranilic acid-derived molecules inspired by known kinase inhibitors such as Tranilast and AAL993. A particularity of our set of molecules is the piperazine fragment included in their core structure. The synthesized anthranilamide-piperazine conjugates were then tested against the following kinases: EGFR (wt, L858R and T790M/L858R), Abl_T315I, Akt1 (PKBα), Akt2 (PKBβ) and Aurora B. These tests indicated that unfortunately none of the compounds inhibited the kinases enough to warrant further extension of these libraries.

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Experimental

General Experimental Information

¹H NMR and ¹³C NMR spectra were recorded on Bruker 300, Bruker DRX 400, Varian Inova 400 or Varian Inova 300 spectrometers at the frequency indicated. Infra-red spectra were recorded on Bruker IFS 25, Bruker Vector 22 or Thermo Nicolet Nexus 470 fourier transform spectrometers. Mass spectra were recorded on a Kratos MS 9/50, VG 70E MS or VG 70 SEQ mass spectrometer or alternatively a Waters API Q-TOF Ultima, GCT Premier or SYNAPT G2 mass spectrometer. Prior to being evaluated for HRMS all compounds were checked by LCMS for a purity of >80 %. Macherey-Nagel kieselgel 60 (particle size 0.063–0.200 mm) was used for conventional silica gel chromatography. All solvents used for reactions and chromatography were distilled prior to use. Reactions were performed under a blanket of inert gas (Ar or N₂) unless specified. Melting points are uncorrected.

General Experimental Procedure for the Synthesis of N-phenylbenzamides **13a–m** from Benzoic Acid **12**

4,5-Dimethoxy-2-nitrobenzoic acid **12** (0.23 g, 1.0 mmol) was dissolved in SOCl₂ (1 mL) and the reaction was stirred at 75 °C for 3 h under an Ar atmosphere. The excess SOCl₂ was then removed under reduced pressure using a liquid nitrogen trap. CH₂Cl₂ (5 mL) was then added to the remaining residue, followed by the addition of the substituted aniline (1.1 mmol, 1.1 mol equiv.) and NEt₃ (0.41 mL, 3.0 mmol) and the reaction was stirred at RT, under Ar for 18 h. The organic solvent was removed under reduced pressure and EtOAc (20 mL) was added. The organic fraction was washed sequentially with HCl (10 mL, 1 M), H₂O (20 mL), aq. NaOH (10 mL, 2 M) and H₂O (20 mL). The organic fraction was then dried (MgSO₄) and removed *in vacuo*. The desired amide was then obtained after column chromatography on flash silica (eluent: 40–50 % EtOAc/cyclohexane or as indicated). If required, recrystallization was also performed to provide purified compounds.

N-(3-Bromophenyl)-4,5-dimethoxy-2-nitrobenzamide **13a**

Obtained as a yellow-coloured semi-solid after purification by way of column chromatography (Eluent: 50 % EtOAc/cyclohexane); yield 73 %; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.69 (s, 1H), 8.03 (d, *J* = 1.7 Hz, 1H), 7.73 (s, 1H), 7.62–7.56 (m, 1H), 7.35–7.31 (m, 3H), 3.94 (s, 3H), 3.93 (s, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 164.4, 153.1, 149.1, 140.6, 138.6, 130.8, 126.9, 126.3, 121.8, 121.5, 118.3, 111.1, 107.3, 56.7, 56.4; HRMS Calculated 381.0081 (M⁺ + H) for C₁₅H₁₄BrN₂O₅, found 381.0085.

N-(3-Chloro-4-fluorophenyl)-4,5-dimethoxy-2-nitrobenzamide 13c

Obtained as a yellow-coloured solid after purification by way of column chromatography (Eluent: 40 % EtOAc/cyclohexane), followed by recrystallization from EtOAc; yield 56 %; mp 218–220 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 10.72 (s, 1H), 7.97 (dd, J = 6.8, 2.6 Hz, 1H), 7.72 (s, 1H), 7.56–7.53 (m, 1H), 7.44–7.40 (m, 1H), 7.31 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 164.3, 153.4 (d, J = 243.6 Hz), 153.1, 149.1, 138.6 (d, J = 3.0 Hz), 136.2, 126.8, 120.9, 119.9 (d, J = 6.8 Hz), 119.2 (d, J = 18.1 Hz), 117.2 (d, J = 21.5 Hz), 111.1, 107.3, 56.7, 56.4; HRMS Calculated 357.0462 (M^+ + H) for $\text{C}_{15}\text{H}_{13}^{37}\text{ClFN}_2\text{O}_5$, found 357.0463.

N-(3,4-Dimethoxyphenyl)-4,5-dimethoxy-2-nitrobenzamide 13e

Obtained as a yellow-coloured solid after purification by way of column chromatography (Eluent: 70 % EtOAc/cyclohexane), followed by recrystallization from EtOAc; yield 58 %; mp 185–188 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.92 (s, 1H), 7.48 (s, 1H), 7.23 (d, J = 2.4 Hz, 1H), 6.97–6.90 (m, 2H), 6.76 (d, J = 8.7 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 166.2, 153.9, 149.2, 148.9, 146.4, 138.9, 132.1, 127.8, 113.2, 111.8, 110.6, 107.4, 105.7, 56.8, 56.6, 56.2, 55.9; HRMS Calculated 363.1187 (M^+ + H) for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_7$, found 363.1188.

N-(3-Ethynylphenyl)-4,5-dimethoxy-2-nitrobenzamide 13f

Obtained as a yellow-coloured solid after purification by way of column chromatography (Eluent: 10 % MeOH/EtOAc); yield 68 %; mp 179–181 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 10.60 (s, 1H), 7.85 (s, 1H), 7.71 (s, 1H), 7.63 (d, J = 9.4 Hz, 1H), 7.39–7.35 (m, 1H), 7.30 (s, 1H), 7.22 (d, J = 7.6 Hz, 1H), 4.20 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 164.3, 153.1, 149.0, 139.2, 138.6, 129.3, 127.1, 126.9, 122.4, 122.1, 120.1, 111.1, 107.3, 83.3, 80.7, 56.7, 56.4; HRMS Calculated 327.0981 (M^+ + H) for $\text{C}_{17}\text{H}_{15}\text{N}_2\text{O}_5$, found 327.0980.

2-[4-(4,5-Dimethoxy-2-nitrobenzoyl)-1-piperazinyl]pyrimidine 13 g

Obtained as white crystals (recrystallized from EtOAc); yield 94 %; mp 232–234 °C; ^1H NMR (400 MHz, CDCl_3) δ 8.30 (d, J = 4.8 Hz, 2H), 7.72 (s, 1H), 6.77 (s, 1H), 6.55–6.51 (m, 1H), 3.98 (s, 3H), 3.97 (s, 3H), 3.99–3.95 (m, 6H), 3.25 (t, J = 5.3, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 166.8, 161.5, 157.7, 154.3, 149.2, 137.9, 126.9, 110.5, 109.07, 107.3, 56.7, 56.5, 46.5, 43.5, 43.1, 41.8; HRMS Calculated 374.1460 (M^+ + H) for $\text{C}_{17}\text{H}_{20}\text{N}_5\text{O}_5$, found 374.1459.

1-(3,4-Dichlorophenyl)-4-(4,5-dimethoxy-2-nitrobenzoyl)piperazine 13h

Obtained as a pale yellow foam (deemed pure enough after work-up); yield quantitative; ^1H NMR (400 MHz, CDCl_3) δ 7.69 (s, 1H), 7.52 (d, J = 8.9 Hz, 1H), 6.91 (d, J = 2.9 Hz, 1H), 6.74 (s, 1H), 6.70 (dd, J = 2.9, 8.9 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.30–3.01 (m, 8H); ^{13}C NMR (101 MHz, CDCl_3) δ 166.5, 154.3, 150.2, 149.2, 137.8, 132.9, 130.5, 126.5, 123.2, 118.0, 116.1, 109.0, 107.2, 60.4, 56.5, 48.8, 48.6, 46.2, 41.5; HRMS Calculated 440.0773 (M^+ + H) for $\text{C}_{19}\text{H}_{20}\text{Cl}_2\text{N}_3\text{O}_5$, found 440.0775.

1-(4,5-Dimethoxy-2-nitrobenzoyl)-4-methylpiperazine 13i

Obtained as a pale yellow foam (deemed pure enough after work-up); yield 96 %; ^1H NMR (400 MHz, CDCl_3) δ 7.69 (s, 1H), 6.73 (s, 1H), 3.95 (br s, 6H), 3.19 (t, J = 5.1 Hz, 2H), 2.56–2.48 (m, 6H), 2.30 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 166.3, 154.2, 149.1, 137.8, 127.0, 109.1, 107.2, 56.7, 56.5, 54.6, 54.1, 46.6, 46.0, 41.7; HRMS Calculated 310.1398 (M^+ + H) for $\text{C}_{14}\text{H}_{20}\text{N}_3\text{O}_5$, found 310.1398.

1-Benzoyl-4-(4,5-dimethoxy-2-nitrobenzoyl)piperazine 13j

Obtained as pale yellow crystals (recrystallized from EtOAc);

yield 76 %; mp 185–187 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.69 (br s, 1H), 7.40 (br s, 5H), 6.74 (s, 1H), 4.10–4.00 (br m, 1H), 4.00–3.95 (br m, 1H), 3.96 (br s, 6H), 3.75–3.50 (br m, 4H), 3.30–3.15 (br m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.6, 166.7, 154.4, 149.3, 137.8, 135.0, 130.1, 128.6, 127.0, 126.4, 108.9, 107.3, 56.7, 56.5, 46.7 (br), 42.0 (br); HRMS Calculated 400.1496 (M^+ + H) for $\text{C}_{20}\text{H}_{22}\text{N}_3\text{O}_6$, found 400.1503.

1-(4,5-Dimethoxy-2-nitrobenzoyl)-4-(2-phenylethyl)piperazine 13k

Obtained as a pale yellow semi-solid (purified by silica gel column chromatography: 10 % MeOH-EtOAc); yield 68 %; ^1H NMR (400 MHz, CDCl_3) δ 7.69 (s, 1H), 7.33–7.09 (m, 5H), 6.74 (s, 1H), 3.95 (br s, 6H), 3.95–3.90 (br m, 1H), 3.79–3.71 (br m, 1H), 3.21 (t, J = 5.1 Hz, 2H), 2.81–2.74 (br m, 2H), 2.71–2.54 (br m, 4H), 2.45–2.33 (br m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 166.3, 154.2, 149.0, 139.9, 137.8, 128.6, 128.3, 127.0, 126.1, 109.1, 107.2, 60.0, 56.6, 56.5, 52.7, 52.1, 46.6, 41.8, 33.5; HRMS Calculated 400.1859 (M^+ + H) for $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_5$, found 400.1867.

2-[4-(4,5-Dimethoxy-2-nitrobenzoyl)-1-piperazinyl]ethanol 13l

Obtained as a pale yellow oil; yield 71 %, (deemed pure enough after work-up); ^1H NMR (400 MHz, CD_3OD) δ 7.78 (s, 1H), 6.97 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.91–3.84 (br m, 1H), 3.78–3.72 (br m, 1H), 3.69 (t, J = 5.1 Hz, 2H), 3.32–3.27 (m, 2H), 2.67 (t, J = 5.1 Hz, 2H), 2.58 (t, J = 5.8 Hz, 2H), 2.52–2.45 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.0, 156.1, 151.0, 139.3, 127.5, 110.6, 108.5, 61.2, 59.8, 57.4, 57.0, 54.1, 53.6, 47.9, 42.8; HRMS Calculated 340.1504 (M^+ + H) for $\text{C}_{15}\text{H}_{22}\text{N}_3\text{O}_6$, found 340.1503.

N-{2-[4-(4,5-Dimethoxy-2-nitrobenzoyl)-1-piperazinyl]ethyl}-N,N-dimethylamine 13m

Obtained as a yellow oil; yield 55 %, (deemed pure enough after work-up); ^1H NMR (400 MHz, CDCl_3) δ 7.69 (s, 1H), 6.73 (s, 1H), 3.77 (s, 6H), 3.93–3.87 (br m, 1H), 3.76–3.70 (br m, 1H), 3.19 (t, J = 5.1 Hz, 2H), 2.67–2.60 (br m, 1H), 2.56–2.52 (br m, 1H), 2.51–2.47 (br m, 2H), 2.43–2.36 (br m, 4H), 2.22 (s, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 166.3, 154.2, 149.0, 137.8, 127.0, 109.1, 107.2, 56.8, 56.7, 56.5, 56.4, 53.2, 52.6, 46.6, 45.8, 41.8; HRMS Calculated 367.1978 (M^+ + H) for $\text{C}_{17}\text{H}_{27}\text{N}_4\text{O}_5$, found 367.1976.

General Procedure 1: Synthesis of 2-Amino-N-phenylbenzamides 14 by way of the Pd/C Reduction of 2-Nitro-N-phenylbenzamides 13

The 2-nitro-N-phenylbenzamides 13 (~0.5 mmol) were dissolved in EtOH (5 mL), after which 5 % Pd/C (10 % by mass) was added carefully. This was followed by the addition of ammonium formate (5. Mol equiv.), after which the reaction mixture was heated at reflux, with stirring, for 90 min. After cooling to RT, the mixture was filtered through a Celite plug and the filter cake washed with EtOH (3 $\times$ 5 mL). The solvent was removed to afford the anilines 14, which in general were judged by ^1H NMR spectroscopy to be pure enough for biochemical evaluation; if not, flash silica gel column chromatography was employed. See individual descriptions for specific experimental details below.

2-Amino-N-(3-bromophenyl)-4,5-dimethoxybenzamide 14a

For this particular compound, the general reduction described above resulted in the formation of 14b in which the aromatic bromide had also been removed. An alternative reduction method, involved the treatment of 13a (0.060 g, 0.16 mmol) in EtOH (2 mL) with elemental iron (0.044 g, 0.80 mmol) with HCl (12 M, 0.7 mL) for 5.5 h at reflux. H_2O (10 mL) was then added, after which the mixture was basified by the addition of NaOH solution (2 M). Extraction with EtOAc (3 $\times$ 5 mL), wash-

ing with brine (10 mL), drying with Na_2SO_4 and removal of solvent under reduced pressure, afforded a mixture of the desired product and starting material. Silica gel column chromatography (50 % EtOAc/hexanes) then resulted in **14a** as a yellow semi-solid (0.010 g, 17 %), in addition to starting material **13a** (20 %). The NMR spectra of **14a** compared well with that published in the literature.¹² ^1H NMR (400 MHz, CD_3OD) δ 7.98–7.94 (m, 1H), 7.63–7.57 (m, 1H), 7.28 (d, J = 1.2 Hz, 1H), 7.27–7.26 (m, 2H), 6.46 (s, 1H), 3.87 (s, 3H), 3.85 (s, 3H).

2-Amino-4,5-dimethoxy-N-phenylbenzamide **14b**

For this particular compound, use of the general procedure described above resulted in removal of the bromine atom and **14b** was thus obtained as a beige solid (quantitative yield). The NMR spectra of **14b** compared well with that published in the literature.³⁷ ^1H NMR (400 MHz, CD_3OD) δ 7.66 (dd, J = 7.7, 0.9 Hz, 2H), 7.44–7.41 (m, 2H), 7.33 (br s, 1H), 7.21–7.19 (m, 1H), 6.50 (br s, 1H), 3.94 (s, 3H), 3.93 (s, 3H).

2-Amino-N-(3-chloro-4-fluorophenyl)-4,5-dimethoxybenzamide **14c**

For this particular compound, the general reduction described above resulted in the formation of **14c** in which the aromatic chloride had also been removed (see spectroscopic description below). An alternative reduction method, involved the treatment of **13c** (0.070 g, 0.20 mmol) in EtOH (2 mL) with elemental iron (0.10 g, 2.0 mmol added in two portions at start of reaction and after 5.5 h) with HCl (12 M, 0.8 mL) for 28 h at reflux. H_2O (10 mL) was then added, after which the mixture was made basic by the addition of NaOH solution (2 M). Extraction with EtOAc (3 $\times$ 5 mL), washing with brine (10 mL), drying with Na_2SO_4 and removal of solvent under reduced pressure, afforded a mixture of the desired product and starting material. Silica gel column chromatography (50 % EtOAc/hexanes) then resulted in **14c** as a light yellow-coloured semi-solid (0.022 g, 35 %). The NMR spectra of **14c** compared well with that published in the literature.¹² ^1H NMR (400 MHz, CD_3OD) δ 7.93–7.89 (m, 1H), 7.60–7.57 (m, 1H), 7.30 (s, 1H), 7.27–7.21 (m, 1H), 6.49 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H).

2-Amino-N-(4-fluorophenyl)-4,5-dimethoxybenzamide **14d**

Obtained as pale yellow oil (purified by silica gel column chromatography: 50 % EtOAc-hexane), yield 86 %; ^1H NMR (400 MHz, CD_3OD) δ 7.60–7.53 (m, 2H), 7.22 (s, 1H), 7.09–7.01 (m, 2H), 6.40 (s, 1H), 4.82 (s, 3H), 3.82 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 168.8, 160.4 (d), 154.0, 145.9, 141.1, 134.8 (d), 123.8 (d), 115.1 (d), 112.6 (d), 107.9, 101.3, 57.3, 55.6; HRMS Calculated 291.1140 (M^+ + H) for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_5\text{F}$, found 291.1140.

2-Amino-N-(3,4-dimethoxyphenyl)-4,5-dimethoxybenzamide **14e**

Obtained as an off-white foam (purified by silica gel column chromatography: EtOAc), yield 43 %; ^1H NMR (400 MHz, CD_3OD) δ 7.28 (d, J = 2.4 Hz, 1H), 7.22 (s, 1H), 7.09 (dd, J = 8.6, 2.4 Hz, 1H), 6.89 (d, J = 8.7 Hz, 1H), 6.40 (s, 1H), 4.82 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.9, 155.1, 150.3, 147.4, 147.3, 141.8, 133.6, 115.3, 114.0, 113.2, 108.5, 108.2, 101.8, 57.7, 56.7, 56.4, 56.1; HRMS Calculated 333.1445 (M^+ + H) for $\text{C}_{17}\text{H}_{21}\text{N}_2\text{O}_5$, found 333.1446.

2-Amino-N-(3-ethynylphenyl)-4,5-dimethoxybenzamide **14f**

Obtained as an off-white semi-solid (purified by silica gel column chromatography: 50 % EtOAc-hexane), yield 43 %, ^1H NMR (400 MHz, CD_3OD , alkyne proton not observed in spectrum) δ 7.90–7.89 (m, 1H), 7.74–7.73 (m, 1H), 7.46–7.39 (m, 1H), 7.37–7.32 (m, 2H), 6.53 (s, 1H), 3.98 (s, 3H), 3.97 (s, 3H); ^{13}C NMR

(101 MHz, CD_3OD) δ 169.5, 154.7, 146.9, 141.4, 139.7, 129.4, 128.3, 125.6, 123.6, 122.7, 113.3, 108.0, 101.6, 84.0, 78.1, 57.5, 56.1; HRMS Calculated 297.1234 (M^+ + H) for $\text{C}_{17}\text{H}_{17}\text{N}_2\text{O}_3$, found 297.1235.

4,5-Dimethoxy-2-([4-(2-pyrimidinyl)-1-piperazinyl]carbonyl)phenylamine **14g**

Obtained as a pale yellow semi-solid, (purified by silica gel column chromatography: 5 % MeOH/EtOAc); yield 40 %; ^1H NMR (400 MHz, CDCl_3) δ 8.31 (d, J = 4.7 Hz, 2H), 6.66 (s, 1H), 6.52 (t, J = 4.7 Hz, 1H), 6.26 (s, 1H), 4.27 (br s, 2H), 3.88–3.84 (m, 4H), 3.84 (s, 3H), 3.76 (s, 3H), 3.71–3.62 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.4, 161.5, 157.7, 151.7, 141.3, 141.1, 112.2, 110.4, 110.0, 101.0, 56.7, 55.8, 45.1 (br), 43.9; HRMS Calculated 344.1717 (M^+ + H) for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_3$, found 344.1718.

2-([4-(3,4-Dichlorophenyl)-1-piperazinyl]carbonyl)-4,5-dimethoxyphenylamine **14h**

Obtained as a pale beige-coloured oil, (purified by silica gel column chromatography: EtOAc), yield 69 %; ^1H NMR (400 MHz, CD_3OD) δ 7.29 (d, J = 8.9 Hz, 1H), 7.06 (d, J = 2.9 Hz, 1H), 6.86 (dd, J = 9.0, 2.9 Hz, 1H), 6.72 (s, 1H), 6.45 (s, 1H), 3.79 (s, 3H), 3.84 (s, 3H), 3.84–3.80 (m, 4H), 3.24–3.14 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD , one aliphatic carbon not observed in spectrum) δ 172.0, 153.5, 152.1, 142.7, 142.4, 133.6, 131.6, 123.2, 118.6, 117.1, 114.3, 111.5, 102.2, 57.6, 56.2, 49.4 (br); HRMS Calculated 410.1033 (M^+ + H) for $\text{C}_{19}\text{H}_{22}\text{N}_3\text{O}_3\text{Cl}_2$, found 410.1031.

4,5-Dimethoxy-2-[(4-methyl-1-piperazinyl)carbonyl]phenylamine **14i**

Obtained as a pale yellow oil which slowly solidified to a semi-solid; yield quantitative; ^1H NMR (400 MHz, CD_3OD) δ 6.69 (s, 1H), 6.45 (s, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.64–3.59 (m, 4H), 2.53–2.40 (m, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD , one aliphatic carbon not observed in spectrum) δ 180.8, 162.3, 151.4, 151.2, 122.9, 120.4, 110.9, 66.4, 65.0, 64.7, 54.8; HRMS Calculated 280.1656 (M^+ + H) for $\text{C}_{14}\text{H}_{22}\text{N}_3\text{O}_3$, found 280.1656.

2-([4-(Benzoyl-1-piperazinyl)carbonyl]-4,5-dimethoxyphenylamine **14j**

Obtained as a light yellow oil, (purified by silica gel column chromatography: 5 % MeOH/EtOAc); yield 93 %; ^1H NMR (400 MHz, CDCl_3) δ 7.38 (s, 5H), 6.60 (s, 1H), 6.23 (s, 1H), 4.48 (s, 2H), 3.79 (s, 3H), 3.74 (s, 3H), 3.77–3.44 (m, 8H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.6, 151.9, 141.3, 141.1, 135.1, 130.0, 128.5, 127.0, 112.1, 109.4, 101.0, 56.7, 55.7, 47.4 (br), 45.2 (br); HRMS Calculated 370.1761 (M^+ + H) for $\text{C}_{20}\text{H}_{24}\text{N}_3\text{O}_4$, found 370.1762.

4,5-Dimethoxy-2-([4-(2-phenylethyl)-1-piperazinyl]carbonyl)phenylamine **14k**

Obtained as a yellow oil (purified by silica gel column chromatography: 10 % MeOH/EtOAc); yield 75 %; ^1H NMR (400 MHz, CD_3OD) δ 7.34–7.05 (m, 5H), 6.69 (s, 1H), 6.45 (s, 1H), 3.78 (s, 3H), 3.72 (s, 3H), 3.65–3.61 (m, 4H), 2.84–2.77 (m, 2H), 2.68–2.61 (m, 2H), 2.60–2.58 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD , one aliphatic carbon not observed in spectrum) δ 171.8, 153.5, 142.6, 142.4, 141.0, 129.7, 129.5, 127.2, 114.2, 111.6, 102.2, 61.1, 57.6, 56.2, 54.1, 45.7 (br), 33.9; HRMS Calculated 370.2125 (M^+ + H) for $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_3$, found 370.2127.

2-[4-(2-Amino-4,5-dimethoxybenzoyl)-1-piperazinyl]ethanol **14l**

Pale yellow oil; yield 96 %; ^1H NMR (400 MHz, CD_3OD) δ 6.69 (s, 1H), 6.44 (s, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.76–3.63 (m, 6H), 2.74–2.68 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD , one aliphatic carbon not observed) δ 171.8, 153.5, 142.7, 142.3, 114.2, 111.3, 111.1, 102.1, 60.8, 59.1, 57.6, 56.2, 54.2, 45.2 (br), 45.0 (br); HRMS Calculated 310.1761 (M^+ + H) for $\text{C}_{15}\text{H}_{24}\text{N}_3\text{O}_4$, found 310.1763.

General Synthesis of [(Pyridinylmethyl)amino]benzoates **16** and **17**

The methyl 2-aminobenzoates **15a** or **15b** (5–7 mmol) were dissolved in MeOH (96 %, ~50 mL), to which 4-pyridine carboxaldehyde (1.3 mol equiv.) was added. After the addition of AcOH (0.6 mL) the reaction mixture was stirred at RT for 3–5 days under a N₂ atmosphere. NaCNBH₃ (1.3 mol equiv.) was subsequently added in small increments over a period of 30 min and the reaction mixture left to stir for a further 3–4 h. The solvent was then removed under reduced pressure to afford a gummy residue. EtOAc (8 mL) was used to dissolve the residue after which the organic phase was washed sequentially with aqueous NaHCO₃ (sat., 100 mL) and brine (100 mL). The organic layer was then dried (Na₂SO₄), filtered and reduced under vacuum. The resulting residue was purified with silica gel column chromatography (70 % EtOAc-hexane) to afford semi-solids from which the pure products **16** or **17** were obtained by recrystallization (1:1 EtOAc/hexane) – see below for individual descriptions of products obtained.

Methyl 4,5-dimethoxy-2-[(4-pyridinylmethyl)amino]benzoate **16**

Obtained as a white solid; yield 65 %; mp 142–144 °C; ¹H NMR (300 MHz, CDCl₃): δ 8.54 (d, *J* = 5.4 Hz, 2H), 8.24 (br t, *J* = 4.9 Hz, 1H), 7.40 (s, 1H), 7.28 (d, *J* = 5.4 Hz, 2H), 5.94 (s, 1H), 4.47 (d, *J* = 5.7 Hz, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 3.70 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 168.6, 155.2, 150.1, 148.5, 147.6, 139.7, 121.9, 113.8, 101.6, 94.9, 56.5, 55.6, 51.4, 46.3; HRMS Calculated 303.1339 (M⁺ + H) for C₁₆H₁₉N₂O₄, found 303.1333.

Methyl 2-[(pyridin-4-ylmethyl)amino]benzoate **17**

For this particular compound, use of the general procedure described above on substrate **15b**, resulted in the product **17** being obtained as a white solid (65 %). The NMR spectra of **17** compared well with that published in the literature.⁹ ¹H NMR (300 MHz, CDCl₃): δ 8.52 (d, *J* = 6.0 Hz, 2H), 8.30–8.26 (m, 1H), 7.92 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H), 7.24–7.39 (m, 3H), 6.61–6.57 (m, 1H), 6.44 (d, *J* = 8.5 Hz, 1H), 4.46–4.42 (m, 2H), 3.87 (s, 3H).

General Description Involving the Synthesis of Piperazin-1-yl{2-[(pyridin-4-ylmethyl)amino]phenyl}methanones **18** and **19**, from Methyl [(pyridinylmethyl)amino]benzoates **16** and **17**:³⁸

A solution of AlMe₃ in toluene (1.5 mol equiv., 2 M) was added to the methyl benzoate **16** or **17** (1.0 mmol) dissolved in toluene (3.5 mL) in a round-bottomed flask. The appropriate piperazine (1.4 mol equiv.) was then added, together with an additional volume of toluene (3.5 mL). The reaction mixture was then stirred at RT for 1 h, before being stirred under heating at 110 °C for an additional 1 h. The solvent was then removed under reduced pressure. EtOAc (10 mL) was then used to dissolve the residue, after which the organic phase was washed sequentially with aqueous NaHCO₃ (sat., 50 mL) and brine (50 mL). The organic layer was dried (Na₂SO₄), filtered and removed under reduced pressure, resulting in a dark yellow oil. This residue was purified by silica gel column chromatography (90 % EtOAc/MeOH) to obtain the desired products **18** or **19**, for which the details are given below.

4,5-Dimethoxy-2-(1-piperazinylcarbonyl)-N-(4-pyridinylmethyl)aniline **18a**

Obtained as a pale yellow solid; yield 61 %; mp 109–111 °C; ¹H NMR (300 MHz, CDCl₃, one proton not observed in spectrum) δ 8.54 (d, *J* = 6.0 Hz, 2H), 7.30 (d, *J* = 6.0 Hz, 2H), 6.71 (s, 1H), 6.06 (s, 1H), 5.85 (br t, *J* = 6.0 Hz, 1H), 4.37 (d, *J* = 6.0 Hz, 2H), 3.78 (s, 3H), 3.69 (s, 3H), 3.63 (br t, *J* = 5.9 Hz, 4H), 2.90 (br t,

J = 5.9 Hz, 4H); ¹³C NMR (75 MHz, CDCl₃) δ 170.4, 151.8, 149.9, 148.9, 142.6, 140.1, 122.0, 113.3, 109.8, 97.1, 57.0, 55.6, 47.0, 46.4 (2C); HRMS Calculated 357.1921 (M⁺ + H) for C₁₉H₂₅N₄O₃, found 357.1920.

2-[(4-Ethyl-1-piperazinyl)carbonyl]-4,5-dimethoxy-N-(4-pyridinylmethyl)aniline **18b**

Obtained as a pale yellow solid; yield 51 %; mp 55–56 °C, ¹H NMR (300 MHz, CDCl₃) δ 8.55 (d, *J* = 6.0 Hz, 2H), 7.28 (d, *J* = 6.0 Hz, 2H), 6.71 (s, 1H), 6.05 (s, 1H), 5.87 (br t, *J* = 6.1 Hz, 1H), 4.37 (d, *J* = 6.1 Hz, 2H), 3.78 (s, 3H), 3.72–3.68 (m, 7H), 2.50–2.43 (m, 6H), 1.09 (t, *J* = 6.0 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 170.4, 152.0, 150.1, 148.9, 142.9, 140.1, 122.0, 113.5, 109.7, 97.2, 57.1, 55.7, 53.1, 52.3, 47.1, 45.5 (br), 12.0; HRMS Calculated 385.2234 (M⁺ + H) for C₂₁H₂₉N₄O₃, found 385.2235.

4,5-Dimethoxy-N-(4-pyridinylmethyl)-2-[(4-(2-pyridinyl)-1-piperazinyl)carbonyl]aniline **18c**

Obtained as an orange oil; yield 83 %; ¹H NMR (300 MHz, CDCl₃): δ 8.68 (d, *J* = 4.5, 2H), 8.22 (br d, *J* = 5.2 Hz, 1H), 7.55–7.50 (m, 1H), 7.30–7.28 (d, *J* = 6.0 Hz, 2H), 6.78–6.76 (m, 1H), 6.71–6.67 (m, 2H), 6.07 (s, 1H), 6.03–5.99 (m, 1H), 4.44 (d, *J* = 7.4 Hz, 2H), 3.79–3.77 (m, 7H), 3.70 (s, 3H), 3.64–3.60 (m, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 170.8, 159.1, 152.2, 150.1, 148.7, 148.0, 143.2, 140.1, 137.7, 122.0, 114.0, 113.5, 109.3, 107.3, 97.3, 57.0, 55.7, 47.1, 45.6, 45.2; HRMS Calculated 434.2187 (M⁺ + H) for C₂₄H₂₈N₅O₃, found 434.2184.

4,5-Dimethoxy-N-(4-pyridinylmethyl)-2-[(4-(4-pyridinyl)-1-piperazinyl)carbonyl]aniline **18d**

Obtained as a dark yellow oil; yield 22 %; ¹H NMR (300 MHz, CDCl₃): δ 8.54 (d, *J* = 4.8 Hz, 2H), 8.31 (d, *J* = 5.3 Hz, 2H), 7.31 (d, *J* = 5.2 Hz, 2H), 6.74 (s, 1H), 6.69 (br s, 2H), 6.08 (br s, 2H), 4.39 (br s, 2H), 3.80–3.79 (br M, 7H), 3.71 (s, 3H), 3.42 (br s, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 170.9, 154.7, 152.4, 150.1, 150.0, 148.7, 143.4, 140.1, 122.0, 113.6, 108.7, 108.6, 97.2, 57.1, 55.7, 47.0, 46.2, 44.7; HRMS Calculated 434.2187 (M⁺ + H) for C₂₄H₂₈N₅O₃, found 434.2183.

4,5-Dimethoxy-N-(4-pyridinylmethyl)-2-[(4-(2-pyridinyl)-1-piperazinyl)carbonyl]aniline **18e**

Obtained as a dark yellow oil; yield 83 %; ¹H NMR (300 MHz, CDCl₃): δ 8.54 (d, *J* = 4.5 Hz, 2H), 8.34 (d, *J* = 4.8 Hz, 2H), 7.30 (d, *J* = 6.0 Hz, 2H), 6.75 (s, 1H), 6.56–6.53 (m, 1H), 6.07 (s, 1H), 6.03–5.99 (m, 1H), 4.39 (d, *J* = 5.3 Hz, 2H), 3.92–3.89 (br M, 4H), 3.79 (s, 3H), 3.76–3.72 (m, 4H), 3.70 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 170.8, 161.6, 157.8, 152.1, 150.0, 148.8, 143.1, 140.2, 122.0, 113.5, 110.5, 109.3, 97.2, 57.0, 56.2, 47.1, 45.3, 44.0; HRMS Calculated 435.2139 (M⁺ + H) for C₂₃H₂₇N₆O₃, found 435.2135.

4,5-Dimethoxy-2-[(4-(2-phenylethyl)-1-piperazinyl)carbonyl]-N-(4-pyridinylmethyl)aniline **18f**

Obtained as a yellow oil; yield 45 %; ¹H NMR (400 MHz, CDCl₃) δ 8.53 (d, *J* = 5.8 Hz, 2H), 7.28 (d, *J* = 4.9 Hz, 4H), 7.22–7.18 (m, 4H), 6.70 (s, 1H), 6.04 (s, 1H), 4.36 (s, 2H), 3.77 (d, *J* = 3.2 Hz, 4H), 3.68 (d, *J* = 4.0 Hz, 6H), 2.83–2.79 (m, 2H), 2.68–2.63 (m, 2H), 2.57–2.54 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 170.8, 152.3, 150.9, 150.4, 149.32, 143.2, 140.4, 140.2, 129.1, 128.8, 126.6, 122.4, 113.8, 109.9, 97.5, 60.6, 57.4, 56.1, 53.8, 47.5, 33.8; HRMS Calculated 461.2547 (M⁺ + H) for C₂₇H₃₃N₄O₃, found 461.2543.

2-(1-Piperazinylcarbonyl)-N-(4-pyridinylmethyl)aniline **19a**

Obtained as a pale yellow solid; yield 54 %; mp 84–86 °C, ¹H NMR (300 MHz, CDCl₃) δ 8.53 (d, *J* = 6.0 Hz, 2H), 7.26 (d, *J* = 6.0 Hz, 2H), 7.18–7.10 (m, 2H), 6.72–6.67 (m, 1H), 6.47 (d, *J* = 9.0 Hz, 1H), 5.76 (br t, *J* = 6.1, 1H), 4.39 (d, *J* = 6.1 Hz, 2H), 3.64 (br

s, 4H), 2.91–2.87 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3 , one carbon not observed in aliphatic region of spectrum) δ 170.2, 150.0, 148.6, 146.4, 130.9, 128.0, 121.9, 119.3, 116.5, 111.9, 46.5, 46.4 (br); HRMS Calculated 297.1710 ($\text{M}^+ + \text{H}$) for $\text{C}_{17}\text{H}_{21}\text{N}_4\text{O}$, found 297.1709.

2-[(4-Ethyl-1-piperazinyl)carbonyl]-N-(4-pyridinylmethyl)aniline **19b**

Obtained as a yellow solid; yield 45 %; mp 142–144 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.54–8.52 (m, 2H), 7.27 (d, $J = 5.9$ Hz, 2H), 7.18–7.10 (m, 2H), 6.68 (t, $J = 5.9$ Hz, 1H), 6.47 (d, $J = 9.0$ Hz, 1H), 5.78 (br t, $J = 6.0$ Hz, 1H), 4.38 (d, $J = 6.0$ Hz, 2H), 3.69 (br s, 4H), 2.50–2.42 (m, 6H), 1.11 (t, $J = 6.0$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3 , one carbon not observed in aliphatic region) δ 170.0, 150.0, 148.6, 146.4, 131.0, 128.1, 122.0, 119.2, 116.5, 111.9, 53.0, 52.3, 46.4, 11.9; HRMS Calculated 325.2023 ($\text{M}^+ + \text{H}$) for $\text{C}_{19}\text{H}_{25}\text{N}_4\text{O}$, found 325.2021.

N-(4-Pyridinylmethyl)-2-{[4-(2-pyridinyl)-1-piperazinyl]carbonyl}aniline **19c**

Obtained as a yellow solid; yield 30 %; mp 62–64 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.52 (d, $J = 5.3$ Hz, 2H), 8.20 (d, $J = 3.7$ Hz, 1H), 7.50–7.48 (m, 1H), 7.25 (d, $J = 4.6$ Hz, 2H), 7.20–7.14 (m, 2H), 6.73–6.61 (m, 3H), 6.49 (d, $J = 8.2$ Hz, 1H), 5.88–5.85 (m, 1H), 4.37 (d, $J = 5.8$ Hz, 2H), 3.79 (br s, 4H), 3.61 (br s, 4H); ^{13}C NMR (75 MHz, CDCl_3 , one aliphatic carbon not observed in spectrum) δ 170.5, 159.1, 150.0, 148.5, 148.1, 146.6, 137.7, 131.2, 128.2, 121.9, 118.9, 116.6, 114.1, 112.1, 107.3, 46.4, 45.7; HRMS Calculated 374.1981 ($\text{M}^+ + \text{H}$) for $\text{C}_{22}\text{H}_{24}\text{N}_5\text{O}$, found 374.1975.

N-(4-Pyridinylmethyl)-2-{[4-(4-pyridinyl)-1-piperazinyl]carbonyl}aniline **19d**

Obtained as a pale yellow solid; yield 34 %; mp 78–80 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.54 (br s, 2H), 8.33 (br s, 2H), 7.30–7.14 (br m, 4H), 6.70 (br s, 3H), 6.52 (d, $J = 5.9$ Hz, 1H), 5.96 (br s, 1H), 4.40 (br s, 2H), 3.83 (br s, 4H), 3.43 (br s, 4H); ^{13}C NMR (75 MHz, CDCl_3 , one aliphatic carbon not observed in spectrum) δ 170.6, 154.6, 150.4, 150.0, 148.4, 146.8, 131.5, 128.2, 121.9, 118.2, 116.5, 112.2, 108.7, 46.4, 46.3; HRMS Calculated 374.1981 ($\text{M}^+ + \text{H}$) for $\text{C}_{22}\text{H}_{24}\text{N}_5\text{O}$, found 374.1983.

Activity Based Assay for IC_{50} Determination

IC_{50} determinations for EGFR wt, L858R and T790M/L858R (purchased from Invitrogen: PV3872, PV4128, PV4879) were measured with the HTRF KinEASE-TK assay from Cisbio according to the manufacturer's instructions. After 2 hours of pre-incubation with the tested inhibitor and 50 nM of an artificial biotinylated substrate peptide (TK-substrate), EGFR was allowed to phosphorylate the latter by adding an ATP concentration corresponding to its K_M (30 μM for EGFR wt, 60 μM for EGFR L858R and 30 μM for EGFR T790M/L858R, previously determined using the same assay and EGFR constructs). After completion of the reaction, an antiphosphotyrosine antibody labelled with Europium cryptate and streptavidin labelled with the fluorophore XL665 were added. The FRET between Europium cryptate and XL665 was measured to quantify the phosphorylation of the substrate peptide (Tecan Safire 2 plate reader, excitation at 317 nm, readout at 620 nm -Eu-labelled antibody- and 665 nm -XL665 labelled streptavidin- after 60 μs lag time). The quotient of both intensities for reactions made with eight different inhibitor concentrations (including no inhibitor) were plotted against inhibitor concentrations and fit to a Hill 4-parameter equation to determine IC_{50} values (IDBS XLfit). Each reaction was performed in duplicate, and at least three independent determinations of each IC_{50} were made.

According to the instructions given for EGFR testing, Akt1 wt

(120 pM, Millipore, Lot # D8MN034U-L), Akt2 wt (501 pM, Invitrogen, Lot # PV3184_28770N) and $\Delta\text{PH-Akt1}$ (Millipore, Lot # 1600485-E), respectively were pre-incubated with the respective inhibitors (eight different concentrations) in a dark wet chamber for 1 h at RT. The phosphorylation reaction was started by adding both ATP (50 μM for Akt1, 65 μM for both Akt2 and $\Delta\text{PH-Akt1}$) and STK-substrate 3 (250 nM for Akt1, 300 nM for both Akt2 and $\Delta\text{PH-Akt1}$). After incubation for 45 min (Akt1), 20 min (Akt2) or 17 min ($\Delta\text{PH-Akt1}$), respectively the reaction was stopped and further incubated for 1 h at RT. Each well was excited at 317 nm and emission was measured at 620 nm and 665 nm with a 60 μs delay using a Tecan infinite M1000 plate reader.

References and Notes

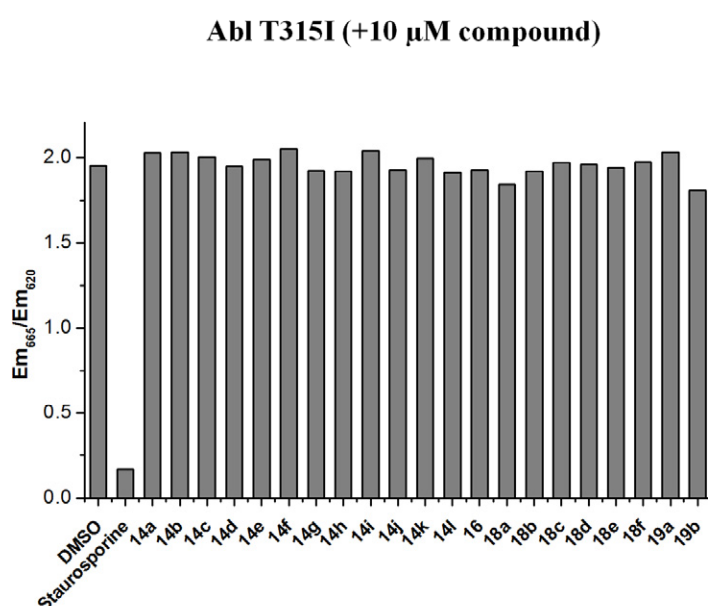
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- 34 Both compounds were resent to Dundee for retesting and this time activities measured were significantly less [IC₅₀s: **14a** (40 % @ 10 μ M) and **14h** (69 % @ 10 μ M)].
- 35 See <http://www.reactionbiology.com> for information concerning this commercial screening platform.
- 36 Extrapolation of the Reaction Biology dose-response data for Aurora B would suggest an IC₅₀ of 459 μ M for **14a**, although the Dundee screen would imply an IC₅₀ of about 10 μ M. This discrepancy might be explained by the ATP-K_m of the Aurora B used by Reaction Biology, which is about one order of magnitude higher than that of the Dundee Aurora B.
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Figure S1. Compounds **14a–l**, **16**, **18a–f** and **19a & b** tested on Abl T315I at 10 μ M compound concentrations. **A)** Final emission ratios (Em_{665}/Em_{620}) determined for the respective compounds and controls. **B)** Normalized inhibition rates with respect to DMSO (100%) and Staurosporine (0%).

A)



B)

Abl T315I	
Compound	Remaining activity (%)
DMSO	100
Staurosporine	0
14a	104
14b	104
14c	103
14d	100
14e	102
14f	105
14g	98
14h	105
14i	102
14j	99
14k	98
14l	98
16	99
18a	94
18b	98
18b	101
18d	100
18e	99
18f	101
19a	104
19b	92

Abl T315I (0.3 ng/well), purchased from Invitrogen (Lot#39639B, PV3866), was measured with the KinEASE-TK assay from Cisbio according to the manufacturer's instructions. A biotinylated poly-Glu-Tyr substrate peptide was phosphorylated and after completion of the reaction, an anti-phosphotyrosine antibody labeled with europium cryptate and streptavidin labeled with the fluorophore XL665 were added. FRET between europium cryptate and XL665 was measured to quantify the phosphorylation of the substrate peptide. ATP concentrations were set at the K_M value (6 μ M) and 250 nM TK-substrate were used. Kinase and inhibitor were preincubated for 30 min before the reaction was started by addition of ATP and substrate peptide. A Tecan infinite M1000 plate reader was used to measure the fluorescence of the samples at 620 nm (Eu-labeled antibody) and 665 nm (XL665 labeled streptavidin) 60 μ s after excitation at 317 nm. The experiment was performed in duplicates using plain DMSO as negative control (100% remaining activity) and 10 μ M Staurosporine as positive control (0% remaining activity).

Figure S2. Profiling of **14a** at 10 μ M compound concentration.

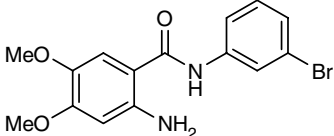
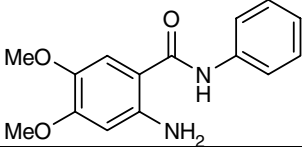
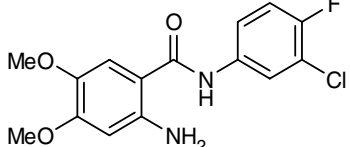
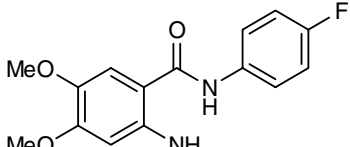
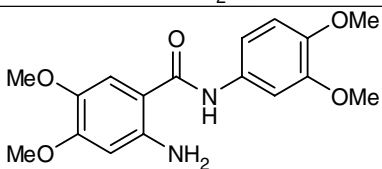
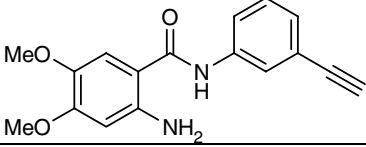
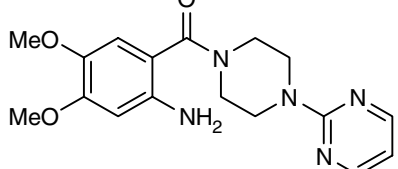
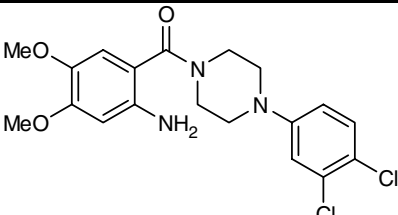
14a					
Kinase	rem. activity (%)	Stdev	Kinase	rem. activity (%)	Stdev
PKB β	17	0	RSK1	97	2
HER4	43	19	MLK1	97	3
Aurora B	51	11	PAK6	98	3
SRPK1	53	11	MST4	98	4
PIM2	65	0	CK2	99	8
RSK2	67	3	AMPK	99	5
FGF-R1	70	11	EPH A2	99	1
MAPKAP-K3	70	3	IRAK4	100	1
EPH-B3	71	0	CAMKK β	100	7
CHK2	74	5	ROCK 2	100	2
PLK1	74	3	PAK4	100	2
MINK1	75	2	PKB α	101	1
CAMK1	75	5	PHK	101	11
PIM1	78	2	IR-HIS	101	23
DYRK3	79	1	MELK	102	2
TTK	79	1	p38 δ MAPK	102	9
VEG-FR	80	13	RIPK2	103	2
BRSK2	83	8	PRK2	103	7
PKD1	83	1	CDK2-Cyclin A	103	0
TBK1	85	1	p38 γ MAPK	104	5
DYRK2	86	1	CHK1	104	1
IGF-1R	86	14	IKK β	104	18
MST2	88	1	p38 β MAPK	105	6
MNK1	88	9	PKC ζ	105	3
HIPK3	88	12	ERK8	105	8
MKK1	89	7	BRSK1	106	9
PIM3	90	25	PDK1	106	0
MARK3	91	4	ERK1	106	12
BTK	91	9	PAK2	107	16
PAK5	91	3	S6K1	107	8
HIPK2	92	9	Src	107	2
Aurora A	92	3	JNK2	107	4
MARK4	92	5	LKB1	108	3
NUAK1	93	6	IRR	108	7
GSK3 β	93	9	ERK2	109	6
DYRK1A	94	1	CSK	109	5
NEK6	94	6	MLK3	111	1
YES1	94	4	PRAK	111	23
PKA	94	1	Lck	113	6
PKC α	94	4	MARK2	114	1
NEK2a	94	6	CK1	114	13
SmMLCK	95	8	JNK3	115	5
GCK	96	3	MAPKAP-K2	115	5
SGK1	96	1	IKK ϵ	116	9
JNK1	96	3	SYK	119	4
HIPK1	97	0	MNK2	119	7
MSK1	97	14	EF2K	123	1
p38 α MAPK	97	7			

Figure S3. Profiling of **14h** at 10 μ M concentration.

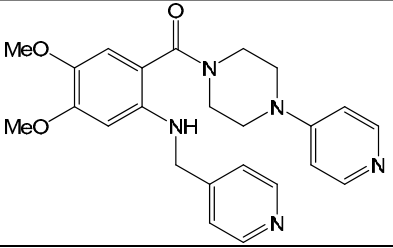
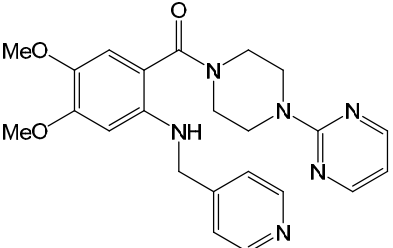
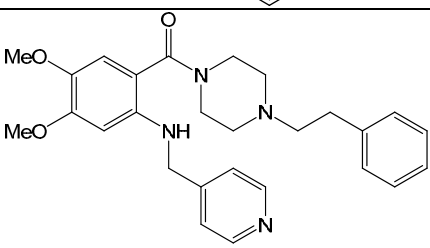
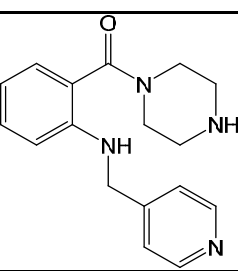
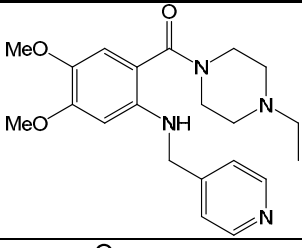
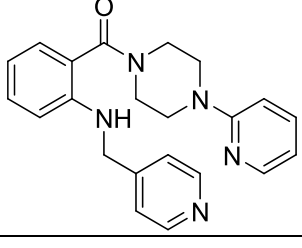
14h					
Kinase	rem. activity (%)	Stdev	Kinase	rem. activity (%)	Stdev
CAMK1	60	9	Lck	86	6
Aurora B	61	18	TBK1	86	2
PKB β	64	4	p38 β MAPK	86	6
MNK1	67	12	IRAK4	86	5
CHK2	71	8	MAPKAP-K2	87	1
HER4	71	10	MKK1	87	12
MAPKAP-K3	72	9	ROCK 2	87	5
PIM1	73	9	IGF-1R	88	0
FGF-R1	74	3	JNK1	88	6
NUAK1	74	3	IKK ϵ	88	10
SmMLCK	75	2	PKD1	88	15
HIPK3	76	1	CK2	88	1
PIM2	76	1	CK1	89	4
MINK1	77	1	DYRK1A	89	3
YES1	78	13	S6K1	89	7
PLK1	78	6	NEK2a	89	7
BRSK2	78	3	EPH-B3	90	3
RSK2	79	2	DYRK3	90	3
p38 δ MAPK	80	2	RIPK2	90	1
PAK6	80	9	MLK3	90	2
BTK	80	3	CAMKK β	90	1
MST2	81	8	AMPK	91	3
PKA	82	3	PAK2	91	5
VEG-FR	82	5	p38 α MAPK	91	1
SRPK1	82	15	IR-HIS	91	16
SYK	82	5	IKK β	91	3
ERK1	82	2	SGK1	92	11
HIPK1	82	6	HIPK2	92	12
MARK2	82	11	MST4	92	3
NEK6	82	4	RSK1	93	0
DYRK2	82	1	MSK1	94	3
MELK	83	17	JNK2	94	3
PIM3	83	16	IRR	95	3
MLK1	83	15	CDK2-Cyclin A	95	12
BRSK1	83	8	PRK2	96	2
PRAK	83	0	PKC α	97	5
JNK3	83	9	LKB1	97	0
PAK4	84	3	PDK1	97	11
Src	84	2	MARK3	97	13
PAK5	84	0	ERK2	98	2
MNK2	84	2	Aurora A	98	9
GSK3 β	84	7	p38 γ MAPK	99	2
CHK1	84	2	PKC ζ	99	1
ERK8	85	1	EF2K	103	1
GCK	85	1	PHK	104	6
CSK	85	4	MARK4	109	24
EPHA2	86	5	PKB α	121	5
TTK	86	2			

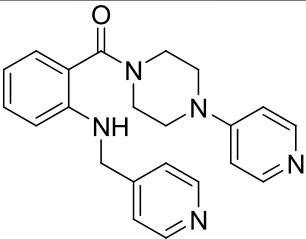
14a and **14h** were tested on 95 kinases at 10 μ M concentrations using a radiometric (^{33}P - γ -ATP) filter-binding assay conducted at the MRC Protein Phosphorylation Unit of the University of Dundee (<http://www.kinase-screen.mrc.ac.uk/>). ATP concentrations were chosen individually for each kinase to be at their respective ATP- K_m . The remaining activity of each kinase was normalized and is represented in %.

Figure S4. Table containing testing of “library 1” (compounds **14a-l**) and “library 2” (compounds **18a-f** and **19a-d**) against kinases Akt1 and Akt2. [n.i. = no inhibition up to 100 μ M. All experiments were conducted in duplicate in three independent measurements (n = 3)].

structure	name	no	% activity @ 100 μ M			IC ₅₀ / μ M		
			Akt1	Δ PH-Akt1	Akt2	Akt1	Δ PH-Akt1	Akt2
	WvO-1b	14a	79 $\pm$ 8	102 $\pm$ 8	128 $\pm$ 9	n.i.		n.i.
	WvO-1c	14b	82 $\pm$ 13	109 $\pm$ 11	120 $\pm$ 14			
	WvO-2b	14c	72 $\pm$ 7	112 $\pm$ 10	120 $\pm$ 5	n.i.	n.i.	n.i.
	WvO-2c	14d	101 $\pm$ 25	108 $\pm$ 11	123 $\pm$ 2			
	WvO-3b	14e	104 $\pm$ 13	110 $\pm$ 13	130 $\pm$ 10	n.i.		n.i.
	WvO-4b	14f	69 $\pm$ 7	104 $\pm$ 8	128 $\pm$ 12	n.i.		n.i.
	WvO-5b	14g	101 $\pm$ 11	102 $\pm$ 4	127 $\pm$ 3	n.i.		n.i.
	WvO-10b	14h	85 $\pm$ 19	101 $\pm$ 7	109 $\pm$ 2	n.i.		n.i.

	WvO-8b	14i	85 ± 19	106 ± 12	122 ± 5			
	WvO-6b	14j	83 ± 12	110 ± 4	122 ± 1		n.i.	
	WvO-9b	14k	80 ± 19	100 ± 15	125 ± 5	n.i.		n.i.
	WvO-11b	14l	93 ± 18	103 ± 11	122 ± 12			
	WvO-HFK1	16	92 ± 13	105 ± 8	126 ± 1			
	WvO-SC3	18a	100 ± 11	89 ± 17	128 ± 22			
	WvO-SC2	18b	109 ± 13	102 ± 4	108 ± 13	n.i.		n.i.
	WvO-HFK3	18c	91 ± 14	86 ± 15	134 ± 7	n.i.		n.i.

	WvO-HFK4	18d	66 ± 6	72 ± 12	95 ± 8	n.i.	n.i.	n.i.
	WvO-HFK5	18e	$83 \pm 0,5$	106 ± 3	151 ± 5			
	WvO-HFK13	18f	93 ± 5	101 ± 4	89 ± 4	n.i.		n.i.
	WvO-SC4	19a	113 ± 8	103 ± 7	98 ± 4	n.i.		n.i.
	WvO-SC5	19b	90 ± 16	103 ± 2	87 ± 1	n.i.		n.i.
	WvO-HFK4-2	19c	100 ± 19	104 ± 10		n.i.		n.i.

	WvO- HFK4- 1	19d	81 ± 20	105 ± 6		n.i.		n.i.