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Azastilbenes: a cut-off to p38 MAPK inhibitors†

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Inhibitors with vicinal 4-fluorophenyl/4-pyridine rings on a five- or six-membered heterocyclic ring are known to inhibit the p38 mitogen-activated protein kinase (MAPK), which is a potential target for rheumatoid arthritis and several different types of cancer. Several substituted azastilbene-based compounds with vicinal 4-fluorophenyl/4-pyridine rings were designed using computational docking, synthesized, and evaluated in a cell-free radiometric p38α assay. The biochemical evaluation shows that the best inhibition (down to 110 nM) is achieved for azastilbene-based compounds having an isopropylamine substituent in the 2-position of the pyridine ring. The inhibition of p38 signaling in human breast cancer cells was observed for two of the compounds.

Introduction

The mitogen-activated protein kinases (MAPKs) are essential regulators for signal transduction pathways and play crucial roles in cellular processes such as transcription, apoptosis and differentiation.[1] The MAP kinase p38 responds to environmental stress (*e.g.* UV radiation, osmotic shock and mechanical stress) and is involved in the production of cytokines (IL-1 and TNFα), which are implicated in chronic inflammatory diseases.[2] Consequently, inhibition of the p38 MAP kinase with small organic molecules could provide an effective therapy for the chronic treatment of these autoimmune diseases, and therefore, large efforts have been made in order to synthesize and evaluate different inhibitors of p38 kinase.[3] Further, recently the p38 kinase has also been suggested to be a target for treatments of several different cancers, *e.g.* such as breast cancer, colon cancer, and ovarian cancer.[3f,4]

One class of selective p38 inhibitors is the pyridinylimidazole-based compounds that are recognized by their vicinal 4-fluoro-phenyl/pyridyl rings.[5] Since then, several different p38 inhibitors with the 4-fluoro-phenyl/pyridinyl motif have been developed using different ring scaffolds, such as five-membered pyrazoles,[6] isoxazoles,[7] triazoles,[8] and six-membered pyrimidines,[9] quinolinones,[10] and chromones[11] and several of these compounds have shown to be highly potent and inhibit the p38 kinase activity at nanomolar concentrations (Fig. 1).

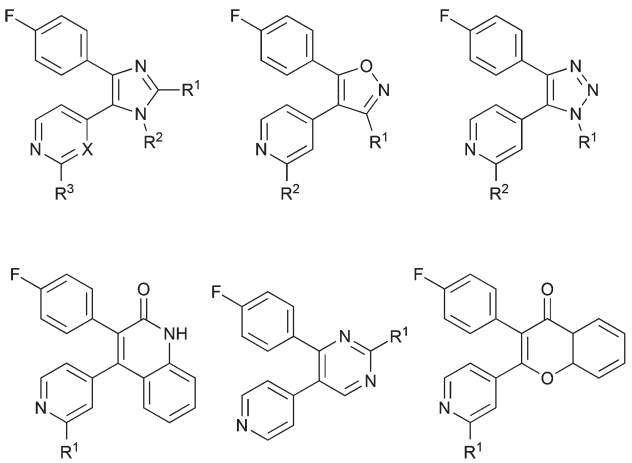

Fig. 1 p38 inhibitors with vicinal 4-fluorophenyl/4-pyridine rings.

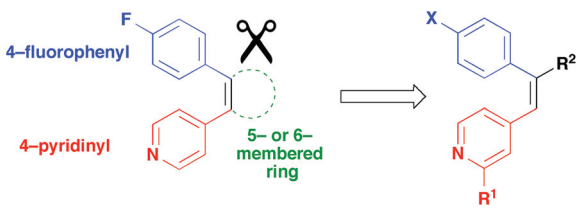

Fig. 2 A cut-off of the core ring leads to azastilbene compounds.

In order to determine the importance of the core ring system in the scaffold, we wanted to explore if it is possible to reduce the core of the scaffold from a five- or six-membered ring to a double bond and still keep the strong inhibitory effect observed for previous inhibitors with the 4-fluorophenyl/pyridinyl motif (Fig. 2).

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†Electronic supplementary information (ESI) available: General experimental procedure, characterization of synthesized compounds, ^{1}H NMR and ^{13}C NMR spectra. See DOI: 10.1039/c3ob27449g

Here we wish to present the design, synthesis, and biological evaluation of p38α inhibitors based on the azastilbene scaffold with a 4-fluoro-phenyl/pyridinyl motif.

Results and discussion

Design

Several inhibitors with a 4-fluoro-phenyl/pyridinyl motif are potent inhibitors of the p38α kinase and have been co-crystallized with the p38α kinase (for example with the **SB203580** inhibitor, IC_{50} = 48 nM). The binding of the inhibitor **SB203580** to the p38α kinase involves hydrogen bonding to the hinge region with the nitrogen of the pyridine ring and between the imidazole nitrogen and the amino group of Lys53 (Fig. 3A). In addition, the 4-fluorophenyl ring of the inhibitor interacts with an additional hydrophobic pocket and the other aryl group extends into the phosphate-binding region where a π–π-stacking with Tyr35 is possible (Fig. 3A). The aim of the present work is to investigate if it is possible to deconstruct the inner core ring of the imidazole scaffold and to replace it with a C=C double bond motif and still obtain binding affinity, and thus, inhibition of the p38α kinase. However, the removal of the imidazole ring also removes the possibility to hydrogen bond to the Lys53 in the active site, which is important for the activity as shown in previous work.[8b,12] Therefore, we also wanted to introduce a hydrogen bond acceptor group (*e.g.* ester or carboxylic acid) in the double bond of the azastilbene (R^2 in Fig. 1B). Further, the introduction of an amino group in the 2-position (R^1 in Fig. 1B) of the pyridine ring can also increase the binding affinity of the p38 inhibitors as seen in previous studies.[8a,13] Several azastilbenes were docked into the ATP binding site of p38α kinase (PDB: 1a9u) using Glide (XP)[14] in the Schrödinger Suite[15] in order to identify different compounds that bind to the ATP binding site in a similar way to **SB203580**.

The docking result suggests that several different azastilbenes (yellow in Fig. 3C) mimic the binding mode of the known imidazole-based **SB203580** inhibitor (blue in Fig. 3C), *i.e.* the inhibitor hydrogen bonds to the hinge region and the phenyl ring interacts with the hydrophobic pocket (Fig. 3D). The docking result shows that compounds with a hydrogen bond acceptor group in the double bond of the azastilbene, such as an ester or a carboxylic acid group, can form a hydrogen bond to Lys53. The result also suggests that the introduction of an amine (–NH_2 or –NH-i-Pr) in the 2-position of the pyridine ring could lead to increased inhibition capacity due to the formation of an extra hydrogen bond to Met109, which has previously been observed experimentally.[8a,13] These promising docking results prompted us to synthesize and evaluate several different azastilbenes as p38α inhibitors.

Chemistry

The synthesis of the azastilbenes is depicted in Scheme 1. The azastilbenes **6a–c** were obtained in moderate yields (32–50%)

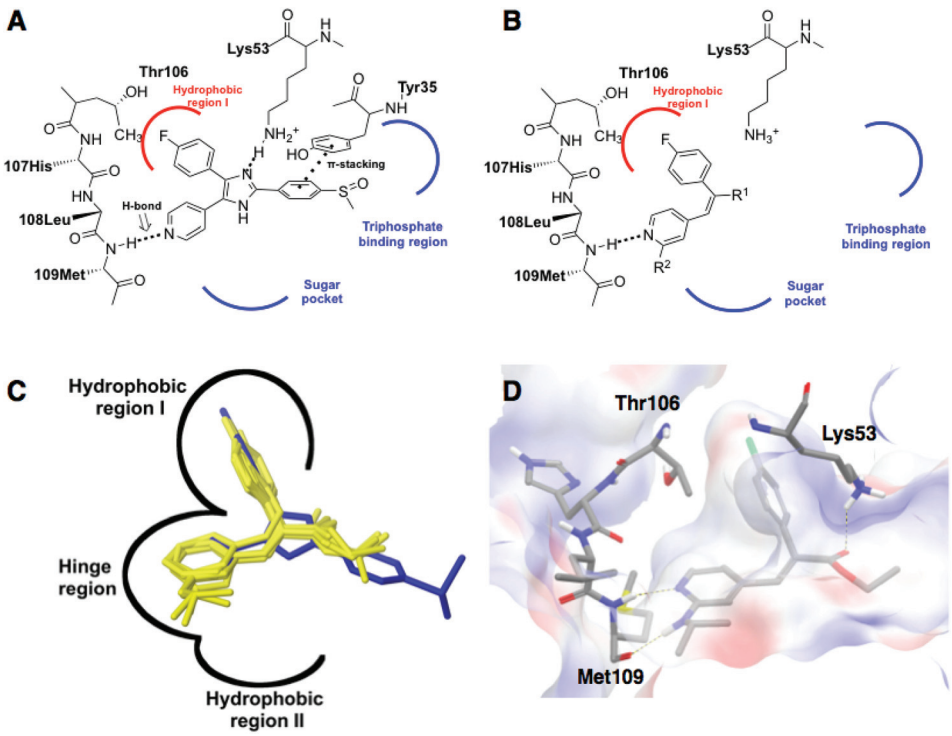
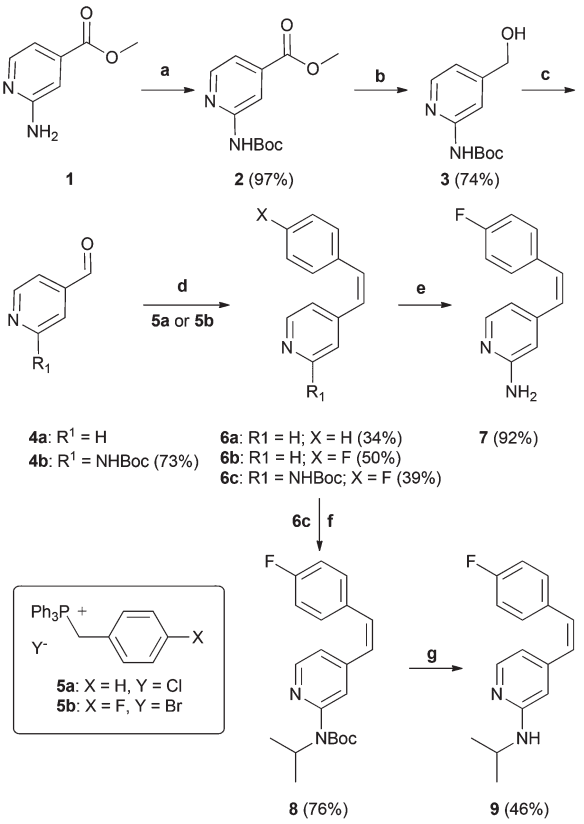


Fig. 3 (**A**) Schematic picture of the binding of the **SB203580** inhibitor to the ATP pocket. (**B**) Schematic binding of azastilbenes to the ATP pocket. (**C**) The docking results suggest a similar binding mode for the known imidazole-based **SB203580** inhibitor (blue) and several different azastilbenes (yellow), *i.e.* hydrogen bonding to the hinge region and interaction of a phenyl ring in the hydrophobic pocket. (**D**) Binding mode of compound **16c** into the ATP pocket. The docking suggests hydrogen bonding to the hinge region and to Lys53 and an interaction of the aromatic group into the hydrophobic pocket.



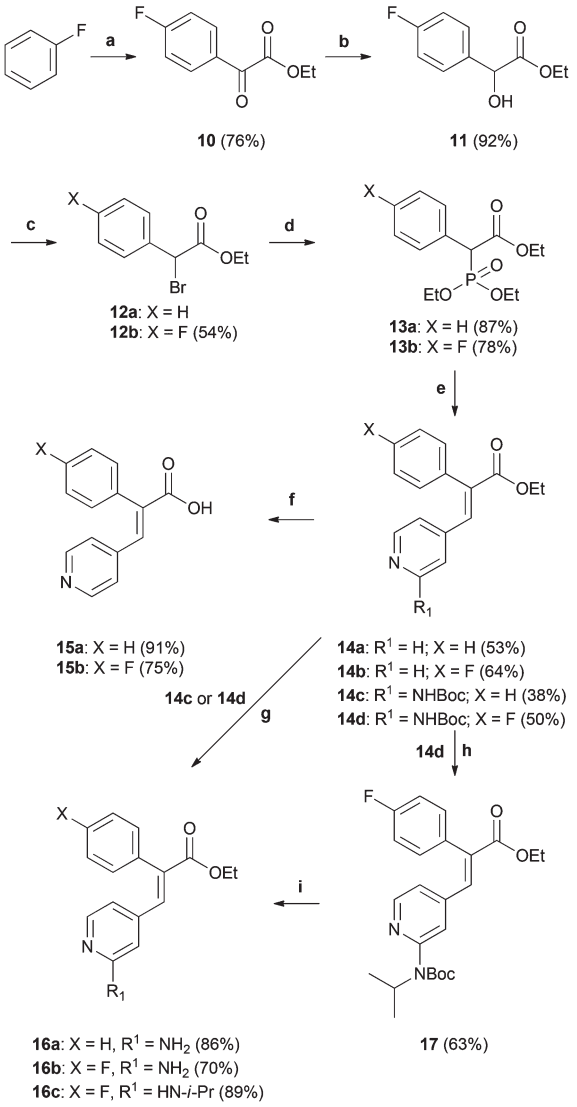
Scheme 1 (a) Boc_2O, DMAP, *t*-BuOH, RT. (b) $NaBH_4$, $CaCl_2$, EtOH, RT. (c) Dess–Martin periodinane, DCM, RT. (d) LiOH, 2-propanol, MW (60–80 °C). (e) Compound **6c**, TFA, DCM. (f) NaH, 2-iodopropane, DCM, 0 °C. (g) TFA, DCM.

via a Wittig reaction between the aldehydes **4a–b** and two different triphenylphosphonium salts **5a–b** using lithium hydroxide monohydrate as a base in 2-propanol.[16] In the reaction, both the (*Z*)- and the (*E*)-isomers were formed and the pure (*Z*)-isomers could be separated and isolated using flash chromatography. The (*Z*)-stereochemistry of the products **6a–c** was determined by comparison of the size of the coupling constants of the two protons in the double bond (see ESI†).

Compound **7** was obtained *via* the removal of the Boc-protecting group by treatment with TFA in DCM (92%). Compound **9** was synthesized *via* alkylation of **6c** using 2-iodopropane yielding **8** followed by subsequent deprotection by TFA.

The aldehyde **4b**, used in the Wittig reaction, was prepared in a three-step procedure, starting with the Boc-protection of the amino group[17] (97%), followed by the reduction of the ester to the corresponding benzylic alcohol using an $NaBH_4$–$CaCl_2$-mixture in ethanol (74%).[18] The alcohol **3** was then converted to the aldehyde **4b** using Dess–Martin periodinane in dichloromethane in good yield (73%).[19]

The azastilbenes **14a–d**, having an ester group or a carboxylic acid group in the double bond, were prepared *via* a Horner–Wadsworth–Emmons reaction using two different phosphonates **13a–b** and the aldehydes **4a–b** using triethylamine as a base in the presence of lithium chloride in acetonitrile (Scheme 2). In the original procedure by Ianni *et al.*,[20]



Scheme 2 (a) 2-Chloro-2-oxoacetate, $AlCl_3$, DCM, 0 °C. (b) $NaBH_4$, EtOH, −30 °C. (c) PBr_3, $CHCl_3$, RT. (d) $P(OEt)_3$, reflux. (e) **4a–b**, NEt_3, LiCl, MeCN, RT. (f) LiOH, THF–water (4 : 1), RT. (g) TFA, DCM. (h) NaH, 2-iodopropane, 0 °C. (i) TFA, DCM.

DBU was employed as a base for the synthesis of 2-aryl-substituted cinnamic acid esters from 2-aryl-substituted phosphonoacetates with excellent (*E*)-selectivity. However, using this procedure for aldehydes **4a–b** in the reaction, no stereoselectivity was observed.‡ Several different organic amine bases, such as DIPEA, (−)-quinine, 1-methylpiperidine, and triethylamine, were tested in the HWE reaction (Table 1, entries 2–4). Among these bases, triethylamine showed the best stereoselectivity (3 : 1) in a small test scale reaction.

‡The *E*/*Z* stereochemistry of the azastilbene-based compounds **14a–17** was determined by COSY and NOESY NMR experiments for compound **14b** (see ESI†). Further, the *E*/*Z* ratio was determined by comparing the characteristic peaks of the alkenyl proton, which are located around 7.7–7.9 ppm and 6.8 ppm for the (*E*)-isomers and (*Z*)-isomers, respectively.

Table 1 Stereoselectivity of the HWE reaction for different organic bases[a]

Entry	Prod.	X	R^1	R^2	Base	*E* : *Z*	Conv
1	**14a**	H	H	COOEt	DBU	1 : 1	Full
2	**14a**	H	H	COOEt	DIPEA	2.3 : 1	Full
3	**14a**	H	H	COOEt	Quinine	2.7 : 1	Full
4	**14a**	H	H	COOEt	1-MP[b]	2.4 : 1	Full
5	**14a**	H	H	COOEt	Et_3N	3 : 1	Full

[a] The reaction was performed in small scale (0.1 mmol aldehyde) according to general procedure A and the stereoselectivity and conversion were determined by ^{1}H NMR-analysis of the crude reaction mixture. [b] 1-Methylpiperidine.

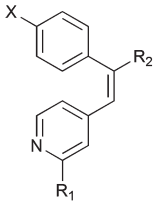
Under the modified conditions, compounds **14a–d** were afforded in moderate yields (38–64%) with a stereoselectivity ranging from 1.6 to 2.2. The phosphonate **13b** was prepared in a 4-step procedure starting with a Friedel–Crafts acylation of fluorobenzene with ethyl 2-chloro-2-oxoacetate in the presence of anhydrous aluminium trichloride (76%), followed by the reduction of the ketone carbonyl group to an alcohol using sodium borohydride (92%).[20] The alcohol **11** was converted to the alkyl halide **12b** using phosphorus tribromide (54%). The phosphonates **13a–b** were prepared from the alkylhalides **12a–b** *via* a Michaelis–Arbuzov reaction in the presence of triethyl phosphite (87% and 78%, respectively).[20] Compounds **14a–b** were hydrolyzed to the corresponding carboxylic acids **15a–b** using LiOH in a THF–water solution in good yields (91% and 75%, respectively). Compounds **16a–b** were obtained by removal of the Boc-protecting group using TFA in DCM. Compound **16c** was obtained *via* a base-promoted alkylation using 2-iodopropane yielding **17**, followed by a deprotection by TFA.

Inhibition of p38α MAPK for azastilbene compounds

The inhibitory potencies of the compounds **6a–b**, **7**, **9**, **14a–b**, **15a–b**, and **16a–c** were evaluated using a commercial radiometric p38 assay.[21] The result shows that of the parent azastilbenes compound **6a** only moderately inhibits the p38 activity (4.8 μM). The result also indicates that the fluorine atom in the 4-position of the phenyl ring and the NH_2-group of the pyridine ring increases the inhibitory activity for compounds **6b** and **7** (Table 2, entries 2 and 3). The introduction of an amine in the 2-position of the pyridine ring may result in another possible hydrogen bond interaction to the hinge region, which is seen from the docking studies (see the ESI†).

Especially, the introduction of an isopropylamine in the 2-position of the pyridine ring, leading to compound **9**, gives a 20-fold increase of the inhibitory capacity (110 nM). The isopropyl substituent influences the basicity of the amino group and thereby also increases the strength of the hydrogen bond to the hinge region and could be one reason for the large increase in binding affinity of compound **9** compared to **7**. Secondly, the sterically more demanding isopropyl group also interacts with the hydrophobic region II (see ESI†), which also may be a reason for the increased binding. The addition of the ester functionality in the double bond did not lead to any increase of the inhibition of p38α for compounds **14a–b** and **16a–b**. The hydrolysis of the ester group to a carboxylic acid leads to a 4-fold decrease in inhibition for compounds **15a–b**. This shows that the ester group or carboxylic acid at the double bond does not contribute to binding affinity and reveals that the hydrogen bond interaction to Lys53 does not play an important role in the azastilbene series. Compound **16c**, having an ester group in the double bond, a fluorine atom in the 4-position of the phenyl ring, and an isopropylamine in the 2-position of the pyridine ring also inhibits the p38α kinase more strongly with an IC_{50}-value of 140 nM. These inhibition results for compounds **9** and **16c** suggest that the core ring system of the inhibitors having a 4-fluoro-phenyl/pyridinyl motif can be replaced with the structurally more simple double bond and still obtain good inhibitory capacity. However, it should be noted that the inhibition capacity for these azastilbene compounds is lower compared to other known imidazole-based p38 inhibitors with vicinal isopropylaminopyridine and fluorobenzene substituents (down to 2 nM IC_{50}-value).[5d]

Table 2 IC_{50}-values for synthesized compounds **6a–b**, **7**, **9**, **14a–b**, **15a–b**, and **16a–c** in p38α MAP kinase inhibition



Entry	Cmpd.	X	R^1	R^2	IC_{50}-value (μM)
1	**6a**	H	H	H	4.8
2	**6b**	F	H	H	2.0
3	**7**	F	NH_2	H	1.7
4	**9**	F	i-Pr-NH	H	0.11
5	**14a**	H	H	COOEt	5.1
6	**14b**	F	H	COOEt	2.0
7	**15a**	H	H	COOH	18.3
8	**15b**	F	H	COOH	8.2
9	**16a**	H	NH_2	COOEt	2.4
10	**16b**	F	NH_2	COOEt	1.5
11	**16c**	F	i-Pr-NH	COOEt	0.14

Compounds **9** and **16c** were also evaluated in a cell-based assay with human derived MCF7 breast cancer cells as previously described.[11]

Anisomycin-induced activation of p38 and phosphorylation of its downstream target, heat shock protein 27 (Hsp27), were inhibited at doses as low as 0.1 μM and maximal inhibition was observed at 1–5 μM (Fig. 4). The result shows that the azastilbenes **9** and **16c** inhibit the downstream signaling of p38 despite their slightly decreased activity in the radiometric assay compared to the other known p38 inhibitors.

Conclusions

We have developed two synthetic routes yielding substituted azastilbenes and the synthesized compounds have been

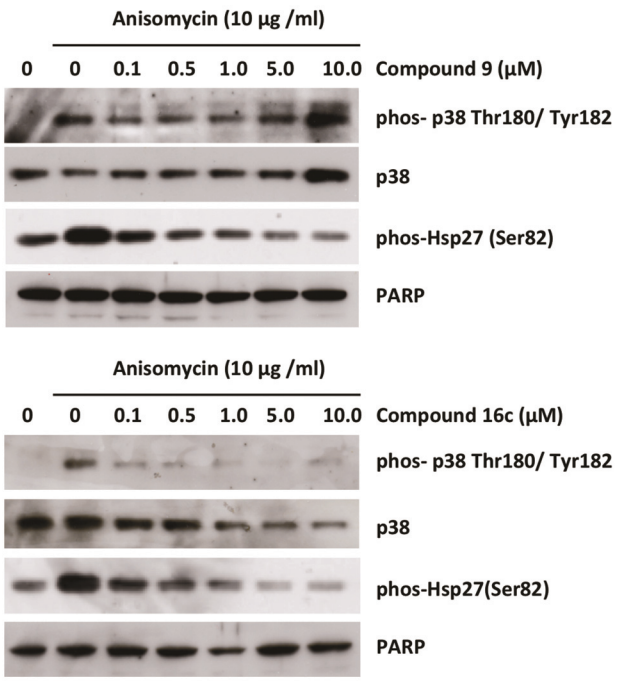


Fig. 4 Compounds **9** and **16c** inhibit p38 activation and signaling in human breast cancer cells. MCF7 cells were preincubated with the indicated doses of **9** and **16c** for 30 min and then exposed to 10 μg ml^{-1} anisomycin for another 30 min. Total cell lysates were resolved by SDS–PAGE. Membranes were probed with antibodies directed against phosphorylated and total p38 and phosphorylated Hsp27 (Ser82). A polyclonal antibody directed against PARP was used as a loading control.

evaluated as p38α inhibitors in a radiometric cell-free assay and in MCF7 breast cancer cells. The first strategy was based on the Wittig reaction and gave (*Z*)-azastilbenes and the second synthetic approach yielded (*E*)-diarylacrylates *via* a Horner–Wadsworth–Emmons reaction. The inhibition data show that the two best inhibitors, having a fluorine atom in the 4-position of the phenyl ring, and an isopropylamine in the 2-position of the pyridine ring strongly reduce the activity of p38α (111 nM and 137 nM, respectively). This suggests that the core ring system of the 4-fluoro-phenyl/pyridinyl inhibitors can be replaced with the structurally more simple double bond and still obtain good inhibitory capacity. In spite of a decrease in IC_{50}-values due to the truncation of the inhibitors, the two best azastilbene inhibitors decreased the downstream signaling of p38α in human breast cancer cells.

Experimental section

General

All reagents were purchased from Sigma-Aldrich and were used without further purification. ^{1}H NMR (399.97 MHz) and ^{13}C NMR (100.58 MHz) spectra were recorded on a Varian Unity 400 spectrometer, using solvent residual peaks (^{1}H: $CDCl_3$: δ 7.26 ppm, DMSO-d_6: δ 2.50 ppm; ^{13}C: $CDCl_3$: δ 77.0 ppm, DMSO-d_6: δ 39.52 ppm) as an indirect reference to TMS. Chemical shifts and literature NMR shifts were used to assign the NMR spectra of the synthesised compounds. Flash column chromatography was performed using Merck silica gel (0.04–0.06 mm). Thin layer chromatography (TLC) was performed on ALUGRAM® SIL G/UV_{254} plates (0.2 mm), using UV-light (254 nm) for visualization. IR spectra were recorded on a Perkin-Elmer Spectrum One (ATR Technique). HPLC analysis was performed using a Young Lin (YL-9100) system equipped with a Kromasil 5-CellCoat column and using hexane and isopropanol (flow: 0.5 ml min^{-1}) as eluents and UV-detection (220 nm and 254 nm).

Synthesis

2-*tert*-Butoxycarbonylamino-isonicotinate (2).[18] To a solution of methyl 2-aminopyridin-4-carboxylate (2.5 g, 16.7 mmol) in acetone (10 ml) and *tert*-butanol (30 ml), DMAP (115 mg, 0.94 mmol) and di-*tert*-butyl dicarbonate (10.5 mg, 48.1 mmol) were added under vigorous stirring. The reaction mixture was stirred at room temperature for 15 hours. The solution was diluted with pentane (36 ml), cooled in the refrigerator for 3 hours and filtered to yield **2** as a white powder (4.1 g, 97%). ^{1}H NMR (DMSO-d_6): δ 10.11 (s, 1H), 8.43 (d, J = 5.1 Hz, 1H), 8.33 (s, 1H), 7.45 (d, J = 5.1 Hz, 1H), 3.89 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (DMSO-d_6): δ 165.2, 153.5, 152.7, 149.0, 138.6, 117.0, 111.2, 80.0, 52.7, 28.0.

***N*-[4-Hydroxymethyl)-2-pyridinyl]-1,1,1-dimethylethylester (3).**[22] To a solution of compound **2** (1.20 g, 4.76 mmol) in ethanol (18 ml) were added sodium borohydride (540 mg, 14.3 mmol) and calcium chloride (792 mg, 7.14 mmol). After stirring at room temperature for 7 hours, the solution was quenched with water (20 ml) and extracted with dichloromethane (20 ml). The precipitate was filtered through Celite, washed with dichloromethane (4 × 20 ml). The organic phase was washed with brine (20 ml) and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (1 : 1) as an eluent, yielding **3** as a colorless solid (789 mg, 74%). ^{1}H NMR ($CDCl_3$): δ 9.65 (s, 1H), 8.25 (d, J = 4.8 Hz, 1H), 7.93 (s, 1H), 6.96 (d, J = 4.8 Hz, 1H), 4.68 (s, 2H), 3.44 (s, 1H), 1.50 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 153.1, 153.0, 152.9, 147.7, 115.8, 109.8, 81.0, 63.7, 28.4.

2-[*N*,*N*-Mono-(*tert*-butoxycarbonyl)amino]-pyridyl-4-aldehyde (4b).[22] To a solution of Dess–Martin periodinane (2.7 g, 6.4 mmol) in dichloromethane (24 ml), compound **3** (1.3 g, 5.8 mmol) in dichloromethane (24 ml) was added. After stirring for 15 hours, the solution was quenched by saturated $NaHCO_3$ (aq., 30 ml) and saturated $Na_2S_2O_3$ (aq., 30 ml), followed by extraction with dichloromethane (3 × 50 ml). The combined organic phases were washed with water (40 ml), dried with magnesium sulfate, filtered, and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (1 : 1) as an eluent, yielding **4b** as a white solid (939 mg, 73%). ^{1}H NMR ($CDCl_3$): δ 10.32 (s, 1H), 10.00 (s, 1H), 8.52 (d, J = 4.8 Hz, 1H), 8.44 (s, 1H), 7.50 (dd, J = 1.2 Hz, J = 4.8 Hz, 1H), 1.53 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 191.6, 154.6, 152.8, 148.7, 144.0, 114.8, 113.8, 81.5, 28.3.

(*Z*)-4-Styryl-pyridine (6a). To a solution of benzyltriphenylphosphonium chloride **5a** (350 mg, 0.90 mmol) in isopropanol (3 ml) was added lithium hydroxide monohydrate (35 mg, 1.4 mmol) under vigorous stirring. The solution was stirred at room temperature for 15 min. 4-Pyridinecarboxaldehyde (0.078 ml, 0.82 mmol) was added and the solution was heated by microwave irradiation at 80 °C for 3 hours. The solution was quenched with water (30 ml) and extracted with ethyl acetate (50 ml × 3). The combined organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The mixture was purified through flash column chromatography using pentane–ethyl acetate (1 : 1) as an eluent to yield **6a** as a yellow oil (52 mg, 34%). ^{1}H NMR ($CDCl_3$): δ 8.49 (d, J = 6.0 Hz, 2H), 7.2–7.25 (m, 5H, Ar), 7.14 (d, J = 5.98 Hz, 2H), 6.82 (d, J = 12.2 Hz, 1H), 6.53 (d, J = 12.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 149.9, 144.9, 136.2, 134.0, 128.8, 128.5, 127.9, 127.6, 123.5. IR (cm^{-1}): 3054, 3022, 1631, 1594. HRMS (ESI) Calcd for $C_{13}H_{12}N^+$: 182.0964, found: 182.0963.

(*Z*)-4-(4-Fluorostyryl)pyridine (6b). To a solution of (4-fluorobenzyl)-triphenylphosphonium bromide **5b** (407 mg, 0.90 mmol) in isopropanol (3 ml) was added lithium hydroxide monohydrate (35 mg, 1.4 mmol) under vigorous stirring. The solution was stirred at room temperature for 15 min. 4-Pyridinecarboxaldehyde (0.078 ml, 0.82 mmol) was added and the solution was heated by microwave at 80 °C for 3 hours. The solution was quenched with water (30 ml) and extracted with ethyl acetate (50 ml × 3). The combined organic layer was dried with anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The mixture was purified through flash column chromatography using pentane–ethyl acetate (1 : 1) as an eluent to yield **6b** as a yellow oil (82 mg, 50%). ^{1}H NMR ($CDCl_3$): δ 8.42 (dd, J = 1.6 Hz, J = 4.4 Hz, 2H), 7.10–7.14 (m, 2H), 7.03 (dd, J = 1.6 Hz, J = 4.4 Hz, 2H), 6.86–6.90 (m, 2H), 6.67 (d, J = 12.4 Hz, 1H), 6.43 (d, J = 12.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 162.2 (d, J_{CF} = 247.4 Hz), 149.9, 144.7, 132.7, 132.1 (d, J_{CF} = 3.1 Hz), 130.5 (d, J_{CF} = 8.0 Hz), 127.5, 123.4, 115.4 (d, J_{CF} = 21.1 Hz). IR (cm^{-1}): 3070, 3048, 3023, 2981, 1630, 1594, 1506. HRMS (ESI) Calcd for $C_{13}H_{11}FN^+$: 200.0870, found: 200.0894.

***tert*-Butyl [(*Z*)-4-(4-fluorostyryl)pyridin-2-yl] carbamate (6c).** To a solution of (4-fluoro-benzyl)-triphenylphosphonium bromide **5b** (564 mg, 2.54 mmol) in isopropanol (4.5 ml), lithium hydroxide monohydrate (107 mg, 2.56 mmol) was added under vigorous stirring. The solution was stirred at room temperature for 15 min. Compound **4b** (1.25 g, 2.76 mmol) was added and the solution was heated by microwave irradiation at 60 °C for 1 hour. The solution was quenched with water (50 ml) followed by extraction with ethyl acetate (100 ml × 3). The organic layer was dried with magnesium sulfate, filtered, and evaporated under reduced pressure. The mixture was purified through silica gel using pentane–ethyl acetate (1 : 1) as an eluent, yielding **6c** as a white powder (310 mg, 39%). ^{1}H NMR ($CDCl_3$): δ 10.21 (s, 1H), 8.18 (d, J = 5.25 Hz, 1H), 7.94 (s, 1H), 7.17–7.21 (m, 2H), 6.89–6.93 (m, 2H), 6.67–6.72 (m, 2H), 6.52 (d, J = 12.2 Hz, 1H), 1.52 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 162.3 (d, J_{CF} = 247.4 Hz), 153.7, 153.1, 147.6, 147.4, 132.5, 132.4 (d, J_{CF} = 14.1 Hz), 130.8 (d, J_{CF} = 8.0 Hz), 128.3, 117.8, 115.4 (d, J_{CF} = 21.1 Hz), 112.6, 80.8, 28.4. IR (cm^{-1}): 3193, 2965, 1716, 1603, 1565, 1529, 1506. HRMS (ESI) Calcd for $C_{18}H_{20}FN_2O_2^+$: 315.1503, found: 315.1521.

(*Z*)-4-(4-Fluorostyryl)-pyridin-2-amine (7). To a solution of compound **6c** (210 mg) in dry dichloromethane (1.5 ml), trifluoroacetic acid (1.5 ml) was added. The solution was stirred for 2 hours at room temperature. The solvent was removed by flushing nitrogen gas to the solution and NaOH (2 M, 1 ml) was added to basify the solution. The solution was extracted with dichloromethane (8 ml × 5) and the organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure. The target molecule was obtained through silica gel using dichloromethane–methanol (9 : 1) as an eluent, yielding **7** as a powder (132 mg, 92%). ^{1}H NMR ($CDCl_3$): δ 7.82 (d, J = 5.2 Hz, 1H), 7.16–7.20 (m, 2H), 6.88–6.93 (m, 2H), 6.62 (d, J = 12.5 Hz, 1H), 6.31–6.43 (m, 3H), 4.56 (broad s, 2H). ^{13}C NMR ($CDCl_3$): δ 162.1 (d, J_{CF} = 248.7 Hz), 158.8, 147.9, 146.9, 132.4 (d, J_{CF} = 4.0 Hz), 132.0, 130.7 (d, J_{CF} = 18.1 Hz), 128.1 (J_{CF} = 6.0 Hz), 115.3 (d, J_{CF} = 21.1 Hz), 114.1, 108.2. IR (cm^{-1}): 3453, 3298, 3132, 2924, 1638, 1597, 1541, 1505. HRMS (ESI) Calcd for $C_{13}H_{12}FN_2^+$: 215.0979, found: 215.0962.

(*Z*)-*tert*-Butyl 4-(4-fluorostyryl)pyridin-2-yl(isopropyl)carbamate (8). To a solution of compound **6c** (400 mg, 1.3 mmol) in DMF (3.9 ml) at 0 °C, sodium hydride (69 mg, 2.9 mmol) was added under vigorous stirring in an inert atmosphere. After stirring for 30 min at 0 °C, 2-iodopropane (0.3 ml, 3.0 mmol) was added dropwise. The solution was stirred for 30 min at 0 °C and brought to room temperature over 4 hours, followed by addition of water (50 ml), extraction of diethyl ether (5 × 50 ml) and washing with diluted hydrochloric acid (0.1 M, 50 ml), saturated sodium hydrogen carbonate (50 ml) and brine (50 ml). The organic layer was dried with magnesium sulfate, and concentrated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **8** as a yellow liquid (240 mg, 76%). ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J = 5.2 Hz, 1H), 7.15–7.18 (m, 2H), 6.92–6.90 (m, 4H), 6.71 (d, J = 12.2 Hz, 1H), 6.49 (d, J = 12.2 Hz, 1H), 4.40–4.47 (m, 1H), 1.39 (s, 1H), 1.17 (d, J = 6.8 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 162.1 (d, J_{CF} = 246.3 Hz), 154.3, 154.2, 148.4, 146.2, 132.6, 132.0 (d, J_{CF} = 3.0 Hz), 130.5 (d, J_{CF} = 7.6 Hz), 127.3, 123.6, 121.3, 115.4 (d, J_{CF} = 21.4 Hz), 80.2, 49.2, 28.3, 21.3. IR (cm^{-1}): 2974, 1696, 1596, 1540, 1507. HRMS (ESI) Calcd for $C_{21}H_{26}FN_2O_2^+$: 357.1973, found: 357.1976.

(*Z*)-4-(4-Fluorostyryl)-*N*-isopropylpyridin-2-amine (9). To a solution of compound **8** (180 mg, 0.5 mmol) in anhydrous dichloromethane (0.8 ml), trifluoroacetic acid (0.8 ml, 9.7 mmol) was added. After stirring for 3 hours at room temperature, the excess reagent was removed by flushing nitrogen gas to the solution, followed by addition of saturated sodium hydrogen carbonate for basification. The solution was extracted with dichloromethane (5 × 10 ml) and the combined organic layer was dried with magnesium sulfate, filtered, and evaporated under reduced pressure. The target molecule was

obtained by flash column chromatography using pentane–ethyl acetate (8 : 2) as an eluent, yielding **9** as a yellow oil (60 mg, 46%).[1]H NMR ($CDCl_3$): δ 7.93 (d, J = 5.2 Hz, 1H), 7.20–7.26 (m, 2H), 6.90–6.95 (m, 2H), 6.91 (d, J = 8.8 Hz, 1H), 6.36–6.43 (m, 2H), 6.17 (s, 1H), 4.42 (broad s, 1H), 3.66–3.70 (m, 1H), 1.14 (d, J = 6.0 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 162.0 (d, J_{CF} = 247.2 Hz), 158.4, 148.2, 146.2, 132.5, 131.5, 130.7 (d, J_{CF} = 7.6 Hz), 128.6 (d, J_{CF} = 1.5 Hz), 115.2 (d, J_{CF} = 21.1 Hz), 112.6, 106.0, 43.0, 23.0. IR (cm^{-1}): 3256, 2967, 1599, 1547, 1505. HRMS (ESI) Calcd for $C_{16}H_{18}FN_2^+$: 257.1449, found: 257.1445.

Ethyl 2-(4-fluorophenyl)-2-oxoacetate (10).[23] To a solution of fluorobenzene (1.87 ml, 20.0 mmol) in anhydrous dichloromethane (40 ml) at 0 °C, ethyl oxalylchloride (3.35 ml, 30.0 mmol) was added under nitrogen. The solution was stirred for 10 min under inert conditions followed by addition of anhydrous aluminum chloride (5.10 g, 38.2 mmol) in small portions over 15 min. After stirring for 4 hours, crushed ice (150 g) and concentrated hydrochloric acid (100 ml) were added. The solution was extracted with dichloromethane (3 × 25 ml) and the organic layer was washed with sodium hydroxide (0.1 M, 30 ml) and brine (2 × 40 ml), dried with magnesium sulfate, filtered and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **10** as a yellow oil (2.9 g, 74%). ^{1}H NMR ($CDCl_3$): δ 8.04–8.08 (m, 2H), 7.15–7.19 (m, 2H), 4.43 (q, J = 7.2 Hz, 2H), 1.41 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 184.7, 166.9 (d, J_{CF} = 259.0 Hz), 163.5, 133.1 (d, J_{CF} = 9.8 Hz), 129.1, 116.4 (d, J_{CF} = 22.1 Hz), 62.6, 14.2.

Ethyl 2-(4-fluorophenyl)-2-hydroxyacetate (11).[23] Sodium borohydride (330 mg, 8.7 mmol) was added to a solution of compound **10** (3.4 g, 17.3 mmol) in ethanol (31 ml) under inert conditions at −30 °C. After stirring for 1 hour, the solution was quenched with hydrochloric acid (10%) until the evolution of gas stopped. Water (100 ml) was added and the solution was extracted with ethyl acetate (5 × 30 ml). The organic layer was washed with brine (2 × 45 ml), dried with magnesium sulfate, filtered, and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (6 : 4) as an eluent, affording **11** as a colorless oil (3.2 g, 92%). ^{1}H NMR ($CDCl_3$): δ 7.35–7.38 (m, 2H), 6.96–7.00 (m, 2H), 5.11 (s, 1H), 4.12–4.18 (m, 3H), 1.15 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 173.4, 162.9 (d, J_{CF} = 246.4 Hz), 134.3 (d, J_{CF} = 7.8 Hz), 128.3 (d, J_{CF} = 8.4 Hz), 115.3 (d, J_{CF} = 21.4 Hz), 72.2, 62.1, 13.8.

Ethyl 2-bromo-2-(4-fluorophenyl)acetate (12b).[24] To a solution of compound **11** (1.2 g, 6.1 mmol) in chloroform (25 ml), phosphorus tribromide (0.35 ml, 3.7 mmol) was added under inert conditions at 0 °C. After stirring for 7 hours, the solution was quenched with water (12 ml) and extracted with ethyl acetate (5 × 17 ml). The organic layer was washed with brine (2 × 17 ml), dried with magnesium sulfate, filtered, and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **12b** as a yellow oil (850 mg, 54%). ^{1}H NMR ($CDCl_3$): δ 7.53–7.56 (m, 2H), 7.02–7.06 (m, 2H), 5.32 (s, 1H), 4.23 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 168.2, 163.1 (d, J_{CF} = 248.1 Hz), 131.9 (d, J_{CF} = 3.8 Hz), 130.7 (d, J_{CF} = 9.1 Hz), 115.9 (d, J_{CF} = 22.0 Hz), 62.1, 45.9, 13.8. IR (cm^{-1}): 2984, 1738, 1508, 1222, 1139, 1023, 839.

Ethyl 2-(diethylphosphoryl)-2-phenylacetate (13a).[20] Ethyl alpha-bromophenylacetate **12a** (3.0 ml, 17.1 mmol) was heated to reflux with triethyl phosphite (1.65 ml, 9.62 mmol) for 4 hours, followed by addition of water (40 ml) and extraction with ethyl acetate (5 × 80 ml). The organic layer was washed with brine (2 × 40 ml), dried with magnesium sulfate, filtered and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (1 : 1) as an eluent, yielding **13a** as a colorless oil (4.5 g, 87%). ^{1}H NMR (DMSO-d_6): δ7.51 (d, J = 7.2 Hz, 2H), 7.31–7.37 (m, 3H), 4.60 (d, J_{PH} = 24.0 Hz, 1H), 3.83–4.17 (m, 6H), 1.20 (t, J = 18.1 Hz, 6H), 1.06 (t, J = 18.1 Hz). ^{13}C NMR (DMSO-d_6): δ 167.4, 129.7 (d, J_{CP} = 6.1 Hz), 131.5, 128.2 (d, J_{CP} = 2.3 Hz), 127.6 (d, J_{CP} = 2.2 Hz), 62.6 (d, J_{CP} = 6.9 Hz), 62.4 (d, J_{CP} = 6.8 Hz), 61.2, 50.5 (d, J_{CP} = 131.2 Hz), 16.1 (d, J_{CP} = 5.3 Hz), 16.0 (d, J_{CP} = 6.0 Hz), 13.9.

Ethyl 2-(diethoxyphosphoryl)-2-(4-fluorophenyl)acetate (13b).[25] Compound **12b** (650 mg, 2.5 mmol) and triethyl phosphite (0.48 ml, 2.8 mmol) were heated under reflux for 5 hours, followed by addition of water (6 ml) and extraction with ethyl acetate (5 × 12 ml). The organic layer was washed with brine (2 × 6 ml), dried with magnesium sulfate, filtered and evaporated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethylacetate (1 : 1) as an eluent, yielding **13b** as a colorless oil (620 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 7.44–7.46 (m, 2H), 6.96–7.00 (m, 2H), 3.89–4.20 (m, 7H), 1.13–1.24 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 167.5, 162.5 (dd, J_{CF} = 246.0 Hz, J_{CP} = 3.0 Hz), 131.3 (dd, J_{CF} = 8.3 Hz, J_{CP} = 6.8 Hz), 126.7 (dd, J_{CF} = 3.0 Hz, J_{CP} = 8.3 Hz), 115.4 (dd, J_{CF} = 21.3 Hz, J_{CP} = 2.2 Hz), 63.3 (d, J_{CP} = 6.9 Hz), 63.1 (d, J_{CP} = 6.8 Hz), 51.4 (d, J_{CP} = 135.1 Hz), 16.3 (d, J_{CP} = 6.1 Hz), 16.2 (d, J_{CP} = 6.1 Hz), 14.0.

General procedure for synthesis of α,β-disubstituted α,β-unsaturated esters (procedure A)

To a solution of anhydrous lithium chloride (3 eq.) in acetonitrile, phosphonate (1 eq.) and triethylamine (3 eq.) were added. The solution was stirred for 10 min at room temperature, followed by addition of aldehyde (1.1 eq.). After stirring for 3 hours, DBU (0.3 eq.) was added and the solution was quenched with an aqueous solution of citric acid (13 ml, 10%) for 10 min and extracted with ethyl acetate (5 × 5 ml). The organic layer was washed with brine (3 × 5 ml), dried with magnesium sulfate, filtered and evaporated under reduced pressure. The target molecule was obtained by flash column chromatography using pentane–ethyl acetate as an eluent.

(*E*)-Ethyl 2-phenyl-3-(pyridin-4-yl)acrylate (14a). Lithium chloride (720 mg, 17 mmol), compound **13a** (1.7 g, 5.7 mmol), triethylamine (2.4 ml, 17 mmol), and isonicotinaldehyde (0.6 ml, 6.3 mmol) were reacted in acetonitrile (18 ml) according to the general procedure A. The mixture was purified by flash column chromatography using pentane–ethyl acetate

(1 : 1) as an eluent, yielding **14a** as a yellow oil (764 mg, 53%). ^{1}H NMR ($CDCl_3$): δ 8.35 (d, J = 5.6 Hz, 2H), 7.67 (s, 1H), 7.15–7.67 (m, 3H), 7.13–7.15 (m, 2H), 6.81 (d, J = 6.4 Hz, 2H), 4.23 (q, J = 7.2 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 167.3, 145.0, 142.3, 137.3, 137.0, 134.8, 129.6, 128.7, 128.4, 124.2, 61.7, 14.3. IR (cm^{-1}): 2981, 1709, 1592, 1245, 1175. HRMS (ESI) Calcd for $C_{16}H_{16}NO_2^+$: 254.1176, found 254.1178.

(*E*)-Ethyl 3-(pyridin-4-yl)-2-(4-fluorophenyl)acrylate (14b). Lithium chloride (115 mg, 2.7 mmol), compound **13b** (300 mg, 0.9 mmol), triethylamine (0.38 ml, 2.7 mmol), and isonicotinaldehyde (0.1 ml, 1.2 mmol) were reacted in acetonitrile (4 ml) according to the general procedure A. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **14b** as a yellow powder (156 mg, 64%). ^{1}H NMR ($CDCl_3$): δ 8.40 (d, J = 6.0 Hz, 2H), 7.69 (s, 1H), 7.11–7.15 (m, 2H), 7.00–7.04 (m, 2H), 6.85 (d, J = 6.0 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 1.27 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.8, 162.7 (d, J_{CF} = 228.9 Hz), 150.0, 142.1, 137.3, 136.2, 131.5 (d, J_{CF} = 7.5 Hz), 130.6 (d, J_{CF} = 3.8. Hz), 124.0, 115.8 (d, J_{CF} = 22.0 Hz), 61.7, 14.2. IR (cm^{-1}): 2982, 1711, 1594, 1509, 1233, 1176. HRMS (ESI) Calcd for $C_{16}H_{15}FNO_2^+$: 272.1081, found: 272.1089.

(*E*)-Ethyl 3-(2-(*tert*-butoxycarbonylamino)pyridin-4-yl)-2-phenyl-acrylate (14c). Lithium chloride (157 mg, 3.7 mmol), ethyl compound **13a** (370 mg, 1.2 mmol), triethylamine (0.52 ml, 3.7 mmol), and aldehyde **4b** (300 mg, 1.3 mmol) were reacted in acetonitrile (4 ml) according to the general procedure A. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **14c** as a yellow powder (390 mg, 38%). ^{1}H NMR ($CDCl_3$): δ 9.65 (s, 1H), 7.97 (d, J = 5.2 Hz, 1H), 7.93 (s, 1H), 7.75 (s, 1H), 7.19–7.33 (m, 3H), 7.17–7.19 (m, 2H), 6.29 (dd, J = 1.2 Hz, J = 5.2 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 1.50 (s, 9H), 1.31 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 167.0, 153.2, 152.8, 146.9, 145.1, 137.6, 137.1, 134.8, 129.7, 128.6, 128.3, 117.9, 114.2, 80.9, 61.4, 28.4, 14.3. IR (cm^{-1}): 2976, 1724, 1709, 1568, 1531, 1250, 1158. HRMS (ESI) Calcd for $C_{21}H_{25}N_2O_4^+$: 369.1809, found: 369.1816.

(*E*)-Ethyl 3-(2-(*tert*-butoxycarbonylamino)pyridin-4-yl)-2-(4-fluorophenyl) acrylate (14d). Lithium chloride (115 mg, 2.7 mmol), compound **13b** (300 mg, 0.9 mmol), triethylamine (0.38 ml, 2.7 mmol), aldehyde **4b** (230 mg, 1.1 mmol) and DBU (0.1 ml, 0.63 mmol) were reacted in acetonitrile (4 ml) according to the general procedure A. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **14d** as a white powder (184 mg, 50%). ^{1}H NMR ($CDCl_3$): δ 9.60 (broad s, 1H), 8.05 (d, J = 5.2 Hz, 1H), 7.87 (s, 1H), 7.75 (s, 1H), 7.14–7.18 (m, 2H), 7.00–7.04 (m, 2H), 6.34 (dd, J = 1.2 Hz, J = 5.2 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 1.49 (s, 9H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.9, 162.8 (d, J_{CF} = 247.9 Hz), 153.3, 152.8, 147.4, 144.8, 138.1, 136.0, 131.6 (J_{CF} = 7.6 Hz), 130.7 (d, J_{CF} = 3.0 Hz), 118.0, 115.7 (d, J = 21.1 Hz), 113.8, 81.0, 61.7, 28.4, 14.3. IR (cm^{-1}): 2975, 1722, 1707, 1567, 1530, 1511, 1224, 1156. HRMS (ESI) Calcd for $C_{21}H_{24}FN_2O_4^+$: 387.1715, found: 387.1718.

(*E*)-2-Phenyl-3-(pyridin-4-yl)acrylic acid (15a). To a solution of compound **14a** (220 mg, 0.87 mmol) in water (2 ml) and THF (8 ml), LiOH (167 mg, 7.0 mmol) was added. After stirring for 2 days, the solvent was evaporated under reduced pressure, purified by flash column chromatography using chloroform–MeOH–acetic acid (90 : 10 : 1) as an eluent. The residue acetic acid was removed by washing the solid material with hot ethanol, yielding **15a** as a white powder (179 mg, 91%). ^{1}H NMR (DMSO-d_6): δ 13.0 (bs, 1H), 8.39 (d, J = 5.2, 1H), 7.70 (s, 1H), 7.37–7.39 (m, 3H), 7.16–7.18 (m, 2H), 6.95 (d, 2H). ^{13}C NMR (DMSO-d_6): δ167.7, 149.6, 142.0, 137.7, 136.1, 135.3, 129.2, 128.5, 128.0, 123.8. IR (cm^{-1}): 2396, 1702, 1598, 1233, 1207. HRMS (ESI) Calcd for $C_{14}H_{12}NO_2^+$: 226.0863, found: 226.0870.

(*E*)-2-(4-Fluorophenyl)-3-(pyridin-4-yl)acrylic acid (15b). To a solution of compound **14b** (40 mg, 0.14 mmol) in water (2 ml) and THF (8 ml), LiOH (28 mg, 1.7 mmol) was added. After stirring for 2 days, the solvent was evaporated under reduced pressure, purified by flash column chromatography using chloroform–MeOH–acetic acid (90 : 10 : 1) as an eluent. The residue acetic acid was removed by washing the solid material with hot ethanol, yielding **15b** as a white powder (27 mg, 75%). ^{1}H NMR (DMSO-d_6): δ 13.04 (broad s, 1H), 8.43 (d, J = 4.8 Hz, 2H), 7.73 (s, 1H), 7.20–7.22 (m, 4H), 6.98 (d, J = 4.8 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 167.5, 161.8 (d, J_{CF} = 245.2 Hz), 149.7, 141.9, 136.6, 136.4, 131.6 (d, J_{CF} = 8.4 Hz), 131.5 (d, J_{CF} = 3.1 Hz), 123.8, 115.4 (d, J_{CF} = 21.4 Hz). IR (cm^{-1}): 2382, 1698, 1602, 1512, 1227, 1211. HRMS (ESI) Calcd for $C_{14}H_{11}FNO_2^+$: 244.0768, found: 244.0770.

(*E*)-Ethyl 3-(2-aminopyridin-4-yl)-2-phenyl acrylate (16a). To a solution of compound **14c** (175 mg, 0.5 mmol) in anhydrous dichloromethane (1.5 ml), trifluoroacetic acid (0.7 ml, 9.1 mmol) was added. After stirring for 7 hours at room temperature, the excess reagent was removed by flushing nitrogen gas over the solution, followed by addition of saturated sodium hydrogen carbonate for basification. The solution was extracted with dichloromethane (5 × 10 ml) and the combined organic layer was dried with magnesium sulfate, filtered and evaporated under reduced pressure. The mixture was purified by flash column chromatography using dichloromethane–methanol (9 : 1) as an eluent, yielding **16a** as a yellow powder (109 mg, 86%). ^{1}H NMR ($CDCl_3$): δ 7.85 (d, J = 5.6 Hz, 1H), 7.61 (s, 1H), 7.34–7.36 (m, 3H), 7.18–7.20 (m, 2H), 6.20 (d, J = 5.6 Hz, 1H), 6.13 (s, 1H), 4.28 (q, J = 7.2 Hz, 4H), 1.30 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 167.2, 158.6, 148.0, 144.0, 137.7, 136.5, 135.1, 129.6, 128.5, 128.2, 114.6, 109.5, 61.5, 14.3. IR (cm^{-1}): 3428, 3159, 2986, 1703, 1633, 1597, 1246, 1169. HRMS (ESI) Calcd for $C_{16}H_{17}N_2O_2^+$: 269.1285, found: 269.1279.

(*E*)-Ethyl 3-(2-aminopyridine-4-yl)-2-(4-fluorophenyl)acrylate (16b). To a solution of compound **14d** (120 mg, 0.3 mmol) in anhydrous dichloromethane (1.5 ml), trifluoroacetic acid (0.5 ml, 21 mmol) was added. After stirring for 4 hours at room temperature, the excess reagent was removed by flushing nitrogen gas to the solution, followed by addition of saturated sodium hydrogen carbonate for basification. The solution was extracted with dichloromethane (5 × 10 ml) and the combined

organic layer was dried with magnesium sulfate, filtered and evaporated under reduced pressure. The mixture was purified by flash column chromatography using dichloromethane–methanol (9 : 1) as an eluent, yielding **16b** as a yellow powder (62 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 7.87 (d, J = 5.2 Hz, 1H), 7.62 (s, 1H), 7.15–7.18 (m, 2H), 7.02–7.06 (m, 2H), 6.20 (d, J = 5.2 Hz, 1H), 6.15 (s, 1H), 4.32 (broad s, 2H), 4.27 (q, J = 7.2 Hz, 2H), 1.30 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 167.1, 162.7 (d, J_{CF} = 248.1 Hz), 158.7, 148.3, 144.0, 138.2, 135.5, 131.6 (d, J_{CF} = 8.4 Hz), 131.0 (d, J_{CF} = 3.8 Hz), 115.7 (d, J_{CF} = 22.0 Hz), 114.6, 109.3, 61.7, 14.3. IR (cm^{-1}): 3426, 3165, 2990, 1699, 1641, 1594, 1508, 1246, 1155. HRMS (ESI) Calcd for $C_{16}H_{16}FN_2O_2^+$: 287.1190, found: 287.1190.

(*E*)-Ethyl 2-(4-fluorophenyl)-3-(2-(isopropylamino)pyridin-4-yl)acrylate (16c). To a solution of compound **17** (100 mg, 0.23 mmol) in anhydrous dichloromethane (0.7 ml), trifluoroacetic acid (0.36 ml, 4.7 mmol) was added. After stirring for 3 hours at room temperature, the excess reagent was removed by flushing nitrogen gas to the solution, followed by addition of saturated sodium hydrogen carbonate for neutralization. The solution was extracted with dichloromethane (5 × 20 ml) and the combined organic layer was dried with magnesium sulfate, filtered and evaporated under reduced pressure. The target molecule was obtained by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **17** as a yellow oil (68 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 7.89 (d, J = 5.6 Hz, 1H), 7.64 (s, 1H), 7.17–7.20 (m, 2H), 7.03–7.17 (m, 2H), 6.16 (d, J = 5.6 Hz, 1H), 5.96 (s, 1H), 4.30 (broad s, 1H), 4.27 (d, J = 7.2 Hz, 2H), 3.46–3.54 (m, 1H), 1.30 (t, J = 7.2 Hz, 3H), 1.10 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 167.0, 162.5 (d, J_{CF} = 247.1 Hz), 148.4, 143.2, 138.7, 134.9, 131.5 (d, J_{CF} = 8.4 Hz), 115.5 (d, J_{CF} = 21.4 Hz), 113.3, 107.0, 61.5, 43.1, 22.9, 14.2. IR (cm^{-1}): 3254, 2969, 1710, 1598, 1508, 1232, 1158. HRMS (ESI) Calcd for $C_{19}H_{22}FN_2O_2^+$: 329.1660, found: 329.1677.

3-[2-(*tert*-Butoxycarbonyl-isopropyl-amino)-pyridin-4-yl]-2-(4-fluoro-phenyl)-acrylic acid ethyl ester (17). To a solution of compound **14d** (100 mg, 0.26 mmol) in DMF at 0 °C (1.0 ml), sodium hydride (14 mg, 0.58 mmol) was added under vigorous stirring in an inert atmosphere. After stirring for 20 min at 0 °C, 2-iodopropane (0.06 ml, 0.60 mmol) was added dropwise. The solution was stirred for 30 min at 0 °C and brought to room temperature over 4 hours, followed by addition of water (10 ml) and extraction of diethyl ether (5 × 10 ml). The organic phase was washed with diluted hydrochloric acid (0.1 M, 10 ml), saturated sodium hydrogen carbonate (10 ml) and brine (10 ml). The organic layer was dried with magnesium sulfate and concentrated under reduced pressure. The mixture was purified by flash column chromatography using pentane–ethyl acetate (9 : 1) as an eluent, yielding **17** as a yellow liquid (70 mg, 63%). ^{1}H NMR ($CDCl_3$): δ 8.27 (d, J = 5.2 Hz, 1H), 7.71 (s, 1H), 7.14–7.18 (m, 2H), 7.01–7.06 (m, 2H), 6.84 (d, J = 0.8 Hz, 1H), 6.15 (d, J = 0.8 Hz, J = 5.2 Hz, 1H), 4.39–4.42 (m, 1H), 4.28 (q, J = 7.2 Hz, 2H), 1.29–1.40 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 166.8, 162.8 (d, J_{CF} = 248.1 Hz), 154.6, 154.2, 148.5, 143.6, 137.3, 136.2, 131.6 (d, J_{CF} = 8.3 Hz), 130.7 (d, J_{CF} = 4.6 Hz), 124.5, 121.6, 115.9 (d, J_{CF} = 21.4 Hz), 80.5, 61.8, 49.5, 28.5, 21.4, 14.3. IR (cm^{-1}): 2976, 1698, 1596, 1510, 1222, 1162. HRMS (ESI) Calcd for $C_{24}H_{30}FN_2O_4^+$: 429.2184, found: 429.2204.

Biological evaluation

Cell lines. MCF7 breast cancer cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in humidified 5% CO_2.

Reagents. p38 inhibitors were diluted in DMSO (10 mM) and stored at 4 °C. Stock solutions were freshly diluted to the appropriate concentration prior to every experiment. Anisomycin was from Sigma (Sigma-Aldrich, Stockholm, Sweden). 10 mg ml^{-1} stock solutions in DMSO were stored at −20 °C. Antibodies directed against phospho-Hsp27 (Ser82), phospho-p38 (Thr180/Tyr182) and p38 were from Cell signalling Technology (*In Vitro* Sweden AB, Stockholm, Sweden). Antibodies directed against PARP were from Santa Cruz Biotechnology (Heidelberg, Germany).

Cell treatments. MCF7 cells were seeded to approximately 80% confluency and allowed to recover for at least 16 h. The cultures were then pre-treated for 30 min with the desired inhibitor concentration, followed by the addition of 10 μg ml^{-1} anisomycin for another 30 min.

Immunoblotting. Cells treated as indicated were washed with phosphate buffered saline (PBS) and lysed directly in ice-cold HEPES buffer [50 mM HEPES (pH 7.5), 10 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 10% (v/v) glycerol, 1% (v/v) Triton X-100 and a cocktail of protease inhibitors (Roche Diagnostics Scandinavia AB, Bromma, Sweden)] at 4 °C for 30 min. Lysates were clarified by centrifugation (13 000 rpm for 15 min at 4 °C) and the supernatants then either analyzed immediately or stored at −80 °C. Equivalent amounts of protein (20–50 μg) from total cell lysates were resolved by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked in blocking buffer [5% (w/v) nonfat dried milk, 150 mM NaCl, 10 mM Tris (pH 8.0) and 0.05% (v/v) Tween 20]. Proteins were detected by incubation with primary antibodies at appropriate dilutions in blocking buffer overnight at 4 °C. Blots were then incubated at room temperature with horseradish peroxidase-conjugated secondary antibody. Bands were visualized by enhanced chemiluminescence (Supersignal West Pico; Pierce, Nordic Biolabs AB, Täby, Sweden) followed by exposure to autoradiography film (General Electric Bio-Sciences, Uppsala, Sweden).

Docking of ligands

Molecular modeling was performed using the Schrödinger Package, Maestro interface version 9.0 (r211).[15] The structure of p38 in complex with the inhibitor **SB203580** (PDB 1A9U) was utilized for the study. The p38 structure and compounds **6a–b**, **7**, **9**, **14a–b**, **15a–b**, and **16a–c** were prepared and energy minimized according to the standard procedure. Docking was performed by using Glide v. 5.5 (r211)[14] with extra precision (XP) settings and standard parameters for ligand docking.

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