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BRIEF ARTICLE

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Design, Synthesis, and Biological Evaluation of Chromone-Based p38 MAP Kinase Inhibitors

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Supporting Information

ABSTRACT: 3-(4-Fluorophenyl)-2-(4-pyridyl)chromone derivatives were synthesized and evaluated as p38 MAP kinase inhibitors. Introduction of an amino group in the 2-position of the pyridyl moiety gave p38α inhibitors with IC_{50} in the low nanomolar range (e.g., **8a**, IC_{50} = 17 nm). The inhibitors (**8a** and **8e**) showed excellent selectivity profiles when tested on a panel of 62 kinases, as well as efficient inhibition (**8e**) of p38 signaling in human breast cancer cells.

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 p38 MAP kinase is highly expressed in severe invasive breast cancers and is involved in the regulation of cytokine biosynthesis (IL-1 and TNFα), which is associated with chronic inflammatory diseases such as rheumatoid arthritis, Crohn's disease, and inflammatory bowel syndrome.[2−4] Several small molecule p38α MAPK inhibitors have been shown to block the production of cytokines in vitro and in vivo, e.g., pyridylimidazole derivatives exemplified by SB203580 (Figure 1).[5−7] Furthermore, several natural occurring flavanoids, which contain a chromone framework, were recently reported as p38α inhibitors.[8] We have for a long time been working on the synthesis and functionalization of chromone derivatives[9−14] because of their designation as privileged structures in drug discovery.[15] Hence, in this study we have investigated the use of a 2,3-diarylated chromone scaffold as a starting point for designing p38α inhibitors by using molecular modeling to explore the plausible binding mode in the ATP-binding site of p38α. In this paper we report the design, synthesis, and biological evaluation of 3-(4-fluorophenyl)-2-(4-pyridyl)chromone derivatives as potential p38α inhibitors. In addition, the activity of two chromone inhibitors was tested against 62 different kinases to investigate the selectivity across the human kinome. We also demonstrate that these inhibitors can prevent anisomycin-induced p38 activation and downstream signaling in a human breast cancer cell line.

RESULTS AND DISCUSSION

Structure-Based Design. Docking of diarylated 4-fluorophenyl/pyridyl chromone derivatives into the ATP binding site of p38α (PDB code 1A9U), using the Schrödinger package (Glide XP mode), was performed to find a suitable substitution pattern on the chromone ring system and to study potential interactions with the active site to obtain inhibitory activity.[7,16,17]

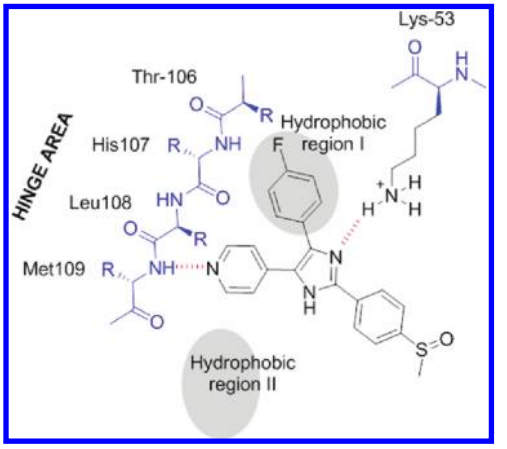

Figure 1. Pyridylimidazole derivative SB203580 acts as a p38α inhibitor by binding to the ATP binding site of p38α.

Compounds that contain the vicinal 4-fluorophenyl/pyridyl motif, e.g., SB203580 (Figure 1), are known to interact with the ATP binding site of the p38α MAP kinase.[7,18−26] A key interaction for these compounds is a hydrogen bond between the pyridin-4-yl nitrogen and the backbone NH group of Met109 in the hinge area.[7,19] Furthermore, to obtain selectivity, the 4-fluorophenyl moiety is placed in a hydrophobic pocket (I), which is guarded by a gatekeeper residue (Thr106).[7,17] It was found that 3-(4-fluorophenyl)-2-(4-pyridyl)chromone derivatives could mimic the same binding mode as SB203580 and that introduction of amino functions in the 2-position of the pyridyl moiety could provide an extra hydrogen bonding interaction to the hinge region (Figure 2).

Furthermore, the R-group on the amine could interact with a secondary hydrophobic pocket (II). However, according to

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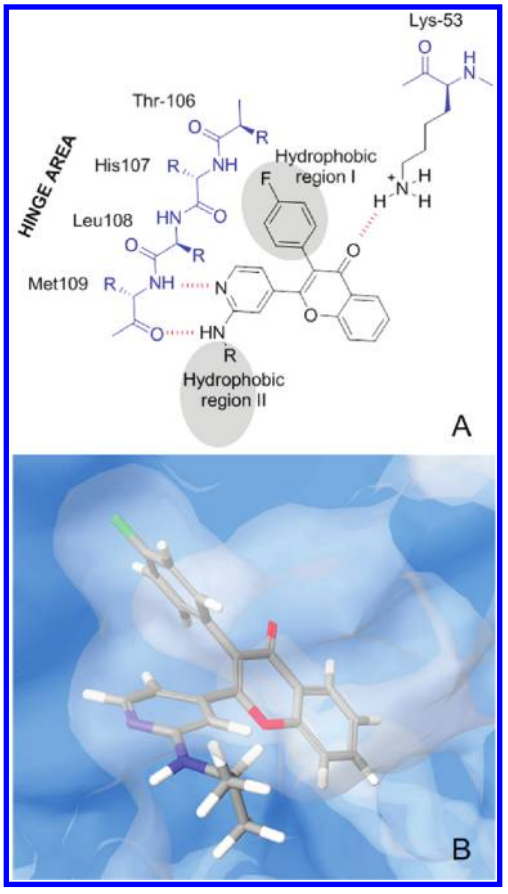


Figure 2. (A) Suggested interactions between 2-(2-amino-4-pyridyl)-3-(4-fluorophenyl)chromones and the ATP-binding site in p38α. (B) Compound **8a** docked into the ATP-binding site of p38α.

modeling studies, the choice of substituents on the amine did not seem to have any definite significance, as all the tested R-groups, with different size and properties (alkylic, cyclic and aromatic), were orientated in the same fashion into the hydrophobic area. Furthermore, the chromone carbonyl oxygen can interact via hydrogen bonding to the side chain of Lys53 in the active site. On the basis of the modeling studies, we decided to synthesize a small series of nine chromone derivatives that could be expected to bind efficiently to the ATP binding site of p38α.

Chemistry. The target compounds were synthesized from 2′-hydroxyacetophenone **1** and the appropriate acid chloride **2a,b** via esterification to yield esters **3a,b** followed by a Baker−Venkataraman rearrangement to obtain diketones **4a,b** (Scheme 1).[27−29] Thereafter, efficient acid-promoted cyclization afforded the flavone derivatives **5a,b**.[29] Initial attempts to introduce a halogen substituent (bromide or iodide) into the 3-position of **5a,b** were performed using standard conditions, e.g., bromine in pyridine, bromine in acetic acid, or *N*-halosuccinimides (NBS or NIS) in DMF. However, none of the reactions produced the desired products.[29−32] Other attempts using iodine and CAN in acetonitrile or iodine and silver trifluoroacetate in DMF were also performed but unfortunately without any product formation.[33,34] Finally, the screening for viable reaction conditions gave the 3-iodo flavone derivatives **6a,b** using in situ generated LDA and iodine in THF.[35] However, the reaction was not reproducible when using the 2-chloropyridine derivate **5b**. Instead, microwave assisted bromination of **5b** using excess NBS (5 equiv) in DMF gave high and reproducible yields of **6c** (94%). Subsequent Pd-mediated Suzuki coupling was used for the introduction of the 4-fluorophenyl moiety in the 3-position of **6a,b**. However, standard Suzuki coupling protocols gave poor and very low yields of products.[36−39] Compounds **7a** and **7b** were obtained in reasonable yields (47% and 62%, respectively) when using an oxygen-promoted ligand-free procedure with PEG-400 as solvent. This procedure has previously been reported in the literature as a useful protocol for the reaction with aryl chlorides.[40] The final target compounds **8a**−**g** were obtained via a Buchwald−Hartwig amination with various amines in the presence of palladium(II) acetate, 2-(dicyclohexylphosphino)biphenyl or 1,3-bis[diphenylphosphino)-propane], and sodium *tert*-butoxide in toluene.[41−43]

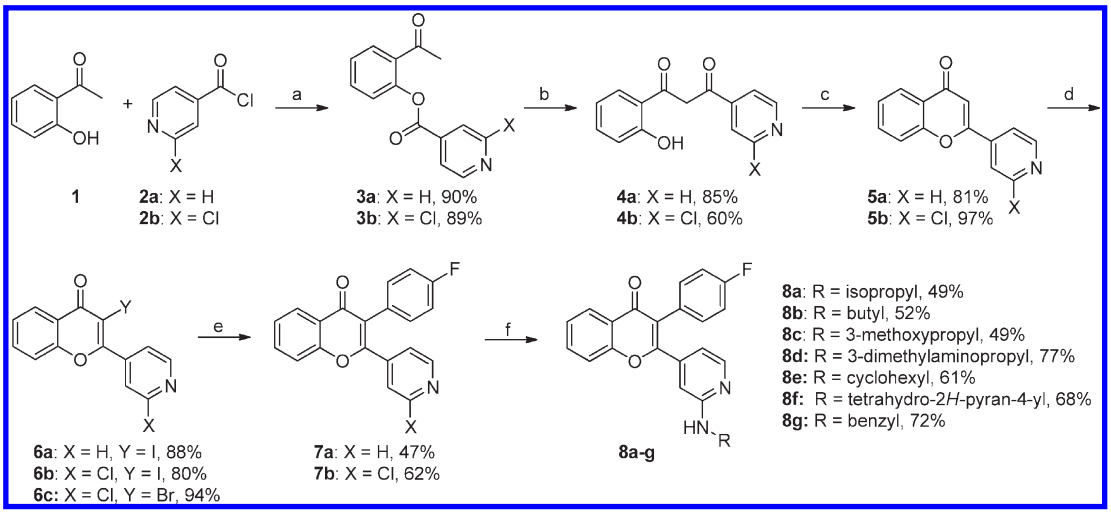
Biological Evaluation. The inhibitory potency of **7a,b** and **8a**−**g** were evaluated using a commercial radiometric p38α assay performed by Millipore KinaseProfiler.[44] The results, summarized in Table 1, showed that all compounds acted as inhibitors of the p38α MAP kinase. Compound **7a** showed moderate activity (IC_{50} = 813 nM) which decreased when replacing the hydrogen in the 2-position on the pyridin-4-yl moiety with a chlorine (**7b**, IC_{50} = 1380 nM). This negative effect on the inhibitory activity can be explained as a result of the electron withdrawing properties of the chlorine that leads to less efficient hydrogen acceptor properties of the pyridine nitrogen. Introduction of secondary amino functions in the 2-position of the pyridyl moiety, with the possibility of an extra hydrogen bonding interaction to the hinge area, gave good inhibitory activities for most of the compounds (**8a**−**c** and **8e**−**g**, IC_{50} = 17−45 nM). In contrast, introduction of a diamine, such as in **8d**, gave decreased activity (IC_{50} = 761 nM), which unexpectedly suggests that a polar group in a hydrophobic pocket is unfavorable for the inhibitory activity.

Two of the inhibitors, **8a** and **8e** (at 0.8 μM), were chosen for a selectivity screen against a panel of 62 kinases, which were selected to represent the complete human kinome and kinases closely related to the p38 MAP kinase.[44] Only three kinases, p38α, p38β, and JNK3, were strongly inhibited (0−25% remaining kinase activity) (for IC_{50}, see Supporting Information), whereas most of the other kinases in the selected panel were not at all or barely affected by the inhibitors (76−100% remaining kinase activity) (Supporting Information, Table S2 and Figure S1). These results suggest that **8a** and **8e** have a very good selectivity profile toward the p38 kinase isoforms (α and β) among other human kinases and it is reasonable to believe that the selectivity for the compounds are even higher at lower concentrations (below 0.8 μM).

Compound **8e** was also used to evaluate the efficacy in a cell-based assay with human derived MCF-7 breast cancer cells. Anisomycin-induced activation of p38 signaling, as shown for the p38 phosphorylation targets activating transcription factor 2 (ATF2) and heat shock protein 27 (HSP27), was inhibited by doses as low as 0.5 μM, and maximal inhibition was observed at 10 μM (Figure 3). Interestingly, **8e** also inhibits phosphorylation of p38 itself. Importantly, this occurs without affecting the total levels of the kinase (data not shown), ruling out an effect on protein stability of the inhibitor as the mechanism. Furthermore, a supplemental experiment showed that phosphorylation of MKK3 and MKK6, the upstream activators of p38, was unaffected by **8e** (Supporting Information, Figure S2). Thus, the loss of p38 phosphorylation, induced by **8e**, does not appear to result from the inhibition of upstream signaling.

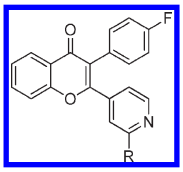
Alone, **8a** and **8e** did not significantly affect the proliferation of MCF-7 or MDA-MB436 cells (Supporting Information Figure 3).

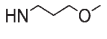
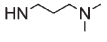
Scheme 1. Synthesis of 2-((2-Amino)-4-pyridyl)-3-(4-fluorophenyl)chromones (8a−g)[a]



[a] Reagents and conditions: (a) pyridine, 0 °C → room temp, 2 h; (b) KOH, pyridine, 100 °C, 15 min, microwave heating; (c) HCl, acetic acid, 60 °C, 30 min, microwave heating or H_2SO_4, EtOH, 100 °C, 15 min, microwave heating; (d) LDA, I_2, THF, −78 °C, 15 min or NBS, DMF, 80 °C, 60 min, microwave heating; (e) 4-fluorophenylboronic acid, K_2CO_3, $Pd(OAc)_2$, PEG-400, 60 °C, 90 min, microwave heating; (f) amine, NaO^tBu, $Pd(OAc)_2$, 2-(dicyclohexylphosphino)biphenyl or 1,3-bis(diphenylphosphino)propane, toluene, 130 °C, 90 min, microwave heating.

Table 1. Biological Activity of 7a,b and 8a−g against the p38 MAP Kinase



Compound	R	IC_{50} (nM) p38α
7a	H	813
7b	Cl	1380
8a		17
8b		21
8c		39
8d		761
8e		34
8f		45
8g		35

Furthermore, neither compound enhanced sensitivity to anisomycin or doxorubicin in MCF-7 cells. However, **8a** and **8e** appeared to suppress the sensitivity of MDA-MB436 cells, harboring a p53 mutation, to doxorubicin. This observation was unexpected, since p38 activity has previously been shown to suppress sensitivity to genotoxic agents in p53 negative cells.[45]

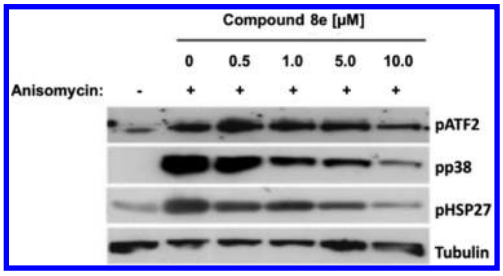


Figure 3. Compound **8e** inhibits p38 activation and signaling in human breast cancer cells. MCF-7 cells were preincubated with the indicated doses of **8e** for 30 min and then exposed to 10 μg/mL anisomycin for another 30 min. Total cell lysates were resolved by SDS−PAGE. Membranes were probed with antibodies directed against phosphorylated ATF2, p38, and HSP27. Tubulin was used as a loading control.

CONCLUSIONS

We have developed an efficient synthetic route for the preparation of 2-(2-aminopyridin-4-yl)-3-(4-fluorophenyl)chromones. Several of the synthesized compounds demonstrate a strong inhibitory activity toward the p38α MAP kinase (e.g., **8a**, IC_{50} = 17 nm). Among them, **8a** and **8e** were shown to be selective inhibitors of the p38 isoforms (α and β), and it was also revealed that **8e** inhibits p38 signaling in human cancer cells. Furthermore, molecular docking suggests that the synthesized chromone-based compounds bind into the ATP binding site of the p38α MAP kinase in a similar fashion as earlier known p38α inhibitors containing the vicinal 4-fluorophenyl/pyridyl motif, e.g., SB203580 (Figure 1). In conclusion, the syntheses of chromone-based compounds represent a promising starting point for the development of novel potent small molecule inhibitors of the p38α kinase.

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EXPERIMENTAL SECTION

General. All reagents and solvents were of analysis or synthesis grade. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX 400 spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$. Chemical shifts are reported in ppm with the solvent residual peak as internal standard ($CHCl_3$ δ 7.26, $CDCl_3$ δ 77.0) The reactions were monitored by thin-layer chromatography (TLC), on silica plated (silica gel 60 F_{254}, E. Merck) aluminum sheets, detecting spots by UV (254 and 365 nm). Flash chromatography was performed manually on Merck silica gel 60 (0.040−0.063 mm) or using a Biotage SP4 Flash instrument with prepacked columns. Solvents THF and toluene were refluxed over sodium/benzophenone and distilled into 4 Å molecular sieves. Melting points were measured in a Büchi melting point B-540 apparatus and are uncorrected. Microwave reactions were carried out in a Biotage Initiator instrument with a fixed hold time using capped vials. Purities of the assayed compounds were established by reversed-phase analytical HPLC and were found to be >95%. The HPLC analysis was performed on a Waters 2690 separations module system using an Atlantis T3, C18 (5 μm, 4.6 mm × 250 mm) column and a Waters 996 photodiode array detector operating at a wavelength between 210 and 400 nm. High-resolution mass spectrometry data (nanospray FT-ICR-MS) were obtained from BioAnser, Sahlgrenska Science Park, Gothenburg, Sweden. Elemental analyses were performed at Kolbe Mikroanalytisches Laboratorium, Mülheim and der Ruhr, Germany.

General Procedure for the Buchwald−Hartwig Cross-Coupling of Pyridyl Chlorides. Synthesis of 8b−g. The aryl chloride (1.0 equiv), $Pd(OAc)_2$ (0.05 equiv), sodium *tert*-butoxide (2.0 equiv), and 1,3-bis(diphenylphosphino)propane (0.1 equiv) were added to a dry microwave vial. The vial was evacuated and backfilled with nitrogen. Toluene (5 mL) was added to the vial, followed by the addition of the appropriate amine (5.0 equiv). The mixture was heated at 130 °C for 90 min in a microwave cavity. The mixture was diluted in dichloromethane and filtered through a layer of Celite.

2-(2-(Butylamino)pyridine-4-yl)-3-(4-fluorophenyl)chromone (8b). The title compound was synthesized according to general procedure B. The crude product was purified by column chromatography (heptane/ethyl acetate, gradient 20% → 40% ethyl acetate). Compound **7b** (100 mg, 0.28 mmol) gave **8b** (57 mg, 52%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, J = 7.3 Hz, 3H), 1.31−1.43 (m, 2H), 1.44−1.55 (m, 2H), 3.00−3.10 (m, 2H), 4.51−4.59 (m, 1H), 6.27 (s, 1H), 6.55 (dd, J = 1.4, 5.3 Hz, 1H), 7.00−7.11 (m, 2H), 7.19−7.31 (m, 2H), 7.43−7.51 (m, 1H), 7.53−7.59 (m, m, 1H), 7.74 (ddd, J = 1.7, 7.2, 8.5 Hz, 1H), 8.04 (d, J = 5.3 Hz, 1H), 8.28 (dd, J = 1.6, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 20.1, 31.4, 41.9, 106.3, 112.1, 115.4 (d, $^2J_{CF}$ = 21.6 Hz, 2C), 118.0, 122.8, 123.4, 125.4, 126.4, 128.2 (d, $^4J_{CF}$ = 3.3 Hz, 1C), 132.7 (d, $^3J_{CF}$ = 8.1 Hz, 2C), 134.1, 141.9, 148.6, 155.9, 158.7, 160.0, 162.5 (d, $^1J_{CF}$ = 248.3, 1C), 177.2. HRMS (FT-ICR-MS): $[M + H]^+$ calcd for $C_{24}H_{22}FN_2O_2$: 389.1659. Found: 389.1658.

ASSOCIATED CONTENT

Supporting Information. Synthesis of **2**−**8**, compound characterization, and biological procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

MAP, mitogen activated protein; IC_{50}, the half maximal inhibitory concentration; MAPK, mitogen activated protein kinase; ATP, adenosine triphosphate; NBS, *N*-bromosuccinimide; NIS, *N*-iodosuccinimide; DMF, *N*,*N*-dimethylformamide; CAN, cerium-(IV) ammonium nitrate; LDA, lithium diisopropylamide; THF, tetrahydrofuran; PEG-400, polyethylene glycol 400; SDS−PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ATF2, activating transcription factor; HSP27, heat shock protein 27

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