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 p38 inhibitors.8 We have for a long time been working on the synthesis and functionalization of chromone derivatives9-14 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

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
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 sizes and properties (alkyl, cyclic and aromatic), were oriented 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 other hand, the chromone carboxylic group is not able to interact with any of the amino groups in the hinge area.

**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.30 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 derivative 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 (IC50 = 813 nM) which decreased when replacing the hydrogen in the 2-position on the pyridin-4-yl moiety with a chlorine (7b, IC50 = 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, IC50 = 17–45 nM). In contrast, introduction of a diamine, such as in 8d, gave decreased activity (IC50 = 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 IC50 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).
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

**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.
**EXPERIMENTAL SECTION**

**General.** All reagents and solvents were of analysis or synthesis grade. H and 13C NMR spectra were recorded on a JEOL JNM-EX 400 spectrometer at 400 and 100 MHz, respectively, in CDCl3. Chemical shifts are reported in ppm with the solvent residual peak as internal standard (CHCl3 δ 7.26, CDCl3 δ 77.0). The reactions were monitored by thin-layer chromatography (TLC), on silica plates (silica gel 60 F254 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/tetrahydrofuran; PEG-400, polyethylene glycol 400; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ATTF2, activating transcription factor; HSP27, heat shock protein 27.

**ABBREVIATIONS USED**

MAP, mitogen activated protein; IC50, 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; ATTF2, activating transcription factor; HSP27, heat shock protein 27.

**REFERENCES**


