European Journal of Medicinal Chemistry 155 (2018) 562–578

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Identification of fused pyrimidines as interleukin 17 secretion inhibitors

Ann Christin Reiersølmoen [a], Jin Han [a, b], Eirik Sundby [c], Bård Helge Hoff [a, *]

[a] Department of Chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, NO-7491, Trondheim, Norway
[b] GlucoSet AS, Richard Birkelandsvei 2B, 7034, Trondheim, Norway
[c] Department of Material Science, Norwegian University of Science and Technology, Høgskoleringen 5, NO-7491, Norway

ARTICLE INFO

Article history:
Received 5 March 2018
Received in revised form
4 June 2018
Accepted 6 June 2018

Keywords:
Interleukin 17 secretion
Interleukin 5
Pyrrolopyrimidine
Thienopyrimidine
Furopyrimidine

ABSTRACT

Inhibiting the interleukin 17 pathway is of interest in a number of autoimmune diseases. Herein, 42 fused pyrimidines have been evaluated as interleukin 17 secretion inhibitors using a phenotypic assay with peripheral blood mononuclear cells. 7*H*-Pyrrolo [2,3-*d*]pyrimidin-4-amines having aryl groups at C-5 or C-6 were found more active than the corresponding thieno- and furopyrimidines. Low cytotoxicity was seen for the most active inhibitors. However, the pyrrolopyrimidines also inhibit interleukin 5 secretion, suggesting that selective interleukin 17 inhibitors should rather be based on furopyrimidines. Profiling towards a panel of 51 kinases and assays towards the retinoic acid receptor-related orphan receptor gamma were performed in order to identify the compounds mode of action.

1. Introduction

Interleukin 17 (IL-17) is a pro-inflammatory cytokine involved in host defence and pathogenic autoimmunity [1]. However, IL-17 is also implicated in the pathology of a number of autoimmune diseases such as arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease [2]. These pathways are highly complex and several classes of lymphocytes secrete IL-17 [1]. Thus, up-stream signalling leading to its secretion can be interrupted by a variety of mechanisms. One known regulator of IL-17 secretion is the retinoic acid receptor-related orphan receptor (ROR), and inhibitors such as GNE-6468 towards the isoform RORc [3] and TAK-828F towards RORγt [4,5] have been developed, Fig. 1. Moreover, clinical trials on psoriasis are ongoing [6–8]. The JAK/STAT pathway, which links to interleukin 23 has also been shown to affect IL-17 levels [9]. Among others, KD025 a selective Rho-associated kinase 2 (ROCK2) inhibitor acting via STAT3 has proved promising as IL-17 secretion inhibitor [10,11]. Other kinase inhibitors have also been evaluated for their effect on IL-17. Imatinib, possessing ABL, KIT, PDGFR, DDR and CSF1R kinase inhibitory properties, reduced IL-17 production in splenocytes [12], Nintedanib decrease IL-17 levels in a prostate cancer mice model [13], while Lapatinib, a dual EGFR/HER2, inhibitor reduced IL-17 levels in a rat arthritis model [14]. In contrast, Ibrutinib an irreversible BTK/ITK inhibitor was found to increase IL-17 production [15]. It has also been found that the biaryl compound 4SC-101, an inhibitor of dihydroorotate dehydrogenase, blocks IL-17 production in lymphocytes [16,17]. Interleukin 6 (IL-6) has also been found to affect IL-17 production [18]. Fig. 1 shows the structures of some of these small molecular IL-17 secretion inhibitors. Herein, we describe the synthesis of 4,5-disubstituted pyrrolopyrimidines and our initial discovery and investigation on fused pyrimidines as IL-17 secretion inhibitors.

2. Result and discussion

2.1. Design of the study

In a phenotypic screen our previously identified EGFR inhibitor **I** [19], proved to be a reasonable potent IL-17 secretion inhibitor with an IC_{50} of 4.6 μM. Thus, the primary aim was to identify more active analogues based on the pyrimidine core, Fig. 2. Structures investigated in this study includes thieno-, furo- and pyrrolopyrimidines.

* Corresponding author. Department of Chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, NO-7491, Trondheim, Norway.
E-mail addresses: ann.c.reiersolmoen@ntnu.no (A.C. Reiersølmoen), Jin.Han@ntnu.no (J. Han), Eirik.Sundby@ntnu.no (E. Sundby), bard.helge.hoff@chem.ntnu.no (B.H. Hoff).

https://doi.org/10.1016/j.ejmech.2018.06.019
0223-5234/

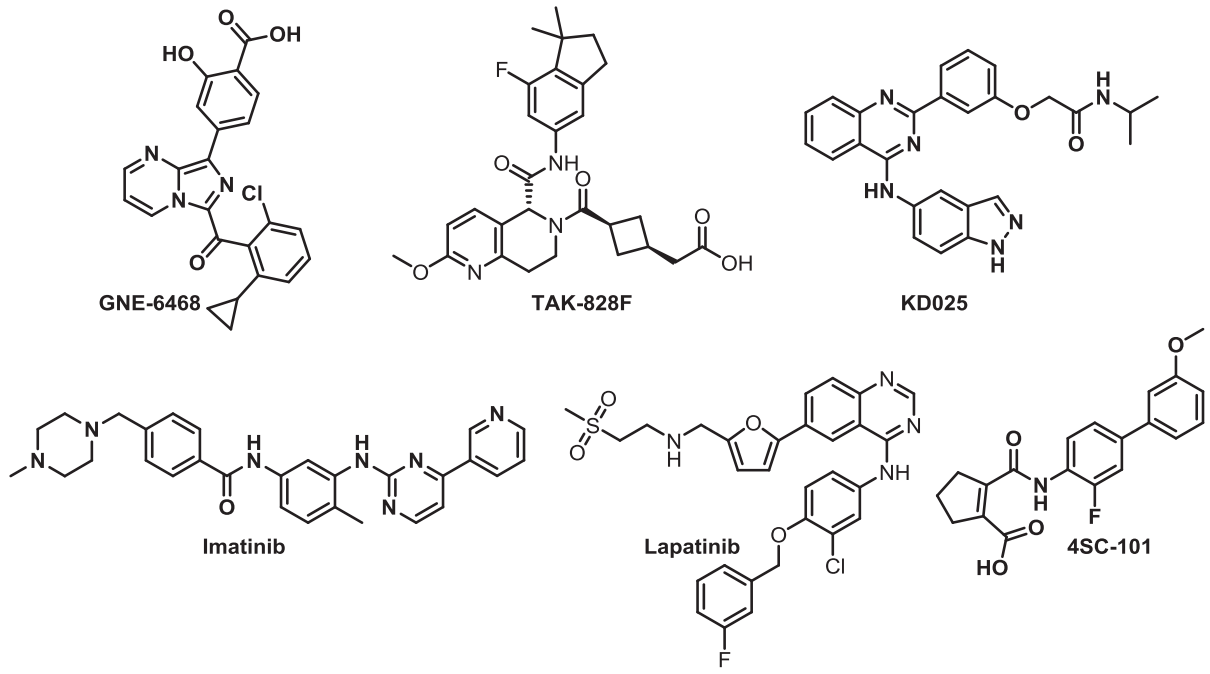


Fig. 1. Small molecular structures regulating IL-17 secretion: GNE-6468: RORc [3]; TAK-828 F: RORγt [5]; KD025: ROCK2 [10,11]; 4SC-101: dihydroorotate dehydrogenase inhibitor [16,17]; Imatinib [12] and Lapatinib [14] with unknown mechanism.

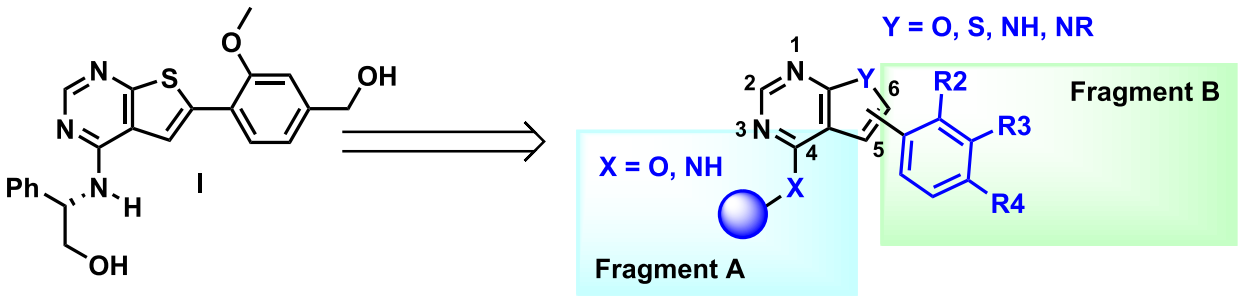


Fig. 2. Lead thienopyrimidine structure **I** and structural variations investigated.

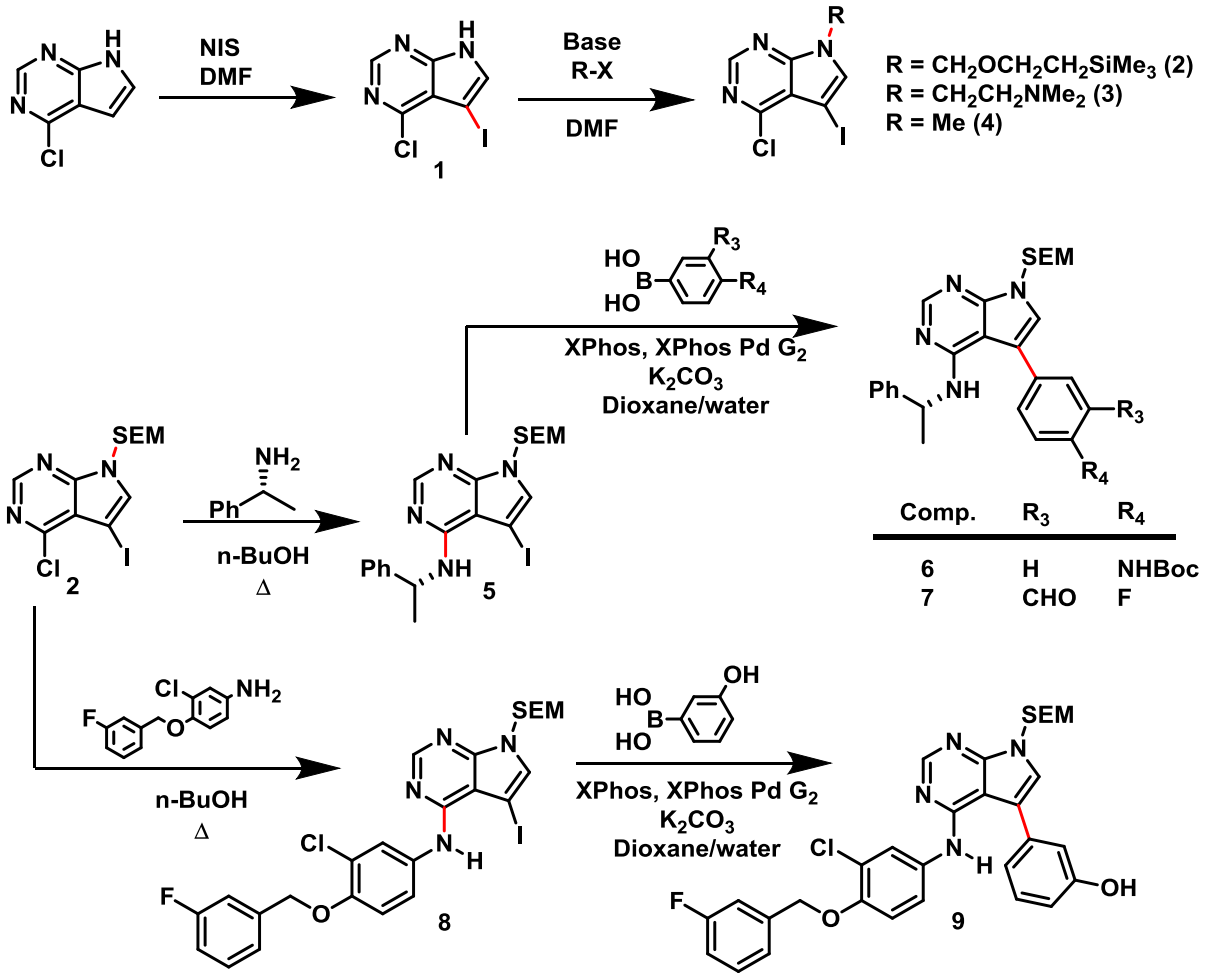
Whereas most of the molecules have the 4,6-disubstituted pattern, we also included 4,5-disubstituted pyrrolopyrimidines to extend the structure-activity relationship data.

2.2. Synthesis

The new 4,6-disubstituted thieno- furo- and 4,6-disubstituted-pyrrolopyrimidines were made mostly as previously described [19–22] (see Supplementary Material), while the preparation methods for the 4,5-disubstituted pyrrolopyrimidines are outlined in Schemes 1–3. To evaluate 5-arylated pyrrolopyrimidines as IL-17 secretion inhibitors, we targeted structures containing variations at N-7, C-4 and C-5. Firstly, 4-chloro-pyrrolo[2,3-*d*]pyrimidine was iodinated at C-5 yielding 5-iodo-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1**) in 81–85% yield. The pyrrole nitrogen was then reacted to give the 2-(trimethylsilyl)ethoxymethyl- (SEM), *N*,*N*-dimethylethylamine- and methyl derivatives **2**-**4**, Scheme 1. Amination of **2** at C-4 with (*R*)-1-phenylethylamine was then performed allowing facile formation of compound **5** in 91% yield. As a note, attempts to react the unprotected derivative **1** under similar conditions lead to decomposition and a number of unidentified products indicating limited stability of the starting material and the product. The following Suzuki cross-couplings with two different arylboronic acids gave isolated yields of 89 and 82%, respectively for the advanced intermediates **6** and **7**. Further, amination of **2** with 3-chloro-4-((3-fluorobenzyl)oxy)aniline resulted in compound **8** in 81% yield. However, in contrast to the Suzuki cross-coupling to **6** and **7**, the reaction to **9** with the XPhos system was extremely sluggish. Later experiments showed that switching to [1,1′-bis(diphenylphosphino)ferrocene]-dichloropalladium (II) as catalyst makes this conversion unproblematic.

Compound **6** was then double deprotected using trifluoroacetic acid (TFA) followed by ammonia in THF to give the aniline derivative **10** (Scheme 2), which was treated with propionyl chloride to furnish the diacylated derivative **11**. As acylated pyrroles are unstable compared to normal amides, hydrolysis at room temperature selectively led to the mono acylated derivative **12**.

The intermediate **7** was reacted under two different conditions. Firstly, a sodium borohydride reduction of the aldehyde function gave the corresponding alcohol **13**. Secondly, compound **7** was treated with *N*,*N*′-dimethylethylenediamine to give an imine derivative, which was reduced to the amine **15**. SEM-deprotection was done by a two-step procedure. The first acidic step yielded a mixture of the target product and the corresponding *N*-



Scheme 1. Synthesis of the advanced intermediates **2-4** and **6**, **7** and **9**.

hydroxymethyl derivative. Then a basic second step converts the *N*-hydroxymethyl compound to the target product. We found that the second basic step in deprotection leading to **14** and **16** was slow and required the addition of more sodium hydroxide to go to completion. The use of other bases such as ammonia or sodium bicarbonate gave inferior results.

Compound **9** was deprotected by the same protocol, yielding compound **17**. Similarly, the intermediates **3** and **4** were reacted in amination and Suzuki cross-coupling reactions to give the target structures **19**, **21**, **23**-**25**, **27** and **29** (See Scheme 3).

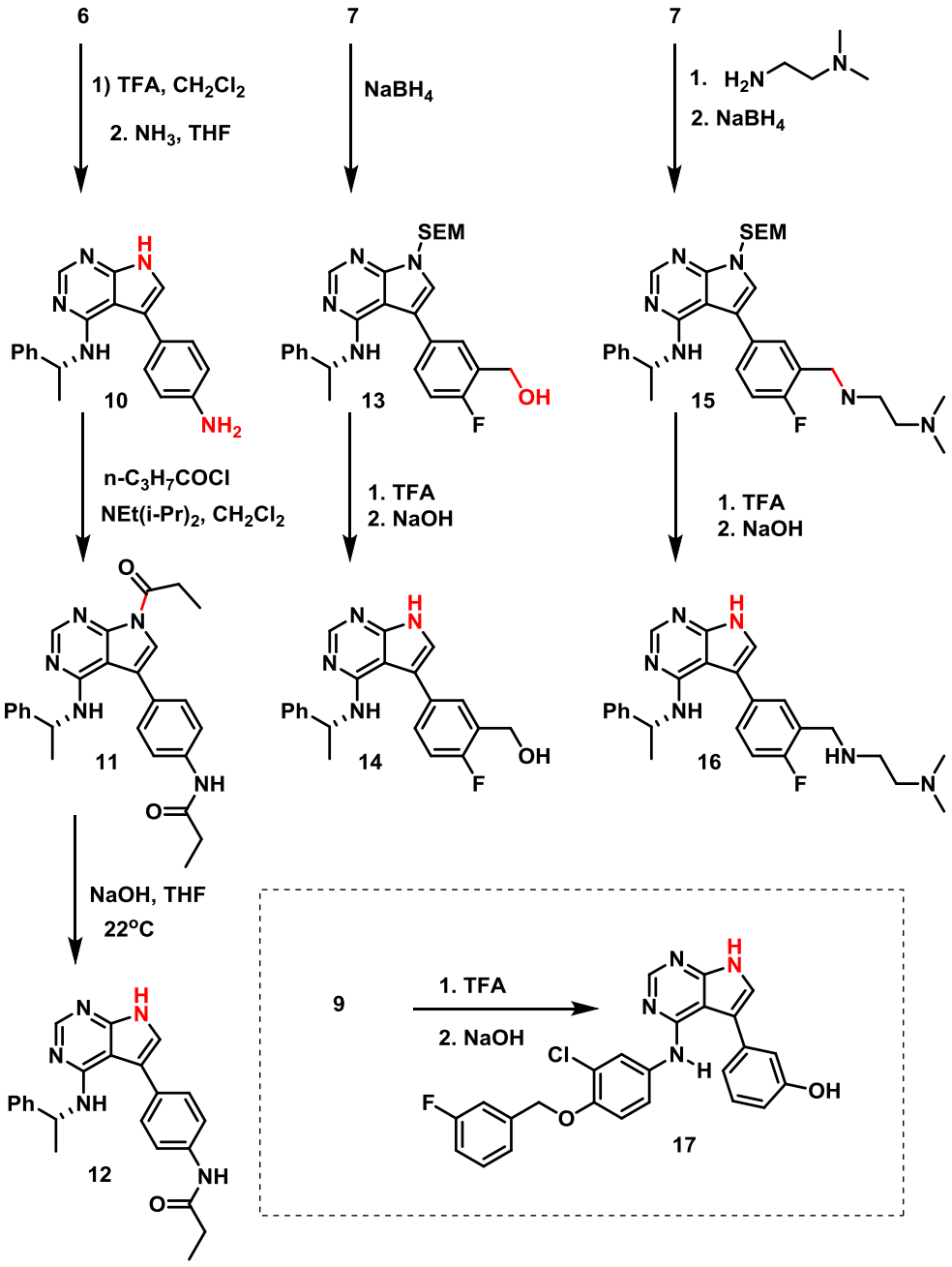
2.3. IL-17 secretion inhibitory activity and SAR

The phenotypic IL-17 secretion inhibitory assay was performed using isolated peripheral blood mononuclear cells (PBMC). Prior to addition of the test compounds the cells were stimulated by a cocktail containing anti-human CD3 antibody, anti-human CD28 antibody, and interleukin 23 (IL-23). The compounds ability to inhibit IL-17 secretion was monitored by a human IL-17 antibody. Fig. 3 shows the inhibition data for the thirteen thienopyrimidines, and nine furopyrimidines evaluated. In the thienopyrimidine series we included structures having different amines and alcohols at C-4 and both hydrophilic and lipophilic substituents in the 6-aryl ring. The most potent compounds had both a hydrogen bond donor in the 4-amino group and in the *para*-position of the 6-aryl ring (compounds **30** and **I**). Lower activity was seen for the 2,6-dimethoxyphenyl analogue **33** and the aniline based structure **32**. An even more pronounced drop in activity was noted for other substitution patterns at C-4 and C-6. Overall, the data revealed that a benzylic amine at C-4, and a hydroxymethyl substituent in the 6-aryl moiety were preferable for inhibiting IL-17 secretion.

Two furopyrimidine showed promising potency (compounds **42** and **49**). They also had polar amine groups at C-4 and bear strong structural resemblance with the most active thienopyrimidines (comp. **30** and **I**). A drop in activity was evident for structures having other amines at C-4 and more lipophilic substituents in the 6-aryl group (compounds **43**-**48** and **50**).

The IL-17 secretion inhibition data for the 4,6- and 4,5-disubstituted pyrrolopyrimidines is summarised in Fig. 4.

Overall, the pyrrolopyrimidine scaffold possessed higher intrinsic activity for inhibiting IL-17 secretion than the thieno- and furopyrimidines. In the 4,6-disubstituted series, most compounds having a hydroxymethyl group in the *para* position of the 6-aryl ring had activity. The highest inhibition was seen for **51** (IC_{50} 1.4 μM) and the aniline based **52** (IC_{50} 2.2 μM). The other aniline containing structures, **54** and **55**, had a low activity ($IC_{50} > 10$ μM). Interestingly, replacing the aromatic ring in the amine part with an adamantly moiety also resulted in appreciable activity (compound **53**). As compound **53** was assayed as its racemate, higher activity is likely to be found for one of the pure enantiomers. *Ortho*-methoxy groups were also tolerated (comp **56-59**), even in the absence of a polar group at the 6-aryl ring and in the 4-amino group.

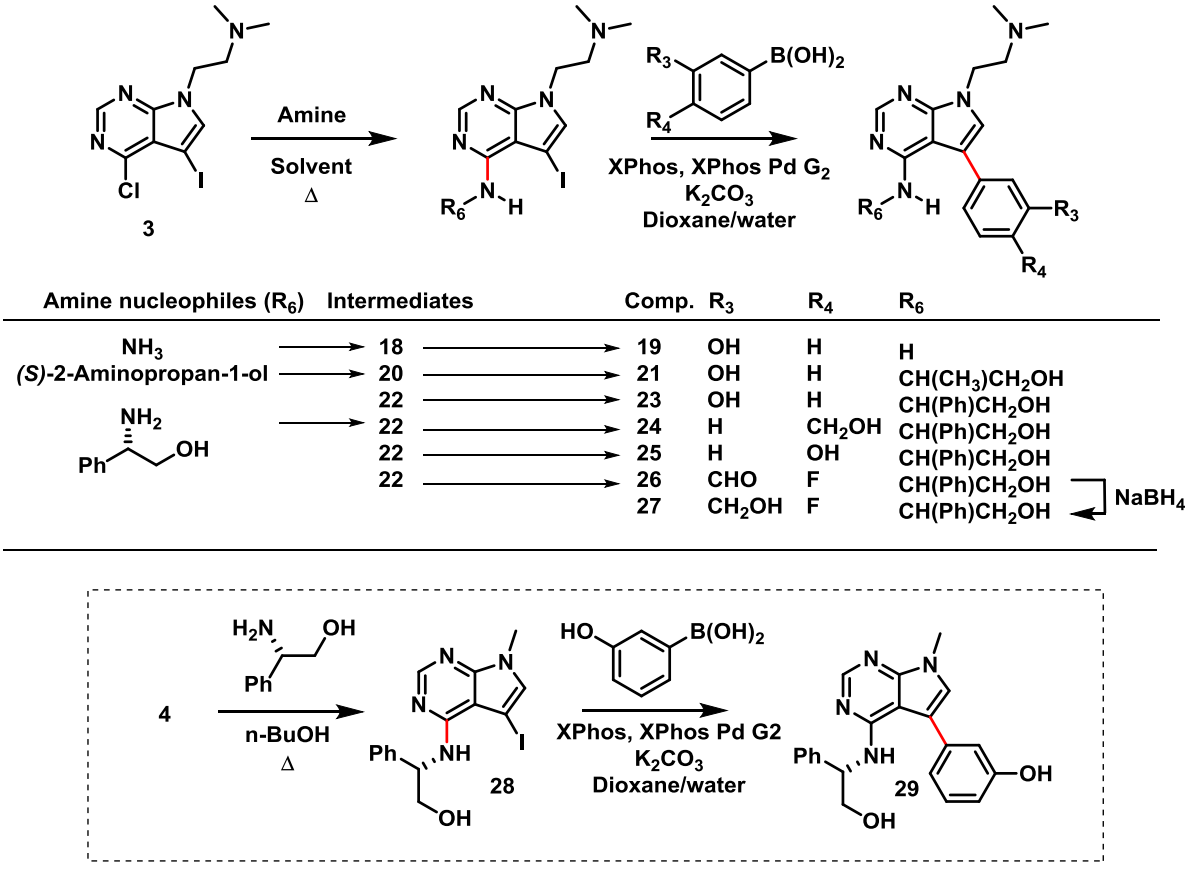


Scheme 2. Methodology used in preparation of derivatives **12, 14, 16** and **17**.

Evaluation of the 5-arylated pyrrolopyrimidines (Fig. 4, lower part) also revealed interesting SAR data. Of the compounds having an *N,N*-dimethylethylene moiety at N-7, the derivative with an unsubstituted amine at C-4, compound **19** was very active, with an IC_{50} of 1.2 μM. Compounds with other type of amines at C-4 (comp. **21** and **23**) or different substituents at the 5-aryl group (comp. **24**, **25** and **27**) were inactive. Possibly, given its different structure, derivative **19** inhibit IL-17 secretion by another mechanism than the other derivatives. When the pyrrole nitrogen was *N*-methylated or unprotected and C-4 contained a benzylamine type substituent a higher inhibitory effect was noticed (comp **12, 14** and **26**). In contrast, a bulky aniline as seen in derivative **17** was not tolerated. Fig. 5 shows degree of inhibition as a function of test concentration of the most potent compounds **12**, **19**, **49** and **51**.

Fig. 6 summaries the SAR information gathered. At C-4 of the core scaffold, amines were more suitable substituents than alcohols. Although, one *meta* substituted aniline proved active, it appers that benzylic type amines with a polar R_1 group are preferable. The pyrrolopyrimidines were found more active than furo- and thienopyrimidine. The reason is probably not due to hydrogen bonding caused by the pyrrole NH, as derivative **29** also showed activity. Generally, employment of the larger *N,N*-dimethylethylamine fragment at the pyrrole function led to loss of activity.

Concerning substituent effects in Fragment B, the aromatic ring can be placed at both C-5 and C-6. Moreover, the data indicate that polar substituents are the preferred substitution. A SAR discontinuity was also observed, as compound **19** possessing an unsubstituted amine at C-4 and an *N,N*-dimethylethylamine at the pyrrole



Scheme 3. Synthesis of the target structures **19**, **21**, **23**-**25**, **27** and **29**.

nitrogen proved active. Possibly, the mode of action of this compound is different to that of the others.

2.4. Selectivity and toxicity profile

Interleukin 5 (IL-5) stimulates B cell growth and increases immunoglobulin secretion by binding to the interleukin-5 receptor [23]. Whereas IL-5 inhibitors are of clinical relevance in treatment of asthma [24,25], it is a possible off target when developing IL-17 secretion inhibitors. To reveal if there were difference between the furo-, thieno-, and pyrrolopyrimidine scaffolds in their ability to inhibit IL-5 secretion, compounds **I**, **32**, **42**, **49**, **51**, and **57**-**59** were assayed for their ability to interfere with IL-5 secretion. The IC_{50} values shown in Fig. 7 indicates that the furo- and thienopyrimidines generally have lower activity as IL-5 secretion inhibitors than the pyrrolopyrimidines. Thus, if IL-17 selective inhibitors are to be prepared further development of furopyrimidines seems most promising.

The cytotoxicity in PBMC cells was evaluated for all the molecules. Generally, the IC_{50} for cytotoxicity was >10 μM. The pyrrolopyrimidines **12**, **19** and **51** were found more cytotoxic than **49**. Of the thienopyrimidines, the derivative **32,** having the aniline fragment from Lapatinib, was found most cytotoxic (IC_{50}: 28 μM), see Fig. 8.

In an attempt to identify mode of action, four of the more potent compounds **12**, **19**, **49** and **51** were assayed in a panel of 51 kinase at 500 nM test concentration. Firstly, the data was used to evaluate the selectivity profile of the compounds by the Gini-method [26], see Fig. 9.

As analysed by this method, compound **12** was least selective towards different kinases, while the others had rather similar average selectivity profile. A more detailed picture of the displayed selectivity is shown in Fig. 10, which highlights those kinases showing at least 20% inhibition towards any of the four compounds. Data for inhibition of the other kinases are shown in the Supplementary File.

None of the compounds inhibited kinases in the JAK-STAT pathway including JAK2, PI3K or AKT (see Supplementary information). Low inhibition was also seen towards IKBβ (Fig. 10), which can affect IL-17 expression via NFKb [9], and ROCK2 acting via STAT3 [10,11].

Compound **19** having a primary amine at C-4 proved to be an efficient RET inhibitor, but also possessing high ABL1, YES1, BTK and SRC activity. RET and Src signalling via STAT3 have been found of importance in oncogenesis [27–29], and this might explain the IL-17 secretion inhibitory properties of compound **19**. On the other hand BTK was also inhibited, which previously have been found to increase IL-17 production [15]. No activity was seen towards the HER family of receptors for this derivative. Compound **51** had lower RET activity and no BTK activity, but displayed good inhibitory properties towards ABL1, YES and SRC. Additionally, it inhibits the HER family of receptors and the colony stimulated growth factor receptor 1 kinase (CSF1R). Interestingly, CSF1R antibodies prohibiting binding of both interleukin-34 and colony stimulated growth factor 1 led to decreased expression of IL-6 [30], a known activator of IL-17 [18]. The 5-arylated pyrrolopyrimidine **12** and the furopyrimidine **49**, displayed low activity towards most of the kinases except EGFR. Although there are no documented links between

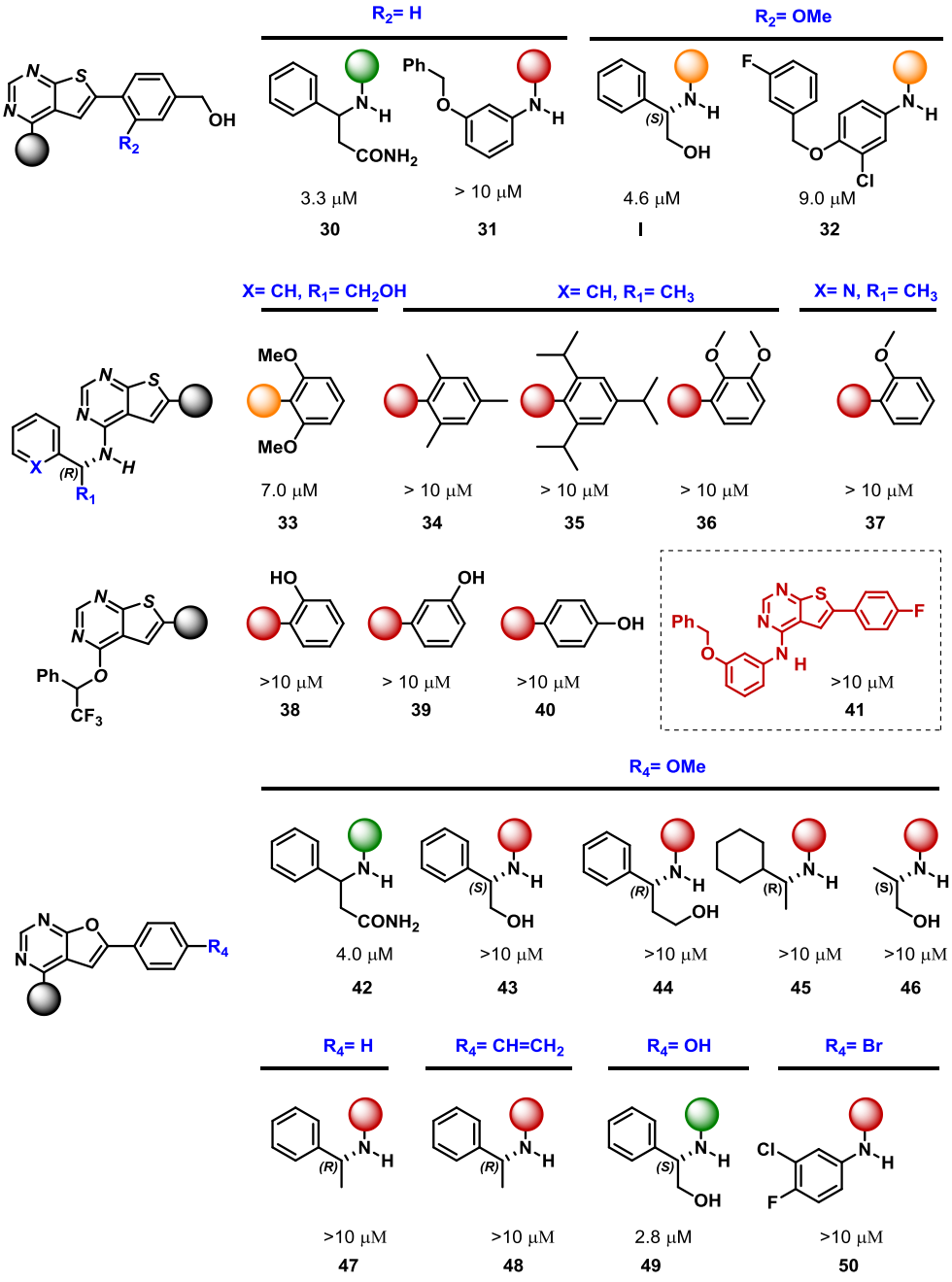


Fig. 3. Thienopyrimidine and furopyrimidine structures assayed for IL-17 secretion inhibition activity. Colour codes: green $IC_{50} \leq 4$ μM; yellow: IC_{50}: 4.1–10 μM; red: $IC_{50} > 10$ μM. For variance in IC_{50} see the Supplementary file. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

EGFR and IL-17 secretion, EGFR is known to signal via STAT3 [31–33], which could explain the activity seen for compounds **12**, **49** and **51**.

One of the known effectors of IL-17 secretion is RORγ. A RORγ assay was performed on 25 of the compounds. The assay includes a control assay, which monitors the compounds ability to interfere with the assay. Of the molecules evaluated, the pyrrolopyrimidine **12** showed some inhibition of RORγ. However, as the inhibition of the control reporter gene was within a factor 2, it can not be firmly concluded that this is the mode of action exerted by compound **12**. Thus, further investigations are needed to understand and confirm the effects of these IL-17 secretion inhibitors.

3. Conclusion

Inhibiting the release of interleukin 17 is a strategy to combat inflammations and autoimmune diseases. Employing in-house library compounds and the synthesis of new materials, we have evaluated 42 compounds for their ability to inhibit interleukin 17 secretion. The most potent derivatives had polar functionality both in the 4-amino side chain and as substituents at the 6-aryl ring. Generally, 7*H*-pyrrolo [2,3-*d*]pyrimidin-4-amines having aryl groups at C-5 or C-6 were found more active than the corresponding thieno- and furopyrimidines. Additionally, one 5-arylated compound 3-(4-amino-7-(2-(dimethylamino)ethyl)-7*H*-pyrrolo

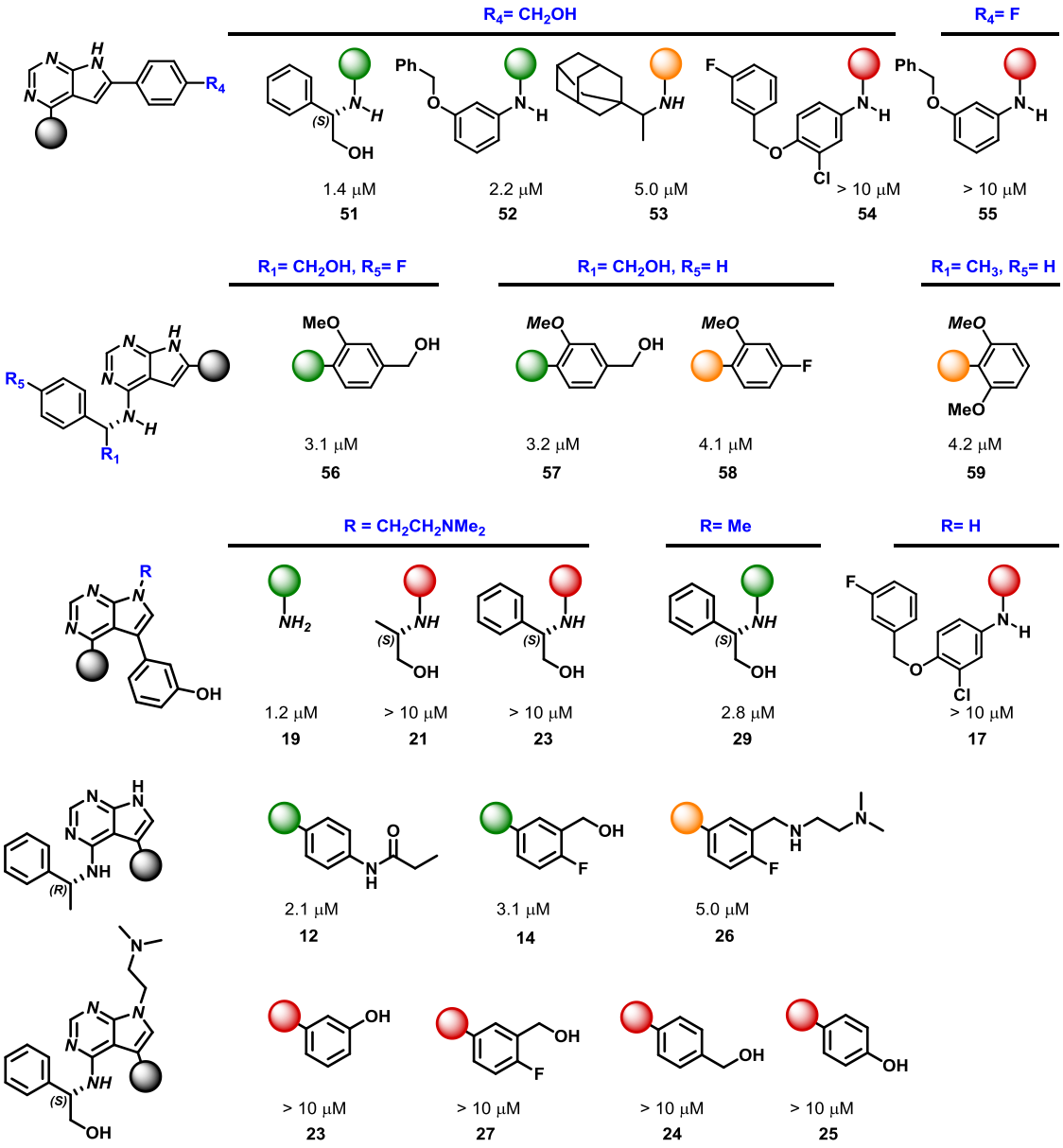


Fig. 4. IL-17 secretion inhibition testing of pyrrolopyrimidines (IC_{50} in μM). Colour codes: green $IC_{50} \leq 4$ μM; yellow: IC_{50}: 4.1–10 μM; red: $IC_{50} > 10$ μM. For variance in IC_{50} see the Supplementary file. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[2,3-*d*]pyrimidin-5-yl)phenol (**19**), with a totally different structure was found active. Thus, most likely two different mechanisms are involved. The cytotoxicity assays performed suggests that the pyrrolopyrimidine based inhibitors are more cytotoxic than the furo- and thienopyrimidines. The pyrrolopyrimidines also inhibit interleukin 5 secretion with equal or better potency. This might indicate that selective interleukin 17 inhibitors should rather be based on furo- or thienopyrimidines. Assay towards the retinoic acid receptor-related orphan receptor gamma indicated that this is not the mode of action for this compound class. In the search for other plausible mechanism, profiling of four different IL-17 secretion inhibitors towards a panel of 51 kinases was performed. The atypical compound **19** proved to be a RET kinase inhibitor, which might exert its effect on IL-17 secretion via STAT3 signalling. Although there are no obvious kinase link to IL-17 secretion for compounds **12**, **49** and **51**, their action might be due to up-stream inhibition of EGFR and effects on STAT3.

4. Experimental

4.1. General

K_2CO_3, $Pd(PPh_3)_4$, $NaBH_4$, *N*-iodosuccinimide, 2-chloro-*N*,*N*-dimethylethan-1-amine hydrochloride, all amines and arylboronic acid were from Sigma-Aldrich. 4-Chloro-7*H*-pyrrolo [2,3-*d*]pyrimidine was made in-house [34]. Compound **I** [19]; **33** and **36** [35]; **34-35** [36]; **37** [20]; **38-40** [37]; **42-49** [21]; **51** and **56-58** [34]; and **59** [22], were prepared previously in the group. Silica-gel column chromatography was performed using silica-gel 60 A from Fluka, pore size 40–63 μm. Celite 545 from Fluka was also used.

4.2. Analyses

^1H and ^{13}C NMR spectra were recorded with Bruker Avance 600 and 400 spectrometers, operating at 600/400 MHz and 150/

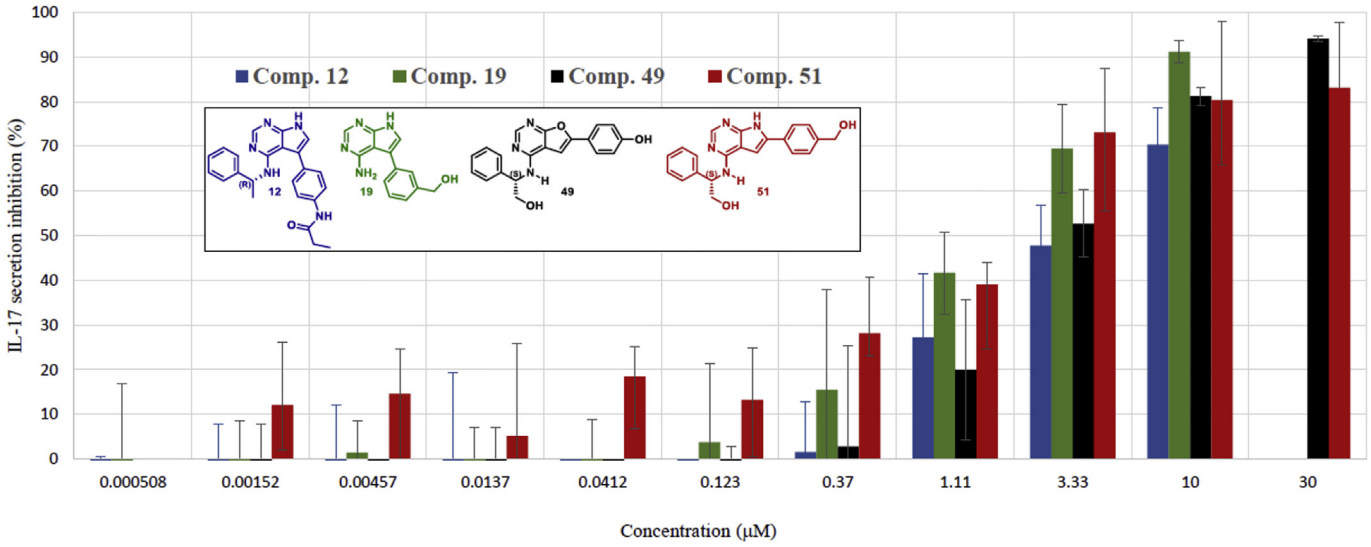


Fig. 5. IL-17 secretion inhibition as a function of inhibitor concentration. Dark blue: comp. **12** (6 × 10 point titration), green: comp. **19** (6 × 10 point titration), black. Comp. **49** (4 × 10 point titration), red: comp. **51** (3 × 10 point titration). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

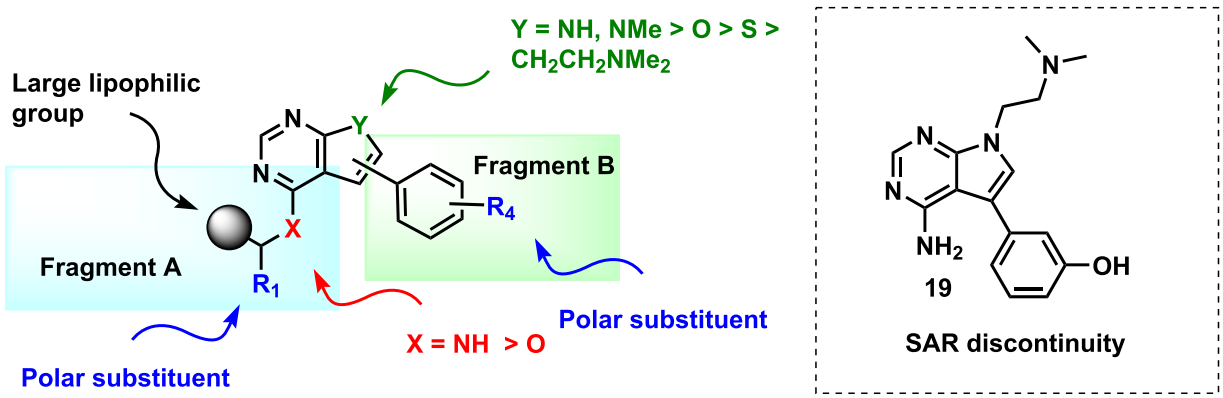


Fig. 6. SAR on IL-17 secretion inhibitors.

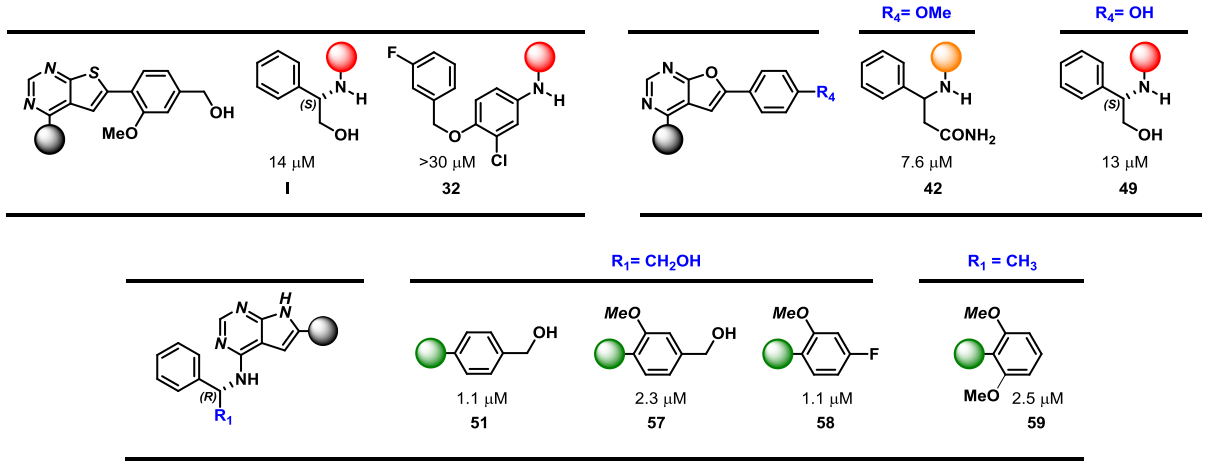


Fig. 7. IL-5 secretion inhibition (IC_{50} in μM) for different scaffolds. Colour codes: green $IC_{50} \leq 4$ μM; yellow: IC_{50}: 4.1–10 μM; red: $IC_{50} > 10$ μM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

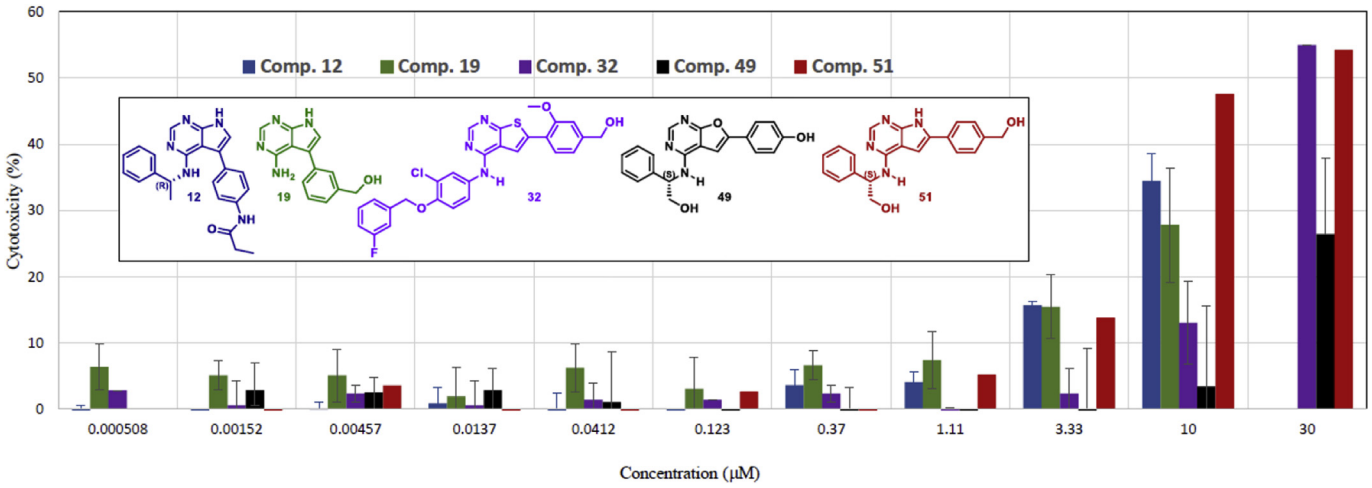


Fig. 8. Examples of cytotoxicity assay for compounds **12**, **19**, **32, 49** and **51**.

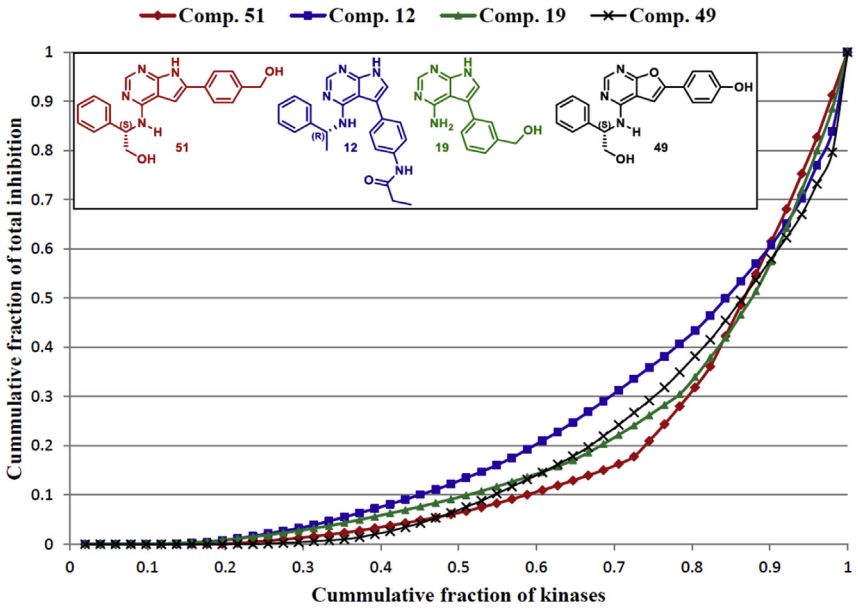


Fig. 9. Gini-plot of compounds **12**, **19**, **49** and **51**, Gini-coefficients: comp **51**: 0.648; comp**. 12**: 0.556; comp. **19**: 0.619; comp. **49**: 0.663.

100 MHz for ^{1}H and ^{13}C, respectively. ^{19}F NMR was performed on a Bruker Avance 500 operating at 564 MHz. For ^{1}H and ^{13}C NMR chemical shifts are in ppm rel. to DMSO-d_6, while for ^{19}F NMR the shift values are relative to hexafluorobenzene. Coupling constants are in hertz. HPLC (Agilent 110-Series) with a G1379A degasser, G1311A Quatpump, G1313A ALS autosampler and a G1315D Agilent detector (230 nm) was used to determine the purity of the synthesised compounds. Conditions: Poroshell C18 (100 × 4.6 mm) column, flow rate 0.8 mL/min, elution starting with water/CH_3CN (90/10), 5 min isocratic elution, then linear gradient elution for 35 min ending at CH_3CN/water (100/0). The software used with the HPLC was Agilent ChemStation. Accurate mass determination (ESI) was performed on an Agilent G1969 TOF MS instrument equipped with a dual electrospray ion source. Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters. Samples were ionized by the use of an ASAP probe, no chromatography separation was used before the mass analysis. FTIR spectra were recorded on a Thermo Nicolet Avatar 330 infrared spectrophotometer. All melting points are uncorrected and measured by a Stuart automatic melting point SMP40 apparatus. Optical rotation was measured with a PerkinElmer Instruments Model 341 Polarimeter.

4.3. Synthesis

4.3.1. General procedure for Suzuki-cross coupling

To a mixture of the selected arylboronic acid (0.610 mmol, 1.2 equiv.), K_2CO_3 (1.53 mmol, 3 equiv.), XPhos (0.0250 mmol, 0.05 equiv.), 2nd generation XPhos pre-catalyst (0.0250 mmol, 0.05 equiv.), and the selected 4-amino-5-iodo-7*H*-pyrrolo [2,3-*d*]pyrimidine (0.511 mmol, 1 equiv.) in 1,4-dioxane (3 mL) was added water (3 mL) under a nitrogen atmosphere. The reaction mixture was stirred at 100 °C until complete conversion. The solvent was removed before water (15 mL) and EtOAc (25 mL) were added, the

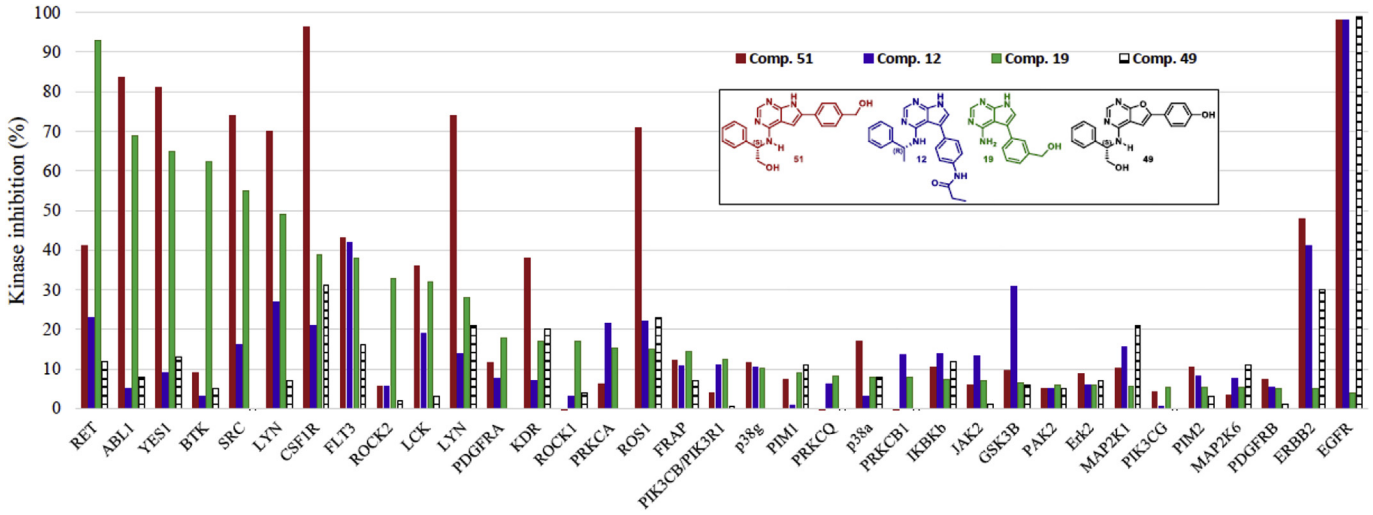


Fig. 10. Degree of inhibition for **12**, **19, 49** and **51** towards 19 of the kinases sorted by the activity displayed by compound **19**.

phases were separated and the water phase was extracted with more EtOAc (3 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated. Purification was as stated for each individual compound.

*4.3.2. 5-Iodo-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (**1**) [38,39]*

N,N-Dimethylformamide (DMF) (47 mL) was added to a mixture of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4.60 g, 29.8 mmol) and *N*-iodosuccinimide (6.82 g, 30.3 mmol) under a nitrogen atmosphere. The solution was stirred at 22 °C for 2.5 h before the mixture was poured into ice water (150 mL). The precipitate was filtered, washed with water and *n*-pentane and dried to give 7.05 g (25.2 mmol, 85%) of **1** as a pale brown powder; mp. 187–188 °C (dec.) (lit [39]. 196–199 °C), $R_f = 0.16$ (*n*-pentane/EtOAc, 10/1). ^{1}H NMR (400 MHz, DMSO-d_6): 12.94 (br s, 1H), 8.59 (s, 1H), 7.94 (d, $J = 2.5$, 1H). The spectroscopic data corresponded well with that reported previously [39].

*4.3.3. 4-Chloro-5-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**2**) [40]*

DMF (66 mL) was added to a mixture of compound **1** (2.00 g, 7.15 mmol) and sodium hydride (243 mg, 10.1 mmol) under nitrogen atmosphere. The reaction mixture was cooled to 0 °C and stirred for 30 min. 2-(Trimethylsilyl)-ethoxymethyl chloride (1.70 mL, 9.58 mmol) was added dropwise over 45 min and stirred at 22 °C for 1.5 h. Water (150 mL) and EtOAc (150 mL) was added to the reaction mixture, the phases were separated, and the water phase was extracted with more EtOAc (2 × 150 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica-gel column chromatography (*n*-pentane/EtOAc, 1/1, $R_f = 0.78$) to give 2.74 g (10.0 mmol, 86%) of a pale yellow powder; mp. 98–104 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 8.69 (s, 1H), 8.13 (s, 1H), 5.60 (s, 2H), 3.51 (t, J = 8.1, 2H), 0.82 (t, J = 8.1, 2H), −0.10 (s, 9H). The spectroscopic data corresponded with that reported previously [40].

*4.3.4. 2-(4-Chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-N,N-dimethylethan-1-amine (**3**)*

Compound **1** (1.48 g, 5.30 mmol), Cs_2CO_3 (3.67 g, 11.3 mmol) and 2-chloro-*N,N*-dimethylethan-1-amine hydrochloride (0.87 g, 6.05 mmol) were dissolved in DMF (50 mL) under a nitrogen atmosphere. The reaction mixture was stirred for 14 h at 35 °C, quenched in saturated $NaHCO_3$-solution (100 mL) and extracted with EtOAc (7 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was isolated by silica-gel column chromatography (CH_2Cl_2/MeOH, 93/7, $R_f = 0.26$). Drying gave 1.45 g (4.13 mmol, 78%) of **3** as a white powder; mp. 80–81 °C; ^{1}H NMR (600 MHz, DMSO-d_6): 8.63 (s, 1H), 8.02 (s, 1H), 4.35 (t, J = 6.3, 2H), 2.66 (t, J = 6.3, 2H), 2.15 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 150.8, 150.5, 150.2, 136.7, 116.0, 58.0, 51.0, 45.0 (2C), 42.4; IR (neat, cm^{-1}): 2940 (w, br), 2764 (w, br), 1584 (s), 1538 (m), 1444 (m), 1335 (s), 1184 (s), 945 (s), 774 (m); HRMS (APCI/ASAP+, *m/z*): found 350.9874 (calcd. $C_{10}H_{13}N_4ClI$, 350.9873, $[M+H]^+$).

*4.3.5. 4-Chloro-5-iodo-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (**4**) [41]*

Compound **1** (151 mg, 0.540 mmol) and Cs_2CO_3 (209 mg, 0.641 mmol) were dissolved in DMF (3 mL) under a nitrogen atmosphere. Methyl iodide (0.54 mL, 1.07 mmol) was added over 30 min. The reaction mixture was stirred for 3 h at 22 °C at which time the reaction mixture was quenched in saturated $NaHCO_3$-solution (30 mL). The aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was isolated by silica-gel column chromatography (CH_2Cl_2/MeOH, 49/1, $R_f = 0.60$). Drying gave 128 mg (0.436 mmol, 81%) of **4** as a white powder, mp. 185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 8.65 (s, 1H), 7.98 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 151.3, 151.2, 150.8, 137.9, 116.5, 51.3, 32.0; HRMS (APCI/ASAP+, *m/z*): found 293.9295 (calcd. $C_7H_6N_4ClI$, 293.9295, $[M+H]^+$). The spectroscopic data corresponded well with that reported previously [41].

*4.3.6. (R)-5-Iodo-N-(1-phenylethyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**5**)*

(*R*)-1-Phenylethylamine (0.93 mL, 7.22 mmol) and *n*-BuOH (20 mL) were added to compound **2** (1.03 g, 2.51 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at reflux for 3.5 h. The reaction mixture was then cooled to 22 °C and concentrated. Water (50 mL) and EtOAc (75 mL) were added and the water phase was extracted with more EtOAc (3 × 100 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (*n*-pentane/EtOAc, 3/1, $R_f = 0.59$) to give **5** as a

yellow oil, 1.14 g (2.30 mmol, 91%); $[\alpha]_D^{20} = -73.4$ (*c* 1.01, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6): 8.19 (s, 1H), 7.60 (s, 1H), 7.44–7.42 (m, 2H), 7.35–7.33 (m, 2H), 7.27–7.23 (m, 1H), 6.53–6.51 (m, 1H), 5.46 (s, 2H) 5.50–5.43 (m, 1H), 3.38 (t, J = 8.1, 2H), 1.57 (d, J = 6.9, 3H), 0.80 (t, J = 8.1, 2H), −0.10 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6):155.9, 153.0, 150.7, 145.1, 130.7, 129.5 (2C), 127.9, 126.7 (2C), 103.5 73.1, 66.5, 51.9, 50.2, 23.8, 18.0, −0.5 (3C); IR (neat, cm^{-1}): 3405 (w), 2947 (w, br), 1593 (s), 1547 (s), 1466 (m), 1075 (s), 928 (m), 831 (s), 694 (s), 651 (m); HRMS (APCI/ASAP+, *m/z*): found 495.1075, (calcd $C_{20}H_{28}N_4OSiI$, 495.1077, $[M+H]^+$).

4.3.7. tert-Butyl-(R)-(4-(4-((1-phenylethyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d] pyrimidin-5-yl) phenyl)carbamate (**6**)

The synthesis was performed as described in Section 4.3.1, and starting with compound **5** (350 mg, 0.707 mmol) and 4-(*N*-Boc-amino)phenylboronic acid (203 mg, 0.856 mmol). The reaction time was 10 min. Purification by silica-gel column chromatography (*n*-pentane/EtOAc, 3/1, $R_f = 0.67$) gave 352 mg (0.629 mmol, 89%) of a white solid; $[\alpha]_D^{20} = -124.7$ (c 1.08, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6): 9.49 (s, 1H), 8.20 (s, 1H), 7.59–7.57 (ap.d, 2H), 7.40–7.31 (ap.d, 2H), 7.37 (s, 1H), 5.76 (s, 2H), 7.31–7.26 (m, 4H), 7.23–7.19 (m, 1H), 5.57–5.56 (m, 1H), 5.39–5.32 (m, 1H), 3.54 (t, J = 8.1, 2H), 1.50 (s, 9H), 1.39 (d, J = 6.9, 3H), 0.83 (t, J = 8.1, 2H), −0.08 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): 155.3, 152.7, 151.8, 150.3, 144.3, 138.8, 128.9 (2C), 128.4 (2C), 127.8, 126.8, 125.7 (2C), 123.0, 118.5 (2C), 115.6, 100.4, 79.2, 72.2, 65.5, 49.5, 28.1 (3C), 23.0, 17.1, −1.4 (3C); HRMS (APCI/ASAP+, *m/z*): found 560.3057, (calcd $C_{31}H_{42}N_5O_3Si$, 560.3057, $[M+H]^+$).

4.3.8. (R)-2-Fluoro-5-(4-((1-phenylethyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl) benzaldehyde (**7**)

The synthesis was performed as described in Section 4.3.1 starting with compound **5** (399 mg, 0.808 mmol) and 4-fluoro-3-formylphenylboronic acid (163 mg, 0.969 mmol). The reaction time was 2 h. Purification by silica-gel column chromatography (*n*-pentane/EtOAc, 3/1, $R_f = 0.53$) and drying gave the product **7** as a yellow oil, 325 mg (0.662 mmol, 82%), $[\alpha]_D^{20} = -143.2$ (c 1.02, DMSO); ^1H NMR (600 MHz, DMSO-d_6): 10.27 (s, 1H), 8.23 (s, 1H), 7.93–7.92 (m, 1H), 7.88–7.86 (m, 1H), 7.58 (s, 1H), 7.54–7.51 (m, 1H), 7.34–7.32 (m, 2H), 7.28–7.26 (m, 2H), 7.21–7.18 (m, 1H), 5.83–5.82 (m, 1H), 5.55 (s, 2H), 5.42–5.37 (m, 1H), 3.55 (t, J = 8.1, 2H), 1.41 (d, J = 6.9, 3H), 0.83 (t, J = 8.1, 2H), −0.08 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): 187.8 (d, J = 4.4), 162.4 (d, J = 257.9), 155.3, 151.9, 150.8, 144.6, 136.3 (d, J = 9.0), 131.4 (d, J = 3.0), 128.7, 128.2 (2C), 126.6, 126.0 (2C), 124.2, 123.8 (d, J = 8.8), 117.4 (d, J = 20.6), 113.9, 100.0, 72.3, 65.6, 49.7, 22.5, 17.1, −1.4 (3C); ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −125.5 (s, dec.); IR (neat, cm^{-1}): 3434 (w), 2956 (w, br), 1699 (m), 1558 (m), 1465 (m), 1252 (m), 1184 (m), 1075 (m), 831 (s), 696 (s); HRMS (APCI/ASAP+, *m/z*): found 491.2272, (calcd $C_{27}H_{32}N_4O_2FSi$, $[M+H]^+$).

4.3.9. N-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-5-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**8**)

3-Chloro-4-((3-fluorobenzyl)oxy)aniline (333 mg, 1.32 mmol) and *n*-BuOH (10 mL) were added to compound **2** (257 mg, 0.630 mmol) under a nitrogen atmosphere. The reaction mixture was refluxed for 2.5 h. Water (15 mL) and EtOAc (15 mL) were added and phase separated. The water phase was extracted with more EtOAc (2 × 15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (*n*-pentane/EtOAc, 3/1, $R_f = 0.65$) and dried to give a light brown oil, 318 mg (0.509 mmol, 81%); ^1H NMR (600 MHz, DMSO-d_6): 8.36 (s, 1H), 8.20 (s, 1H), 7.973–7.966 (m, 1H), 7.74 (s, 1H), 7.56–7.53 (m, 1H), 7.49–7.44 (m, 1H), 7.33–7.29 (m, 2H), 7.25–7.23 (m, 1H), 7.20–7.15 (m, 1H), 5.51 (s, 2H), 5.24 (s, 2H), 3.51 (t, J = 8.1, 2H), 0.83 (t, J = 8.1, 2H), −0.08 (s, 9H); ^{13}C NMR (150 MHz, DMSO- d_6): 162.2 (d, J = 243.7), 153.4, 151.6, 150.3, 149.3, 139.7 (d, J = 7.9), 132.9, 131.2, 130.6 (d, J = 8.9), 123.3 (d, J = 2.2), 122.7, 121.3, 120.9, 114.7 (d, J = 20.6), 114.6, 114.0 (d, J = 21.9), 103.7, 72.2, 69.4 (d, J = 1.5), 65.7, 51.0, 17.1, −1.4 (3C); ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −115.4 (s, dec.); IR (neat, cm^{-1}): 3377 (w), 2940 (w, br), 1605 (s), 1564 (s), 1501 (s), 1465 (s), 1247 (m), 1081 (s), 831 (s); HRMS (APCI/ASAP+, *m/z*): found 625.0690, (calcd $C_{25}H_{28}N_4O_2FClSi$, 625.0699, $[M+H]^+$).

4.3.10. 3-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d] pyrimidin-5-yl)phenol (**9**)

Compound **9** was made as described in Section 4.3.1 starting with compound **8** (111 mg, 0.178 mmol) and (3-hydroxyphenyl) boronic acid (156 mg, 0.250 mmol). The reaction mixture was stirred at 100 °C for 67 h without going to completion. Silica-gel column chromatography (*n*-pentane/EtOAc, 13/7, $R_f = 0.65$) and drying gave the product **9** as a clear oil, 51 mg (0.087 mmol, 45%); ^1H NMR (600 MHz, DMSO-d_6): 9.66 (s, 1H), 8.40 (s, 1H), 7.89 (d, J = 2.7, 1H), 7.57 (s, 1H), 7.56 (s, 1H), 7.47–7.44 (m, 1H), 7.34–7.27 (m, 4H), 7.19–7.15 (m, 2H), 6.99–6.97 (m, 1H), 6.95–6.94 (m, 1H), 6.82–6.80 (m, 1H), 5.59 (s, 2H), 5.21 (s, 2H), 3.58 (t, J = 8.1, 2H), 0.85 (t, J = 8.1, 2H), −0.07 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): 162.2 (d, J = 245.2),157.9, 153.5, 151.3, 150.9, 148.9, 139.7 (d, J = 7.8), 135.4, 133.5, 130.5 (d, J = 8.2), 130.2, 124.5, 123.3 (d, J = 3.3), 122.0, 121.3, 120.1, 119.2, 115.9, 115.3, 114.8, 114.7 (d, J = 22.0), 114.3, 114.0 (d, J = 22.0), 101.1, 72.3, 69.4, 65.7, 17.2, −1.4 (3C); ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −115.4 (s, dec.); IR (neat, cm^{-1}): 3362 (w), 2914 (w, br), 1607 (m), 1563 (m), 1496 (m), 1470 (m), 1446 (m), 1289 (m), 1216 (m), 1082 (s), 1015 (m), 841 (m), 784 (s), 681 (m), 587 (m); HRMS (APCI/ASAP+, *m/z*): found 590.1907, (calcd $C_{31}H_{32}N_4O_3FSiCl$, 590.1916, $M^{\cdot+}$).

4.3.11. (R)-5-(4-Aminophenyl)-N-(1-phenylethyl)-7H-pyrrolo-[2,3-d]pyrimidin-4-amine (**10**)

Compound **6** (334 mg, 0.597 mmol) was double deprotected by mixing with CH_2Cl_2 (10 mL) and TFA (3 mL, 39.3 mmol) under a nitrogen atmosphere. The mixture was stirred at 50 °C for 3 h before the solvent was removed and MeOH (15 mL) and NH_3-solution (15 mL, 25%) was added. The reaction mixture was stirred 18 h before the solvent was removed. Work-up and purification by silica-gel column chromatography (EtOAc, $R_f = 0.20$) gave 167 mg (0.503 mmol, 86%) of a yellow powder, mp. 138–140 °C; $[\alpha]_D^{20} = -198.0$ (c 0.99, $CHCl_3$). ^1H NMR (400 MHz, DMSO-d_6): 11.63 (s, 1H), 8.10 (s, 1H), 7.32–7.19 (m, 5H), 7.16–7.13 (ap.d, 2H), 7.04–7.05 (m, 1H), 6.68–6.66 (ap.d, 2H), 5.51–5.49 (m, 1H), 5.39–5.32 (m, 1H), 5.21 (s, 2H), 1.37 (t, J = 6.9, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 155.3, 151.3, 150.3, 147.9, 144.5, 129.5 (2C), 128.4 (2C), 126.7, 125.7 (2C), 121.9, 118.8, 115.9, 114.1 (2C), 100.5, 49.1, 23.3; IR (neat, cm^{-1}): 3403 (w), 3112 (w, br), 1584 (s), 1465 (m); HRMS (APCI/ASAP+, *m/z*): found 330.1714, (calcd $C_{20}H_{20}N_5$, 330.1719, $[M+H]^+$).

4.3.12. (R)-N-(4-(4-((1-Phenylethyl)amino)-7-propionyl-7H-pyrrolo-[2,3-d]pyrimidin-5-yl)phenyl)propionamide (**11**)

Compound **10** (155 mg, 0.470 mmol) was dissolved in CH_2Cl_2 (3 mL) and *N*,*N*-diisopropylethylamine (0.100 ml, 0.560 mmol) and cooled to 0 °C. Propinoyl chloride (0.0450 mL, 0.520 mmol) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred for 2 h before the reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL). EtOAc (50 mL) was added, the phases separated, and the water phase was extracted with more

EtOAc (3 × 30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. Silica-gel column chromatography (EtOAc, $R_f = 0.65$) gave 154 mg (0.350 mmol, 74%) of **11** as a white powder, mp. 95–97 °C; $[\alpha]_D^{20} = -136.2$ (c 1.02, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6): 10.04 (s, 1H), 8.35 (s, 1H), 7,68 (s, 1H), 7.76–7.74 (ap.d, 2H), 7.46–7.49 (ap.d, 2H), 7.31–7.20 (m, 5H), 5.67–5.65 (m, 1H), 5.37.5.39 (m, 1H), 3.45 (q, J = 7.2, 2H), 2.37 (q, J = 7.5, 2H), 1.38 (d, J = 6.9, 3H), 1.19 (t, J = 7.3, 3H), 1.11 (t, J = 7.5, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 172.3, 172.2, 155.5, 153.1, 150.4, 143.9, 139.4, 129.1 (2C), 128.4 (2C), 127.1, 126.9, 125.7 (2C), 119.3, 119.2 (2C), 118.7, 103.0, 49.7, 31.0, 29.6, 22.8, 9.6, 8.5; IR (neat, cm^{-1}): 3418 (w), 3294 (w, br), 2971 (w), 2353 (w), 1720 (w), 1595 (s), 1543 (s), 1403 (m), 1278 (m); HRMS (APCI/ASAP+, *m/z*): found 442.2238, (calcd $C_{26}H_{28}N_5O_2$, 442.2243, $[M+H]^+$).

4.3.13. (R)-N-(4-(4-((1-Phenylethyl)amino)-7H-pyrrolo[2,3-d] pyrimidin-5-yl)phenyl)propionamide (**12**)

The diacylated compound **11** (95.7 mg, 0.217 mmol) was dissolved in THF (4 mL) and NaOH (0.15 mL, 5 M) under a nitrogen atmosphere and stirred at 22 °C for 2 h. The mixture was diluted in water (10 mL) and EtOAc (20 mL), the phases were separated and the water phase was extracted with more EtOAc (3 × 20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. Purification by silica-gel column chromatography (CH_2Cl_2/MeOH, 9/1, $R_f = 0.57$) gave 66.7 mg (0.173 mmol, 80%) of a white soild, mp. 126.4–128.4 °C; HPLC purity: 92%, $t_R = 19.4$ min; $[\alpha]_D^{20} = -189.5$ (c 1.02, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6): 11.81 (s, 1H), 9.97 (s, 1H), 8.14 (s, 1H), 7.72–7.70 (ap.d, 2H), 7.43–7.41 (ap.d, 2H), 7.32–7.26 (m, 4H), 7.23–7.19 (m, 1H), 7.21–7.20 (m, 1H), 5.48–5.46 (m, 1H), 5.40–5.33 (m, 1H), 2.35 (q, J = 7.5, 2H), 1.39 (d, J = 6.9, 3H), 1.11 (t, J = 7.5, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 172.0, 155.2, 151.5, 150.7, 144.5, 138.3, 129.5, 128.9 (2C), 128.4 (2C), 126.8, 125.7 (2C), 119.9, 119.3 (2C), 115.1, 100.1, 49.3, 29.5, 23.0, 9.7; IR (neat, cm^{-1}): 3423 (w), 3016 (w, br), 1579 (s), 1533 (m), 1464 (m), 1299 (w); HRMS (APCI/ASAP+, *m/z*): found 386.1987 (calcd. $C_{23}H_{24}N_5O$, 386.1981 $[M+H]^+$).

4.3.14. (R)-(2-Fluoro-5-(4-((1-phenylethyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl) phenyl)methanol (**13**)

Sodium borohydride (35 mg, 0.917 mmol) was added to compound **7** (128 mg, 0.247 mmol) dissolved in a mixture of MeOH (7.5 mL) and THF (15 mL) under a nitrogen atmosphere. The reaction mixture was stirred at 22 °C for 3 h at which time the solvent was removed. Water (20 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Purification by silica-gel column chromatography (CH_2Cl_2/MeOH, 19/1, $R_f = 0.16$) gave **13** as a clear oil, 113 mg (0.230 mmol, 93%), $[\alpha]_D^{20} = -106.7$ (c 0.92, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 8.28 (s, 1H), 7.64–7.62 (m, 1H), 7.51 (s, 1H), 7.42–7.40 (m, 1H), 7.33–7.29 (m, 4H), 7.28–7. 25 (m, 1H), 7.24–7.21 (m, 1H), 5.92 (s, 1H), 5.56 (s, 2H), 5.36–5.32 (m, 1H), for CH_2-OH AB-system: $\delta_A = 4.64$ (1H), $\delta_B = 4.59$ (1H), $J_{AB} = 13.9$, 3.56 (t, J = 8.1, 2H), 1.45 (d, J = 6.9, 3H), 0.84 (t, J = 8.1, 2H), −0.07 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): 158.9 (d, J = 244.8), 154.2, 150.3, 149.7, 143.7, 130.1 (d, J = 2.2), 129.8 (d, J = 15.5), 128.8 (d, J = 4.6), 128.6 (d, J = 8.7), 128.4 (2C), 126.9, 125.9 (2C), 124.0, 115.7, 115.6 (d, J = 22.0), 100.0, 72.5, 65.7, 56.6 (d, J = 3.4), 50.1, 22.5, 17.1, −1.4 (3C); ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −124.4 (s, dec.); IR (neat, cm^{-1}): 3382 (w), 2951 (w, br), 1579 (m), 1455 (m), 1174 (m), 1081 (m), 831 (s), 691 (s); HRMS (APCI/ASAP+, *m/z*): found 493.2431 (calcd. $C_{27}H_{34}N_4O_2FSi$, 493.2435, $[M+H]^+$).

4.3.15. (R)-(2-Fluoro-5-(4-((1-phenylethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenyl)methanol (**14**)

Compound **13** (108 mg, 0.226 mmol) was dissolved in CH_2Cl_2 (10 mL) and TFA (1 mL) under a nitrogen atmosphere and stirred at 45 °C for 3 h. The solvent was removed before THF (8 mL) and NaOH (0.34 mL, 2.0 M) were added. The mixture was stirred for 19 h before additional NaOH (0.34 mL, 2.0 M) was added, and the mixture was stirred for 3 additional hours. Then the solvent was removed, water (20 mL) was added, and the mixture extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/MeOH, 10/1, $R_f = 0.37$) to give the product **14** as a pale powder, 51 mg (0.148 mmol, 65%), mp. 97–98 °C (dec.); HPLC purity: 92%, $t_R = 18.1$ min; $[\alpha]_D^{20} = -186.7$ (c 1.00, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 11.86 (s, 1H), 8.15 (s, 1H), 7.62–7.61 (m, 1H), 7.42–7.40 (m, 1H), 7.32–7.27 (m, 4H), 7.26–7.23 (m, 2H), 7.22–7.19 (m, 1H), 5.47–5.45 (m, 1H), 5.37–5.36 (m, 1H), 5.35–5.32 (m, 1H), for CH2-OH ABX-system: $\delta_A = 4.64$ (1H), $\delta_B = 4.57$ (1H), $J_{AB} = 3.9$, $J_{AX} = 5.6$, $J_{BX} = 5.4$, 1.41 (d, J = 6.9, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): 158.6 (d, J = 244.5), 155.1, 151.5, 150.8, 144.5, 131.2 (d, J = 3.3), 129.6 (d, J = 15.4), 128.9 (d, J = 5.5), 128.7 (d, J = 7.8), 128.3 (2C), 126.7, 125.9 (2C), 120.3, 115.4 (d, J = 21.0), 114.5, 99.9, 56.7 (d, J = 3.3), 49.5, 22.8; ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −125.1 (s, dec.); IR (neat, cm^{-1}): 3114 (s, br), 1577 (s), 1470 (m), 1229 (m), 1109 (m), 1015 (m), 794 (m), 694 (s); HRMS (APCI/ASAP+, *m/z*): found 362.1538, (calcd $C_{21}H_{19}N_4OFSi$, 362.1543 [M+].

4.3.16. (R)-N^1-(2-Fluoro-5-(4-((1-phenylethyl)amino)-7-((2-(trimethylsilyl)-ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl) benzyl)-N^2,N^2-dimethylethane-1,2-diamine (**15**)

Compound **7** (165 mg, 0.335 mmol) was dissolved in CH_2Cl_2 (3 mL) before N^1,N^1-dimethylethane-1,2-diamine (0.11 mL, 1.01 mmol) was added. The mixture was stirred at 22 °C until full conversion to the imine was indicated by ^{1}H NMR spectroscopy (4 h). The solvent was removed before the crude product was dissolved in MeOH (8 mL). $NaBH_4$ (40 mg, 1.07 mmol) was added and the mixture was stirred until full conversion was obtained (2 h). Water (15 mL) was added and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1.5, $R_f = 0.44$) to give **15** as a light brown oil, 160 mg (0.284 mmol, 85%), $[\alpha]_D^{20} = -102.0$ (c 1.00, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 8.22 (s, 1H), 7.60–7.58 (m, 1H), 7.43 (s, 1H), 7.41–7.38 (m, 1H), 7.31–7.27 (m, 5H), 7.22–7.20 (m, 1H), 5.54 (s, 2H), 5.54–5.52 (m, 1H), 5.38–5.33 (m, 1H), for CH_2-NH AB-system: $\delta_A = 3.79$ (1H), $\delta_B = 3.74$ (1H), $J_{AB} = 14.3$, 3.54 (t, J = 8.1, 2H), 2.59 (t, J = 6.3, 2H), 2.29 (t, J = 6.3, 2H), 2.07 (s, 6H), 1.41 (d, J = 6.9, 3H), 0.83 (t, J = 8.1, 2H), −0.08 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): 159.7 (d, J = 244.5), 155.2, 151.9, 150.4, 144.3, 130.2 (d, J = 3.3), 130.5 (d, J = 5.6), 128.5 (d, J = 7.6), 128.4 (2C), 128.3, 126.8, 125.8 (2C), 123.4, 115.6 (d, J = 22.9), 115.0, 100.2, 72.2, 65.5, 58.6, 49.6, 46.4, 46.0 (d, J = 1.8), 45.1 (2C), 22.7, 17.1, −1.4 (3C): ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −124.1 (s, dec.); IR (neat, cm^{-1}): 3425 (w), 2813 (w, br), 1587 (s), 1563 (s), 1463 (s), 1246 (m), 1189 (m), 1079 (s), 838 (s), 791 (s); HRMS (APCI/ASAP+, *m/z*): found 563.3320, (calcd $C_{31}H_{44}N_6OFSi$, 563.3330, $[M+H]^+$).

4.3.17. (R)-(2-Fluoro-5-(4-((1-phenylethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenyl)methanol (**16**)

Compound **15** (120 mg, 0.213 mmol) was dissolved in CH_2Cl_2 (10 mL) and TFA (1.2 mL) under a nitrogen atmosphere. The

reaction mixture was stirred at 45 °C for 3 h before the solvent was removed. THF (8 mL) and NaOH (2.2 mL, 2.0 M) were added under a nitrogen atmosphere. The reaction mixture was stirred for 4 days, where additional NaOH was added after 16 h (1 mL, 2.0 M) and 48 h (1 mL, 2.0 M). Then the mixture was concentrated, and re-dissolved in THF (4 mL) and NaOH (1 mL, 2.0 M) and stirred for one more day in order to reach full conversion. The solvent was removed and water (20 mL) was added and extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1, $R_f = 0.29$) to give the product **16** as a beige powder, 45 mg (0.104 mmol, 49%), mp. 65–66 °C; HPLC purity >99%, $t_R = 19.5$ min; $[\alpha]_D^{20} = -76.2$ (c 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6): 11.86 (s, 1H), 8.15 (s, 1H), 7.59–7.56 (m, 1H), 7.42–7.38 (m, 1H), 7.30–7.27 (m, 4H), 7.24–7.19 (m, 3H), 5.45–5.43 (m, 1H), 5.38–5.32 (m, 1H), for CH_2-NH AB-system: $\delta_A = 3.78$ (1H), $\delta_B = 3.73$ (1H), $J_{AB} = 12.0$, 2.58 (t, J = 6.3, 2H), 2.29 (t, J = 6.3, 2H), 2.07 (2, 6H), 1.41 (d, J = 6.7, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 159.5 (d, J = 244.8), 155.1, 151.5, 150.8, 144.5, 131.2 (d, J = 3.7), 130.3 (d, J = 4.9), 128.6 (d, J = 8.0), 128.3 (2C), 128.2 (d, J = 15.8), 126.7, 125.9 (2C), 120.3, 115.5 (d, J = 22.2), 114.5, 100.0, 58.6, 49.4, 46.4, 46.1 (d, J = 2.6), 45.2 (2C), 22.8; ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −124.6 (s, dec.); IR (neat, cm^{-1}): 3423 (w), 2816 (w, br), 1569 (s), 1480 (m), 1221 (m), 1101 (m), 795 (m), 696 (s); HRMS (APCI/ASAP+, *m/z*): found 433.2513, (calcd $C_{25}H_{30}N_6F$, 433.2516, $[M+H]^+$).

4.3.18. 3-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenol (**17**)

Compound **9** (47 mg, 0.079 mmol) was dissolved in CH_2Cl_2 (5 mL) and TFA (0.5 mL, 6.55 mmol). The reaction mixture was stirred at 40 °C for 3 h before the reaction mixture was cooled and concentrated in vacuo. THF (5 mL) and aqueous NaOH (1.2 mL, 2 M, 2.37 mmol) were added and the mixture was stirred at 22 °C for 23 h. Water (20 mL) and EtOAc (20 mL) were added and phase separated. The aqueous phase was extracted with more EtOAc (3 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was isolated by silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1, $R_f = 0.44$). Drying gave **17** as a white powder, 33 mg (0.071 mmol, 89%), mp. 180–181C ° (dec.); HPLC purity: 98%, $t_R = 24.5$ min; ^{1}H NMR (600 MHz, DMSO-d_6): 12.06 (s, 1H), 9.63 (s, 1H), 8.33 (s, 1H), 7.920–7.915 (m, 1H), 7.48 (s, 1H), 7.47–7.43 (m, 1H), 7.37 (s, 1H), 7.33–7.31 (m, 1H), 7.30–7.28 (m, 3H), 7.18–7.15 (m, 2H), 6.99–6.97 (m, 1H), 6.953–6.946 (m, 1H), 6.81–6.79 (m, 1H), 5.20 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6):162.2 (d, J = 243.0), 157.9, 153.3, 151.4, 150.9, 148.7, 139.7 (d, J = 6.7), 136.1, 133.7, 130.5 (d, J = 7.7), 130.2, 123.3 (d, J = 2.2), 121.7, 121.4 (2C), 119.8, 119.3, 115.43, 115.40, 114.8, 114.7 (d, J = 20.8), 114.0, (d, J = 22.1), 113.99, 100.8, 69.5; ^{19}F NMR (376 MHz, DMSO-d_6, C_6F_6): −115.4 (s, dec.); IR (neat, cm^{-1}): 3362 (w), 2917 (w, br), 1590 (m), 1567 (m), 1476 (m), 1446 (m), 1266 (m), 1216 (m), 1059 (m), 781 (s); HRMS (APCI/ASAP+, *m/z*): found 461.1173, (calcd $C_{25}H_{19}N_4O_2ClF$, 461.1181, $[M+H]^+$).

4.3.19. 7-(2-(Dimethylamino)ethyl)-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**18**)

Compound **3** (310 mg, 0.884 mmol) was dissolved in ammonia solution (7 mL, 2 M in MeOH) and stirred at 80 °C. The reaction was left for a total of 5 days, with addition of more ammonia/MeOH (2 mL, 2 M) after two days and four days (3 mL, 2 M). Then the solvent was removed in vacuo. Water (20 mL) was added and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1, $R_f = 0.17$) and drying gave 167 mg (0.504 mmol, 59%) of a white powder, mp. 148–149 °C (dec); ^{1}H NMR (600 MHz, DMSO-d_6): 8.08 (s, 1H), 7.48 (s, 1H), 6.56 (br s, 2H), 4.18 (t, J = 6.4, 2H), 2.58 (t, J = 6.4, 2H), 2.15 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 157.1, 151.7, 149.6, 129.7, 102.8, 58.3, 49.3, 45.0 (2C), 41.7; IR (neat, cm^{-1}): 3460 (m), 3065 (w), 2764 (w), 1642 (m), 1584 (s), 1548 (s), 1475 (m), 1325 (m), 1268 (m), 1065 (m), 940 (m), 790 (m), 748 (s); HRMS (APCI/ASAP+, *m/z*): found 332.0371, (calcd $C_{10}H_{15}N_5I$, 332.0372, $[M+H]^+$).

4.3.20. 3-(4-Amino-7-(2-(dimethylamino)ethyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenol (**19**)

Compound **19** was prepared as described in Section 4.3.1 starting with compound **18** (53 mg, 0.159 mmol) and (3-hydroxyphenyl)boronic acid (38 mg, 0.276 mmol). The reaction time at 100 °C was 15 min. Purification by silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1.5, $R_f = 0.19$) gave the product **19** as a beige powder upon drying, 44 mg (0.132 mmol, 80%), mp. 81–82 °C; HPLC purity >99%, $t_R = 6.9$ min; ^{1}H NMR (400 MHz, DMSO-d_6): 9.58 (s, 1H), 8.14 (s, 1H), 7.33 (s, 1H), 7.28–7.24 (m, 1H), 6.87–6.84 (m, 2H), 6.76–6.73 (m, 1H), 6.11 (br s, 2H), 4.25 (t, J = 6.5, 2H), 2.67 (t, J = 6.5, 2H), 2.20 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): 157.8, 157.7, 151.5, 150.2, 136.1, 130.0, 123.5, 119.0, 115.1 (2C), 113.7, 99.7, 58.4, 45.1 (2C), 41.4; IR (neat, cm^{-1}): 2764 (w, br), 1621 (m), 1579 (s), 1455 (m), 1288 (m), 1179 (m), 862 (m), 779 (s), 701 (m), 644 (m); HRMS (APCI/ASAP+, *m/z*): found 298.1665, (calcd $C_{16}H_{20}N_5O$, 298.1668, $[M+H]^+$).

4.3.21. (R)-2-((7-(2-(Dimethylamino)ethyl)-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)propan-1-ol (**20**)

Compound **3** (234 mg, 0.667 mmol) was dissolved in *n*-BuOH (5 mL) under a nitrogen atmosphere before (*R*)-2-aminopropan-1-ol (0.15 mL, 1.97 mmol) was added. The mixture was stirred for 4 h at reflux before the solvent was removed in vacuo. Water (10 mL) and EtOAc (10 mL) were added. After phase separation, the water phase was extracted with more EtOAc (5 × 20 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/MeOH, 17/3, $R_f = 0.20$) to give 244 mg (0.627 mmol, 95%) of a white powder, mp. 105–106 °C; $[\alpha]_D^{20} = -11.0$ (c 1.01, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 8.18 (s, 1H), 7.47 (s, 1H), 6.38–6.37 (m, 1H), 5.01 (t, J = 5.2, 1H), 4.35–4.29 (m, 1H), 4.26–4.24 (m, 2H), 3.54–3.52 (m, 2H), 2.77 (br s, 2H), 2.28 (s, 6H), 1.23–1.22 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): 155.9, 152.2, 149.6, 129.8, 103.2, 64.4, 58.4, 49.7, 47.6, 45.1 (2C), 41.7, 17.9; IR (neat, cm^{-1}): 3388 (m), 2864 (w, br), 1704 (w), 1586 (s), 1553 (s), 1486 (m), 1460 (m), 1316 (m), 1202 (m), 1142 (m), 1059 (m), 942 (m), 758 (m), 657 (m); HRMS (APCI/ASAP+, *m/z*): found 390.0792, (calcd. $C_{13}H_{21}N_5OI$, 390.0791, $[M+H]^+$).

4.3.22. (R)-3-(7-(2-(Dimethylamino)ethyl)-4-((1-hydroxypropan-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenol (**21**)

Compound **21** was made as described in Section 4.3.1 staring with compound **20** (80 mg, 0.206 mmol) and (3-hydroxyphenyl) boronic acid (35 mg, 0.252 mmol). The reaction time was 5 min. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10:1.5, $R_f = 0.32$). Residual palladium was removed through an additional silica-gel plug (CH_2Cl_2/MeOH, 9/1, $R_f = 0.04$). Drying gave 49 mg (0.138 mmol, 67%) of a white powder, mp. 78 °C; HPLC purity: 98%, $t_R = 5.9$ min; $[\alpha]_D^{20} = -30.5$ (c 1.09, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 9.55 (s, 1H), 8.20 (s, 1H), 7.32 (s, 1H), 7.26 (t, J = 7.9, 1H), 6.89–6.88 (m,

1H), 6.85–8.84 (m, 1H), 6.76–6.67 (m, 1H), 5.48–5.46 (m, 1H), 4.76 (t, J = 5.3, 1H), 4.29–4.27 (m, 1H), 4.27–4.25 (m, 2H), 3.44–3.37 (m, 2H), 2.70–2.67 (m, 2H), 2.21 (s, 6H), 1.11–1.12 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): 158.3, 156.1, 151.8, 150.1, 136.5, 130.5, 123.7, 119.4, 115.6, 115.4, 114.3, 100.3, 64.5, 58.9, 47.8, 45.5 (2C), 41.9, 17.9; IR (neat, cm^{-1}): 3395 (w), 2927 (w, br), 1593 (s), 1563 (s), 1473 (s), 1279 (m), 1186 (m), 1045 (m), 868 (m), 777 (s), 644 (m); HRMS (APCI/ASAP+, *m/z*): found 356.2085, (calcd $C_{19}H_{26}N_5O_2$, 356.2087, $[M+H]^+$).

4.3.23. (S)-2-((7-(2-(Dimethylamino)ethyl)-5-iodo-7H-pyrrolo [2,3-d]pyrimidin-4-yl)amino)-2-phenylethan-1-ol (**22**)

n-BuOH (20 mL) was added to compound **3** (503 mg, 1.43 mmol) and (*S*)-2-amino-2-phenylethan-1-ol (591 mg, 4.28 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at reflux for 7 h before the *n*-BuOH was removed by evaporation. Water (75 mL) and EtOAc (100 mL) were added and phase separated. The water phase was extracted with more EtOAc (5 × 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Silica-gel column chromatography (CH_2Cl_2/MeOH, 9/1, $R_f = 0.22$) gave 578 mg (1.28 mmol, 89%) of a white powder, mp. 124–125 °C (dec.); $[\alpha]_D^{20} = -96.7$ (c 1.14, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 8.09 (s, 1H), 7.51 (s, 1H), 7.40–7.39 (m, 2H), 7.34–7.31 (m, 2H), 7.24–7.22 (m, 1H), 7.01–7.00 (m, 1H), 5.40–5.37 (m, 1H), 5.21 (t, J = 4.9, 1H), 4.19 (t, J = 6.4, 2H), 3.86–3.83 (m, 1H), 3.75–3.72 (m, 1H), 2.59 (t, J = 6.4, 2H), 2.15 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 155.3, 151.5, 149.2, 141.6, 129.7, 128.2 (2C), 126.7, 126.6 (2C), 103.0, 64.8, 58.3, 55.0, 48.9, 45.0 (2C), 41.7; IR (neat, cm^{-1}): 3366 (w), 2774 (w, br), 1579 (s), 1548 (s), 1299 (m), 1060 (m), 925 (m), 779 (m), 701 (s); HRMS (APCI/ASAP+, *m/z*): found 452.0943, (calcd. $C_{18}H_{23}N_5OI$, 452.0947, $[M+H]^+$).

4.3.24. (S)-3-(7-(2-(Dimethylamino)ethyl)-4-((2-hydroxy-1-phenylethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenol (**23**)

Compound **23** was made as decribed in Section 4.3.1 starting with compound **22** (87 mg, 0.193 mmol) and (3-hydroxyphenyl) boronic acid (36 mg, 0.264 mmol). Silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1, $R_f = 0.28$) and drying gave 67 mg (0.161 mmol, 84%) of a beige powder, mp. 112–113 °C (dec.); HPLC purity > 99%, $t_R = 15.0$ min; $[\alpha]_D^{20} = -135.4$ (c 1.10, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 9.59 (s, 1H), 8.14 (s, 1H), 7.36 (s, 1H), 7.30–7.27 (m, 5H), 7.23–7.20 (m, 1H), 6.96–6.95 (m, 1H), 6.93–6.92 (m, 1H), 6.79–6.77 (m, 1H), 6.07–6.06 (m, 1H), 5.32–5.29 (m, 1H), 4.92 (t, J = 5.0, 1H), 4.26 (t, J = 6.4, 2H), 3.71–3.68 (m, 1H), 3.59–3.55 (m, 1H), 2.66 (t, J = 6.4, 2H), 2.19 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 157.9, 155.5, 151.2, 149.6, 141.4, 136.1, 130.1, 128.1 (2C), 126.7 (3C), 123.4, 119.0, 115.3, 114.9, 113.9, 100.1, 64.7, 58.5, 55.6, 45.1 (2C), 41.5; IR (neat, cm^{-1}): 3397 (w, br), 2925 (w, br), 1595 (m), 1569 (m), 1288 (m), 1065 (m), 873 (m), 784 (m), 696 (s); HRMS (APCI/ASAP+, *m/z*): found 418.2240, (calcd $C_{24}H_{28}N_5O_2$, 418.2243, $[M+H]^+$).

4.3.25. (S)-2-((7-(2-(Dimethylamino)ethyl)-5-(4-(hydroxymethyl) phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-phenylethan-1-ol (**24**)

Compound **24** was made as described in Section 4.3.1 starting with **22** (110 mg, 0.244 mmol) and (4-(hydroxymethyl)phenyl) boronic acid (36 mg, 0.236 mmol). The reaction time was 20 min. Silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1, $R_f = 0.28$) gave 82 mg (0.191 mmol, 95%) of a white powder, mp. 72–73 °C; HPLC purity: 96%, $t_R = 13.8$ min; $[\alpha]_D^{20} = -32.5$ (c 1.17, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6): 8.14 (s, 1H), 7.52–7.51 (m, 2H), 7.45–7.43 (m, 2H), 7.39 (s, 1H), 7.30–7.26 (m, 4H), 7.22–7.19 (m, 1H), 5.99–5.98 (m, 1H), 5.34–5.31 (m, 1H), 5.25 (t, J = 5.7, 1H), 4.97 (t, J = 5.0, 1H), 4.57 (d, J = 5.7, 2H), 4.26 (t, J = 6.6, 2H), 3.74–3.70 (m, 1H), 3.58–3.54 (m, 1H), 2.66 (t, J = 6.6, 2H), 2.18 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 155.6, 151.3, 149.8, 141.5, 141.1, 133.2, 128.2 (2C), 128.1 (2C), 127.2 (2C), 126.8, 126.7 (2C), 123.7, 114.7, 100.1, 64.7, 62.7, 58.6, 55.6, 45.2 (2C), 41.6; IR (neat, cm^{-1}): 3397 (w, br), 2821 (w, br), 1579 (s), 1465 (m), 1351 (m), 1190 (m), 1034 (m), 790 (m), 696 (s); HRMS (APCI/ASAP+, *m/z*): found 432.2395, (calcd $C_{25}H_{30}N_5O_2$, 432.2400, $[M+H]^+$).

4.3.26. (S)-4-(7-(2-(Dimethylamino)ethyl)-4-((2-hydroxy-1-phenylethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenol (**25**)

Compound **25** was made as described in Section 4.3.1 starting with **22** (91 mg, 0.201 mmol) and (4-hydroxyphenyl)boronic acid (38 mg, 0.277 mmol). The reaction time was 20 min. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1.5, $R_f = 0.20$). Residual palladium was removed through an additional silica-gel plug (CH2Cl2:MeOH, 9:1, $R_f = 0.02$). The product **25** was obtained as a white powder, 55 mg (0.132 mmol, 67%), mp. 116–117 °C, HPLC purity: 99%, $t_R = 10.5$ min; $[\alpha]_D^{20} = -164.8$ (c 0.99, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 9.53 (s, 1H), 8.11 (s, 1H), 7.35–7.33 (m, 2H), 7.30–7.27 (m, 5H), 7.22–7.20 (m, 1H), 6.89–6.88 (m, 2H), 5.97–5.96 (m, 1H), 5.32–5.30 (m, 1H), 4.95 (t, J = 5.1, 1H), 4.24 (t, J = 6.4, 2H), 3.72–3.69 (m, 1H), 3.57–3.54 (m, 1H), 2.65 (t, J = 6.4, 2H), 2.18 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 156.5, 155.6, 151.1, 149.4, 141.5, 129.7 (2C), 128.1 (2C), 126.7, 126.6 (2C), 125.2, 122.8, 115.9 (2C), 114.8, 100.3, 64.6, 58.5, 55.4, 45.1 (2C), 41.5; IR (neat, cm^{-1}): 2940 (w, br), 1590 (s), 1466 (m), 1272 (m), 1229 (m), 1065 (m), 838 (m), 794 (m), 701 (s); HRMS (APCI/ASAP+, *m/z*): found 418.2238, (calcd. $C_{24}H_{28}N_5O_2$, 418.2243, $[M+H]^+$).

4.3.27. (S)-2-((7-(2-(Dimethylamino)ethyl)-5-(4-fluoro-3-(hydroxymethyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl) amino)-2-phenylethan-1-ol (**27**)

First, a Suzuki cross-coupling was performed as described in Section 4.3.1 using compound **22** (114 mg, 0.252 mmol) and (4-fluoro-3-formylphenyl)boronic acid (48 mg, 0.286 mmol). The reaction mixture was run for 8 min, followed by normal work-up. The crude benzaldehyde product **26** was then dissolved in THF (10 mL) and MeOH (5 mL) before $NaBH_4$ (32.1 mg, 0.846 mmol) was added. The mixture was stirred for 2 h before brine (30 mL) was added. The water phase was extracted with EtOAc (3 × 30 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1, $R_f = 0.30$) and drying gave 63 mg (0.140 mmol, 56%) of a white powder, mp. 80–81 °C, HPLC purity: 95%, $t_R = 13.9$ min; $[\alpha]_D^{20} = -163.6$ (c 0.99, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 8.13 (s, 1H), 7.67–7.65 (m, 1H), 7.46–7.44 (m, 1H), 7.40 (s, 1H), 7.31–7.26 (m, 5H), 7.21–7.19 (m, 1H), 5.96–5.95 (m, 1H), 5.35 (t, J = 5.6, 1H), 5.30–5.28 (m, 1H), 4.99 (t, J = 5.1, 1H), for CH_2-OH ABX - system: $\delta_A = 4.66$ (1H), $\delta_B = 4.60$ (1H), $J_{AB} = 13.8$, $J_{AX} = 5.6$, $J_{BX} = 5.7$, 4.27 (t, J = 6.4, 2H), 3.73–3.70 (m, 1H), 3.61–3.59 (m, 1H), 2.67 (t, J = 6.3, 2H), 2.19 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 158.7 (d, J = 243.1), 155.5, 151.3, 149.7, 141.5, 130.9 (d, J = 3.3), 129.7 (d, J = 15.3), 128.9 (d, J = 5.4), 128.4 (d, J = 8.3),128.1 (2C), 126.7 (2C), 126.6, 123.6, 115.6, (d, J = 22.0), 113.9, 100.0, 64.6, 58.4, 56.8 (d, J = 4.0), 55.6, 45.1 (2C), 41.5; 19 F NMR (376 MHz, DMSO-d_6, C_6F_6): −124.4 (s, dec.); IR (neat, cm^{-1}): 3405 (w), 2827 (w, br), 1593 (s), 1567 (m), 1466 (m), 1343 (m), 1189 (m), 1028 (m), 794 (m), 697 (s); HRMS (APCI/ASAP+, *m/z*): found 450.2298, (calcd $C_{25}H_{29}N_5O_2F$, 450.2305, $[M+H]^+$).

4.3.28. (S)-2-((5-Iodo-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl) amino)-2-phenylethan-1-ol (**28**)

Compound **4** (111 mg, 0.378 mmol) and (*S*)-2-amino-2-phenylethan-1-ol (158 mg, 1.15 mmol) were dissolved in *n*-BuOH

(5 mL) under a nitrogen atmosphere. The reaction mixture was stirred at reflux for 5 h before the solvent was removed. Water (10 mL) and EtOAc (10 mL) were added and the phases were separated. The water phase was extracted with more EtOAc (3 × 10 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Silica-gel column chromatography (CH_2Cl_2/MeOH, 9/1, $R_f = 0.59$) gave 147 mg (0.373 mmol, 98%) of a beige powder; mp. 156–157 °C (dec.); $[\alpha]_D^{20} = -157.7$ (c 0.99, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): 8.10 (s, 1H), 7.46 (s, 1H), 7.39–7.38 (m, 2H), 7.33–7.31 (m, 2H), 7.24–7.21 (m, 1H), 7.01–7.00 (m, 1H), 5.40–5.38 (m, 1H), 5.20 (t, J = 5.0, 1H), 3.87–3.83 (m, 1H), 3.76–3.72 (m, 1H), 3.68 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): 155.3, 151.6, 149.4, 141.5, 130.4, 128.2 (2C), 126.7, 126.6 (2C), 103.1, 64.7, 55.0, 48.7, 31.1; IR (neat, cm^{-1}): 3377(w, br), 2925 (w, br), 1590 (s), 1548 (s), 1475 (m), 1299 (m), 1065 (m), 930 (m), 961 (s); HRMS (APCI/ASAP+, *m/z*): found 395.0367, (calcd $C_{15}H_{16}N_4OI$, 395.0369 $[M+H]^+$).

4.3.29. 3-(4-Amino-7-(2-(dimethylamino)ethyl)-7H-pyrrolo[2,3-d] pyrimidin-5-yl)phenol (**29**)

Compound **29** was made as described in Section 4.3.1 starting with compound **28** (53 mg, 0.159 mmol) and (3-hydroxyphenyl) boronic acid (38 mg, 0.276 mmol). The reaction time was 15 min. Silica-gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25% aq. solution), 80/10/1.5, $R_f = 0.19$) gave 44 mg (0.132 mmol, 80%) of a beige powder, mp. 81–82 °C; HPLC purity >99%, $t_R = 6.9$ min; $[\alpha]_D^{20} = -143.4$ (c 0.90, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6): 9.58 (s, 1H), 8.14 (s, 1H), 7.33 (s, 1H), 7.28–7.24 (m, 1H), 6.87–6.84 (m, 2H), 6.76–6.73 (m, 1H), 6.11 (br s, 2H), 4.25 (t, J = 6.5, 2H), 2.67 (t, J = 6.5, 2H), 2.20 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): 157.8, 157.7, 151.5, 150.2, 136.1, 130.0, 123.5, 119.0, 115.1 (2C), 113.7, 99.7, 58.4, 45.1 (2C), 41.4; IR (neat, cm^{-1}): 2764 (w, br), 1621 (m), 1579 (s), 1455 (m), 1288 (m), 1179 (m), 862 (m), 779 (s), 701 (m), 644 (m); HRMS (APCI/ASAP+, *m/z*): found 298.1665, (calcd. $C_{16}H_{20}N_5O$, 298.1668, $[M+H]^+$).

4.4. In vitro assays

4.4.1. Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC's) were isolated from whole blood buffy coats by first combining fresh buffy coats (obtained from the Indiana blood center) with equal volumes of phosphate buffered saline (PBS). PBS/buffy coat solution (35 mL) was then gently overlaid onto 15 mL of ficol in 50 mL conical tubes. Following centrifugation for 30 min at 500xG (with slow acceleration and deceleration) the top layer of plasma was discarded and the layer of cells along the ficol interface was collected and pooled (five donors per 250 mL conical tube). Each 250 mL tube was filled to the top with room temperature RPMI-1640 media. Tubes were spun for 10 min at 500xG (with slow acceleration and deceleration), media was removed by aspiration and the wash step was repeated. Cells were then resuspended in ice cold Recovery Cell Culture Freezing Medium from Life Technologies (Catalog number 12648-010) on ice. The cell concentration is adjusted to 66.7 million cells/mL. Cell are slow frozen at -1 °C/minute in vials with 100 million cells and stored in liquid nitrogen.

4.4.2. Stimulation of IL-17 secretion and compound addition

PBMC's from 20 to 30 combined donors were brought out of thaw by re-suspending with 1 mL of complete media (RPMI-1640 containing 30 mM HEPES, 100 units/mL penicillin, 100 μg/mL streptomycin, 3.25 mM L-Glutamine, 0.2 μM beta-mercaptoethanol, and 10% fetal bovine serum) followed by the dropwise addition of 2 mL, 4 mL, 8 mL, and finally 16 mL of complete media with gentle swirling. Cells were spun down for 5 min and the cell pellet was resuspended in complete media. Clumps of cells are broken up by running the cell solution through a 23 gauge syringe needle and a 40 μM cell strainer. One hundred thousand cells per well are added to 384 well polystyrene tissue culture treated flat bottomed plates in a total of 30 μL. Stimulation cocktail containing anti-human CD3 antibody, anti-human CD28 antibody, and IL23 (all produced in-house) and compounds prepared in complete media were added to the cells simultaneously in a total volume of 30 μL. The final concentration of added stimulants was 160 ng/mL, 500 ng/mL, and 5 ng/mL for anti-CD3 antibody, anti-CD28 antibody, and IL-23 respectively and 0.3% for DMSO. Plates were sealed with Aera-Seal® sealing film and incubated for 48 h at 37 °C, 95% humidity, and 5% $C0_2$.

4.4.3. IL-17 ELISA and analysis

Following the incubation period, the plates were spun at 200 × g for 5 min. Supernatants were diluted one to two in 1% BSA/PBS and tested for IL-17 with a human IL-17 ELISA kit from R&D system (catalog #D317E) according to the protocol provided with the kit. Absorbance at 492 nm was measured with the Envision multi-label plate reader. Absorbance at 492 were converted to concentration of IL-17 based on the IL-17 standard curve as shown:

$$\text{pg/mL IL-17} = IC_{50} \times [[(\text{Top-Bottom})/(\text{A492-Bottom})]\text{-}1]^{(1/-\text{Hill})}$$

IC_{50}' for inhibition of IL-17 secretion was calculated based on converted values using a standard 4-parameter fit with maximum inhibition determined from the average values of wells with no added stimulants nor compounds and minimum inhibition from the average values of wells with stimulants alone and no added compound.

4.4.4. Cell viability testing and analysis

Equal volumes of Cell TiterGlo® cell viability testing reagent (Promega Cat# G7573) were added to the cells remaining in the plates, and following a 15 min incubation with gentle shaking at room temperature luminescence was measured with the Envision multi-label plate reader. Percent cell death was calculated by setting 100% activity (cell death) to zero luminescence units and minimum activity (max number of viable cells) as the average luminescence units of wells containing stimulants alone and no added compound. IC_{50} were calculated using a standard four parameter fit.

4.4.5. IL-5 ELISA and analysis

Following the incubation period, the plates were spun at 200 × g for 5 min. Undiluted supernatants were tested for IL-5 with a human IL-5 ELISA kit from R&D system (catalog #DY205) according to the protocol provided with the kit. Absorbance was measured with the Envision multi-label plate reader. Absorbance values were converted to concentration of IL-5 based on the IL-5 standard curve as shown:

$$\text{pg/mL IL-5} = IC_{50}{}^{*}[[(\text{Top-Bottom})/(\text{Abs-Bottom})]\text{-}1](1/\text{-Hill})$$

IC50's for inhibition of IL-5 secretion was calculated based on converted values using a standard 4-parameter fit with maximum inhibition determined from the average values of wells with no added stimulants nor compounds and minimum inhibition from the average values of wells with stimulants alone and no added compound.

4.4.6. HEK293 RORγ reporter-receptor assay

As an indicator of inverse agonist activity, a retinoic acid receptor (RAR)-related orphan receptor gamma (RORγ) receptor-

reporter assay (RORg-GAL4/pGL4.31) was performed in human embryonic kidney (HEK293) cells. HEK293 cells were co-transfected using Fugene™ reagent. A reporter plasmid containing a GAL4 binding domain and a minimal adenoviral promoter upstream of a firefly luciferase gene was co-transfected with a plasmid constitutively expressing a human RORγ ligand binding domain fused to yeast GAL4 DNA binding domain. Cells were transfected in T150 cm^2 flasks in MEM media without FBS. After 18 h incubation, transfected cells were trypsinized, plated in 96-well microtiter plates in 3:1 DMEM-F12 media containing 10% FBS, incubated for 4 h and then exposed to various concentrations of test compounds ranging from about 0.05 nM to 10 μM. After 18 h of incubations with compounds, cells were lysed and luciferase activity was quantified using standard techniques. Data was fitted to a four parameter-fit logistics to determine IC_{50} values.

HEK293 RORγ reporter-receptor pGL3 control assay: An assay used to determine non-specific activity in the HEK293 RORγg reporter-receptor assay was performed using a pGL3 control vector that contains an SV40 promoter and enhancer sequences upstream of a firefly luciferase gene. This assay uses the same methods as the previous HEK293 RORγ reporter-receptor assay.

4.4.7. *In vitro kinase profiling*

The compounds were supplied in a 10 mM DMSO solution, and enzymatic kinase inhibition potency was determined by Invitrogen (LifeTechnology) using their Z′-LYTE® assay technology [42], at 500 nM in duplicates. ATP concentration used was equal to K_m, except when this service was not provided and other concentrations had to be used.

Acknowledgements

The cell data was generated by Lilly Open Innovation Drug Discovery (OIDD) and supplied by courtesy of Eli Lilly and Company, and used with Lilly's permission. Susana Villa Gonzalez is thanked for the HRMS experiments. Roger Aarvik is acknowledged for technical assistance and Kristin Uhlving Larsen for synthetic work.

Author contributions

The synthetic work was mainly performed by A. C. Reiersølmoen and some compounds were synthesised by J. Han. B. Hoff planed the work and wrote the paper. E. Sundby assisted during the writing process.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.06.019.

References

[1] W. Jin, C. Dong, IL-17 cytokines in immunity and inflammation, Emerg. Microb. Infect. 2 (2013) e60.
[2] B.W. Kirkham, A. Kavanaugh, K. Reich, Interleukin-17A: a unique pathway in immune-mediated diseases: psoriasis, psoriatic arthritis and rheumatoid arthritis, Immunology 141 (2014) 133–142.
[3] B.P. Fauber, A. Gobbi, K. Robarge, A. Zhou, A. Barnard, J. Cao, Y. Deng, C. Eidenschenk, C. Everett, A. Ganguli, J. Hawkins, A.R. Johnson, H. La, M. Norman, G. Salmon, S. Summerhill, W. Ouyang, W. Tang, H. Wong, Discovery of imidazo[1,5-α]pyridines and -pyrimidines as potent and selective RORc inverse agonists, Bioorg. Med. Chem. Lett 25 (2015) 2907–2912.
[4] R.I. Olsson, Y. Xue, S. von Berg, A. Aagaard, J. McPheat, E.L. Hansson, J. Bernstroem, P. Hansson, J. Jirholt, H. Grindebacke, A. Leffler, R. Chen, Y. Xiong, H. Ge, T.G. Hansson, F. Narjes, Benzoxazepines achieve potent suppression of IL-17 release in human T-helper 17 (TH17) cells through an Induced-fit binding mode to the nuclear receptor RORγ, ChemMedChem 11 (2016) 207–216.
[5] A. Shibata, K. Uga, T. Sato, M. Sagara, K. Igaki, Y. Nakamura, A. Ochida, M. Kono, J. Shirai, S. Yamamoto, M. Yamasaki, N. Tsuchimori, Pharmacological inhibitory profile of TAK-828F, a potent and selective orally available RORγt inverse agonist, Biochem. Pharmacol. 150 (2018) 35–45.
[6] C. Gege, Retinoid-related orphan receptor gamma t (RORγt) inhibitors from Vitae Pharmaceuticals (WO2015116904) and structure proposal for their Phase I candidate VTP-43742, Expert Opin. Ther. Pat. 26 (2016) 737–744.
[7] C. Gege, RORγt inhibitors as potential back-ups for the phase II candidate VTP-43742 from Vitae Pharmaceuticals: patent evaluation of WO2016061160 and US20160122345, Expert Opin. Ther. Pat. 27 (2017) 1–8.
[8] S.H. Smith, C.E. Peredo, Y. Takeda, T. Bui, J. Neil, D. Rickard, E. Millerman, J.-P. Therrien, E. Nicodeme, J.-M. Brusq, V. Birault, F. Viviani, H. Hofland, A.M. Jetten, J. Cote-Sierra, Development of a topical treatment for psoriasis targeting RORγ: from bench to skin, PLoS One 11 (2016) e0147979/0147971–e0147979/0147918.
[9] M.-L. Cho, J.-W. Kang, Y.-M. Moon, H.-J. Nam, J.-Y. Jhun, S.-B. Heo, H.-T. Jin, S.-Y. Min, J.-H. Ju, K.-S. Park, Y.-G. Cho, C.-H. Yoon, S.-H. Park, Y.-C. Sung, H.-Y. Kim, STAT3 and NF-κB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice, J. Immunol. 176 (2006) 5652–5661.
[10] A. Zanin-Zhorov, J.M. Weiss, M.S. Nyuydzefe, W. Chen, J.U. Scher, R. Mo, D. Depoil, N. Rao, B. Liu, J. Wei, S. Lucas, M. Koslow, M. Roche, O. Schueller, S. Weiss, M.V. Poyurovsky, J. Tonra, K.L. Hippen, M.L. Dustin, B.R. Blazar, C.-j. Liu, S.D. Waksal, Selective oral ROCK2 inhibitor down-regulates IL-21 and IL-17 secretion in human T cells via STAT3-dependent mechanism, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 16814–16819.
[11] A. Zanin-Zhorov, J.M. Weiss, A. Trzeciak, W. Chen, J. Zhang, M.S. Nyuydzefe, C. Arencibia, S. Polimera, O. Schueller, J. Fuentes-Duculan, K.M. Bonifacio, N. Kunjravia, I. Cueto, J. Soung, R.M. Fleischmann, A. Kivitz, M. Lebwohl, M. Nunez, J. Woodson, S.L. Smith, R.F. West, M. Berger, J.G. Krueger, J.L. Ryan, S.D. Waksal, Cutting edge: selective oral ROCK2 inhibitor reduces clinical scores in patients with *psoriasis vulgaris* and normalizes skin pathology via concurrent regulation of IL-17 and IL-10, J. Immunol. 198 (2017) 3809–3814.
[12] N. Akashi, I. Matsumoto, Y. Tanaka, A. Inoue, K. Yamamoto, N. Umeda, Y. Tanaka, T. Hayashi, D. Goto, S. Ito, K. Sekiguchi, T. Sumida, Comparative suppressive effects of tyrosine kinase inhibitors imatinib and nilotinib in models of autoimmune arthritis, Mod. Rheumatol. 21 (2011) 267–275.
[13] L.F. Alves, R. Frenedoso da Silva, V.H.A. Cagnon, Nintedanib effects on delaying cancer progression and decreasing COX-2 and IL-17 in the prostate anterior lobe in TRAMP mice, Tissue Cell 50 (2018) 96–103.
[14] M. Ozgen, S.S. Koca, A. Karatas, A.F. Dagli, F. Erman, B. Gundogdu, K. Sahin, A. Isik, Lapatinib ameliorates experimental arthritis in rats, Inflammation 38 (2015) 252–259.
[15] G. Natarajan, C. Terrazas, S. Varikuti, S. Oghumu, J.A. Dubovsky, J.C. Byrd, A.R. Satoskar, Ibrutinib enhances IL-17 response by modulating the function of bone marrow derived dendritic cells, OncoImmunology 5 (2016) e1057385.
[16] L.R. Fitzpatrick, L. Deml, C. Hofmann, J.S. Small, M. Groeppel, S. Hamm, S. Lemstra, J. Leban, A. Ammendola, 4SC-101, a novel immunosuppressive drug, inhibits IL-17 and attenuates colitis in two murine models of inflammatory bowel disease, Inflamm. Bowel Dis. 16 (2010) 1763–1777.
[17] L.R. Fitzpatrick, E. Stonesifer, J.S. Small, K.T. Liby, The synthetic triterpenoid (CDDO-Im) inhibits STAT3, as well as IL-17, and improves DSS-induced colitis in mice, Inflammopharmacology 22 (2014) 341–349.
[18] L. Zhou, I.I. Ivanov, R. Spolski, R. Min, K. Shenderov, T. Egawa, D.E. Levy, W.J. Leonard, D.R. Littman, IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways, Nat. Immunol. 8 (2007) 967–974.
[19] S. Bugge, S.J. Kaspersen, S. Larsen, U. Nonstad, G. Bjørkøy, E. Sundby, B.H. Hoff, Structure-activity study leading to identification of a highly active thienopyrimidine based EGFR inhibitor, Eur. J. Med. Chem. 75 (2014) 354–374.
[20] S. Bugge, I.U. Moen, K.O. Kragseth Sylte, E. Sundby, B.H. Hoff, Truncated structures used in search for new lead compounds and in a retrospective analysis of thienopyrimidine-based EGFR inhibitors, Eur. J. Med. Chem. 94 (2015) 175–194.
[21] J. Han, S.J. Kaspersen, S. Nervik, K.G. Nørsett, E. Sundby, B.H. Hoff, Chiral 6-aryl-furo[2,3-d]pyrimidin-4-amines as EGFR inhibitors, Eur, J. Med. Chem. 119 (2016) 2778–2799.
[22] J. Han, S. Henriksen, K.G. Nørsett, E. Sundby, B.H. Hoff, Balancing potency, metabolic stability and permeability in pyrrolopyrimidine-based EGFR inhibitors, Eur. J. Med. Chem. 124 (2016) 583–607.
[23] T. Yanagibashi, M. Satoh, Y. Nagai, M. Koike, K. Takatsu, Allergic diseases: from bench to clinic - contribution of the discovery of interleukin-5, Cytokine 98 (2017) 59–70.
[24] E. Leung, K. Al Efraij, J.M. FitzGerald, The safety of mepolizumab for the treatment of asthma, Expert Opin. Drug Saf. 16 (2017) 397–404.
[25] T.V. Saco, A.N. Pepper, R.F. Lockey, Benralizumab for the treatment of asthma, Expet Rev. Clin. Immunol. 13 (2017) 405–413.
[26] P.P. Graczyk, Gini coefficient: a new way to express selectivity of kinase inhibitors against a family of kinases, J. Med. Chem. 50 (2007) 5773–5779.
[27] I. Plaza-Menacho, L. Mologni, N.Q. McDonald, Mechanisms of RET signaling in cancer: current and future implications for targeted therapy, Cell. Signal. 26 (2014) 1743–1752.
[28] Y. Qian, S. Chai, Z. Liang, Y. Wang, Y. Zhou, X. Xu, C. Zhang, M. Zhang, J. Si, F. Huang, Z. Huang, W. Hong, K. Wang, KIF5B-RET fusion kinase promotes cell growth by multilevel activation of STAT3 in lung cancer, Mol. Canc. 13 (2014), 176/171–176/111.

[29] J.J. Schuringa, K. Wojtachnio, W. Hagens, E. Vellenga, C.H.C.M. Buys, R. Hofstra, W. Kruijer, MEN2A-RET-induced cellular transformation by activation of STAT3, Oncogene 20 (2001) 5350–5358.
[30] S. Garcia, L.M. Hartkamp, B. Malvar-Fernandez, I.E. van Es, H. Lin, J. Wong, L. Long, J.A. Zanghi, A.L. Rankin, E.L. Masteller, B.R. Wong, T.R.D.J. Radstake, P.P. Tak, K.A. Reedquist, Colony-stimulating factor (CSF) 1 receptor blockade reduces inflammation in human and murine models of rheumatoid arthritis, Arthritis Res. Ther. 18 (2016), 75/71–75/14.
[31] S.P. Gao, K.G. Mark, K. Leslie, W. Pao, N. Motoi, W.L. Gerald, W.D. Travis, W. Bornmann, D. Veach, B. Clarkson, J.F. Bromberg, Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas, J. Clin. Invest. 117 (2007) 3846–3856.
[32] K.M. Quesnelle, A.L. Boehm, J.R. Grandis, STAT-mediated EGFR signaling in cancer, J. Cell. Biochem. 102 (2007) 311–319.
[33] J. Wu, W. Liu, J.P. Williams, N. Ratner, EGFR-Stat3 signalling in nerve glial cells modifies neurofibroma initiation, Oncogene 36 (2017) 1669–1677.
[34] S.J. Kaspersen, J. Han, K.G. Nørsett, L. Rydså, E.B. Kjøbli, S.G. Bjørkøy, E. Sundby, B.H. Hoff, Identification of new 4-*N*-substituted 6-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amines as highly potent EGFR-TK inhibitors with Src-family activity, Eur. J. Pharmaceut. Sci. 59 (2014) 69–82.
[35] S. Bugge, A.F. Buene, N. Jurisch-Yaksi, I.U. Moen, E.M. Skjoensfjell, E. Sundby, B.H. Hoff, Extended structure-activity study of thienopyrimidine-based EGFR inhibitors with evaluation of drug-like properties, Eur. J. Med. Chem. 107 (2016) 255–274.
[36] S. Bugge, S.J. Kaspersen, E. Sundby, B.H. Hoff, Route selection in the synthesis of C-4 and C-6 substituted thienopyrimidines, Tetrahedron 68 (2012) 9226–9233.
[37] J. Han, E. Sundby, B.H. Hoff, Solvent selection in synthesis of 4-(1-arylfluoroethoxy)quinazolines and thienopyrimidines, J. Fluorine Chem. 153 (2013) 82–88.
[38] J.S. Pudlo, M.R. Nassiri, E.R. Kern, L.L. Wotring, J.C. Drach, L.B. Townsend, Synthesis, antiproliferative, and antiviral activity of certain 4-substituted and 4,5-disubstituted 7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-d]pyrimidines, J. Med. Chem. 33 (1990) 1984–1992.
[39] Y. Song, H. Ding, Y. Dou, R. Yang, Q. Sun, Q. Xiao, Y. Ju, Efficient and practical synthesis of 5'-deoxytubercidin and its analogues via vorbruggen glycosylation, Synthesis (2011) 1442–1446.
[40] J.L. Henderson, B.L. Kormos, M.M. Hayward, K.J. Coffman, J. Jasti, R.G. Kurumbail, T.T. Wager, P.R. Verhoest, G.S. Noell, Y. Chen, E. Needle, Z. Berger, S.J. Steyn, C. Houle, W.D. Hirst, P. Galatsis, Discovery and preclinical profiling of 3-[4-(morpholin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl]benzonitrile (PF-06447475), a highly potent, pelective, brain penetrant, and in vivo active LRRK2 kinase inhibitor, J. Med. Chem. 58 (2015) 419–432.
[41] S.M. Maddox, C.J. Nalbandian, D.E. Smith, J.L. Gustafson, A practical Lewis base catalyzed electrophilic chlorination of arenes and heterocycles, Org. Lett. 17 (2015) 1042–1045.
[42] B.A. Pollok, B.D. Hamman, S.M. Rodems, L.R. Makings, Optical probes and assays, in: Aurora Biosciences Corporation, 2000. WO 2000066766 A1.