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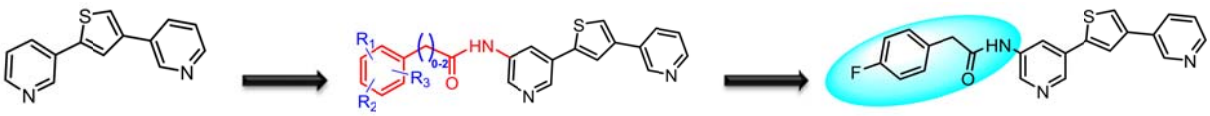
Graphical Abstract



C4

Dyrk1A; IC_{50} = 300 nM
Dyrk1B; IC_{50} = 300 nM

R_1, R_2, R_3 = alkyl, halogen, methoxy

31b

Dyrk1A; IC_{50} = 14.3 nM
Dyrk1B; IC_{50} = 383 nM

Development of Novel 2,4-Bispyridyl Thiophene–based Compounds as Highly Potent and Selective Dyrk1A Inhibitors. Part I: Benzamide and Benzylamide Derivatives

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Abstract.

The protein kinase Dyrk1A modulates several processes relevant to the development or progression of Alzheimer's disease (AD), e. g. through phosphorylation of tau protein, amyloid precursor protein (APP) as well as proteins involved in the regulation of alternative splicing of tau pre-mRNA. Therefore, Dyrk1A has been proposed as a potential target for the treatment of AD. However, the co-inhibition of other closely related kinases of the same family of protein kinases (e.g. Dyrk1B and Dyrk2) or kinases from other families such as Clk1 limits the use of Dyrk1A inhibitors, as this may cause unpredictable side effects especially over long treatment periods. Herein, we describe the design and synthesis of a series of amide functionalized 2,4-bispyridyl thiophene compounds, of which the 4-fluorobenzyl amide derivative (**31b**) displayed the highest potency against Dyrk1A and remarkable selectivity over closely related kinases (IC_{50}: Dyrk1A = 14.3 nM; Dyrk1B = 383 nM, Clk1 > 2µM). This degree of selectivity over the frequently hit off-targets has rarely been achieved to date. Additionally, **31b** inhibited Dyrk1A in intact cells with high efficacy (IC_{50} = 79 nM). Furthermore, **31b** displayed a high metabolic stability *in vitro* with a half-life of 2h. Altogether, the benzamide and benzylamide extension at the 2,4-bispyridyl thiophene core improved several key properties, giving access to compound suitable for future *in vivo* studies.

Keywords: Dyrk1A, Alzheimer's disease, bispyridyl thiophenes amides, selectivity, metabolic stability

1. Introduction

Dual-specificity tyrosine-regulated kinases (Dyrks) are a family of eukaryotic kinases that belong to a larger super family known as the CMGC group. [1] The Dyrk family consists of five mammalian subtypes including Dyrk1A, 1B, 2, 3, and 4. [2] Dyrk1B is highly expressed in some types of cancers, where it is claimed to exert an anti-apoptotic role in tumour cells by mediating some survival signals. [3] For Dyrk2, several controversial roles were reported in cancer biology: this isoenzyme was found to be amplified and overexpressed in lung and esophageal adenocarcinoma as well as gastrointestinal stromal tumours. [4, 5] However, a previous study proposed that a Dyrk2 inactivation contributes to the invasiveness of human tumours due to the decreased levels of phosphorylated c-Jun and c-Myc transcription factors as well as the lowered activation of the p53 tumour suppressor protein. [6, 7] Comparably little information exists on the remaining Dyrk isoforms. Dyrk3, which is most closely related to Dyrk2 among the Dyrk family, was shown to attenuate erythropoiesis by efficiently inhibiting NFAT transcriptional activation. [8] Dyrk4, for which few reports exist, was found expressed in human brain during neuronal differentiation. [9] Dyrk1A, the most studied isoform of this family, also has a well-established neurodevelopmental role, as confirmed by the examination of Dyrk1A expression in the developing mouse brain. [10] In humans, Dyrk1A was identified in the Down syndrome (DS) critical region (DSCR) of chromosome 21 [11, 12] as a potential candidate gene causing the DS phenotype. DS individuals are characterized by mental retardation, cognitive as well as cranio-facial characteristics, congenital heart disease, an increased risk of childhood leukemia, and an early onset of Alzheimer´s Disease (AD). [13] In DS individuals, the 1.5-fold overexpression of Dyrk1A is believed to cause an imbalance in the neurodevelopmental processes such as neurogenesis and neuronal differentiation, [14-18] eventually resulting in reduced brain size,

neuronal deficits and altered neuronal morphology. Especially the early onset of AD was attributed to an increased phosphorylation of the tau protein and presenilin-1 by Dyrk1A. [19]

Importantly, Dyrk1A was found to be involved in the regulation of alternative splicing of pre-mRNA, which is crucial for controlling embryonic development, cell growth and apoptosis. [20] The splicing process is catalyzed by a macromolecular machine called spliceosome which is controlled by protein phosphorylation. [21] Splice site recognition is determined mainly through the interaction of serine-arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins with specific exonic and intronic pre-mRNAsequences. [22] Dyrk1A was reported to phosphorylate several SR proteins, thus modulating their activity. [23, 24] In addition, Dyrk1A is known to phosphorylate the non-SR protein SF3b1 which plays an important role in the basic splicing reaction since it is detected only in functional spliceosomes. [25]

Among the important SR proteins phosphorylated by Dyrk1A are SRp55, SC35 and ASF, which regulate the inclusion of exon 10 in the tau mRNA in the brain. [23, 24, 26] Indeed, overexpression of Dyrk1A led to the increase of 3R-tau with incorporated exon 10 in the neonatal brains of Ts65Dn mice, a model of Down syndrome. [27] In the same study, inhibition of Dyrk1A restored the balance of 3R/4R tau protein by preventing the inclusion of exon 10 in the tau mRNA. The relatively increased amount of 3R-tau relative to 4R-tau facilitates tau aggregation, one of the main hallmarks of AD. [28] Not surprisingly, increased Dyrk1A activity in DS brains and Dyrk1A overdosage models was found to change further transcript compositions, e. g. in the mRNAs of neuroligin and of acetylcholinesterase variants, thus producing key synaptic proteins with unusual features. [29]

In addition, Dyrk1A was reported to phosphorylate tau protein and APP directly [1, 13, 30], thus promoting tau aggregation and formation of amyloid plaques, the main molecular hallmarks of

sporadic as well as DS-linked AD neurodegeneration. [31, 32] A recent study proved that normalization of the gene dosage of Dyrk1A could reduce several phenotypes of AD in a mouse model of DS. [33] Another group showed that Dyrk1A inhibition could improve AD pathologies in mouse model. [34] Altogether, there is ample evidence that Dyrk1A is a promising target in AD [27, 33, 34] and possibly in splicing–related disorders, [35] although the latter indication has scarcely been explored up to now.

Any therapeutic use of Dyrk1A inhibitors requires high selectivity over closely related kinases in the Dyrk family, such as Dyrk1B and Dyrk2, as well as frequently co-inhibited kinases from other families, including Clks, to prevent unwanted adverse effects. However, most of the previously published Dyrk1A inhibitors affected other kinases known to maintain or promote cell proliferation, mainly Clk1/4, haspin, and CK2. [31, 36-39] Indeed, several Dyrk1A inhibitors, including harmine, [40] meridianin derivatives [41] and pyrido[2,3-d]pyrimidines, [42] were reported to affect cell growth at low µM concentrations, which could well be explained by off-target activities. With respect to cancer, multi-targeted inhibitors might even have advantages over specific ones; for instance, in a study by Zhou et al., 7-azaindole–based compounds that simultaneously targeted Dyrk1A, Dyrk1B and Clk1, efficiently blocked cell proliferation and migration of glioblastoma cells. [43] In contrast, potentially cytotoxic effects of Dyrk inhibitors are expected to limit the therapeutic applicability in neurodegenerative diseases, where the cell viability is already affected by harmful peptide and protein aggregates.

In a previous report, we introduced bispyridyl thiophenes as a novel scaffold for the inhibition of Dyrk and Clk kinases. [38] While the overall selectivity of these inhibitors was promising, they did not show a pronounced preference among the close homologues Dyrk1A/B, Dyrk2 or

Clk1/4; only slight selectivity shifts could be achieved by the introduction of different small substituents; the most potent compound **C29** (compound **29** in ref. [38]) is depicted in Fig. 1**.**

In the current study, we present new derivatives of the bispyridyl thiophene scaffold with a crucial amide extension that greatly enhanced the potency and selectivity of the compounds for Dyrk1A as well as the metabolic stability *in vitro*.

2. Results and Discussion

Compound design. We previously described the development of a series of ATP-competitive, bisheterocyclic substituted thiophenes based on the original hit compound **C4** (compound **4** in ref. [38])**,** depicted in Fig. 1 (IC_{50}s: Dyrk1A = 300 nM; Dyrk1B = 300 nM; Dyrk2 = 300 nM). The optimized compound **C29** displayed higher potency against Dyrk1A, [38] however, still lacked selectivity for Dyrk1A over the closely related kinases Dyrk1B and Dyrk2 as well as Clk1.

Small substituents at one of the pyridine rings of **C4**, such as methyl, methoxy, cyano or halogens effected only a slight modulation of the Dyrk subtype selectivity; however, it was found that particularly at the *meta* position, diverse substituents were tolerated without affecting the potency. Hence we surmised that an amino function, which would give access to a focused library of amides, could also be installed on the pyridine ring. In light of the good physicochemical properties and the low molecular weight (238.31 g/mol) of the core structure **C4,** [38] the attachment of benzamide, sulfonamide and benzylamide moieties was deemed acceptable to enhance binding affinity and selectivity whilst keeping molecular weight and lipophilicity in a drug-like range.

Based on our docking simulation (Fig. 2), the two important hydrogen bonds with the conserved Lys188 and the hinge region residue Leu241 as well as the CH–π interactions involving Lys188, Leu241 and the Phe238 benzene ring were not expected to be compromised by the probe amino function that was added to ring A of **C4**. In accordance, the potency of the resulting compound **2** was even somewhat higher than that of **C4** (Table 1).

Firstly, further aminopyridine derivatives of **C4** were synthesized as probe compounds to identify the optimum position with respect to potency and selectivity. Similar to what was observed with other substituents, the amino function was best tolerated in the 3-position of ring A (Compound **2**, Table 1), however, it did not improve the selectivity over Dyrk1B. Interestingly, the amino function could be introduced at both pyridine rings (ring A and B) with basically the same outcome (cf. compound **35**, Table 1), indicating that it was equally tolerated at each end of the ATP binding pocket or, as also suggested by our docking simulation (Fig. 2), that the inhibitor could flip in the binding site by 180 degrees without loss of affinity due to the rather symmetric shape of the 2,4-bispyridyl scaffold. Shifting the pyridine nitrogen of ring B by incorporating 4-pyridyl (compound **3)** almost abolished the potency, indicating that the added 3-amino did not induce a new binding mode that would favour or tolerate another position of the acceptor nitrogen in pyridine ring B (cf. Fig. 1). Eventually, we selected probe compound **2** as basis for further modifications.

Since in both possible binding orientations, the amino function pointed to the borders of the ATP binding pocket, molecule extensions utilizing an amide linkage could potentially address several side chains and/or backbone peptide functions of unexploited residues located at the borders of the ATP binding pocket (encircled in yellow, Fig. 2). These included the Ile165 and Tyr243 side chains at the hinge region side (Fig. 2A) as well as the Phe170 and Asp307 residues located at

the opposite edge of the pocket (Fig. 2B). Using a diversification strategy involving an extension of probe compound **2** through amide and sulfonamide couplings, we aimed at enhancing both the potency towards Dyrk1A and the selectivity over other kinases, in particular Dyrk1B, Dyrk2, Clk1 and haspin.

Chemistry. The synthesis of the planned amide functionalized bispyridyl thiophene compounds was accomplished through a four-step synthesis as summarized in Scheme 1. Firstly, a Miyaura reaction was carried out on 2/3-amino-5-bromopyridine and bis(pinacolato)diboron in presence of potassium acetate and Pd(dppf)Cl_2 as a catalyst to afford the corresponding boronic acid pinacol ester. Next, the produced boronic acid derivative became accessible by a consecutive Suzuki cross coupling reaction with 2,4-dibromothiophene in the presence of Cs_2CO_3 and palladium-tetrakis(triphenylphosphine) to give 4-bromothiophen-2-yl pyridine amines (compounds **C**-**D**). In the third step and starting from compound **D**, a coupling reaction took place with diverse acid chlorides or benzenesulfonyl chlorides to yield a series of amides in addition to one sulfonamide derivative, respectively. The final reaction was a second Suzuki reaction to synthesize the bispyridyl thiophene functionalized amides (**4b**-**34b**). This was done using 3-pyridine boronic acid and Pd(dppf)Cl_2 in presence of Na_2CO_3 as a base. It is worth mentioning that the poor yield of the later Suzuki reaction hindered diversification via amide coupling as a last step. Few compounds (**1**-**3**) were synthesized by direct coupling of the 4-bromothiophen-2-yl pyridine amine intermediates (**C**-**D**) with 3/4-pyridine boronic acids (Scheme 1). To synthesize the inverted positional isomer of compound **2**, compound **E** was synthesized by coupling 2,4-dibromothiophene with 3-pyridine boronic acid, followed by coupling with compound **B** to yield compound **35** (Scheme 2).

Biological evaluation.

The inhibitory activity of the synthesized compounds was evaluated against recombinant Dyrk1A by screening at 250 nM. For compounds showing more than 60% inhibition, the IC_{50} values were determined (Tables 1 and 2). In order to obtain an early indication of the selectivity, all new compounds were tested in parallel against Dyrk1B, the most closely related isoenzyme.

In the present study, we generally focused on the installation of an aromatic system, that could engage in several of the potential interactions with the residues indicated in Fig. 2.

Structure-Activity-Relationships (SAR). Evaluation of the carboxamide *vs.* sulfonamide extension. We started with the attachment of a plain phenyl ring through an amide linkage to obtain the benzamide derivative **4b**. When compared to the probe compound **2**, **4b** gave a slightly reduced inhibition without enhancement of selectivity over Dyrk1B (Tables 1 and 2). However, this did not argue for a poor steric fit of the benzoyl moiety, because the amide coupling strongly decreased the mesomeric effect of the amino function on the pyridyl, thus weakening the acceptor strength of the ring nitrogen (cf. Fig. 3A). Considering that this caused a partial loss of affinity, we concluded that the benzoyl moiety was overall tolerated.

In contrast, the substitution of the amide function in **4b** with a sulfonamide moiety yielded a totally inactive analogue (**5b**), clearly indicating that the carboxamide group was much more preferred by the binding site (Table 2).

Evaluation of electron–donating substituents. In an attempt to improve the potency of compound **4b**, different electron–donating as well as electron–withdrawing groups were tested as substituents on the phenyl ring. Firstly, mono-substitution by electron–donating groups was investigated, starting with the addition of the lipophilic methyl group in the *ortho*, *meta* and *para*

positions. The *o*- and *m*-toluoyl derivatives (compounds **6b** and **7b**) showed slightly lower inhibitory activity than compound **4b** while the *p*-methyl substitution in **8b** proved to be more favourable (IC_{50} = 51.4 nM), raising the potency over that of the probe compound **2** (IC_{50} = 101 nM, Table 2). In addition, **8b** retained the selectivity over Dyrk1B. To confirm the optimum substitution position, the more polar and stronger electron–donating methoxy group was explored in the same manner. However, the methoxy group effected leveling of potency between the *ortho*-, *meta*- and *para*-regioisomers, as all of the compounds showed similar degrees of inhibition (compounds **9b**-**11b**, inhibition between 53.5 and 59.5%, Table 2). There was only a marginal preference for the *para*-position. Altogether, it could be concluded that the *para*-position is superior in case of electron–donating groups, but that the potential activity–enhancing effect of the methoxy substituent might be counteracted by steric clashes. To probe the available space surrounding the *para*-position, the bulky, lipophilic and electron donating *tert*-butyl group was introduced (**12b**); this modification abolished the inhibitory activity against Dyrk1A, suggesting that substituents which are essentially larger than methyl – such as methoxy and particularly *t*-butyl – are likely to produce a steric clash with the ATP binding pocket. Of note, the methoxy–substituted congeners **9b** and **10b** exhibited diminished activity toward Dyrk1B (Table 2), indicating that the variation of substituents at the benzoyl moiety permits to modulate the selectivity.

Evaluation of electron–withdrawing substituents. The preference of the benzoyl *para* position, as found with the electron–donating substituents, was not observed when electron–withdrawing groups were explored. In light of the finding described above, we restricted to substituents that did not largely exceed the size of a methyl group. First, we analyzed the effect of a chloro substituent; among the three positional isomers, the *o*-chlorobenzamide analogue **13b**

(IC_{50}= 90.5 nM, Table 2) was clearly more active than the *m*- or *p*-chloro isomers (**14b** and **15b**, respectively) and represented the most potent mono-substituted benzamide analogue. By examining Dyrk1B inhibition, **13b** showed considerable preference for Dyrk1A over Dyrk1B. Similarly, the smaller fluoro substituent was also tested; on average, the three positional isomers showed a higher activity than the chlorinated analogues (cf. **16b**–**18b**, Table 2), which might be attributable to the lower steric demand of the fluorine, in particular at the *meta*- and *para*-positions. Additionally, all fluoro derivatives possessed superior selectivity for Dyrk1A over Dyrk1B. However, the most active *o*–fluorobenzamide derivative **16b** (IC_{50}= 106.7 nM) did not fully reach the potency level of the chlorinated congener **13b**. Finally, we investigated whether the trifluoromethyl group could boost the activity stronger than fluorine, which was, however, not the case (compounds **19b**-**21b**, Table 2); all three regioisomers displayed lower inhibitory activity than compound **4b.** Altogether, there was no clear correlation between the inhibitory activity and the modulation of the electron density by the substituents in the benzamide class. Most probably, the influence of the substituents on potency is governed by more than one parameter, e. g. by direct interaction with the binding site, modulation of the electron density, conformational stabilizations, and potential steric interference. Various substituents, in particular methyl, chlorine and fluorine, favourably enhanced the selectivity for Dyrk1A over Dyrk1B, which had not been observed with the unsubstituted benzamide **4b**.

Effect of multiple substitutions. Assuming that some of the favourable effects observed with different substituents at several ring positions could be additive, disubstitution patterns were investigated using combinations of the substituents described above. Initially, disubstitution using identical groups was investigated, starting with the 2,3-dichlorobenzamide analogue (compound **22b**) which led to a decrease in the inhibitory activity compared with the benzamide

derivative **4b** and with the *o*-chloro derivative **13b**, confirming the deleterious effect of the *m*-chloro on potency. On the other hand, the 2,4-difluoro congener **23b** showed a great improvement in the inhibitory activity with an IC_{50} of 49 nM, indicating that the effect of combining two fluorine atoms is indeed additive. However, changing the nature of substituents to 2-chloro-4-fluoro (compound **24b**) decreased the inhibitory activity below that of the unsubstituted analogue **4b**, again confirming that the electron withdrawing properties of the halogens do not play a major role, as they would have been additive. Similarly, the 3-chloro-4-fluoro substitution was detrimental to the activity (compound **25b**). Consequently, the compound design was extended to include a combination of the favourable 2-fluorine with an electron–donating 5-methoxy group (**26b**). **26b** showed a further improved activity with an IC_{50} of 37.3 nM, indicating that the 2-fluoro substitution can favourably be combined with other substituents to enhance the potency. Fortunately, the resulting disubstituted compounds **23b** and **26b** also exhibited a good selectivity towards Dyrk1A over its closely related isoform Dyrk1B. Prompted by the favourable effects especially by the difluorination, we tested whether a trifluorinated analogue, bearing another fluorine in *ortho*, would even show higher activity. Indeed, compound **27b** with the 2,4,6-trifluoro substitution proved to be the most potent inhibitor among the benzamide series (IC_{50} = 23.5 nM). Moreover, this improvement of potency of **27b** was accompanied by a remarkable 13-fold increase in selectivity over Dyrk1B. Somewhat unexpectedly, the 3-fluoro in the 2,3,4-trifluoro–substituted isomer (**28b**), completely neutralized the activity–enhancing effects of the other fluorine substituents.

Changing the nature of the benzamide ring. To decouple effects of electron withdrawing effects of substituents from potential secondary effects such as steric clashes, we replaced the benzamide phenyl by the electron deficient 4-pyridyl (**29b)**, which was supposed to mimic the 4-

fluoro substitution in **18b**. Indeed, the inhibitory activity of **29b** followed the same trend as **18b**: Reaching an IC_{50} of 107 nM, **29b** was more active than the unsubstituted benzamide **4b**, but less active than the 4-methyl–substituted analogue **8b** (IC_{50} = 51.4 nM). However, since the absence of a *p*-substituent in **29b** diminished the selectivity toward Dyrk1B (compare with the 4-fluorobenzamide derivative **18b**) no further 4-pyridyl derivatives were synthesized. In many cases, placing substituents at the benzamide favourably reduced the inhibitory activity against Dyrk1B, in particular methoxy (**9b-11b**), 2-chloro (**13b**) and fluoro (**16b-19b**).

Insertion of a spacer in some benzamide derivatives (homologation). In our last set of modifications, alkyl spacers were introduced between the amide group and the phenyl ring. Compared with its direct benzamide homologue **4b**, the benzyl amide derivative **30b** displayed a marked improvement in the inhibitory activity to reach an IC_{50} of 14.9 nM, which was even slightly superior to the most potent benzamide derivative **27b**. However, the introduction of a 4-fluoro substituent (analogous to **18b**) did not further increase the potency toward Dyrk1A (**31b**, IC_{50} = 14.3 nM), and substitution of the benzyl by the electron–donating 3-methoxy strongly diminished the activity.

The main advantage of the benzylamide *vs.* the benzamide group was that it did not concomitantly enhance the activity towards Dyrk1B, thus increasing the selectivity factor to almost 27 for **31b** (IC_{50} ratio Dyrk1B/Dyrk1A). Further elongation of the spacer by one methylene unit to afford compounds **33b** and **34b** did not give any indication that further homologations could be beneficial for the inhibitory activity.

Prediction of the binding mode of 31b to Dyrk1A. As described above, the addition of a benzamide and especially of a benzylamide moiety to the basic bispyridyl thiophene scaffold led to a boost of potency against Dyrk1A. In order to predict a potential binding mode to the ATP

pocket of Dyrk1A, the most potent compound, **31b**, was docked in the coordinates from PDB entry 5A3X. [44] As depicted in Fig. 3A, our docking study predicted two new interactions that are brought about by the benzylamide group: an H-bond between the amide NH and the Asp307 carboxylate, and a CH–π interaction (edge-to-face) between the phenyl ring and Phe170 at the Dyrk1A ATP binding pocket. Moreover, the latter interaction forces the benzyl group to sterically occlude this part of the pocket, thus shielding the H-bond between Asp307 and the amide NH from competing water molecules (Fig. 3B). Taken together, this ensemble of effects might explain the 21-fold increase in potency resulting from the addition of the benzylamide group to the basic scaffold (cf. Table 1).

Extended selectivity profiling of 31b.

As mentioned earlier, one of our major objectives was to improve the selectivity of the previous bispyridyl thiophene compound class for Dyrk1A. After confirming its superior selectivity over Dyrk1B, our most potent inhibitor **31b** was further evaluated for its selectivity against an extended panel of kinases that were frequently reported as being co-inhibited by chemically diverse classes of Dyrk1A inhibitors (Table 3). Of note, **31b** showed only negligible inhibition of Clk1, which was frequently hit by previous Dyrk1A inhibitors published by us and others. [37-39, 45-47] In addition, compound **31b** showed no or only moderate inhibitory activity towards the other frequently reported off-target kinases such as CDK5/p25, CK1 delta, Dyrk2, HIPK1, TRKB, PIM1, MLCK2, SRPK1 and STK17A (Table 3). Also considering the remarkable selectivity towards the closest homologue, Dyrk1B, **31b** was found to be one the most selective Dyrk1A inhibitors described to date. However, it still inhibited the known off-target haspin, with about 2.5-fold lower potency than Dyrk1A. Haspin is a histone H3 kinase and required for cell proliferation; hence chemical inhibition of intracellular haspin leads to cell growth arrest. [48] However, we did not observe strong effects on HeLa cell proliferation with **31b** (see below for details).

How was selectivity over Clk1 achieved? Of note, while the core compound **C4** had shown the same affinity to both Dyrk1A and Clk1, [38] the benzylamide extension decreased the potency of **31b** against Clk1 below that of the parent compound C**4**. Although all residues except Leu167 (corresponds to Ile165 in Dyrk1A) are conserved in Clk1, small spatial shifts of the backbone and the side chains are detectable in the published X-ray structures of the two kinases and may account for the affinity difference. A docking simulation revealed that the small topology differences translated into a less efficient binding of **31b** to Clk1 than to Dyrk1A, as reflected by

the reduction in number of CH–π bonds (6 vs. 8) and H-bonds (2 vs. 3) (cf. Fig. S1, Supplementary Information). In accordance with the observed structure–selectivity relationships, the benzyl extension was predicted to have a key role in selectivity tuning towards Dyrk1A: in contrast to that of Dyrk1A, the ATP pocket of Clk1 did not allow favourable interaction of the 4-fluorobenzyl ring with Phe172 (corresponding to Phe170 in Dyrk1A, cf. Fig. 3A) inside the pocket; instead, the CH–π interaction is only possible with Gly170 from the glycine–rich loop, which can only occur at the expense of some interactions inside the pocket (Fig. S1, Supplementary Information).

Inhibition of Dyrk1A by 31b in HeLa cells.

To assess the ability of our most potent analogue **31b** to inhibit Dyrk1A in intact cells, we tested its effect on the Dyrk1A-catalysed phosphorylation of splicing factor 3b1 (SF3b1). Phosphorylation of SF3b1 on Thr434 correlated with the cellular activity of endogenous Dyrk1A. [25] HeLa cells were transiently transfected with a GFP-SF3b1 expression vector and treated with **31b**. As can be seen in Fig. 4, **31b** inhibited the Dyrk1A–catalyzed phosphorylation of SF3b1 at Thr434 with high efficacy; a significant reduction of phosphorylated SF3b1 was already noted at 30 nM (IC_{50} = 79 nM, Fig. 4).

Evaluation of cytotoxicity in HeLa cells.

Selective Dyrk1A inhibitors are not expected to induce cell growth arrest, since Dyrk1A activity was even reported to diminish cell proliferation in several cell types. [49-51] Therefore, we evaluated our most potent compound, **31b**, for cytotoxicity, which would indicate potential off-target effects or non-specific toxicity. The cell viability assay was performed using HeLa cells, in which the inhibition of intracellular Dyrk1A had been quantified for the same compound (see

above). As shown in Table 4, **31b** exerted minimal cytotoxicity up to a concentration of 3 µM, at which almost full inhibition of cellular Dyrk1A was observed before (cf. Fig. 4, IC_{50} = 79 nM). A weak inhibition of cell proliferation was noted at 10 µM; this could be due to the inhibition of haspin, which might become effective in the cells at this higher concentration. In accordance, chemical inhibition of haspin in HeLa cells was previously shown to induce cell cycle arrest. [48] In the former study, compound HSD972, possessing an IC_{50} of 12 nM against purified haspin, inhibited HeLa cell proliferation with an IC_{50} of 4.8 µM.

Altogether, our results corroborated that – at least in HeLa cells – inhibition of Dyrk1A does not affect cell proliferation. It is therefore likely that the effects of previously published Dyrk1A inhibitors on cell growth of HeLa or other cell lines (e. g. harmine, IC_{50} with HeLa cells: 8 µM [40]) were rather caused by co-inhibition of Dyrk1B, haspin or other kinases.

Evaluation of the metabolic stability.

We further assessed the suitability of two of the most potent analogues (**27b** and **31b**) for a potential application *in vivo*. To this end, we tested their phase I and phase II metabolic stability against S9 fraction from human liver homogenate. Different samples were taken at various time points, and the remaining fraction of parent compound was determined by LC-MS/MS. As shown in Table 5, both **27b** and **31b** exhibited a metabolic half-life of about 2h. Even considering that the metabolic stability assays with our previous series were performed using rat liver microsomes, this was a substantial improvement to the non-amide extended precursor series; for instance, **C29** (Fig. 1) had shown a half-life of only 27 min. [38] Thus, both the benzamide and the benzylamide modification yielded compounds, **27b** and **31b**, respectively with promising metabolic stability, permitting to test them in future *in vivo* studies.

Calculation of key physicochemical properties. In order to assess whether the most potent compound **31b** might be able to enter the CNS, we calculated some physicochemical parameters that were found to have a predictive value in retrospective studies. [52, 53] In our previous study, the calculated values were found in good agreement with the corresponding experimental values for this compound class. [38] It can be concluded that the values for **31b** (Table 6) are still in a good range with respect to a potential application in the CNS, although the clogP is above the average value found for CNS drugs. However, in general, the lipophilicity reported for CNS drugs tends to be higher than that of non-CNS drugs; for instance, in the survey from Mahar Doan et al. the respective clogP median values were 3.43 for CNS drugs (range 0.16–6.59), and 2.78 for non-CNS drugs (range: -2.81–6.09).

3. Conclusions

The current work presented a series of novel Dyrk1A inhibitors bearing a crucial amide extension. Various benzamide derivatives were synthesized by probing mono-, di- and trisubstitutions with distinct electron–modulating properties. Finally, the insertion of a spacer was tested, which resulted in a dramatic improvement in potency as observed with compounds **30b** and **31b** (IC_{50} = 14.9 nM and 14.3 nM, respectively). Importantly, **31b** also showed a remarkable selectivity over the closely related kinases Dyrk1B and Clk1, which has rarely been reported for previous Dyrk1A inhibitors (IC_{50}; Dyrk1B = 383 nM, Clk1 > 2µM). Since Dyrk1A and Clk1 share several splicing factors as substrates, including AF2/ASF, SC35, and SRp55, [23, 24, 26] our selective inhibitor **31b** will allow to dissect Dyrk1A– from Clk1–mediated splicing regulations; moreover, potential side effects due to a synergistic modulation of SR protein activities through Dyrk1A/Clk1 co-inhibition will be avoided in potential therapeutic trials.

Furthermore, **31b** successfully inhibited Dyrk1A–mediated phosphorylation of SF3b1 in HeLa cells in the two-digit nanomolar range (IC_{50} = 79 nM) while no cytotoxic effects were noted at 3 µM, and only marginal effects at 10 µM, thus providing an appropriate toxicity window for *in vivo* applications. The absence of cytotoxic side effects is especially important when a prolonged treatment of neurodegenerative diseases is envisaged, where the cell viability is already compromised by harmful peptide and protein aggregates.

The metabolic stability was also found to be significantly improved by the new benzamide and benzylamide modification of the previous scaffold. Altogether, the new modification led to an optimization of the key parameters potency, selectivity and metabolic stability, thus allowing to envisage *in vivo* studies in the next step.

4. Experimental Section

Chemistry. Solvents and reagents were obtained from commercial suppliers and used as received. Melting points were determined on a Stuart SMP3 melting point apparatus. All final compounds had a percentage purity of at least 95%, and this could be verified by means of HPLC coupled with mass spectrometry. Mass spectra (HPLC−ESIMS) were obtained using a TSQ quantum (Thermo Electron Corp.) instrument prepared with a triple quadrupole mass detector (Thermo Finnigan) and an ESI source. All samples were injected using an autosampler (Surveyor, Thermo Finnigan) by an injection volume of 10 µL. The MS detection was determined using a source CID of 10 V and carried out at a spray voltage of 4.2 kV, a nitrogen sheath gas pressure of 4.0×10^5 Pa, a capillary temperature of 400 °C, a capillary voltage of 35 V, and an auxiliary gas pressure of 1.0×10^5 Pa. The stationary phase used was an RP C18 NUCLEODUR 100-3 (125 mm × 3 mm) column (Macherey & Nagel). The solvent system

consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). The HPLC method used a flow rate of 400 μL/min. The percentage of B started at 5%, was increased up to 100% during 7 min, was kept at 100% for 2 min, and was flushed back to 5% in 2 min and was kept at 5% for 2 min. A Bruker DRX 500 spectrometer was used to obtain the ^{1}H NMR and ^{13}C NMR spectra. The chemical shifts are referenced to the residual protonated solvent signals.

General synthetic procedures and experimental details for some key compounds.

Procedure A, Procedure for synthesis of Compounds A – D. A mixture of 5 mmol of the appropriate amino-5-bromo pyridine and 1.96 gm (20 mmol) of potassium acetate and 0.18 gm (0.25 mmol) of Pd(dppf)Cl_2 and 5.08 gm (20 mmol) of bis(pinacolato)diboron in dioxane was heated to reflux under argon for 2 hours to yield compounds **A** and **B**. The mixture was left to attain room temperature and then filtered under vacuum. Without further purification, a reaction flask containing the filtrate was charged with 6.5 gm (20 mmol) of Cs_2CO_3, 0.29 gm (0.25 mmol) of palladium-tetrakis(triphenylphosphine) and 6 mmol of 2,4-dibromothiophene together with 30% water in a Suzuki coupling reaction. The reaction was left to reflux under argon for 3.5 hours. The mixture was concentrated *in vacuo.* The residue was partitioned between 150 mL ethyl acetate and 50 mL brine solution and then the aqueous layer was re-extracted using 3 portions of 100 mL ethyl acetate. The organic layers were collected and the volume was reduced under reduced pressure. Afterwards the product was purified by CC to yield compounds **C-D**.

Procedure B, General procedure for amide synthesis (4a, 6a-34a). A solution of 0.18 gm (0.7 mmole) of compound **D** dissolved in acetone was treated with 0.84 mmol of the appropriate acid chloride followed by the addition of 0.12 gm (1.05 mmol) of TEA. The reaction mixture was left

to stir at room temperature for 2 hours which was followed by evaporation of solvent *in vacuo* and the product was purified by CC.

Procedure C, General procedure for synthesis of compounds 1-3, 4b-34b, 35. The bromo derivative was added to a suspension of 4 equiv of Na_2CO_3 and 5 mmol% of $Pd(dppf)Cl_2$ in dioxane/water mixture. This was followed by the addition of the pyridine boronic acid derivative. The reaction was heated to reflux for 2 hours under argon atmosphere. The solvent was removed *in vacuo.* Small amount of brine solution was added and extraction was done using ethyl acetate (3 x 50 mL). The ethyl acetate portions were collected and the volume was reduced *in vacuo*. Afterwards the product was purified by CC.

5-(4-Bromothiophen-2-yl)pyridin-2-amine (C). The compound was synthesized according to procedure **A** using 2-amino-5-bromopyridine: yield: 42%. The product was purified by CC (ethyl acetate/petroleum ether 7:3); ^{1}H NMR (500 MHz, DMSO) δ 8.23 (d, *J* = 2.5 Hz, 1H), 7.64 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.51 (d, *J* = 1.4 Hz, 1H), 7.32 (d, *J* = 1.4 Hz, 1H), 6.47 (d, *J* = 8.7 Hz, 1H), 6.27 (s, 2H).; ^{13}C NMR (126 MHz, DMSO) δ 159.71, 144.87, 143.27, 134.41, 123.36, 120.71, 117.24, 109.61, 107.94; MS (ESI) m/z = 254.79 $(M + H)^+$

5-(4-Bromothiophen-2-yl)pyridin-3-amine (D). The compound was synthesized according to Procedure **A** using 3-amino-5-bromopyridine: yield 80%. The product was purified by CC (ethyl acetate); ^{1}H NMR (500 MHz, DMSO) δ 8.08 (d, *J* = 2.0 Hz, 1H), 7.91 (d, *J* = 2.5 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.51 (d, *J* = 1.5 Hz, 1H), 7.16 – 7.05 (m, 1H), 5.50 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 144.98, 142.31, 136.28, 133.61, 128.34, 126.16, 123.47, 115.68, 109.89; MS (ESI) m/z = 254.82 $(M+H)^+$

3-(4-Bromothiophen-2-yl)pyridine (E). The title compound was synthesized by reacting 0.5 gm (4 mmol) 3-pyridine boronic acid with 1.2 gm (4.8 mmol) 2,4-dibromothiophene in presence of 5.2 gm (16 mmol) Cs_2CO_3 and 0.14 gm (0.2 mmol) palladium-tetrakis(triphenylphosphine) in dioxane/water. The mixture was left to stir under reflux in inert conditions for 3 hours. The solvent was concentrated *in vacuo*, a small amount of brine solution was added to the residue and extracted with ethyl acetate (3 X 50 mL). The organic layers were collected and the solvent was evaporated *in vacuo*: yield 77%. The product was purified by CC (ethyl acetate/petroleum ether 6:4); ^{1}H NMR (500 MHz, DMSO) δ 8.92 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.54 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.06 (ddd, *J* = 8.0, 2.4, 1.6 Hz, 1H), 7.78 (d, *J* = 1.4 Hz, 1H), 7.72 (d, *J* = 1.5 Hz, 1H), 7.46 (ddd, *J* = 8.0, 4.8, 0.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 149.17, 146.14, 141.14, 132.72, 128.55, 127.15, 124.28, 124.08, 110.15; MS (ESI) = 239.80 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)benzamide (4a).** The compound was synthesized according to procedure **B** using benzoyl chloride: yield 79%. The product was purified by CC (ethyl acetate/petroleum ether 7:3);^{1}H NMR (400 MHz, DMSO) δ 10.59 (s, 1H), 8.94 (d, *J* = 2.0 Hz, 1H), 8.71 (d, *J* = 1.9 Hz, 1H), 8.48 (t, *J* = 2.1 Hz, 1H), 8.05 – 7.98 (m, 2H), 7.70 (d, *J* = 1.2 Hz, 1H), 7.66 – 7.49 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 166.51, 141.67, 141.58, 136.57, 134.55, 132.49, 129.50, 129.16, 128.96, 128.20, 127.54, 124.85, 123.73, 110.67; MS (ESI) m/z = 358.85 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)benzenesulfonamide (5a).** The title compound was synthesized through adding 1.4 mmol of the benzenesulfonyl chloride to a stirred solution of 0.18 gm (0.7 mmol) of compound **B** dissolved in pyridine. The reaction was heated to 60°C and left overnight. This was followed by the removal of solvent *in vacuo*: yield 89%. The product was purified by precipitation in ethyl acetate; ^{1}H NMR (500 MHz, DMSO) δ 10.17 (s, 1H), 8.65

(d, *J* = 2.0 Hz, 1H), 8.28 (d, *J* = 2.3 Hz, 1H), 7.72 (t, *J* = 2.2 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 7.64 – 7.56 (m, 6H); ^{13}C NMR (126 MHz, DMSO) δ 148.22, 141.33, 140.14, 138.86, 134.95, 133.40, 129.53, 127.67, 126.75, 125.48, 124.92, 123.51, 110.32; MS (ESI) m/z = 387.94 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-methylbenzamide (6a).** The compound was synthesized according to procedure **B** using *o*-toluoyl chloride: yield 96%. The product was purified by CC (ethyl acetate/petroleum ether 2:3); ^{1}H NMR (400 MHz, DMSO) δ 10.63 (s, 1H), 8.83 (d, *J* = 2.1 Hz, 1H), 8.70 (d, *J* = 2.1 Hz, 1H), 8.47 (s, 1H), 7.80 (d, *J* = 1.4 Hz, 1H), 7.69 (d, *J* = 1.3 Hz, 1H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.47 – 7.38 (m, 1H), 7.33 (t, *J* = 7.4 Hz, 2H), 2.41 (s, 3H);^{13}C NMR (101 MHz, DMSO) δ 168.46, 141.13, 140.99, 140.63, 136.28, 136.19, 135.56, 130.69, 130.09, 128.37, 127.38, 127.12, 125.70, 124.44, 122.55, 110.21, 19.38; MS (ESI) m/z = 372.9 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-methylbenzamide (7a).** The compound was synthesized according to procedure **B** using *m*-toluoyl chloride: yield 87%. The product was purified by CC (ethyl acetate/petroleum ether 2:3); ^{1}H NMR (500 MHz, DMSO) δ 10.53 (s, 1H), 8.93 (d, *J* = 2.3 Hz, 1H), 8.71 (d, *J* = 2.1 Hz, 1H), 8.46 (t, *J* = 2.2 Hz, 1H), 7.82 (s, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.80 – 7.77 (m, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.45 (dd, *J* = 3.9, 1.8 Hz, 2H), 2.42 (s, 3H);^{13}C NMR (126 MHz, DMSO) δ 166.17, 141.22, 141.15, 141.01, 137.86, 136.15, 134.13, 132.62, 128.42, 128.28, 128.19, 127.09, 124.94, 124.41, 123.28, 110.21, 20.96; MS (ESI) m/z = 372.96 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-4-methylbenzamide (8a).** The compound was synthesized according to procedure **B** using *p*-toluoyl chloride: yield 90%. The product was purified by CC (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (500 MHz, DMSO) δ 10.48 (s, 1H),

8.93 (d, *J* = 2.3 Hz, 1H), 8.70 (d, *J* = 2.1 Hz, 1H), 8.47 (t, *J* = 2.2 Hz, 1H), 7.93 (s, 1H), 7.91 (s, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.37 (d, *J* = 7.9 Hz, 2H), 2.40 (s, 3H);^{13}C NMR (126 MHz, DMSO) δ 165.87, 142.19, 141.23, 141.18, 140.95, 136.20, 131.23, 129.05, 128.27, 127.80, 127.09, 124.40, 123.29, 110.21, 21.05; MS (ESI) m/z = 372.89 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-methoxybenzamide (9a).** The compound was synthesized according to procedure **B** using 2-methoxybenzoyl chloride: yield 60%. The product was purified by CC (ethyl acetate/petroleum ether 3:7); ^{1}H NMR (500 MHz, DMSO) δ 10.43 (s, 1H), 8.84 (d, *J* = 2.2 Hz, 1H), 8.69 (d, *J* = 2.1 Hz, 1H), 8.47 (t, *J* = 2.1 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.3 Hz, 1H), 7.68 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.57 – 7.49 (m, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.09 (dd, *J* = 7.7, 7.2 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.26, 156.62, 141.11, 140.97, 140.83, 135.94, 132.54, 129.78, 128.38, 127.16, 124.43, 124.12, 122.68, 120.55, 112.08, 110.19, 55.96; MS (ESI) m/z = 388.95 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-methoxybenzamide (10a).** The compound was synthesized according to procedure **B** using 3-methoxybenzoyl chloride: yield 84%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.53 (s, 1H), 8.92 (d, *J* = 2.3 Hz, 1H), 8.72 (d, *J* = 2.1 Hz, 1H), 8.46 (t, *J* = 2.2 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.55 – 7.52 (m, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.24 – 7.14 (m, 1H), 3.85 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.79, 159.26, 141.30, 141.12, 136.05, 135.50, 129.72, 128.30, 127.13, 124.44, 123.39, 121.54, 119.93, 117.75, 113.06, 110.22, 55.40; MS (ESI) m/z = 388.89 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-4-methoxybenzamide (11a).** The compound was synthesized according to procedure **B** using 4-methoxybenzoyl chloride: yield 72%. The product

was purified by CC (ethyl acetate/petroleum ether 7:3); ^{1}H NMR (500 MHz, DMSO) δ 10.46 (s, 1H), 8.93 (d, *J* = 2.3 Hz, 1H), 8.68 (d, *J* = 2.1 Hz, 1H), 8.46 (t, *J* = 2.2 Hz, 1H), 8.02 (s, 1H), 8.00 (s, 1H), 7.80 (d, *J* = 1.4 Hz, 1H), 7.69 (d, *J* = 1.4 Hz, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 3.85 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.41, 162.26, 141.25, 141.23, 140.78, 136.36, 129.78, 128.25, 127.05, 126.12, 124.36, 123.25, 113.74, 110.20, 55.49; MS (ESI) m/z = 388.95 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-4-(*tert*-butyl)benzamide (12a).** The compound was synthesized according to procedure **B** using 4-*tert*-butylbenzoyl chloride: yield 79%. The product was purified by CC (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (500 MHz, DMSO) δ 10.49 (s, 1H), 8.92 (d, *J* = 2.2 Hz, 1H), 8.70 (d, *J* = 2.1 Hz, 1H), 8.47 (t, *J* = 2.2 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 1.33 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 165.97, 154.96, 141.15, 140.93, 136.19, 131.38, 129.16, 128.26, 127.61, 127.07, 126.43, 125.34, 125.28, 124.39, 123.16, 110.19, 34.73, 30.89; MS (ESI) m/z = 414.92 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-chlorobenzamide (13a).** The compound was synthesized according to procedure **B** using 2-chlorobenzoyl chloride: yield 59%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.88 (s, 1H), 8.80 (d, *J* = 2.2 Hz, 1H), 8.73 (d, *J* = 2.0 Hz, 1H), 8.44 (t, *J* = 2.1 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.74 – 7.66 (m, 1H), 7.66 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.62 – 7.58 (m, 1H), 7.55 (td, *J* = 7.7, 1.7 Hz, 1H), 7.49 (td, *J* = 7.4, 1.2 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 165.60, 141.34, 140.97, 140.44, 136.16, 135.85, 131.54, 129.96, 129.79, 129.06, 128.47, 127.35, 127.24, 124.54, 122.43, 110.25; MS (ESI) m/z = 392.9 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-chlorobenzamide (14a).** The compound was synthesized according to procedure **B** using 3-chlorobenzoyl chloride: yield 65%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.65 (s, 1H), 8.92 (d, *J* = 2.3 Hz, 1H), 8.73 (d, *J* = 2.1 Hz, 1H), 8.44 (t, *J* = 2.2 Hz, 1H), 8.06 (t, *J* = 1.8 Hz, 1H), 7.98 – 7.92 (m, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.71 (qd, *J* = 2.2, 1.0 Hz, 2H), 7.61 (t, *J* = 7.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.61, 141.32, 141.27, 141.04, 136.10, 135.85, 133.32, 131.86, 130.57, 128.33, 127.49, 127.17, 126.62, 124.48, 123.43, 110.24; MS (ESI) m/z = 392.81 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-4-chlorobenzamide (15a).** The compound was synthesized according to procedure **B** using 4-chlorobenzoyl chloride: yield 78%. The product was purified by CC (ethyl acetate/petroleum ether 7:3); ^{1}H NMR (500 MHz, DMSO) δ 10.62 (s, 1H), 8.91 (d, *J* = 2.2 Hz, 1H), 8.72 (d, *J* = 2.0 Hz, 1H), 8.44 (t, *J* = 2.2 Hz, 1H), 8.03 (d, *J* = 1.9 Hz, 1H), 8.02 (s, 1H), 7.81 (d, *J* = 1.3 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.66 (d, *J* = 1.9 Hz, 1H), 7.65 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.98, 141.28, 141.24, 141.07, 136.92, 135.93, 132.82, 129.72, 128.63, 128.32, 127.15, 124.46, 123.42, 110.23; MS (ESI) m/z = 392.89 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-fluorobenzamide (16a).** The compound was synthesized according to procedure **B** using 2-fluorobenzoyl chloride: yield 68%. The product was purified by CC (ethyl acetate/petroleum ether 7:3); ^{1}H NMR (500 MHz, DMSO) δ 11.16 (s, 1H), 9.01 (d, *J* = 2.0 Hz, 1H), 8.91 (d, *J* = 1.9 Hz, 1H), 8.66 (s, 1H), 7.89 (d, *J* = 1.4 Hz, 1H), 7.83 (d, *J* = 1.4 Hz, 1H), 7.77 (td, *J* = 7.5, 1.7 Hz, 1H), 7.69 – 7.62 (m, 1H), 7.39 (dt, *J* = 12.0, 9.0 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 163.58, 159.09 (d, J_{C-F}= 250.4 Hz), 139.63, 138.12, 136.94, 136.79, 133.40 (d, J_{C-F}= 8.5 Hz), 130.10 (d, J_{C-F} = 2.2 Hz), 129.87, 128.32, 125.57,

125.51, 124.69 (d, J_{C-F}= 3.5 Hz), 123.62 (d, J_{C-F}= 14.1 Hz), 116.38 (d, J_{C-F}= 21.5 Hz), 110.41; MS (ESI) m/z = 376.89 $(M+H)^+$

***N*-(5-(4-bromothiophen-2-yl)pyridin-3-yl)-3-fluorobenzamide (17a).** The compound was synthesized according to procedure **B** using 3-fluorobenzoyl chloride: yield 79%. The product was purified by CC (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (500 MHz, DMSO) δ 10.63 (s, 1H), 8.92 (d, *J* = 2.3 Hz, 1H), 8.73 (d, *J* = 2.1 Hz, 1H), 8.45 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.83 – 7.80 (m, 2H), 7.71 (d, *J* = 1.4 Hz, 1H), 7.63 (td, *J* = 8.0, 5.9 Hz, 1H), 7.50 (td, *J* = 8.1, 2.2 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.67, 161.94 (d, J_{C-F} = 244.5 Hz), 141.29 (d, J_{C-F} = 4.4 Hz), 141.04, 136.40 (d, J_{C-F} = 7.0 Hz), 135.85, 130.77 (d, J_{C-F} = 8.0 Hz), 128.32, 127.16, 124.47, 124.01, 123.98, 123.43, 118.97 (d, J_{C-F} = 21.2 Hz), 114.60 (d, J_{C-F} = 23.0 Hz), 110.23; MS (ESI) m/z = 377 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-4-fluorobenzamide (18a).** The compound was synthesized according to procedure **B** using 4-fluorobenzoyl chloride: yield 98%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.57 (s, 1H), 8.91 (d, *J* = 2.3 Hz, 1H), 8.72 (d, *J* = 2.1 Hz, 1H), 8.44 (t, *J* = 2.2 Hz, 1H), 8.12 – 8.05 (m, 2H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.41 (t, *J* = 8.9 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 164.96, 164.34 (d, J_{C-F}= 249.7 Hz), 141.26, 141.12 (d, J_{C-F}= 5.1 Hz), 136.03, 132.10 (d, J_{C-F}= 9.5 Hz), 130.60, 127.13, 124.44, 123.37, 115.62 (d, J_{C-F}= 22.0 Hz), 115.61, 115.44, 110.23; MS (ESI) m/z = 376.98 $(M+H)^+$

***N*-(5-(4-bromothiophen-2-yl)pyridin-3-yl)-2-(trifluoromethyl)benzamide fluorobenzamide (19a).** The compound was synthesized according to procedure **B** using 2-trifluoromethylbenzoyl chloride: yield 86%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H

NMR (500 MHz, DMSO) δ 10.96 (s, 1H), 8.78 (d, *J* = 2.3 Hz, 1H), 8.74 (d, *J* = 2.1 Hz, 1H), 8.40 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.83 – 7.81 (m, 2H), 7.76 (ddd, *J* = 8.2, 7.4, 4.9 Hz, 2H), 7.71 (d, *J* = 1.4 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 166.24, 141.40, 140.90, 140.45, 135.80, 132.69, 132.62, 131.16, 130.46, 129.64, 128.61, 128.47, 127.23, 126.46 (q, J_{C-F}= 5.0, 2.2 Hz), 124.52, 122.48, 110.23; MS (ESI) m/z = 426.93 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-(trifluoromethyl)benzamide fluorobenzamide (20a).** The compound was synthesized according to procedure **B** using 3-trifluoromethylbenzoyl chloride: yield 84%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.78 (s, 1H), 8.93 (d, *J* = 2.3 Hz, 1H), 8.75 (d, *J* = 2.1 Hz, 1H), 8.44 (t, *J* = 2.2 Hz, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 8.23 (d, *J* = 7.8 Hz, 1H), 8.18 (s, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.77 (s, 1H), 7.72 (d, *J* = 1.4 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 166.00, 141.02, 135.78, 135.03, 133.22, 130.10, 129.89, 129.40 (q, J_{C-F}= 3.5 Hz) , 128.58 (q, J_{C-F}= 7.2, 3.6 Hz), 128.36, 127.20, 125.50 (q, J_{C-F}= 3.9 Hz), 124.50, 124.35 (q, J_{C-F}= 7.7, 3.9 Hz), 123.60, 122.71, 110.25; MS (ESI) m/z = 426.88 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-4-(trifluoromethyl)benzamide fluorobenzamide (21a).** The compound was synthesized according to procedure **B** using 4-trifluoromethylbenzoyl chloride: yield 80%. The product was purified by CC (ethyl acetate/petroleum ether 7:3); ^{1}H NMR (500 MHz, DMSO) δ 10.78 (s, 1H), 8.92 (d, *J* = 2.3 Hz, 1H), 8.74 (d, *J* = 2.1 Hz, 1H), 8.46 (t, *J* = 2.2 Hz, 1H), 8.20 (s, 1H), 8.19 (s, 1H), 7.97 (s, 1H), 7.95 (s, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.71 (d, *J* = 1.4 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.93, 141.44, 141.31, 141.01, 137.93, 135.80, 131.87, 128.71, 128.36, 127.21, 126.95, 125.55 (q, J_{C-F}= 7.4, 3.7 Hz), 124.51, 123.49, 110.25; MS (ESI) m/z = 426.93 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2,3-dichlorobenzamide (22a).** The compound was synthesized according to procedure **B** using 2,3-dichlorobenzoyl chloride: yield 78%. The product was purified by CC (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (500 MHz, DMSO) δ 10.98 (s, 1H), 8.76 (dd, *J* = 13.1, 2.2 Hz, 2H), 8.42 (t, *J* = 2.2 Hz, 1H), 7.81 (td, *J* = 4.1, 1.5 Hz, 2H), 7.72 (d, *J* = 1.4 Hz, 1H), 7.64 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.80, 141.52, 140.88, 140.42, 138.40, 135.65, 132.22, 131.75, 128.78, 128.51, 128.16, 127.51, 127.29, 124.57, 122.48, 110.26; MS (ESI) m/z = 426.78 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2,4-difluorobenzamide (23a)** The compound was synthesized according to procedure **B** using 2,4-difluorobenzoyl chloride: yield 75%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.75 (s, 1H), 8.81 (d, *J* = 2.1 Hz, 1H), 8.73 (d, *J* = 2.1 Hz, 1H), 8.41 (s, 1H), 7.91 – 7.78 (m, 2H), 7.71 (d, *J* = 1.4 Hz, 1H), 7.54 – 7.40 (m, 1H), 7.28 (td, *J* = 8.3, 2.3 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 163.73 (dd, J_{C-F} = 251.1, 12.3 Hz), 162.54, 159.70 (dd, J_{C-F} = 253.1, 13.0 Hz), 141.40, 140.96, 140.74, 135.70, 131.87 (dd, J_{C-F} = 10.3, 4.0 Hz), 128.42, 127.23, 124.52, 122.84, 120.79 (dd, J_{C-F} = 14.6, 3.6 Hz), 112.01 (dd, J_{C-F} = 21.8, 3.5 Hz) , 110.24, 104.83 (t, J_{C-F} = 26.2 Hz); MS (ESI) m/z = 394.87 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-chloro-4-fluorobenzamide (24a).** The compound was synthesized according to procedure **B** using 2-chloro-4-fluorobenzoyl chloride: yield 81%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.90 (s, 1H), 8.78 (d, *J* = 2.3 Hz, 1H), 8.74 (d, *J* = 2.1 Hz, 1H), 8.42 (t, *J* = 2.2 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.76 (dd, *J* = 8.6, 6.1 Hz, 1H), 7.71 (d, *J* = 1.4 Hz, 1H), 7.64 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.40 (td, *J* = 8.5, 2.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.81, 162.47 (d, J_{C-F} = 250.5 Hz), 141.40, 140.94, 140.46, 135.77, 132.81, 131.53 (d, J_{C-F} = 10.9 Hz),

131.00 (d, $J_{C\text{-}F}$ = 9.4 Hz), 128.47, 127.25, 124.55, 122.48, 117.26 (d, $J_{C\text{-}F}$ = 25.4 Hz), 114.62 (d, $J_{C\text{-}F}$ = 21.7 Hz), 110.26; MS (ESI) m/z = 410.8$(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-chloro-4-fluorobenzamide (25a).** The compound was synthesized according to procedure **B** using 3-chloro-4-fluorobenzoyl chloride: yield 82%. The product was purified by CC (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (500 MHz, DMSO) δ 10.64 (s, 1H), 8.90 (d, *J* = 2.2 Hz, 1H), 8.73 (d, *J* = 2.1 Hz, 1H), 8.42 (t, *J* = 2.2 Hz, 1H), 8.24 (dd, *J* = 7.1, 2.2 Hz, 1H), 8.03 (ddd, *J* = 7.0, 4.4, 1.9 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.3 Hz, 1H), 7.64 (t, *J* = 8.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 163.72, 159.37 (d, $J_{C\text{-}F}$ = 252.2 Hz), 141.30 (d, $J_{C\text{-}F}$ = 14.2 Hz), 141.02, 135.80, 131.73 (d, $J_{C\text{-}F}$ = 3.5 Hz), 130.28, 129.23 (d, $J_{C\text{-}F}$ = 8.4 Hz), 128.34, 127.18, 124.49, 123.42, 119.91, 119.76, 117.22 (d, $J_{C\text{-}F}$ = 21.6 Hz), 110.25; MS (ESI) m/z = 410.82 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-fluoro-5-methoxybenzamide (26a).** The compound was synthesized according to procedure **B** using 2-fluoro-5-methoxybenzoyl chloride: yield 68%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.72 (s, 1H), 8.83 (d, *J* = 2.2 Hz, 1H), 8.73 (d, *J* = 2.1 Hz, 1H), 8.43 (t, *J* = 2.1 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.32 (t, *J* = 9.3 Hz, 1H), 7.24 (dd, *J* = 5.5, 3.2 Hz, 1H), 7.15 (dt, *J* = 9.0, 3.7 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 163.17, 155.30, 155.28, 153.17 (d, $J_{C\text{-}F}$ = 242.1 Hz), 141.35, 140.86 (d, $J_{C\text{-}F}$ = 33.1 Hz), 135.75, 128.41, 127.22, 124.51, 124.38, 122.79, 118.24 (d, $J_{C\text{-}F}$ = 8.2 Hz), 117.21 (d, $J_{C\text{-}F}$ = 23.8 Hz), 114.10 (d, $J_{C\text{-}F}$ = 2.4 Hz), 110.24, 55.89.

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2,4,6-trifluorobenzamide (27a).** The compound was synthesized according to procedure **B** using 2,4,6-trifluorobenzoyl chloride: yield 69%. The

product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 11.20 (s, 1H), 8.76 (dd, *J* = 4.8, 2.2 Hz, 2H), 8.38 (t, *J* = 2.2 Hz, 1H), 7.82 (d, *J* = 1.4 Hz, 1H), 7.73 (d, *J* = 1.4 Hz, 1H), 7.44 (dd, *J* = 9.3, 7.8 Hz, 2H); MS (ESI) m/z = 412.81$(M+H)^{+}$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2,3,4-trifluorobenzamide (28a).** The compound was synthesized according to procedure **B** using 2,3,4-trifluorobenzoyl chloride: yield 81%. The product was purified by CC (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (500 MHz, DMSO) δ 11.03 (s, 1H), 8.83 (d, *J* = 2.2 Hz, 1H), 8.74 (d, *J* = 2.1 Hz, 1H), 8.42 (t, *J* = 2.1 Hz, 1H), 7.81 (d, *J* = 1.4 Hz, 1H), 7.71 (d, *J* = 1.4 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.25 (td, *J* = 9.2, 1.8 Hz, 1H); MS (ESI) m/z = 412.80 $(M+H)^{+}$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)isonicotinamide (29a).** The compound was synthesized according to procedure **B** using isonicotinyl chloride: yield 71%. The product was purified by CC (ethyl acetate/MeOH 100:7); ^{1}H NMR (500 MHz, DMSO) δ 10.83 (s, 1H), 8.91 (d, *J* = 2.3 Hz, 1H), 8.83 (dd, *J* = 4.4, 1.7 Hz, 2H), 8.76 (d, *J* = 2.1 Hz, 1H), 8.45 (t, *J* = 2.2 Hz, 1H), 7.90 (dd, *J* = 4.4, 1.7 Hz, 2H), 7.82 (d, *J* = 1.4 Hz, 1H), 7.72 (d, *J* = 1.4 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.61, 150.43, 141.61, 141.32, 141.16, 140.96, 135.59, 128.39, 127.24, 124.54, 123.56, 121.56, 110.27; MS (ESI) m/z = 459.97 $(M+H)^{+}$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-phenylacetamide (30a).** The compound was synthesized according to procedure **B** using phenylacetyl chloride: yield 81%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.53 (s, 1H), 8.66 (dd, *J* = 14.6, 2.2 Hz, 2H), 8.32 (t, *J* = 2.2 Hz, 1H), 7.78 (d, *J* = 1.4 Hz, 1H), 7.66 (d, *J* = 1.4 Hz, 1H), 7.37 – 7.31 (m, 4H), 7.26 (ddd, *J* = 8.6, 5.6, 2.7 Hz, 1H), 3.70 (s, 2H); ^{13}C NMR (126

MHz, DMSO) δ 170.01, 141.04, 140.71, 140.05, 136.05, 135.40, 129.21, 128.32, 127.09, 126.65, 124.38, 121.97, 115.90, 110.18, 43.09; MS (ESI) m/z = 372.98 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-(4-fluorophenyl)acetamide (31a).** The compound was synthesized according to procedure **B** using 4-fluorophenyl chloride: yield 84%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.34 (s, 1H), 8.48 (dd, *J* = 10.4, 2.2 Hz, 2H), 8.13 (t, *J* = 2.2 Hz, 1H), 7.60 (d, *J* = 1.4 Hz, 1H), 7.47 (d, *J* = 1.4 Hz, 1H), 7.26 – 7.09 (m, 2H), 6.97 (ddd, *J* = 14.7, 8.4, 5.2 Hz, 2H), 3.52 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 164.59, 161.19 (d, J_{C-F}= 242.3 Hz), 141.06, 140.76, 140.09, 136.04, 131.56 (d, J_{C-F}= 3.0 Hz), 131.16 (d, J_{C-F}= 8.1 Hz), 128.36, 127.12, 124.41, 122.02, 115.04 (d, J_{C-F}= 21.3 Hz), 110.21, 42.06; MS (ESI) m/z = 390.93 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-2-(3-methoxyphenyl)acetamide (32a).** The compound was synthesized according to procedure **B** using 3-methoxyphenyl acetyl chloride: yield 97%. The product was purified by CC (ethyl acetate); ^{1}H NMR (500 MHz, DMSO) δ 10.52 (s, 1H), 8.68 (d, *J* = 2.2 Hz, 1H), 8.65 (d, *J* = 2.1 Hz, 1H), 8.32 (t, *J* = 2.1 Hz, 1H), 7.78 (d, *J* = 1.2 Hz, 1H), 7.66 (d, *J* = 1.2 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.01 – 6.88 (m, 2H), 6.85 – 6.78 (m, 1H), 3.75 (s, 3H), 3.67 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 170.39, 159.26, 141.10, 140.77, 140.10, 136.87, 136.11, 129.41, 128.39, 127.16, 124.45, 122.01, 121.46, 115.06, 112.09, 110.26, 59.80, 55.02; MS (ESI) m/z = 404.84 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-(3-methoxyphenyl)propanamide (33a).** The compound was synthesized according to procedure **B** using 3-(3-methoxyphenyl)propanoyl chloride: yield 92%. The product was purified by CC (ethyl acetate/petroleum ether 3:2); ^{1}H NMR (500 MHz, DMSO) δ 10.29 (s, 1H), 8.65 (dd, *J* = 3.8, 2.2 Hz, 2H), 8.30 (s, 1H), 7.79 (d, *J*

= 1.3 Hz, 1H), 7.66 (d, *J* = 1.3 Hz, 1H), 7.19 (dd, *J* = 13.3, 5.2 Hz, 1H), 6.83 (d, *J* = 6.7 Hz, 2H), 6.76 (dd, *J* = 8.1, 2.3 Hz, 1H), 3.72 (s, 3H), 2.91 (t, *J* = 7.7 Hz, 2H), 2.68 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 171.35, 159.31, 142.56, 141.14, 140.05, 136.07, 129.41, 128.37, 127.10, 124.43, 121.94, 120.48, 113.95, 113.91, 111.47, 110.25, 54.91, 35.17, 30.64; MS (ESI) m/z = 416.9 $(M+H)^+$

***N*-(5-(4-Bromothiophen-2-yl)pyridin-3-yl)-3-(pyridin-3-yl)propanamide (34a).** The compound was synthesized according to procedure **B** using 3-(pyridin-3-yl)propanoyl chloride: yield 82%. The product was purified by CC (DCM/MeOH 100:5); ^{1}H NMR (500 MHz, DMSO) δ 10.31 (s, 1H), 8.64 (d, *J* = 2.1 Hz, 2H), 8.49 (d, *J* = 1.7 Hz, 1H), 8.41 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.28 (t, *J* = 2.2 Hz, 1H), 7.79 (d, *J* = 1.4 Hz, 1H), 7.72 – 7.58 (m, 2H), 7.32 (ddd, *J* = 7.8, 4.8, 0.8 Hz, 1H), 2.95 (t, *J* = 7.5 Hz, 2H), 2.72 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 171.02, 149.59, 147.37, 141.09, 140.65, 140.04, 136.38, 135.96, 135.84, 128.36, 127.09, 124.40, 123.45, 121.97, 110.21, 37.25, 27.63; MS (ESI) m/z = 387.94 $(M+H)^+$

5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-2-amine (1). The compound was synthesized according to procedure **C** to give a yellow solid: yield 12%. The product was purified by CC (DCM/MeOH 100:3); mp161.8-162.8 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.45 (d, *J* = 1.7 Hz, 1H), 8.96 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.83 (d, *J* = 2.1 Hz, 1H), 8.55 (ddd, *J* = 7.9, 2.3, 1.6 Hz, 1H), 8.23 – 8.20 (m, 2H), 8.19 (d, *J* = 1.5 Hz, 1H), 7.86 (ddd, *J* = 7.9, 4.8, 0.8 Hz, 1H), 7.08 (dd, *J* = 8.6, 0.7 Hz, 1H), 6.13 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 170.13, 158.84, 158.00, 155.85, 154.00, 150.13, 145.26, 143.55, 141.88, 134.19, 130.78, 130.03, 129.84, 118.52; MS (ESI) m/z = 253.93 $(M + H)^+$

5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-amine (2). The compound was synthesized according to procedure **C** to give a dark brown solid: yield 32%. The product was purified by CC (DCM/MeOH 100:5); mp134.7-135 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.04 (d, *J* = 1.7 Hz, 1H), 8.52 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.20 – 8.16 (m, 2H), 8.06 (d, *J* = 1.5 Hz, 1H), 8.05 (d, *J* = 1.5 Hz, 1H), 7.91 (d, *J* = 2.3 Hz, 1H), 7.46 (ddd, *J* = 8.0, 4.8, 0.8 Hz, 1H), 7.20 – 7.17 (m, 1H), 5.51 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 148.28, 147.17, 144.98, 142.00, 139.14, 135.91, 133.85, 133.22, 130.55, 129.27, 123.88, 122.80, 122.06, 115.90; MS (ESI) m/z = 253.94 $(M + H)^{+}$

5-(4-(Pyridin-4-yl)thiophen-2-yl)pyridin-3-amine (3). The compound was synthesized according to procedure **C** to give a light brown solid: yield 23%. The product was purified by CC (DCM/MeOH 100:3); mp199.2-202 °C; ^{1}H NMR (500 MHz, DMSO) δ 8.62 (s, 2H), 8.23 (s, 1H), 8.20 (s, 1H), 8.09 (d, *J* = 1.0 Hz, 1H), 7.92 (s, 1H), 7.80 (s, 2H), 7.19 (s, 1H), 5.52 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 150.28, 145.04, 142.19, 141.51, 139.69, 136.00, 133.83, 129.19, 124.12, 122.66, 120.51, 115.90; MS (ESI) m/z = 253.96 $(M + H)^{+}$

***N*-(5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (4b).** The compound was synthesized according to procedure **C** to give a red solid: yield 29%. The product was purified by CC (DCM/MeOH 100:3); mp 88.2-90.5 °C; ^{1}H NMR (500 MHz, DMSO) δ 10.59 (s, 1H), 9.08 (s, 1H), 8.87 (dd, *J* = 53.6, 1.9 Hz, 2H), 8.54 (dd, *J* = 5.1, 2.9 Hz, 2H), 8.25 – 8.12 (m, 3H), 8.07 – 7.96 (m, 2H), 7.67 – 7.61 (m, 1H), 7.58 (ddt, *J* = 8.2, 6.7, 1.3 Hz, 2H), 7.48 (dd, *J* = 7.8, 4.7 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 166.08, 148.39, 147.20, 141.19, 140.92, 140.82, 139.41, 136.12, 134.17, 133.28, 132.05, 130.43, 129.26, 128.54, 127.77, 123.92, 123.74, 123.49, 122.94; MS (ESI) m/z = 358.05 $(M + H)^{+}$

***N*-(5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (5b).** The compound was synthesized according to procedure **C** to give a white solid: yield 29%. The product was purified by CC (DCM/MeOH 100:4); mp 213.4-214.7 °C; ^{1}H NMR (500 MHz, DMSO) δ 10.74 (s, 1H), 9.06 (s, 1H), 8.73 (s, 1H), 8.54 (s, 1H), 8.16 (dd, *J* = 19.5, 8.3 Hz, 4H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.73 (s, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.58 (t, *J* = 7.4 Hz, 2H), 7.48 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 148.41, 147.19, 141.94, 140.47, 140.00, 139.47, 138.93, 134.71, 133.34, 133.27, 130.32, 129.64, 129.51, 126.73, 124.12, 123.88, 123.28, 123.22; MS (ESI) m/z = 393.92 $(M + H)^+$

2-Methyl-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (6b). The compound was synthesized according to procedure **C** to give a brown solid: yield 47%. The product was purified by CC (DCM/MeOH 100:2.5); mp 171.6-173.4 °C;^{1}H NMR (500 MHz, DMSO) δ 10.65 (s, 1H), 9.08 (d, *J* = 1.5 Hz, 1H), 8.83 (s, 1H), 8.81 (d, *J* = 1.7 Hz, 1H), 8.54 (d, *J* = 2.4 Hz, 2H), 8.27 – 8.20 (m, 2H), 8.16 (d, *J* = 1.4 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.49 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.43 (dd, *J* = 10.7, 4.3 Hz, 1H), 7.34 (t, *J* = 7.3 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 168.45, 148.27, 147.09, 141.09, 140.82, 140.27, 139.35, 136.34, 136.20, 135.56, 133.41, 130.69, 130.47, 130.07, 129.32, 127.39, 125.70, 123.95, 123.74, 122.99, 122.71, 19.39; MS (ESI) m/z = 372.08 $(M + H)^+$

3-Methyl-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (7b). The compound was synthesized according to procedure **C** to give a dark grey solid: yield 26%. The product was purified by CC (DCM/MeOH 100:3); mp 103-103.7 °C;^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.96 (d, *J* = 10.0 Hz, 3H), 8.91 (s, 1H), 8.87 (s, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.06 (s, 1H), 8.04 (s, 1H), 7.95 (s, 1H), 7.84 (s, 1H), 7.69 (d, *J* = 4.5 Hz, 2H), 7.67 (d, *J* = 7.0 Hz, 1H), 2.73 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.78, 148.58, 147.57, 142.35, 141.59, 140.50, 139.95,

138.98, 135.52, 134.14, 133.72, 133.28, 131.37, 130.41, 128.90, 128.10, 124.39, 124.31, 123.91, 123.51, 122.28, 24.98; MS (ESI) m/z = 372.08 $(M + H)^+$

4-Methyl-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (8b). The compound was synthesized according to procedure **C** to give a dark grey solid: yield 45%. The product was purified by CC (DCM/MeOH 100:3); mp 185.9-186.9°C; ^{1}H NMR (500 MHz, DMSO) δ 10.50 (s, 1H), 9.09 (s, 1H), 8.94 (s, 1H), 8.82 (s, 1H), 8.54 (s, 2H), 8.22 (d, *J* = 13.0 Hz, 2H), 8.16 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 2H), 7.48 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.83, 148.33, 147.15, 142.12, 141.01, 140.87, 140.84, 139.39, 136.21, 134.38, 133.23, 131.24, 130.48, 129.01, 127.77, 123.93, 123.68, 123.42, 122.88, 21.02; MS (ESI) m/z = 372.02 $(M + H)^+$

2-Methoxy-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (9b). The compound was synthesized according to procedure **C** to give a greyish white solid: yield 44%. The product was purified by CC (DCM/MeOH 100:3); mp 185.9-186.9°C;^{1}H NMR (500 MHz, DMSO) δ 10.44 (s, 1H), 9.10 (s, 1H), 8.81 (s, 2H), 8.55 (s, 2H), 8.22 (s, 2H), 8.15 (s, 1H), 7.69 (d, *J* = 6.2 Hz, 1H), 7.54 (s, 1H), 7.49 (s, 1H), 7.21 (d, *J* = 7.9 Hz, 1H), 7.10 (d, *J* = 6.8 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.24, 156.59, 148.35, 147.17, 141.06, 140.78, 140.46, 139.37, 134.39, 133.25, 132.47, 130.44, 129.74, 129.33, 124.19, 123.92, 123.76, 122.92, 122.80, 120.52, 112.04, 55.94; MS (ESI) m/z = 388.03 $(M + H)^+$

3-Methoxy-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (10b). The compound was synthesized according to procedure **C** to give a white solid: yield 47%. The product was purified by CC (DCM/MeOH 100:2); mp 170.5-171.3°C;^{1}H NMR (500 MHz, DMSO) δ 10.54 (s, 1H), 9.07 (s, 1H), 8.92 (s, 1H), 8.82 (s, 1H), 8.53 (d, *J* = 1.9 Hz, 2H), 8.28 –

8.18 (m, 2H), 8.15 (s, 1H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.55 (s, 1H), 7.48 (dd, *J* = 14.3, 6.4 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.77, 159.26, 148.39, 147.20, 141.22, 140.97, 140.80, 139.40, 136.04, 135.54, 133.27, 130.42, 129.71, 129.24, 123.90, 123.74, 123.55, 122.93, 119.94, 117.74, 113.06, 55.40; MS (ESI) m/z = 388.09 $(M + H)^+$

4-Methoxy-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (11b). The compound was synthesized according to procedure **C** to give a dark brown solid: yield 32%. The product was purified by CC (DCM/MeOH 100:4); mp 210.5-210.8°C;^{1}H NMR (500 MHz, DMSO) δ 10.41 (s, 1H), 9.07 (s, 1H), 8.92 (s, 1H), 8.79 (s, 1H), 8.53 (s, 2H), 8.21 (d, *J* = 9.4 Hz, 2H), 8.15 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 2H), 7.50 – 7.37 (m, 1H), 7.10 (d, *J* = 8.5 Hz, 2H), 3.86 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 165.39, 162.25, 148.38, 147.19, 140.93, 140.89, 139.38, 136.29, 133.26, 130.62, 130.43, 129.77, 129.20, 126.14, 123.90, 123.67, 123.42, 122.88, 113.75, 55.49; MS (ESI) m/z = 388.1 $(M + H)^+$

4-(*tert*-Butyl)-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (12b). The compound was synthesized according to procedure **C** to give a white solid: yield 51%. The product was purified by CC (DCM/MeOH 100:3); mp 205.4-205.9°C;^{1}H NMR (500 MHz, DMSO) δ 10.50 (s, 1H), 9.07 (d, *J* = 1.8 Hz, 1H), 8.92 (d, *J* = 2.3 Hz, 1H), 8.80 (d, *J* = 2.1 Hz, 1H), 8.57 – 8.51 (m, 2H), 8.25 – 8.19 (m, 2H), 8.16 (d, *J* = 1.4 Hz, 1H), 7.96 (d, *J* = 1.8 Hz, 1H), 7.95 (d, *J* = 1.8 Hz, 1H), 7.59 (d, *J* = 1.8 Hz, 1H), 7.58 (d, *J* = 1.8 Hz, 1H), 7.51 – 7.44 (m, 1H), 1.33 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 165.98, 154.95, 148.39, 147.20, 141.07, 140.84, 139.40, 136.20, 134.42, 133.27, 131.45, 130.42, 129.22, 127.65, 125.30, 123.90, 123.71, 123.35, 122.91, 34.75, 30.91; MS (ESI) m/z = 414.04 $(M + H)^+$

2-Chloro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (13b). The compound was synthesized according to procedure **C** to give a brown solid: yield 53%. The product was purified by CC (DCM/MeOH 100:3); mp 159-159.6°C;^{1}H NMR (500 MHz, DMSO) δ 10.89 (s, 1H), 9.08 (s, 1H), 8.82 (d, *J* = 17.7 Hz, 2H), 8.53 (d, *J* = 12.2 Hz, 2H), 8.26 – 8.18 (m, 2H), 8.16 (s, 1H), 7.67 (d, *J* = 7.4 Hz, 1H), 7.55 (t, *J* = 6.8 Hz, 2H), 7.49 (d, *J* = 7.3 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 165.59, 149.14, 148.38, 147.78, 147.20, 141.43, 140.66, 140.09, 139.44, 136.22, 134.41, 133.28, 131.50, 129.96, 129.78, 129.06, 127.34, 123.91, 123.86, 123.03, 122.56; MS (ESI) m/z = 391.96 $(M + H)^+$

3-Chloro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (14b). The compound was synthesized according to procedure **C** to give a dark grey solid: yield 56%. The product was purified by CC (DCM/MeOH 100:3); mp 98.5-100.5°C;^{1}H NMR (500 MHz, DMSO) δ 10.66 (s, 1H), 9.08 (s, 1H), 8.93 (s, 1H), 8.84 (s, 1H), 8.54 (s, 1H), 8.51 (s, 1H), 8.23 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.61 (t, *J* = 7.9 Hz, 1H), 7.48 (dd, *J* = 7.6, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.58, 148.36, 147.17, 141.40, 140.91, 140.72, 139.41, 136.12, 135.88, 133.31, 133.26, 131.83, 130.54, 130.44, 129.30, 127.49, 126.61, 123.92, 123.77, 123.55, 122.97; MS (ESI) m/z = 391.97 $(M + H)^+$

4-Chloro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (15b). The compound was synthesized according to procedure **C** to give a dark grey solid: yield 46%. The product was purified by CC (DCM/MeOH 100:3); mp 196-196.4°C;^{1}H NMR (500 MHz, DMSO) δ 10.63 (s, 1H), 9.07 (s, 1H), 8.91 (s, 1H), 8.82 (s, 1H), 8.60 – 8.47 (m, 2H), 8.29 – 8.17 (m, 2H), 8.15 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.48 (dd, *J* = 7.7, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.95, 148.37, 147.18, 141.33, 140.93, 140.74, 139.41, 136.89, 135.93,

133.25, 132.85, 130.42, 129.72, 129.27, 128.61, 123.90, 123.76, 123.56, 122.95; MS (ESI) m/z = 392.02 $(M + H)^+$

2-Fluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (16b). The compound was synthesized according to procedure **C** to give a grey solid: yield 52%. The product was purified by CC (DCM/MeOH 100:3); mp 169.3-169.6°C;^{1}H NMR (500 MHz, DMSO) δ 10.53 (s, 1H), 9.08 (s, 1H), 8.94 (s, 1H), 8.82 (s, 1H), 8.53 (t, *J* = 2.2 Hz, 2H), 8.25 – 8.19 (m, 2H), 8.15 (d, *J* = 1.4 Hz, 1H), 7.84 (s, 1H), 7.82 – 7.79 (m, 1H), 7.52 – 7.47 (m, 1H), 7.47 – 7.42 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 163.44, 159.00 (d, J_{C-F} = 249.5 Hz), 149.15, 147.19, 141.45, 140.69, 140.35, 139.43, 135.82, 133.31, 133.07 (d, J_{C-F}= 8.4 Hz), 130.04 (d, J_{C-F}= 2.5 Hz),130.43, 129.39, 123.93,124.69 (d, J_{C-F} = 3.3 Hz), 124.20 (d, J_{C-F} = 14.6 Hz), 123.86, 123.03, 122.90.16.32 (d, J_{C-F}= 21.6 Hz); MS (ESI) m/z = 376.05 $(M + H)^+$

3-Fluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (17b). The compound was synthesized according to procedure **C** to give a dark brown solid: yield 41%. The product was purified by CC (DCM/MeOH 100:3); mp 152.1-152.2°C;^{1}H NMR (500 MHz, DMSO) δ 10.63 (s, 1H), 9.08 (s, 1H), 8.92 (s, 1H), 8.83 (s, 1H), 8.52 (s, 2H), 8.30 – 8.17 (m, 2H), 8.16 (s, 1H), 7.85 (dd, *J* = 20.5, 8.6 Hz, 2H), 7.64 (dd, *J* = 13.8, 7.5 Hz, 1H), 7.53 – 7.45 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 164.66, 161.94 (d, J_{C-F}= 244.5 Hz), 148.38, 147.18, 141.40, 140.92, 140.71, 139.41, 136.44 (d, J_{C-F} = 6.9 Hz), 135.86, 133.26, 130.79, 130.75 (d, J_{C-F} = 8.1 Hz), 129.28, 124.00 (d, J_{C-F}= 2.6 Hz), 123.90, 123.78, 123.57, 122.97, 118.94 (d, J_{C-F} = 21.1 Hz), 114.60 (d, J_{C-F}= 23.0 Hz); MS (ESI) m/z = 376.06 $(M + H)^+$

4-Fluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (18b). The compound was synthesized according to procedure **C** to give a brown solid: yield 34%. The product was

purified by CC (DCM/MeOH 100:3); mp 175.1-176°C;^{1}H NMR (500 MHz, DMSO) δ 10.58 (s, 1H), 9.07 (d, *J* = 1.9 Hz, 1H), 8.91 (d, *J* = 2.2 Hz, 1H), 8.81 (d, *J* = 2.0 Hz, 1H), 8.53 (dd, *J* = 4.7, 1.3 Hz, 1H), 8.51 (t, *J* = 2.1 Hz, 1H), 8.21 (dd, *J* = 7.9, 4.7 Hz, 2H), 8.15 (d, *J* = 1.3 Hz, 1H), 8.10 (dd, *J* = 8.8, 5.5 Hz, 2H), 7.47 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 164.94, 164.32 (d, J_{C-F}= 249.8 Hz), 148.37, 147.19, 141.24, 140.85 (d, J_{C-F} = 19.3 Hz) , 139.40, 136.01, 133.26, 130.62, 130.52 (d, J_{C-F} = 26.4 Hz), 130.41, 129.24, 123.88, 123.73, 123.54, 122.92, 115.59, 115.41; MS (ESI) m/z = 376.08 $(M + H)^+$

***N*-(5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)-2-(trifluoromethyl)benzamide (19b).** The compound was synthesized according to procedure **C** to give grey solid: yield 53%. The product was purified by CC (DCM/MeOH 100:3); mp 81.4-83.1°C;^{1}H NMR (500 MHz, DMSO) δ 10.96 (s, 1H), 9.08 (d, *J* = 2.0 Hz, 1H), 8.84 (d, *J* = 1.9 Hz, 1H), 8.78 (d, *J* = 2.1 Hz, 1H), 8.59 – 8.51 (m, 1H), 8.47 (t, *J* = 2.1 Hz, 1H), 8.22 (dd, *J* = 7.1, 4.7 Hz, 2H), 8.16 (d, *J* = 1.2 Hz, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.87 – 7.78 (m, 2H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.48 (dd, *J* = 7.9, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 166.23, 148.34, 147.16, 141.50, 140.59, 140.09, 139.41, 135.81, 135.42, 133.31, 132.69, 130.45, 130.39, 129.43, 128.63, 126.45 (q, J_{C-F} = 8.9, 4.1 Hz), 125.76, 124.79, 123.88, 123.86, 123.04, 122.61; MS (ESI) m/z = 425.97 $(M + H)^+$

***N*-(5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)-3-(trifluoromethyl)benzamide (20b).** The compound was synthesized according to procedure **C** to give light brown solid: yield 47%. The product was purified by CC (DCM/MeOH 100:3); mp 185.6-186.4°C;^{1}H NMR (500 MHz, DMSO) δ 10.79 (s, 1H), 9.08 (s, 1H), 8.93 (s, 1H), 8.85 (s, 1H), 8.54 (d, *J* = 3.9 Hz, 1H), 8.51 (s, 1H), 8.36 (s, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 8.24 (s, 1H), 8.21 (d, *J* = 8.1 Hz, 1H), 8.16 (s, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.83 (t, *J* = 7.7 Hz, 1H), 7.48 (dd, *J* = 7.7, 4.8 Hz, 1H); ^{13}C NMR (126

MHz, DMSO) δ 164.57, 141.28 (d, J_{C-F}= 61.5 Hz), 148.39, 147.19, 140.68, 139.42, 135.78, 135.06, 133.26, 131.96, 130.41, 129.89, 129.30, 129.28 (d, J_{C-F}= 32.2 Hz) , 128.57 (d, J_{C-F}= 3.3 Hz), 125.02, 124.35 (q, J_{C-F}= 7.6, 3.8 Hz) , 123.90, 123.81, 123.73, 122.99, 122.86; MS (ESI) m/z = 426.03 $(M + H)^+$

***N*-(5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)-4-(trifluoromethyl)benzamide (21b).** The compound was synthesized according to procedure **C** to give a greyish white solid: yield 34%. The product was purified by CC (DCM/MeOH 100:3); mp 208.8-210.1°C; ^{1}H NMR (500 MHz, DMSO) δ 10.78 (s, 1H), 9.07 (s, 1H), 8.92 (s, 1H), 8.84 (s, 1H), 8.53 (s, 2H), 8.27 – 8.18 (m, 4H), 8.16 (s, 1H), 7.97 (s, 1H), 7.95 (s, 1H), 7.47 (dd, *J* = 7.6, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.89, 148.38, 147.18, 141.52, 141.52, 140.95, 140.68, 139.41, 137.95, 135.77, 133.25, 131.85, 130.39, 129.29, 128.69, 125.52 (q, J_{C-F} = 3.7 Hz), 123.88, 123.80, 123.62, 122.97; MS (ESI) m/z = 426 $(M + H)^+$

2,3-Dichloro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (22b). The compound was synthesized according to procedure **C** to give dark brown semi-solid: yield 58%. The product was purified by CC (DCM/MeOH 100:3) ; ^{1}H NMR (500 MHz, DMSO) δ 11.01 (s, 1H), 9.18 (s, 1H), 8.92 (s, 2H), 8.64 (s, 1H), 8.51 (d, *J* = 9.2 Hz, 1H), 8.25 – 8.20 (m, 2H), 8.17 (s, 1H), 7.82 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.66 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.53 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 164.79, 148.30, 147.12, 141.51, 140.61, 140.01, 139.49, 138.45, 134.38, 133.24, 132.20, 131.82, 131.71, 130.53, 128.76, 128.15, 127.50, 126.60, 123.93, 123.08, 122.55; MS (ESI) m/z = 426.07 $(M + H)^+$

2,4-Difluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (23b). The compound was synthesized according to procedure **C** to give brown solid: yield 56%. The

product was purified by CC (DCM/MeOH 100:3); mp 210-212.8°C;^{1}H NMR (500 MHz, DMSO) δ 10.77 (s, 1H), 9.08 (s, 1H), 8.83 (d, *J* = 11.0 Hz, 2H), 8.54 (s, 1H), 8.49 (s, 1H), 8.24 (d, *J* = 1.3 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 1.3 Hz, 1H), 7.84 (dd, *J* = 15.1, 8.4 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.28 (td, *J* = 8.5, 2.4 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 163.70 (dd, J_{C-F} = 250.8, 12.2 Hz),159.70 (dd, J_{C-F}= 252.7, 13.0 Hz), 162.52, 149.11, 148.36, 147.75, 147.17, 141.47, 140.62, 140.36, 139.42, 134.39, 133.25, 131.85 (dd, J_{C-F}= 10.5, 4.1 Hz), 123.85, 123.00, 122.96, 120.83 (dd, J_{C-F}= 14.5, 3.6 Hz), 111.98 (dd, J_{C-F}= 21.5, 3.5 Hz), 105.02, 104.81 (d, J_{C-F}= 52.5 Hz); MS (ESI) m/z = 394 $(M + H)^+$

2-Chloro-4-fluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (24b). The compound was synthesized according to procedure **C** to give dark grey solid: yield 50%. The product was purified by CC (DCM/MeOH 100:3); mp 166.6-169°C;^{1}H NMR (500 MHz, DMSO) δ 10.90 (s, 1H), 9.08 (s, 1H), 8.84 (s, 1H), 8.79 (s, 1H), 8.54 (s, 1H), 8.50 (d, *J* = 1.9 Hz, 1H), 8.22 (dd, *J* = 12.9, 4.6 Hz, 2H), 8.16 (d, *J* = 1.2 Hz, 1H), 7.77 (dd, *J* = 8.5, 6.1 Hz, 1H), 7.64 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.48 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.40 (td, *J* = 8.5, 2.4 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.78, 162.43 (d, J_{C-F} = 250.5 Hz), 148.36, 147.17, 141.47, 140.60, 140.08, 139.42, 135.77, 134.40, 133.27, 132.87, 131.51 (d, J_{C-F} = 11.0 Hz), 130.98 (d, J_{C-F} = 9.3 Hz) , 130.40, 123.91, 123.85, 123.03, 122.58, 117.24 (d, J_{C-F} = 25.2 Hz), 114.60 (d, J_{C-F} = 21.6 Hz); MS (ESI) m/z = 409.95 $(M + H)^+$

3-Chloro-4-fluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (25b). The compound was synthesized according to procedure **C** to give a brown solid: yield 44%. The product was purified by CC (DCM/MeOH 100:3); mp 126.8-127.7°C;^{1}H NMR (500 MHz, DMSO) δ 10.66 (s, 1H), 9.15 (s, 1H), 8.93 (d, *J* = 36.4 Hz, 2H), 8.61 (s, 1H), 8.49 (s, 1H), 8.26

(dd, *J* = 7.1, 2.1 Hz, 1H), 8.21 (d, *J* = 11.4 Hz, 2H), 8.15 (s, 1H), 8.05 (ddd, *J* = 8.3, 4.6, 2.2 Hz, 1H), 7.64 (t, *J* = 8.9 Hz, 1H), 7.51 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 163.64, 159.29 (d, J_{C-F}= 252.4 Hz), 148.25, 147.07, 141.31, 141.30, 141.26, 140.80, 140.69, 140.68, 139.42, 133.17, 131.70, 131.69 (d, J_{C-F}= 3.4 Hz), 129.19, 129.16 (d, J_{C-F}= 8.5 Hz), 123.75, 123.45, 122.94, 119.77 (d, J_{C-F}= 18.0 Hz), 117.14 (d, J_{C-F}= 21.5 Hz); MS (ESI) m/z = 409.94 $(M + H)^+$

2-Fluoro-5-methoxy-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (26b). The compound was synthesized according to procedure **C** to give white solid: yield 49%. The product was purified by CC (DCM/MeOH 100:3); mp 181.6-182.9°C;^{1}H NMR (500 MHz, DMSO) δ 10.73 (s, 1H), 9.07 (d, *J* = 1.9 Hz, 1H), 8.83 (d, *J* = 1.9 Hz, 2H), 8.53 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.50 (s, 1H), 8.22 (ddd, *J* = 8.0, 4.6, 1.5 Hz, 2H), 8.16 (d, *J* = 1.3 Hz, 1H), 7.48 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.33 (t, *J* = 9.3 Hz, 1H), 7.26 (dd, *J* = 5.5, 3.2 Hz, 1H), 7.18 – 7.13 (m, 1H), 3.82 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 163.16, 153.19 (d, J_{C-F} = 242.2 Hz), 149.14, 148.37, 147.78, 147.18, 141.46, 140.67, 140.39, 139.41, 135.75, 133.31, 130.41, 129.35, 124.50 (d, J_{C-F} = 16.5 Hz), 123.91, 123.84, , 122.98 (d, J_{C-F} = 11.1 Hz),118.22 (d, J_{C-F} = 8.0 Hz), 117.21 (d, J_{C-F} = 23.8 Hz), 114.11 (d, J_{C-F} = 2.5 Hz), 55.87; MS (ESI) m/z = 405.99 $(M + H)^+$

2,4,6-Trifluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide(27b). The compound was synthesized according to procedure **C** to give dark grey solid: yield 59%. The product was purified by CC (DCM/MeOH 100:3); mp 168.2-170.5°C;^{1}H NMR (500 MHz, DMSO) δ 11.22 (s, 1H), 9.15 (s, 1H), 8.91 (s, 1H), 8.60 (s, 1H), 8.46 (s, 1H), 8.26 (s, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 8.17 (s, 1H), 7.58 – 7.50 (m, 2H), 7.44 (t, *J* = 8.7 Hz, 2H); MS (ESI) m/z = 411.95 $(M + H)^+$

2,3,4-Trifluoro-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)benzamide (28b). The compound was synthesized according to procedure **C** to give a grey solid: yield 49%. The product was purified by CC (DCM/MeOH 100:3); mp 161.6-161.9°C;^{1}H NMR (500 MHz, DMSO) δ 10.89 (s, 1H), 9.08 (s, 1H), 8.83 (d, *J* = 23.4 Hz, 2H), 8.54 (s, 1H), 8.47 (s, 1H), 8.24 (d, *J* = 1.2 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 1.2 Hz, 1H), 7.66 (dd, *J* = 13.5, 6.2 Hz, 1H), 7.57 – 7.51 (m, 1H), 7.48 (dd, *J* = 7.7, 5.0 Hz, 1H); MS (ESI) m/z = 412.13 $(M + H)^+$

***N*-(5-(4-(Pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)isonicotinamide (29b).** The compound was synthesized according to procedure **C** to give a white solid: yield 35%. The product was purified by CC (DCM/MeOH 100:8); mp 152.1-152.3°C;^{1}H NMR (500 MHz, DMSO) δ 10.87 (s, 1H), 8.53 (s, 1H), 8.36 (s, 1H), 8.25 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.17 (s, 1H), 7.96 (s, 2H), 7.58 (s, 1H), 7.50 (s, 2H), 7.17 (s, 1H), 6.56 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 164.59, 150.34, 148.32, 148.18, 147.12, 147.07, 141.61, 141.49, 141.12, 140.93, 140.64, 139.45, 133.24, 123.86, 123.65, 123.04, 121.79, 121.71; MS (ESI) m/z = 359.09 $(M + H)^+$

2-Phenyl-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)acetamide (30b). The compound was synthesized according to procedure **C** to give dark grey: yield 30%. The product was purified by CC (DCM/MeOH 100:3); mp 90.5-91.8°C;^{1}H NMR (500 MHz, DMSO) δ 10.58 (s, 1H), 9.11 (s, 1H), 8.76 (d, *J* = 27.7 Hz, 2H), 8.58 (s, 1H), 8.40 (s, 1H), 8.20 (dd, *J* = 12.7, 4.6 Hz, 2H), 8.14 (d, *J* = 1.2 Hz, 1H), 7.49 (d, *J* = 7.1 Hz, 1H), 7.39 – 7.31 (m, 4H), 7.29 – 7.18 (m, 1H), 3.72 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 170.00, 148.16, 146.99, 140.78, 140.74, 139.65, 139.34, 135.46, 134.48, 133.39, 131.02, 130.71, 129.22, 128.33, 126.65, 124.14, 123.72, 122.97, 122.10, 43.11; MS (ESI) m/z = 372.04 $(M + H)^+$

2-(4-Fluorophenyl)-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)acetamide (31b). The compound was synthesized according to procedure **C** to give a yellowish white solid: yield 34%. The product was purified by CC (DCM/MeOH 100:3.5); mp 172.5-175.1°C;^{1}H NMR (500 MHz, DMSO) δ 10.54 (s, 1H), 9.06 (s, 1H), 8.75 (s, 1H), 8.68 (s, 1H), 8.53 (d, *J* = 4.0 Hz, 1H), 8.39 (t, *J* = 2.1 Hz, 1H), 8.23 – 8.16 (m, 2H), 8.13 (d, *J* = 1.4 Hz, 1H), 7.47 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.43 – 7.35 (m, 2H), 7.22 – 7.12 (m, 2H), 3.72 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 169.92, 161.19 (d, J_{C-F} = 242.3 Hz), 148.33, 147.15, 140.80 (d, J_{C-F} = 14.6 Hz), 139.71, 139.38, 136.05, 133.30, 131.59. 131.62, 131.17 (d, J_{C-F} = 8.1 Hz), 130.41, 129.31, 123.91, 123.73, 122.93, 122.14, 115.04 (d, J_{C-F} = 21.2 Hz), 42.08; MS (ESI) m/z = 390.02 $(M + H)^+$

2-(3-Methoxyphenyl)-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)acetamide (32b). The compound was synthesized according to procedure **C** to give a white solid: yield 26%. The product was purified by CC (DCM/MeOH 100:3); mp 156.9-157.4°C;^{1}H NMR (500 MHz, DMSO) δ 10.53 (s, 1H), 9.06 (d, *J* = 1.7 Hz, 1H), 8.75 (d, *J* = 1.9 Hz, 1H), 8.68 (d, *J* = 2.0 Hz, 1H), 8.52 (d, *J* = 3.7 Hz, 1H), 8.39 (t, *J* = 2.0 Hz, 1H), 8.22 – 8.17 (m, 2H), 8.14 (d, *J* = 1.2 Hz, 1H), 7.46 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.00 – 6.88 (m, 2H), 6.88 – 6.79 (m, 1H), 3.75 (s, 3H), 3.68 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 169.85, 159.20, 148.40, 147.16, 140.81, 140.71, 139.67, 139.36, 136.85, 136.05, 133.24, 130.36, 129.36, 129.27, 123.87, 123.70, 122.89, 122.07, 121.42, 115.03, 112.02, 54.96, 43.17; MS (ESI) m/z = 402.02 $(M + H)^+$

2-(3-Methoxyphenyl)-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)acetamide (33b). The compound was synthesized according to procedure **C** to give a beige solid: yield 26%. The product was purified by CC (DCM/MeOH 100:3); mp 154.3-155.7°C;^{1}H NMR (500 MHz, DMSO) δ 10.30 (s, 1H), 9.06 (d, *J* = 2.1 Hz, 1H), 8.70 (dd, *J* = 45.9, 2.1 Hz, 2H), 8.53 (dd, *J* =

4.7, 1.4 Hz, 1H), 8.37 (t, *J* = 2.1 Hz, 1H), 8.22 – 8.18 (m, 2H), 8.14 (d, *J* = 1.2 Hz, 1H), 7.47 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.20 (t, *J* = 8.1 Hz, 1H), 6.84 (d, *J* = 6.8 Hz, 2H), 6.76 (dd, *J* = 8.3, 2.2 Hz, 1H), 3.72 (s, 3H), 2.92 (t, *J* = 7.7 Hz, 2H), 2.69 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 171.27, 159.26, 148.36, 147.17, 142.54, 140.77, 140.67, 139.63, 139.37, 136.02, 133.25, 130.37, 129.36, 129.26, 123.87, 123.66, 122.88, 122.05, 120.44, 113.87, 111.42, 54.86, 37.70, 30.61; MS (ESI) m/z = 415.96 $(M + H)^+$

3-(Pyridin-3-yl)-*N*-(5-(4-(pyridin-3-yl)thiophen-2-yl)pyridin-3-yl)propanamide (34b). The compound was synthesized according to procedure **C** to give a brown solid: yield 10%. The product was purified by CC (DCM/MeOH/TEA 100:3:1); mp 92.8-94.2°C;^{1}H NMR (500 MHz, DMSO) δ 10.25 (s, 1H), 8.79 (s, 1H), 8.42 (d, *J* = 30.0 Hz, 2H), 8.34 (s, 1H), 8.25 (dd, *J* = 13.8, 4.6 Hz, 2H), 8.09 (s, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.89 (d, *J* = 13.1 Hz, 2H), 7.67 (d, *J* = 7.7 Hz, 1H), 7.24 (dd, *J* = 12.8, 5.5 Hz, 2H), 2.21 (s, 4H); ^{13}C NMR (126 MHz, DMSO) δ 170.94, 147.81, 147.15, 146.55, 145.05, 140.81, 140.64, 139.62, 139.13, 138.94, 137.85, 136.06, 134.00, 130.67, 129.35, 124.48, 124.19, 123.74, 123.22, 122.23, 36.92, 27.55; MS (ESI) m/z = 387.03 $(M + H)^+$

5-(5-(Pyridin-3-yl)thiophen-3-yl)pyridin-3-amine (35) The compound was synthesized according to procedure **C** using **compound B** to give a brown solid: yield 19%. The product was purified by CC (DCM/MeOH 100:4); mp 142.2-142.3 °C; ^{1}H NMR (500 MHz, DMSO) δ 8.98 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.53 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.19 (d, *J* = 1.9 Hz, 1H), 8.12 (ddd, *J* = 8.0, 2.4, 1.6 Hz, 1H), 8.06 (d, *J* = 1.5 Hz, 1H), 7.91 (dd, *J* = 3.9, 2.0 Hz, 2H), 7.47 (ddd, *J* = 8.0, 4.8, 0.9 Hz, 1H), 7.23 (dd, *J* = 2.5, 2.1 Hz, 1H), 5.39 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ

148.70, 146.20, 144.84, 140.48, 140.28, 135.62, 135.03, 132.67, 130.41, 129.58, 124.08, 123.81, 122.09, 116.71; MS (ESI) m/z = 253.84 $(M + H)^+$

Biological Assays. Protein Kinases and Inhibition Assays. Human Dyrk1A was expressed and purified as described earlier. [38] Dyrk1B and Clk1 were purchased from Life Technologies (lot no. 877059G, Catalog no. PV4649 and lot no.1095729A, catalog no. PV3315). Woodtide substrate peptide for Dyrk1A and Dyrk1B (KKISGRLSPIMTEQ) and RS repeat substrate peptide for Clk1 (GRSRSRSRSRSRSRSR) were custom synthesized at the Department of Medical Biochemistry and Molecular Biology, Saarland University, Homburg, Germany. Kinase inhibition assays for Dyrk1A, Dyrk1B, Clk1 were performed as described previously, in the presence of 15 μM ATP. [38] The calculated IC_{50} values are representative of at least two independent determinations. The larger panel of kinases shown in Table 3 was screened by the SelectScreen Kinase Profiling Service, Thermo Fisher Scientific, Paisley, U.K.

Cell-Based Assays. Stock solutions of the inhibitors were prepared in dimethylsulfoxide (DMSO). All effects were compared to vehicle controls which contained DMSO at the respective final concentration in growth medium. Protein kinase activity of endogenous Dyrk1A in HeLa cells was assayed by measuring the phosphorylation of T434 in overexpressed GFP-SF3b1-NT as described previously. [54] Briefly, HeLa cells were transiently transfected in 6-well plates and treated with test compounds for 18 h. Total cellular lysates were subjected to Western blot analysis with the help of a custom-made rabbit antibody for phosphorylated T434 in SF3b1 and a commercial goat antibody for GFP (no. 600-101-215, Rockland Immunochemicals, Gilbertsville, PA, USA). Blots were developed using horseradish peroxidase (HRP)–conjugated secondary antibodies and enhanced chemiluminescent substrates. Signals were quantified using the AIDA Image Analyzer 5.0 program (Raytest, Straubenhardt, Germany). pT434 signals were normalised to total protein levels as determined from GFP immunoreactivity. GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) was used for non-linear curve fitting (Hill slope -1).

Viability assays were performed using a 96-well pate format (20,000–30,000 cells per well). Cells were cultivated for 3 days before cell viability was assessed with the help of a tetrazolium dye assay (XTT assay, AppliChem GmbH, Darmstadt, Germany).

Molecular docking studies. Molecular docking was performed as previously described using MOE. [55]

Metabolic stability in a cell free assay. Evaluation of metabolic stability and determination of half-lives was carried out using human S9 fraction as described previously. [56]

Physicochemical properties calculation. Calculation of key physicochemical properties was performed using ACD/Labs software (ACD/ Percepta 2012, Advanced Chemistry Development, Inc) as described previously. [38]

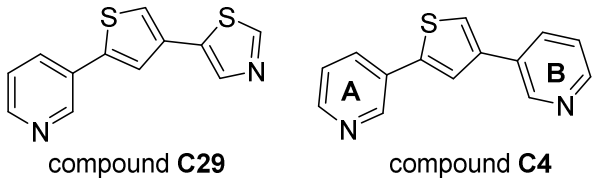

Fig. 1. Previously published Dyrk1A inhibitors.

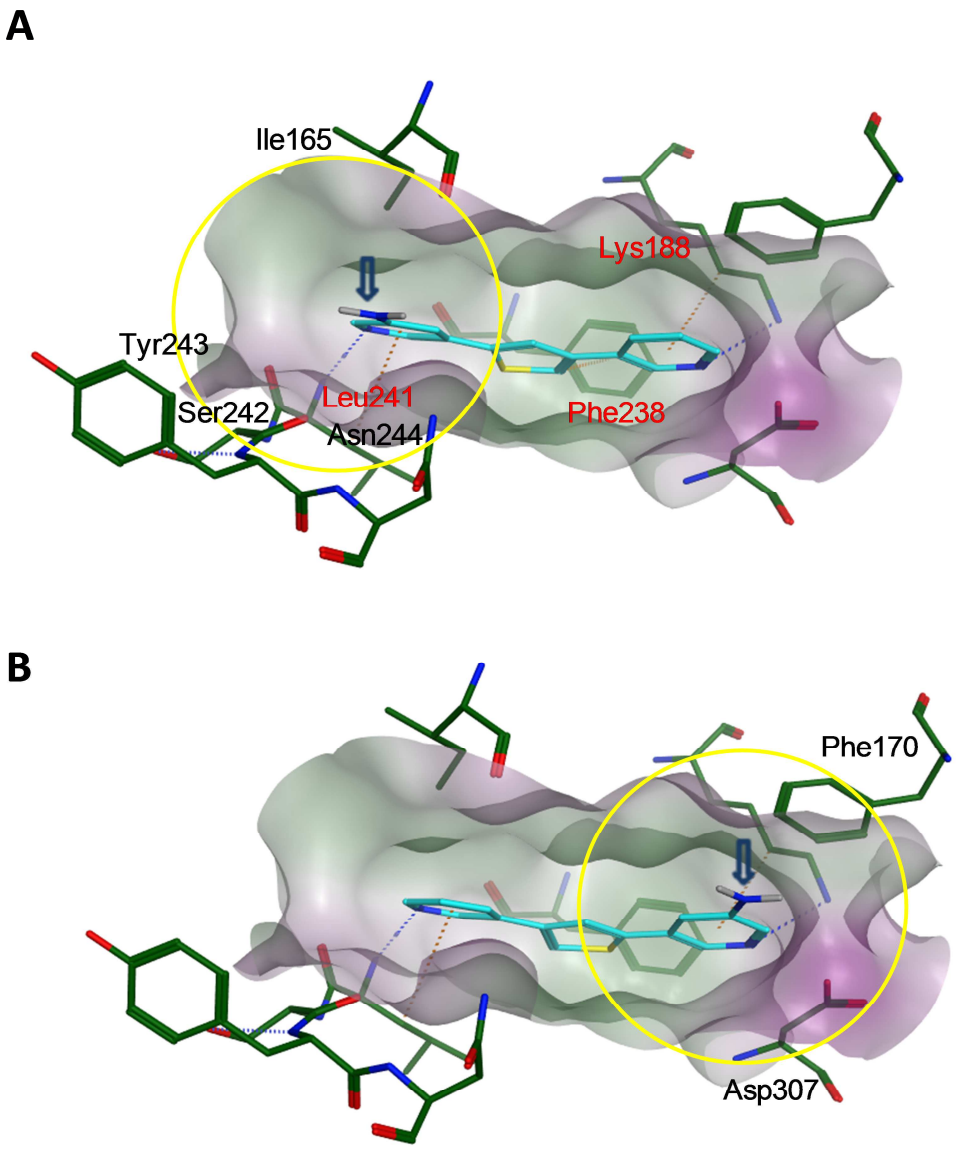

Fig. 2. Extension strategy envisaging amide couplings to probe compound **2**. The amino–functionalized compound **2** (cyan) was docked in the ATP binding pocket of Dyrk1A (derived from PDB entry 3ANR) using MOE. As predicted for the scaffold before, [38] **2** showed the crucial hydrogen bond interactions as well as CH–π interactions with amino acid residues Lys188, Leu241 and Phe238 (exemplarily indicated in A, red letters). Because of the rather symmetric shape of the bispyridyl thiophene scaffold, compound **2** and amide derivatives thereof might bind in two different orientations, with the molecule extension being placed either at the hinge region (A) or at the opposite end of the ATP binding pocket (B). As indicated by the yellow circles (a 4Å radius from probe amino function) in A and B, several H-bond donor/ acceptor functions (e.g., from the peptide backbones of Ile165, Ser242 and Tyr243) in addition to an

acidic (Asp307), polar (Asn244), and an aromatic function (Phe170) are all located within reach of a putative amide–linked molecule extension. Interactions are indicated by dashed lines. In the colour code of the ATP binding pocket surface, green denotes the most lipophilic and magenta the most hydrophilic areas.

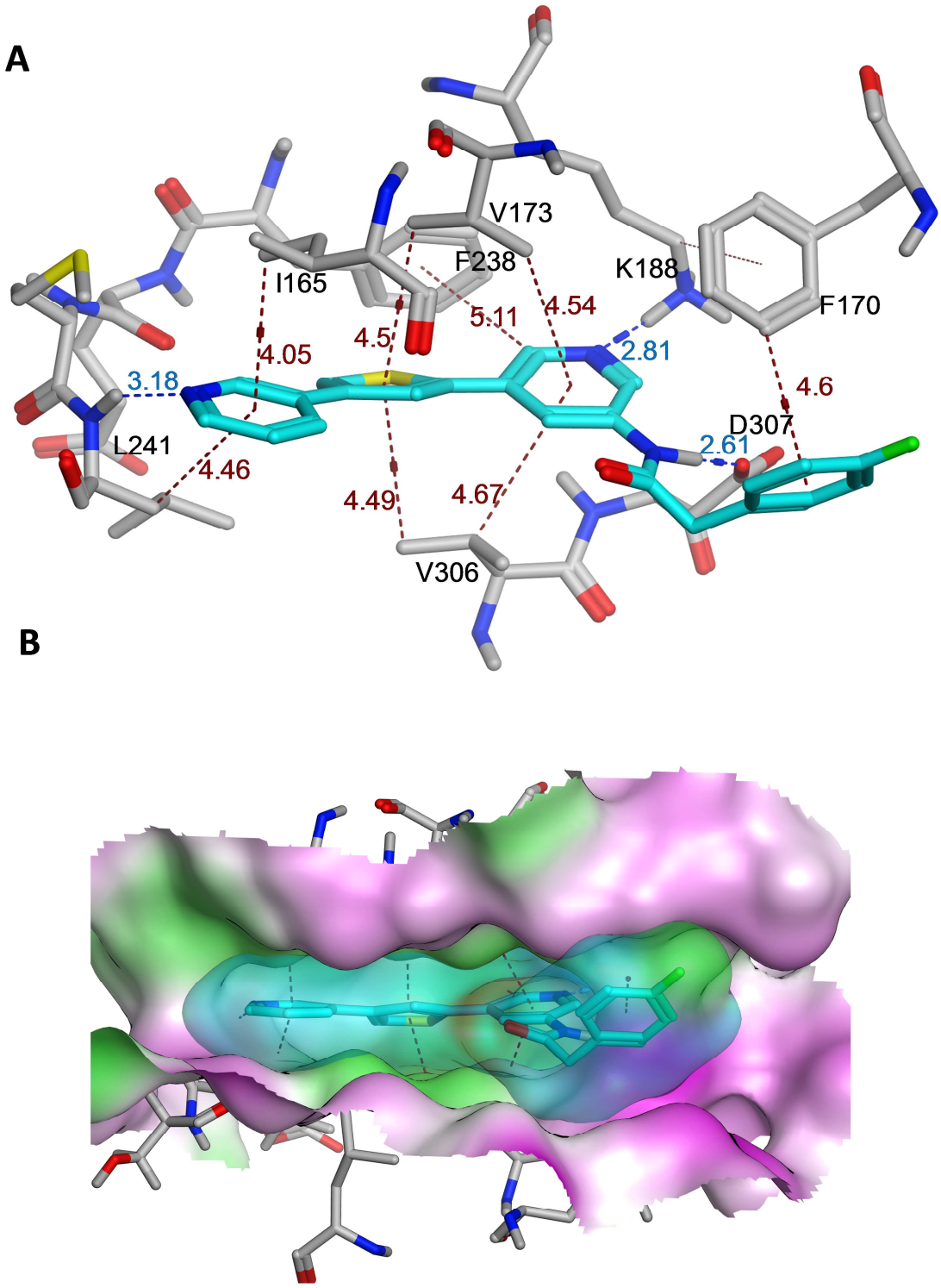

Fig. 3. Predicted binding mode of **31b** in the ATP binding pocket of Dyrk1A. Compound **31b** was docked to the Dyrk1A coordinates derived from the co-crystal structure with harmine (PDB code: 3ANR) using

MOE. (A**)** The pose with the highest score is depicted, showing binding of **31b** (cyan) in the lowest energy conformation of the ligand. Inside the pocket, **31b** is anchored *via* two crucial H-bonds involving the backbone carbonyl of the hinge region residue Leu241, and the conserved Lys188 as a donor. An additional H-bond is formed between the amide NH and Asp307 (H-bonds are indicated by dashed blue lines). Several CH–π interactions additionally enhance the affinity (indicated by brown dashed lines). The assigned numbers denote the distances in Å. (B**)** Through the interaction with Phe170, the 4-fluorobenzyl ring (shown with transparent Connolly surface) occludes the right part of the binding pocket, thus shielding the H-bond between Asp307 and the amide NH from competing water molecules. Surface colours indicate lipophilic (green) and hydrophilic (magenta) areas.

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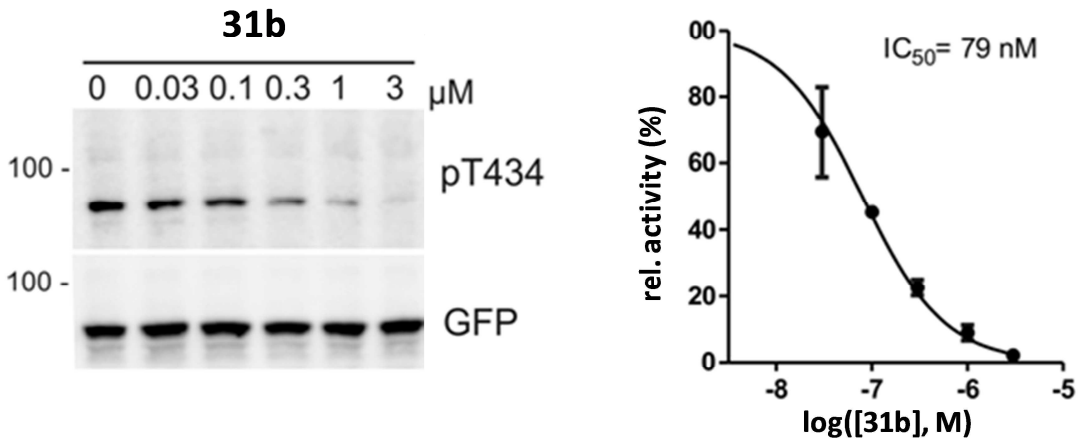
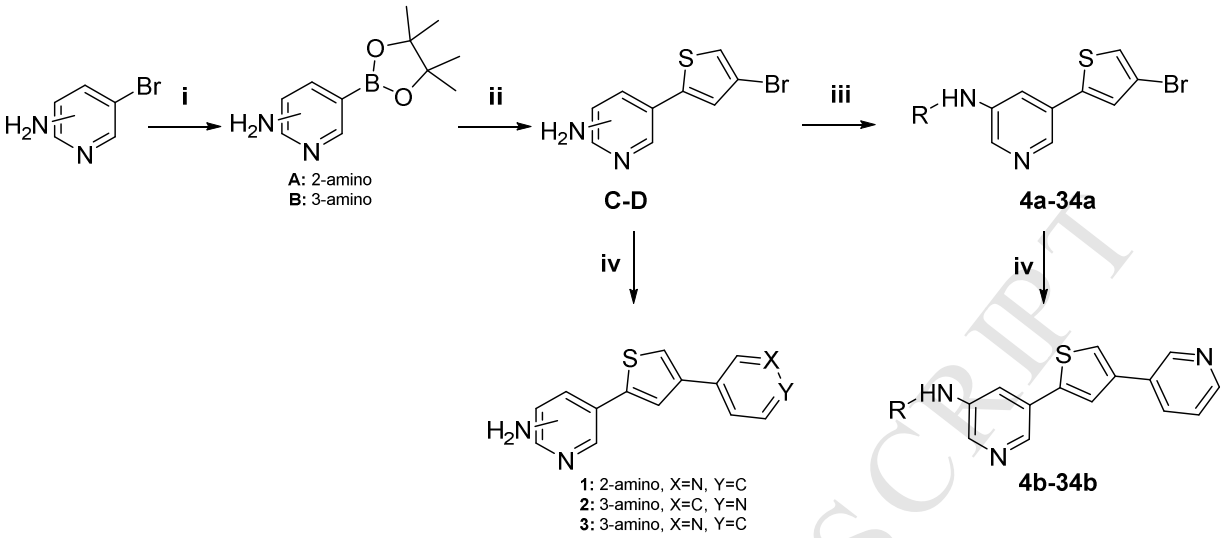
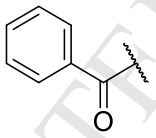
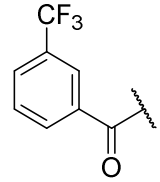
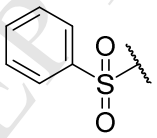
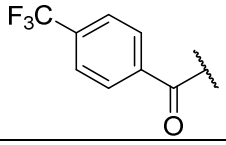
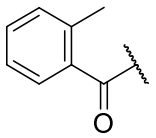
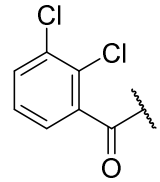
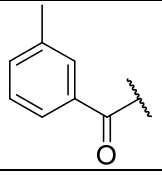
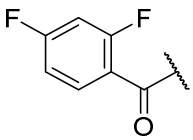
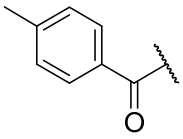
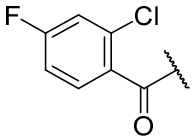
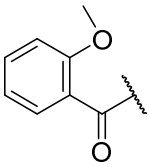
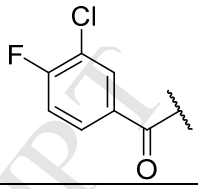
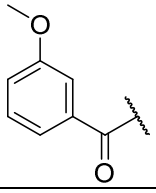
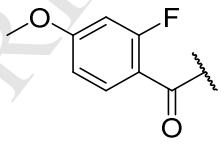
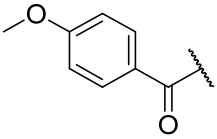
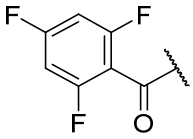
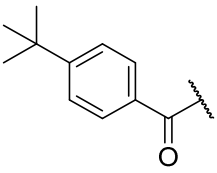
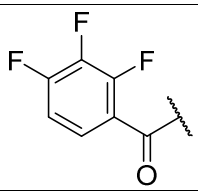
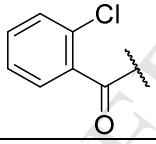
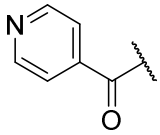
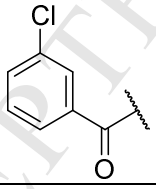
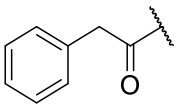
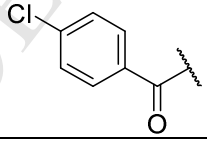
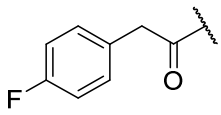
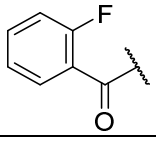
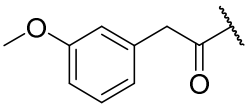
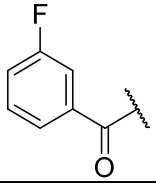
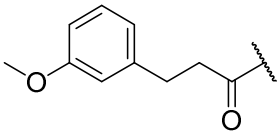


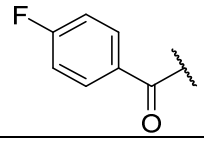
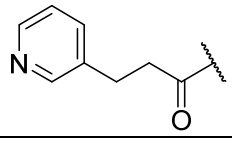
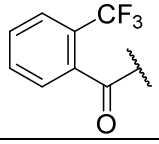
Fig. 4. Inhibition of Dyrk1A activity in cell culture. HeLa cells were transiently transfected with an expression vector for GFP-SF3b1-NT. Cells were treated with variable concentrations of **31b** for 18 h before the phosphorylation of SF3b1 on T434 was measured by immunoblot analysis (left panels). The IC_{50} value for Dyrk1A inhibition was determined from the concentration-response curve fitted to the results of three experiments (means ± SEM). Dyrk1A activity is shown relative to that in vehicle-treated cells

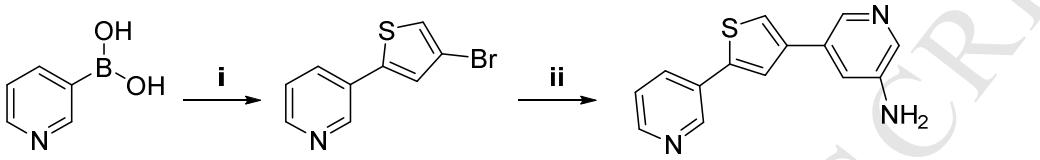


Scheme 1. Reagents and conditions: (i) 4 equiv of CH_3COOK, 4 equiv bis(pinacolato)diboron, 5 mmol% of $Pd(dppf)Cl_2$ in dioxane, reflux 2 hours; (ii) 4 equiv of Cs_2CO_3, 5 mmol% of $Pd(PPh_3)_4$, 1.2 equiv of 2,4-dibromothiophene in dioxane/water, reflux 3.5 hours; (iii) 1.5 equiv Et_3N, 1.2 equiv of the appropriate acid chloride in acetone, room temperature, 2 hours; or 2 equiv of benzenesulfonyl chloride in pyridine, 60°C, overnight; (iv) 4 equiv of Na_2CO_3, 5 mmol% of $Pd(dppf)Cl_2$, 2 equiv of 3/4-pyridine boronic acid in dioxane/water, reflux, 2 hours

Cpd No.	R	No	R
4a		**20a**	
5a		**21a**	
6a		**22a**	
7a		**23a**	

8a		**24a**	
9a		**25a**	
10a		**26a**	
11a		**27a**	
12a		**28a**	
13a		**29a**	
14a		**30a**	
15a		**31a**	
16a		**32a**	
17a		**33a**	

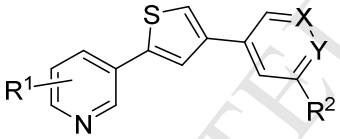
18a		**34a**	
19a			

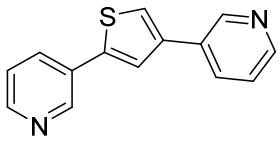


E **35**

Scheme 2. Reagents and conditions: (i) 4 equiv of Cs_2CO_3, 5 mmol% of $Pd(PPh_3)_4$, 1.2 equiv of 2,4-dibromothiophene in dioxane/water, reflux 3 hours; (ii) 4 equiv of Na_2CO_3, 5 mmol% of $Pd(dppf)Cl_2$, 1.5 equiv of compound **B** in dioxane/water, reflux, 2 hours.

Table 1
Inhibition of Dyrk1A and Dyrk1B kinases (compounds **1**-**3**, **35)**



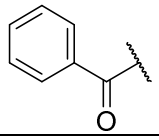
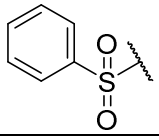
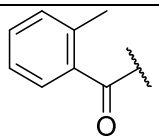
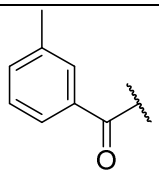
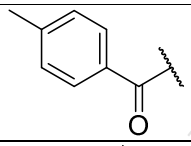
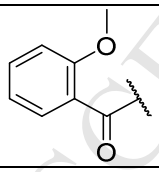
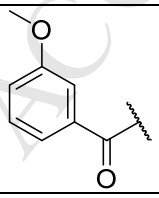
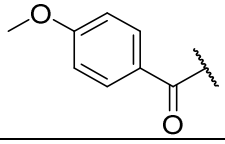


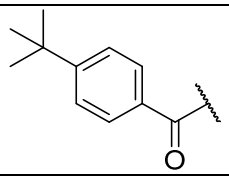
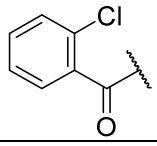
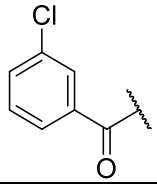
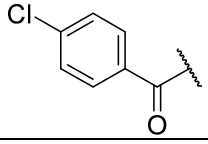
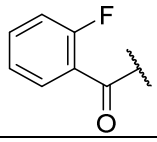
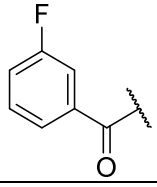
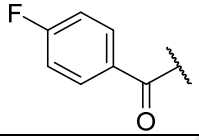
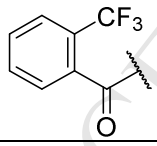
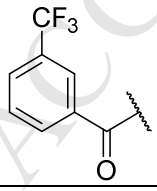
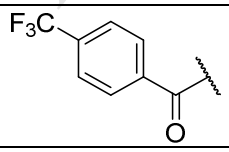
Compound **C4**

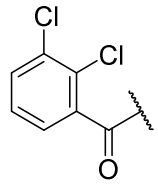
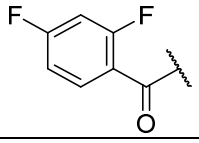
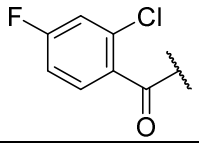
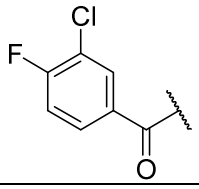
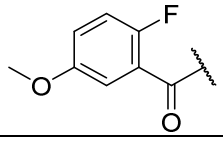
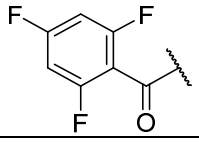
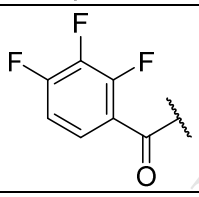
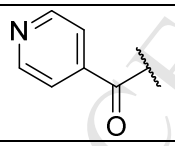
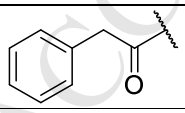
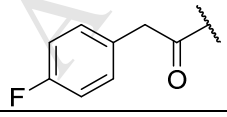
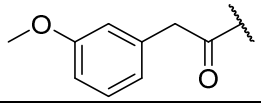
Cpd.No.	R^1	X	Y	R^2	Dyrk1A		Dyrk1B	
					% inhibition at 250 nM[a]	IC_{50} (nM)[a]	% inhibition at 250 nM[a]	IC_{50} (nM)[a]
1	2-NH_2	N	C	H	30.5	614.3	59.5	251.4
2	3- NH_2	N	C	H	64.7	101	65.1	154.3
3	3- NH_2	C	N	H	8.4	ND	21.7	ND
35	H	N	C	NH_2	60	ND	67	130
C4						300		300

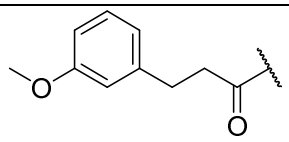
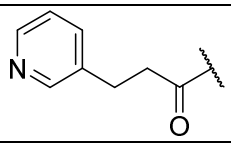
[a] Values are mean values of at least two independent experiments, each done in duplicates; standard deviation <9%; ND: not determined. The ATP concentration in the assay was 15 µM.

Table 2
Inhibition of Dyrk1A and Dyrk1B kinases (**4b**-**34b**)

Cpd.No.	R	Dyrk1A		Dyrk1B	
		% inhibition at 250 nM[a]	IC_{50} (nM)[a]	% inhibition at 250 nM[a]	IC_{50} (nM)[a]
4b		59.5	ND	57.6	ND
5b		9.9	ND	18.8	ND
6b		50.1	ND	39.5	ND
7b		45.2	ND	60.5	ND
8b		60.5	51.4	54.6	ND
9b		57.3	ND	15.4	ND
10b		53.5	ND	29	ND
11b		59.5	ND	25.2	ND

12b		21	ND	3.4	ND
13b		72	90.5	26.3	ND
14b		44.5	ND	59.8	ND
15b		25.9	ND	52.2	ND
16b		65.1	106.7	30	ND
17b		59.4	ND	26.2	ND
18b		62.6	183.2	23.5	ND
19b		37.7	ND	26	ND
20b		46.7	ND	36	ND
21b		8.5	ND	32	ND

22b		42	ND	32	ND
23b		80.2	49	41	ND
24b		54.2	ND	33.6	ND
25b		23.3	ND	5.6	ND
26b		85.2	37.3	43.8	ND
27b		93.1	23.5	38.7	298.5
28b		55.5	ND	25.1	ND
29b		63.7	107	55.1	ND
30b		99.6	14.9	31.2	326.3
31b		86.9	14.3	36.5	383
32b		49.7	ND	48.7	ND

33b		49.7	ND	49.5	ND
34b		10	ND	33.9	ND

[a] Values are mean values of at least two independent experiments, each performed in duplicates; S.D. <10%; the assay was carried out at an ATP conc. of 15 µM; ND: not determined

Table 3
Selectivity profiling of compound **31b**

kinase	**% Inhibition at 2 µM[a] (IC_{50})[b]**
CDK5/p25	0
Clk1	40
Clk2	19
CK1 delta	4
Dyrk1A	87 (14.3 nM)
Dyrk2	40
Haspin	95 (36 nM)
HIPK1	0
MLCK2	9.8
TRKB	10
PIM1	8
SRPK1	2
STK17A	60

[a] The screening list was especially composed to include all kinases that were frequently reported as off-targets for diverse chemical classes of Dyrk inhibitors [38, 45, 57-61]. Screenings were performed as a service at Thermo Fisher Scientific at an ATP concentration of 100 µM; n.i., no inhibition. Data represent mean values of duplicates that differed by less than 12%. [b] IC_{50} values were determined for inhibition values >60% (ATP concentration: 15 µM) and represent mean values of at least two independent experiments, each performed in duplicates; S.D. <10%.

Table 4
Effect of **31b** on HeLa cell growth

	1 µM	**3 µM**	**10 µM**
31b	99±2	97±5	88±2
Staurosporine[a]	33±4		

Viability of treated HeLa cells is given in percent relative to control cells treated with vehicle (means of two experiments with duplicate measurement ± S.D.). [a] Staurosporine is a known inducer of apoptosis and served as a positive control.

Table 5
Metabolic stability of **27b** and **31b** against human liver S9 fraction[a]

Cpd No.	Half-life [min]
27b	123
31b	118
Testosterone*b*	8.5

[a] 10 mg/mL, $NADP^+$ regenerating system, $MgCl_2$, UDPGA, PAPS, [inhibitor] = 0.3 µM, incubation at 37° C, samples taken at 0, 15, 30, 60, 90 and 120 min, determination of the parent compound by LC-MS/MS. [b] included as a positive control for a rapidly metabolized compound.

Table 6
Calculated physicochemical properties of **31b**

Cpd.No.	MW [g/mol]	logP	TPSA [Å^2]	TPSA [Å^2][b]	HBD	HBA
31b	389.45	4.11	83.1	54.88	1	3
C29*a*	244.34	2.48	82.3	25.8	0	2

[a] C29 values are shown for comparison (taken from Ref. [38]) [b] Sulphur was not considered for the calculation of TPSA. Ideal ranges for CNS active drugs: MW: 181-427 g/mol, logP: 0.4-5.1 (median: 2.8), TPSA < 76 Å^2, HBD: 0-1; HBA: 2-3. [62]

Notes

The authors declare no competing financial interest.

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Abbreviations Used

AD, Alzheimer's disease; APP, Amyloid precursor protein; ASF, Alternative splicing factor; CC, Column chromatography; CDK, Cyclin dependant kinases; CID, collision induced dissociation; CK, Casein kinase; Clk, cdc-like kinae; DS, Down's syndrome; Dyrk, Dual specificity tyrosine regulated kinase; GFP, Green fluorescent protein; HBA, Hydrogen bond acceptor; HBD, Hydrogen bond donor; HIPK1, Homeodomain-interacting protein kinase 1; IC_{50}, Half maximal inhibitory concentration, MLCK2, Myosin light chain kinase 2; NFAT, Nuclear factor of activated T cells; $Pd(dppf)Cl_2$, [1, 1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II); PIM1, Proviral integration site for Moloney murine leukemia virus-1; TRKB, Tropomyosin receptor kinase B; SF3b1, Splicing factor 3b1; SRPK1, Serine/arginine-rich protein kinase 1; STK17A, Serine/threonine kinase 17A

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Highlights

- 2,4-Bispyridyl thiophenes aryl and aralkyl amides were developed from the 2,4-bispyridinyl thiophene lead.
- Benzyl amides showed the highest potency against Dyrk1A with excellent selectivity profile vs. Dyrk1B.
- Compound 31b showed a dramatic enhancement of Dyrk1A inhibition (IC_{50} = 14.3 nM)
- 31b could strongly inhibit Dyrk1A in HeLa cells with an IC_{50} of 79 nM.