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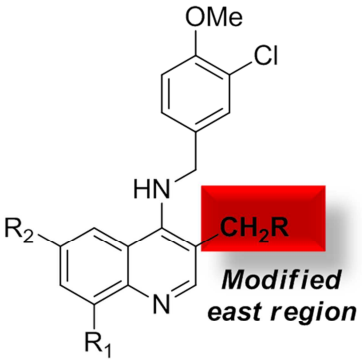

R = F, CH_2F, OCH_2CH_2F

R_1 = ethyl or cyclopropyl

R_2 = CF_3 or CN

New fluorinated probes for PET imaging of the PDE5 enzyme in brain

- Synthesis
- *In vitro* evaluation
- Selection for further ^{18}F labelling

Synthesis and *in vitro* evaluation of new fluorinated quinoline derivatives with high affinity for PDE5: towards the development of new PET neuroimaging probes.

Jianrong Liu,[a] Aurélie Maisonial-Besset,*[a] Barbara Wenzel,[b] Damien Canitrot,[a] Ariane Baufond,[a] Jean-Michel Chezal,[a] Peter Brust,[b] and Emmanuel Moreau[a]

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Keywords:

Quinoline ; fluorination ; Phosphodiesterase 5 ; radiotracer ; positron emission tomography.

Abbreviations used: AD: alzheimer's disease; AllocCl: allyl chloroformate; ATR: attenuated total reflectance; cAMP: cyclic adenosine 3',5'-monophosphate; cGMP: cyclic guanosine 3',5'-monophosphate; CNS: central nervous system; CREB: responsive element binding protein; DAST: (diethylamino)sulfur trifluoride; DIBALH: diisobutylaluminium hydride; DIPEA: *N*,*N*-diisopropylethylamine; DMAP: *N*,*N*-dimethylaminopyridine; DMF: *N*,*N*-dimethylformamide; DMSO: dimethylsulfoxide; EMME: ethoxy methylene malonic diethyl ester; ESI-MS: electrospray ionization mass spectra; HMBC: heteronuclear multiple bond correlation; HRMS: high resolution mass spectroscopy; HSQC: heteronuclear single quantum correlation; IC_{50}: inhibition concentration 50; IR: infrared spectroscopy; Mp: melting point; *n*-BuLi: *n*-butyl lithium; NDs: neurodegenerative disorders; NMR: nuclear magnetic resonance; PDE: phosphodiesterase; PDE5Is: phosphodiesterase 5 inhibitors; PET: positron emission tomography; R_f: retention factor; rt: room temperature; SAR: structure-activity relationship; SPR: structure-property relationship; THF: tetrahydrofuran; TLC: thin layer chromatography; UK: United Kingdom; UV: ultra-violet.

Highlights

New fluorinated inhibitors of the PDE5 enzyme were synthesised and evaluated *in vitro*

Small fluoro-containing alkyl side chains did not alter the inhibitory potency

Compounds **24a, b** appeared to be most promising regarding affinity and selectivity

Compounds **24a, b** were selected for radiolabelling with fluorine-18 and PET imaging

Abstract

The increasing incidence of Alzheimer's disease (AD) worlwide is a major public health problem. Current treatments provide only palliative solutions with significant side effects. Therefore, new efficient treatment options and novel early diagnosis tools are urgently needed. Recently, *s*trong pre-clinical evidences suggested that phosphodiesterase 5 *(*PDE5) may be clinically relevant both as biomarker and drug-target in AD. In this study, we intended to develop a new radiofluorinated tracer for the visualisation of PDE5 in brain using PET imaging. Based on currently known PDE5 inhibitors, a series of novel fluorinated compounds bearing a quinoline core have been synthesised *via* multi-steps reaction pathways. Their affinity for PDE5 and selectivity over other PDE families have been investigated. According to the data collected from this *in vitro* screening, fluorinated derivatives **24a, b** bearing a fluoroethoxy group at the C-3 position of the quinoline core appeared to be the most promising structures and will be further radiolabelled with fluorine-18 for *in vitro* and *in vivo* evaluations as PET radiotracer for neuroimaging of PDE5.

1. Introduction

In the last decades, the neurodegenerative disorders (NDs) became a major concern of public health in industrialized countries. The risk of developing diseases like Alzheimer's, Parkinson's, Huntington's diseases or amyotrophic lateral sclerosis, is clearly increasing with advancing age [1]. The common feature of these pathologies is the progressive deterioration of neuronal activity leading for example to dementia, cognitive and mental degeneration and/or movement disorders and changes in individual personality. Among NDs, Alzheimer's disease (AD) accounts for around 50-75% of diagnosed dementias in elderly people [2, 3]. Currently, 35 million patients are identified worldwide [3] and one case of AD is revealed every 7 second [1, 4]. Early diagnosis of AD is highly challenging [3, 5] and there is no remedial treatment for this pathology. The medical care and treatment of AD patients at the earliest stage of the pathology can considerably reduce the progression rate of the disease and delay the hospitalisation necessity, but most of the time the treatment often begins far too late [3]. Due to the inefficiency of current strategies focused on β-amyloid plaques or neurofibrillary tangles, the identification of new biological targets to offer innovative diagnostic and therapeutic options are urgently needed.

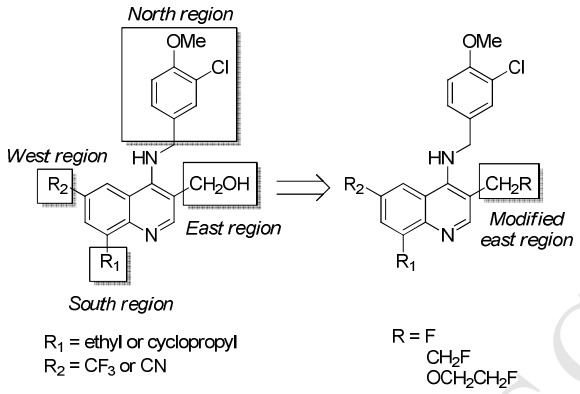
The phosphodiesterases (PDEs) are a large family of enzymes that regulate the intracellular levels of two important second messengers: cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP), by cleaving their phosphodiester bond. To date, 11 subtypes of these enzymes have been identified and classified mainly according to their amino acid sequences [6]. Phosphodiesterase 5 (PDE5) is specific for cGMP and is located in some peripheral organs such as heart, lungs and smooth muscle cells but also in the central nervous system (CNS) and particularly in cerebellar Purkinje cells, hippocampus, caudate and substantia nigra [7-10]. There is currently a growing interest in using inhibitors of PDE5 such as Sildenafil (Viagra®) or Tadalafil (Cialis®), commonly used as treatment for erectile dysfunction or chronic pulmonary hypertension, to reverse or decrease memory damages caused by AD [7, 11]. There are strong pre-clinical evidences suggesting that PDE5 may be both a clinically relevant biomarker and a disease-relevant drug-target in AD. For example, the crucial nitric oxide/cGMP/CREB (responsive element binding protein) signalling pathway is known to be altered in several processes including neurotransmission, synaptic plasticity and memory [12-16]. Restoring normal cGMP levels in the CNS of AD patients by action of PDE5 inhibitors (PDE5Is) could decrease neuroinflammation, improve cerebral blood flow, long-term memory consolidation, and synaptic plasticity [12, 17, 18]. However, the

level of expression, exact role and involvement of this enzyme in AD are still quite unclear and controversial. Therefore we aim at developing radiotracers for clinical positron emission tomography (PET) imaging of PDE5 in brain under normal and pathological conditions. By use of radiotracers with optimal pharmacokinetic profiles in combination with high accuracy, PET imaging should proof valuable in at least two principle areas of interest for basic scientists and clinical investigators: i) the timely availability of diagnostic information i.e. the upfront prediction of disease states, which involve impairments and/or deregulation of the cGMP-PDE5 pathway, and ii) for monitoring and evaluating the disease-progression and response to therapy. Moreover, dynamic PET imaging of PDE5 in intact living subjects should strongly foster the paths to new therapeutic strategies and to new mechanistic insights into the role of PDE5 for biology and pathophysiology of NDs such as AD. To date, there is no radiopharmaceutical approved for the visualisation of PDE5 in clinic [19, 20]. Most of the radiotracers developed at the preclinical level are labelled with carbon-11 and suffer from a lack of specificity toward others PDEs. Only one compound radiolabelled with fluorine-18, which is the best radionuclide for PET imaging purposes, has been reported to date and warrant further preclinical investigations [21-23]. In such a context, we envisaged to develop and evaluate new fluorinated analogues of PDE5 inhibitors as candidates for PET imaging of PDE5 in the CNS.

An extensive study of the literature shows that, on the basis of their structural similarities, PDE5 inhibitors can be grouped as follows: i) cGMP-based derivatives, represented by Sildenafil and Vardenafil [24-26]; ii) β-carboline-derived molecules, represented by Tadalafil; iii) pyrazolopyridine, phtalazine, pyrazolopyridopyridazine, quinoline-based inhibitors; iv) isoquinoline, naphthyridinone and pyridopyrazinone derivatives [27-33]. As the work presented herein is directed toward the development of new radiofluorinated inhibitors for PET imaging of PDE5 in brain, we decided to focus our attention on the heterocyclic scaffold quinoline which presents a high PDE5 potency and selectivity over mainly PDE6 and PDE11 and is described to cross readily the blood-brain barrier (Figure 1) [12]. This lead compound is the result of considerable structure-activity (SAR) and structure-property relationship (SPR) studies which revealed important structural features of this family of PDE5 inhibitors [12, 31]. The quinoline scaffold bearing a hydroxymethyl group at the C-3 position (“east region”) improves the solubility compared to the other core ring systems tested. The binding to the protein is highly dependent on the presence of the benzylamino moiety at the C-4 position (“north region”). Moreover the combination of a cyano or a trifluoromethyl group at the C-6 position (“west region”) and ethyl or cyclopropyl substituents at the C-8 position (“south region”) enhance PDE5 potency and appear to

be the optimal parameters for a high selectivity over others PDEs (based on IC_{50} values).

Figure 1. Strategy of introduction of a fluorine atom on the lead heterocyclic quinoline scaffold presenting a high PDE5 potency and selectivity.



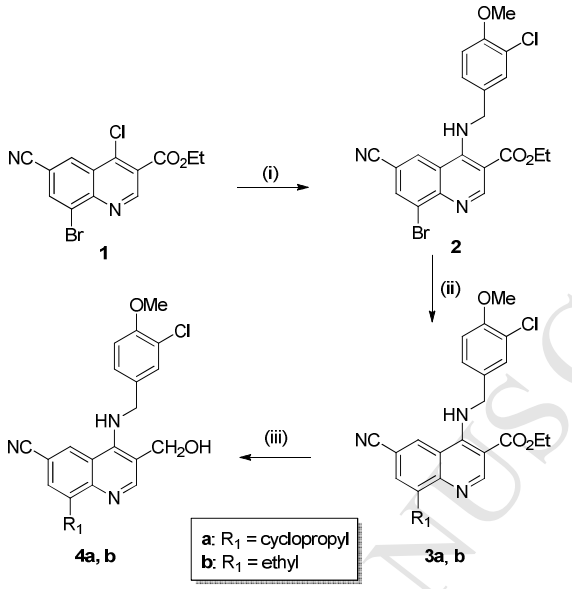
In order to develop a radiotracer for PET imaging of PDE5 according to the SAR studies described above and knowing that fluorine can be considered as a bioisostere of both hydrogen and hydroxyl groups, we firstly decided to slightly modify this lead structure by introducing a fluorine atom on the "east region" of the molecule (Figure 1, see substituents for R). Herein we report, the chemical syntheses and first *in vitro* biological evaluation of a series of new fluorinated PDE5 ligands and the selection of the most promising ones for further labelling with fluorine-18 and evaluation *in vivo*.

2. Results and discussion

2.1. Chemistry: introduction of fluorine at the "east region"

To obtain compounds modified at the "east region" of the quinoline core, we firstly decided to replace the hydroxyl group of the two key compounds **4a**, **b** directly by a fluorine atom (Scheme 1). The synthesis of derivative **4a** was previously described by Fiorito *et al.* [12] in three steps starting from intermediate quinoline **1** (Scheme 1). This synthetic approach was used to produce the second reference alcohol **4b**. Briefly, a nucleophilic aromatic substitution of the chlorine atom at the C-4 position of the quinoline core of **1** using (3-chloro-4-methoxyphenyl)methanamine hydrochloride [29] in *n*-propanol yielded compound **2**. Then, the ethyl and cyclopropyl substituents were introduced at the C-8 position *via* a classical Suzuki coupling in the presence of tetrakis(triphenylphosphine)palladium[0] and caesium carbonate under thermal heating. When using 1.5 equivalents of cyclopropylboronic acid, **3a** was efficiently produced in 70% yield. However, in the case of **3b**, bearing an ethyl group, the concomitant formation of the corresponding debrominated compound **3b1** was observed. Interestingly, a large excess of ethylboronic acid (16 eq.) was mandatory to

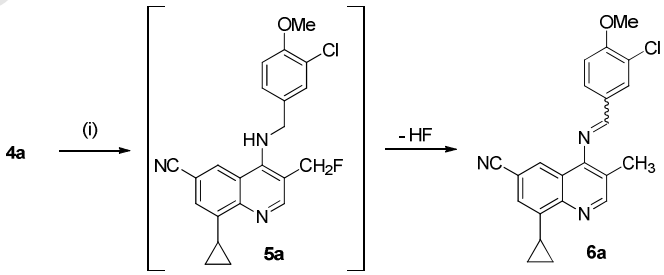
avoid the formation of the by-product and produce preferentially **3b** in 63% yield. To successfully afford **4a**, **b**, reduction of the ester group at the C-3 position into the corresponding hydroxymethyl group was performed using $LiAlH(OtBu)_3$ as a mild reducing agent to avoid the hydrolysis of the cyano group.



Scheme 1. Synthesis of key alcohols intermediates **4a, b**.[a]

[a]Reagents and conditions: (i) (3-Chloro-4-methoxyphenyl)methanamine hydrochloride, DIPEA, *n*-propanol, reflux, 3 h; (ii) Cyclopropylboronic acid or ethylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, reflux, toluene, 24 h; (iii) $LiAlH(tOBu)_3$, THF, 60 °C, 16 h for **4a** or 50 °C, 27 h for **4b**.

To introduce the fluorine atom at the C-3 position, we investigated the fluorination of compound **4a** using diethylaminosulfur trifluoride (DAST) which is a classical reagent to convert straightforward aliphatic alcohols into their corresponding fluorinated derivatives. Under these conditions, the desired fluorinated compound **5a** could not be isolated. Unfortunately, this reaction led to the major imine by-product **6a** bearing a methyl group at the C-3 position (Scheme 2). This was confirmed by NMR analyses (^1H, ^{13}C, HMBC, HSQC) and HRMS analysis (m/z 376.1214, calculated for $C_{22}H_{19}ClN_3O$ m/z 376.1217) (See supplementary material, Figure 1S).

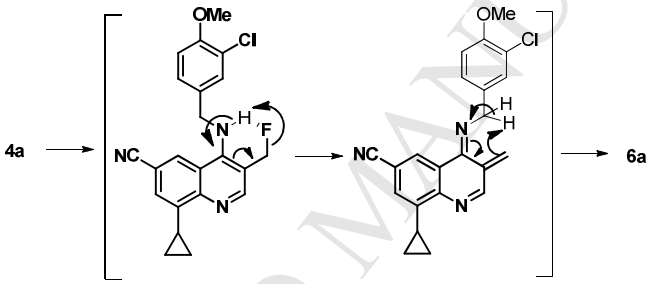


Scheme 2. Formation of **6a**.[a]

[a]Reagents and conditions: (i) DAST, dichloromethane, -60 °C.

We assume that compound **6a** was formed during the purification step of **5a** *via* column chromatography. Indeed, the analysis of the ^{1}H NMR spectrum of the crude product confirmed the presence of **5a** with a doublet at 5.46 ppm, integrating for two protons and presenting a spin-spin coupling between H and F with a coupling constant of 49.2 Hz, typical of a fluoromethyl group (See supplementary material, Figure 2S). To avoid the formation of **6a**, different stationary phases (silica, alumina, RP C18, filtration through a pad of Celite® 545) were tested with various combinations of solvents for elution but remained unsuccessful. Moreover, we observed after one day that the crude compound **5a** was completely converted into **6a** (^{1}H NMR kinetic studies; data not shown). These results clearly demonstrated that the desired compound **5a** is highly unstable. As possible mechanism we propose the elimination of HF as depicted in Figure 2.

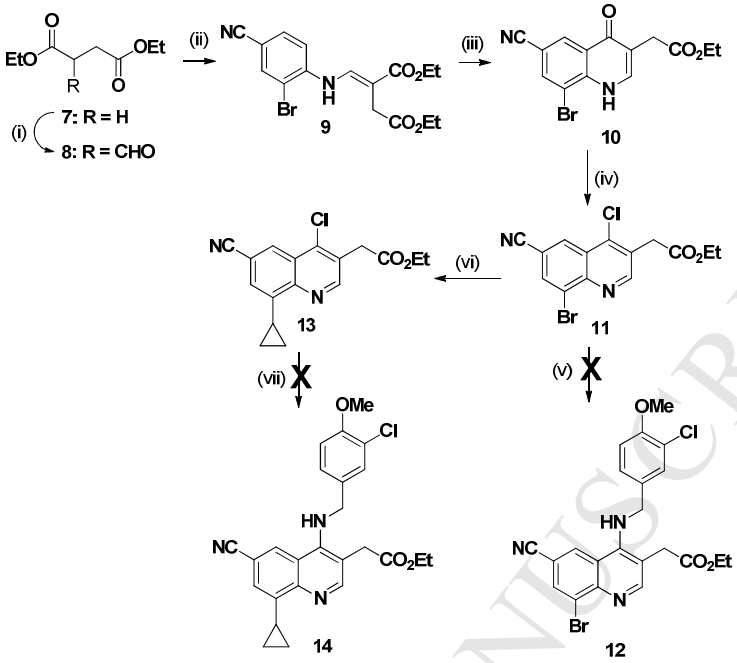
Figure 2. Possible mechanism proposed for the formation of **6a**.



We then tested an alternative synthetic route using a tosylate leaving group. Compound **4a** was treated with 4-toluenesulfonyl chloride in the presence of Et_3N and DMAP in dry dichloromethane at room temperature. As expected, the total conversion of the tosylate into **6a** was observed (See supplementary material, Figure 2S). These findings support our assumption of the instability of these derivatives bearing either a fluoromethyl group (for the reference compounds) or a tosylate leaving group (corresponding precursors for fluorine-18 radiolabelling) at the C-3 position. Consequently, we focused our efforts on the development of quinoline structures bearing a longer alkyl chain such as a fluoroethyl moiety at the C-3 position (Figure 1).

We designed a synthetic pathway based on a Gould-Jacobs reaction as previously developed for the synthesis of starting material **1** (Scheme 3). Firstly, quinolone **10** was obtained in three steps *via* formylation of diethyl succinate **7** according to the procedure described by Holmes *et al.* [34] followed by a condensation with 2-bromo-4-cyanoaniline and a thermal cyclisation in Dowtherm A at 250 °C. Treatment of compound **10** with $POCl_3$ gave the corresponding 4-chloroquinoline **11** in 90% yield. The next step was the aromatic

nucleophilic substitution of the chlorine atom to graft the benzylamino moiety at the C-4 position.



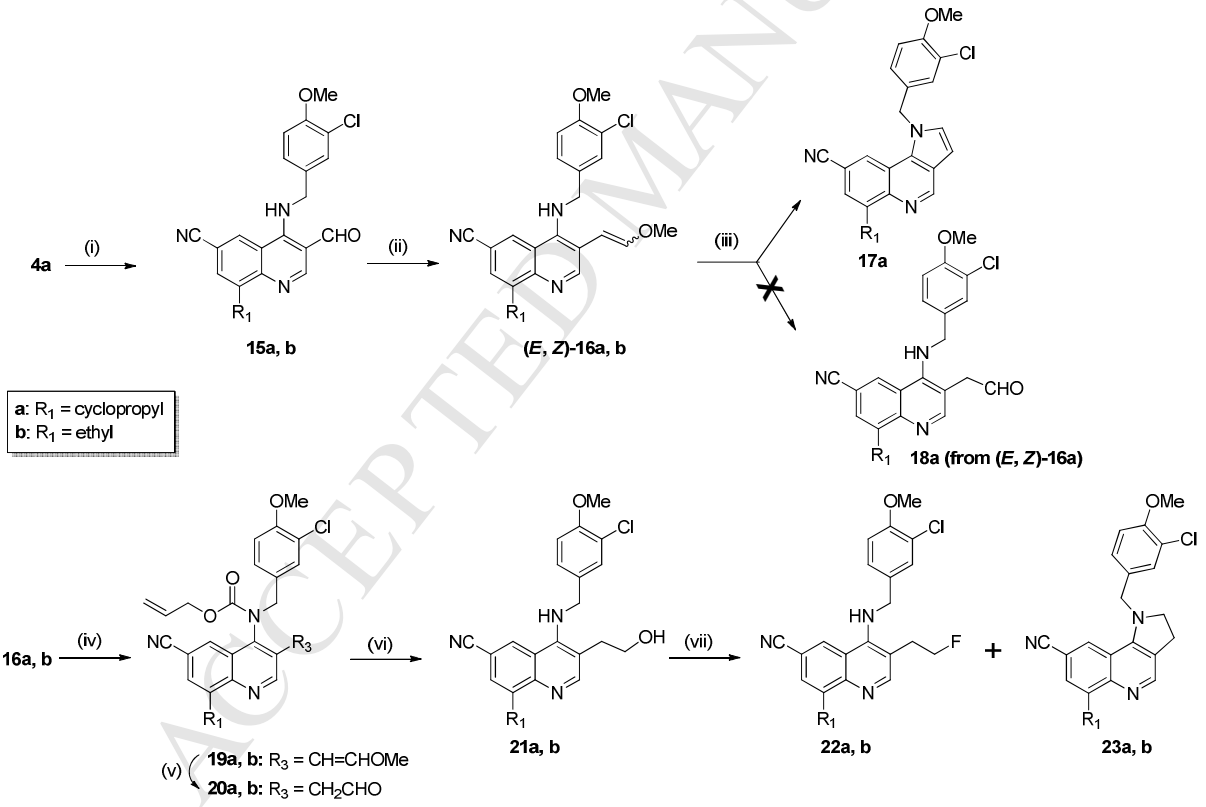
Scheme 3. First synthetic approach tested to introduce a fluoroethyl side chain at the C-3 position of the quinoline scaffold.[a]

[a]Reagents and conditions: (i) Ethyl formate, sodium, Et_2O, 0 °C to rt, 20 h; (ii) 2-bromo-4-cyanoaniline, toluene, reflux, 8 h; (iii) Dowtherm A, 250 °C, 2 h; (iv) $POCl_3$, reflux, 7 h; (v) nucleophilic aromatic substitution under thermal conditions; (vi) Cyclopropylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, reflux, toluene, 22 h; (vii) buchwald reaction under thermal or microwave conditions.

The experimental conditions previously used to obtain compound **2** (Scheme 1) failed to produce **12** even with further modifications of reaction parameters (solvents, temperatures, nature of the base). Obviously, due to the addition of a methylene group between the ester function and the carbon C-3 of the quinoline core, the electron withdrawing effect necessary for the nucleophilic substitution at the C-4 position was suppressed. Therefore, we focused our effort to insert the benzylamino moiety *via* a Buchwald-Hartwig coupling. Given that compound **11** possesses both a chlorine and a bromine atom at C-4 and C-8 positions respectively, we firstly performed the regioselective Suzuki reaction to obtain compound **13** from **11** with a good yield (62%). Then, the Buchwald-Hartwig reaction was performed with compound **13** to introduce the benzylamine derivative at the C-4 position. Despite using several combinations of catalyst, ligand, base and activation *via* thermal or microwave heating, only the degradation of **13** was observed.

Therefore, we were forced to reconsider our synthetic approach and designed a new strategy depicted in Scheme 4. Compound **4a** previously synthesised, was oxidised into its

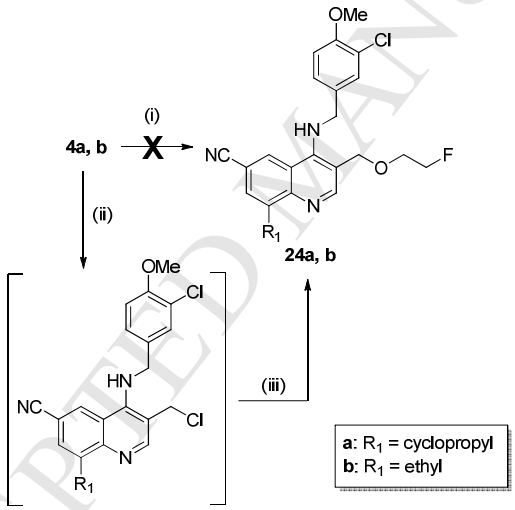
corresponding aldehyde **15a**. Then, we performed a Wittig reaction in the presence of (methoxymethyl)triphenylphosphonium chloride to obtain the mixture of **(*E*, *Z*)-16a**. The cleavage of the methoxy group of **16a** using hydrochloric acid (4 M) unfortunately afforded the cyclic compound **17a** rather than the expected aldehyde **18a**. The formation of the five-membered cycle of **17a** is probably due to the nucleophilic character of the secondary amine at the C-4 position of the quinoline scaffold. Keeping in mind that this amine is an important feature for the binding to the PDE5 enzyme [28] and cannot be blocked as a tertiary amine, we decided to protect this function to avoid the cyclisation. We chose the allyloxycarbonyl protecting group, because of its stability under acidic conditions needed to convert intermediates **19a, b** to the corresponding aldehydes **20a, b**. Afterwards, the aldehyde function was reduced using $LiAlH(OtBu)_3$ to give the alcohols **21a, b** which were finally treated with DAST affording the desired fluorinated compounds **22a, b** in 46% and 15% yield respectively. However, during this reaction we observed the formation of major by-products, identified as the cyclic compounds **23a, b**.



Scheme 4. Modified synthetic approach starting from **4a** and efficiently producing derivatives **22a, b** bearing a fluoroethyl side chain at the C-3 position of the quinoline scaffold.[a]

[a]Reagents and conditions: (i) MnO_2, CH_2Cl_2, rt, 5 h for **15a** or 20 h for **15b**; (ii) (Methoxymethyl)triphenylphosphonium chloride, *n*-Buli, THF, -15 °C to rt, 2 h; (iii) 4 M HCl in H_2O/THF (1/3, v/v), 50 °C, 4 h; (iv) AllocCl, NaH, DMF, rt, 1 h for **19a** and 2 h for **19b**; (v) HCO_2H, 50 °C, 20 h; (vi) $LiAlH(O\,tBu)_3$, THF, 60 °C, 20 h; (vii) DAST, CH_2Cl_2, -78 °C to rt, 3 h for **22a** and 1.5 h for **22b**.

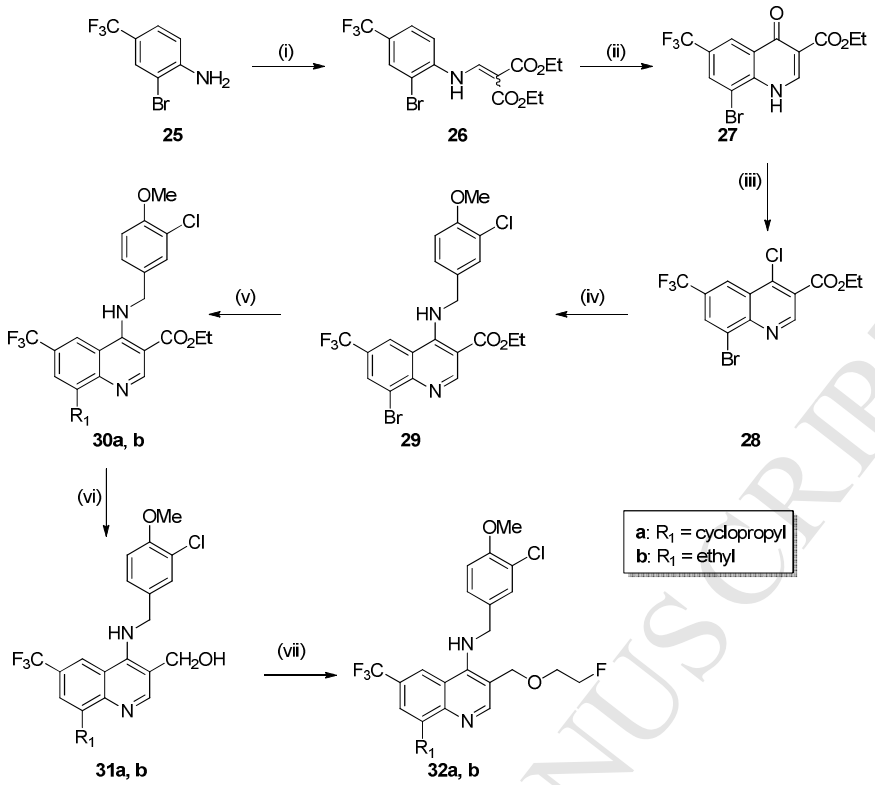
To further exemplify this series of candidates modified at the "east region", we intended to develop fluorinated derivatives (**24a, b** and **29a, b**) *via O*-alkylation of the hydroxymethyl substituent by a 2-fluoroethyl group (Schemes 5 and 6). We firstly intended the direct reaction of **4a** and 2-fluoroethyl-4-methylbenzenesulfonate [35] to obtain **24a** (Scheme 5). However, we observed that the hydroxymethyl group was not reactive enough to substitute the tosylate leaving group, despite modifying experimental conditions (temperature, solvents, bases). Therefore, as we already proved that a halogen atom on the methylene moiety at the C-3 position of the quinoline core is a good leaving group (See Scheme 1 and Figure 2), we decided to convert the alcohol function of **4a** into the corresponding chloromethyl function using thionyl chloride. This chlorinated intermediate was used without purification for the nucleophilic substitution with 2-fluoroethanol to obtain the desired compound **24a** in moderate yield over two steps (37%). These conditions were then applied to compound **4b** to synthesise the fluorinated derivative **24b**.



Scheme 5. Syntheses of compounds **24a, b** starting from **4a, b**.[a]

[a]Reagents and conditions: (i) 2-fluoroethyl-4-methylbenzenesulfonate, basic conditions, THF, rt or reflux; (ii) $SOCl_2$, rt, 30 min; (iii) 2-fluoroethanol, DMF, 80 °C, 27 h for **24a** and 30 h for **24b**.

After the first *in vitro* evaluation of these new fluorinated derivatives (see section "biology: inhibitory effects on selected PDEs") and identification of **24a, b** as most potent analogues, we explored the effect of replacing the cyano function of **24a, b** by a trifluoromethyl group at the "west region" (**32a, b** in Scheme 6).



Scheme 6. Syntheses of analogues **32a**, **b** bearing a trifluoromethyl group at the C-6 position of the quinoline ring.[a]

[a]Reagents and conditions: (i) EMME, toluene, reflux, 11 h; (ii) diphenyl ether, 240 °C, 3 h; (iii) $POCl_3$, 120 °C, 6 h; (iv) (3-chloro-4-methoxyphenyl)meth anamine hydrochloride, DIPEA, *n*-propanol, reflux, 16 h; (v) cyclopropylboronic acid or ethylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, reflux, toluene, 20 h; (vi) DIBALH, CH_2Cl_2, -70 °C to -30 °C, 3 h for **31a** and DIBALH, THF, -60 °C to rt, 16 h for **31b**; (vii) (a) $SOCl_2$, rt, 40 min for **32a** or 30 min for **32b**; (b) 2-fluoroethanol, DMF, 80 °C, 30 h for **32a** and 22 h for **32b**.

The corresponding alcohols **31a, b** were synthesised according to the procedure previously optimised for alcohols **4a, b** (Scheme 6). Quinoline **28** was produced in three steps from commercially available 2-bromo-4-trifluoromethylaniline (**26**) [30]. Then, the aromatic nucleophilic substitution of the chlorine atom at the C-4 position of **28** using (3-chloro-4-methoxyphenyl)methanamine hydrochloride [29] efficiently produced compound **29**. The Suzuki coupling was performed to introduce either ethyl or cyclopropyl group at the C-8 position to obtain **30a, b**. The ester function at the C-3 position was classically reduced using DIBALH to afford alcohols **31a, b** which were converted into their corresponding chlorinated derivatives. The latter were not isolated and reacted immediately with 2-fluoroethanol to yield the desired fluorinated derivatives **32a, b** in 22-29% yields.

2.2. Biology: inhibitory effects on selected PDEs

In order to study the influence of the introduction of a fluorine atom into the lead structures **4a, b** and select the most potent derivative for further development as new radiotracer for PET imaging of PDE5, we evaluated the inhibitory effects of the synthesised fluorinated ligands on human PDE5A1 and a panel of other human PDEs (SB drug discovery, Glasgow, UK). Table 1 summarises the percentage of inhibition of compounds **22a, b**, **24a, b** and **32a, b** compared to lead alcohols **4a, b**. At the beginning, all experiments were performed at ligand concentrations of 100 nM. However, only inhibitory effects on the PDE5 enzyme could be observed under these conditions. Therefore, for all other PDEs the ligand concentration had to be increased to 1 µM in order to achieve analyzable signals. This behaviour already indicated the high specificity of the new derivatives for PDE5. Compounds **22a, b**, and **24a, b** showed a high and selective inhibitory activity on PDE5A1 (>88% at 100 nM vs. 0%-77% at 1 µM for other PDEs). Similar values were obtained with reference alcohols **4a, b** highlighting that the introduction of a fluorine atom at the "east region", i.e. at the C-3 position of the quinoline scaffold, did not significantly affect the affinity of the ligands for the PDE5 enzyme. Only a slightly lower inhibitory activity was observed for products **32a, b** obviously caused by the substitution of a trifluoromethyl group instead of the cyano group at the C-6 position of the quinoline scaffold.

Table 1: Percentage inhibition of selected PDEs by the synthesised compounds; the compounds were studied using a concentration of 100 nM for inhibition of human PDE5A1 and a concentration of 1 µM for inhibition of PDE2A3, PDE3A, PDE4A1, PDE4C2, PDE6AB, PDE9A1, PDE10A1 and PDE11A1; NI: no inhibition observed.

	% Inhibition of PDEs								
	PDE2 A3	PDE3 A	PDE4 A1	PDE4 C2	PDE5 A1	PDE6 AB	PDE9 A1	PDE10 A1	PDE11 A1
4a	15.3	NI	36.9	22.4	84.2	32.4	NI	22.9	24.1
4b	22.4	NI	45.4	40.9	90.3	49.8	NI	40.0	27.2
22a	65.5	NI	44.5	27.3	96.1	26.0	18.0	43.0	17.6
22b	68.4	NI	74.7	40.3	100	26.3	22.5	77.1	42.1
24a	16.0	6.12	13.2	35.9	89.9	54.8	14.8	18.8	15.1
24b	7.49	18.2	9.03	32.4	88.4	60.4	NI	14.1	5.21
32a	12.1	NI	6.5	17.3	57.1	2.36	NI	13.0	3.51
32b	9.74	NI	4.74	21.4	74.6	26.4	40.9	8.5	8.84

The compounds bearing a fluoroethyl chain at the C-3 position of the quinoline appeared to be less selective than derivatives with a fluoroethoxymethyl moiety. For example, **24a** presented a 4-fold lower inhibitory activity on PDE2A3 compared to **22a**. Therefore we focused our attention on compounds **24a, b** and **32a, b** and determined their IC_{50} values for inhibition of PDE5A1. The values obtained were 7.39, 1.86, 358 and 192 nM for **24a, b** and **32a, b** respectively. These results clearly highlight that the substituent at the C-6 position of the quinoline heterocycle influences the binding to the enzyme. As already indicated by the percentage of inhibition values in Table 1, when bearing a trifluoromethyl substituent, the IC_{50}

values reflect a significantly lower affinity for the PDE5A1 enzyme compared to the compounds with a cyano group (358 and 192 nM for **32a, b** vs. 7.39 and 1.86 nM for **24a, b** respectively). Under identical conditions, Sildenafil presented an IC50 value of 6.23 nM.

3. Conclusion

A new series of fluorinated inhibitors of the PDE5 enzyme was synthesised and evaluated *in vitro*. The organic synthesis of six new derivatives was successfully performed. *In vitro* inhibition experiments revealed that the introduction of small fluoro-containing alkyl side chains on the "east region" of the selected lead structures **4a, b** did not alter the inhibitory potency of these compounds towards PDE5. According to the data obtained, the fluorinated derivatives **24a, b** appeared to be most promising regarding affinity and selectivity and were selected for radiolabelling with fluorine-18 and biological evaluations *in vitro* and *in vivo* for PET imaging of PDE5 in the brain [36]. The data collected for [^{18}F]**24a** revealed that this compound was fastly metabolized *in vivo* and formed brain penetrable radiometabolites making it unsuitable for PET imaging of the PDE5 enzyme in the brain [36]. However, all these findings revealed useful for the design of metabolically more stable fluorinated quinoline analogs for further development of PET probes for imaging of PDE5 in brain. This work is currently undertaken in our laboratory.

4. Experimental section

4.1. Materials for Chemical Syntheses

All commercially available reagents and solvents were purchased from the following commercial suppliers: Sigma Aldrich, Acros Organics, Carlo Erba, and Alfa Aesar and were used without further purification. Room temperature (rt) refers to 20-25 ℃. All solvents were dried using common techniques. Air and moisture sensitive reactions were carried out under anhydrous argon atmosphere. Magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4) were used as drying agents. Monitoring of the reaction progresses was performed using thin layer chromatography (TLC) on silica (60 F254) or alumina (gel 60A + F254) plates and visualised with UV light (UV lamp Fisher Bioblock Scientific, 365 nm or 254 nm) or with an appropriate staining agent. Column chromatography was performed on silica gel (Chromagel 60 ACC, 40-63 µm, Carlo Erba Reagents) or on alumina gel (Merck, column chromatographic adsorption analysis ACC. to Brockmann, neutral aluminium oxide 90, standardise, 63-200 µm). Uncorrected melting points (Mp) were measured on an IA9100 Digital Melting Point Apparatus. Infrared spectra (IR) were recorded in the range of 4000-600 cm^{-1} on a

Nicolet IS10 with attenuated total reflectance (ATR) accessory (v: stretch, as: asymmetric, s: symmetric, δ: deformation; op: out of plane). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 instrument (500 MHz for ^{1}H, 125 MHz for ^{13}C), a Bruker Avance 400 instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker Avance 200 instrument (200 MHz for ^{1}H, 50 MHz for ^{13}C). All chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (*J*) are given in Hertz (Hz). Spectral coupling patterns are indicated as follow: s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet, brt: broad triplet and brs: broad singlet. All new compounds were analysed by HRMS (High-Resolution Mass Spectrometry, Waters® Micromass® Q-Tof micro™ Mass Spectrometer, UBP- START, Blaise Pascal University, Clermont-Ferrand, France). The isotope peaks for chlorine and bromine atoms are given with their relative intensities.

4.1.1. Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-ethylquinoline -3-carboxylate (3b). Ethylboronic acid (1.25 g, 16.8 mmol, 16.0 eq.), tetrakis(triphenylphosphine)palladium (0) (122 mg, 0.11 mmol, 0.1 eq.), caesium carbonate (858 mg, 2.63 mmol, 2.5 eq.) were added, under argon and stirring, to a solution of 212 (0.5 g, 1.05 mmol, 1.0 eq.) in anhydrous toluene (20 mL). The resulting mixture was refluxed for 24 h. After cooling to rt, the mixture was filtered on celite® 545, washed with ethyl acetate (100 mL). The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, gradient: ethyl acetate/cyclohexane, 1/9 to 5/5, v/v) to give, by order of elution: the desired product **3b** (270 mg, 0.64 mmol, 60%) as a yellow solid and ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyanoquinoline-3-carboxylate (**3b1**) (150 mg, 0.38 mmol, 36%) as a yellow solid. Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-ethylquinoline-3-carboxylate (**3b**) [30]. R_f (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v): 0.38; Mp: 159-161 ºC; IR (cm^{-1}): 2230 ($\nu_{C\equiv N}$), 1681 ($\nu_{C=O}$), 1577 (δ_{N-H}), 1507 ($\nu_{C=C}$), 1289 (ν_{C-O}), 1190 (ν_{C-O}), 1114 (ν_{C-O}), 1029 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.60 (brs, 1H, NH), 9.26 (s, 1H, H_2), 8.39 (d, 1H, $^{4}J_{H_5-H_7}$ = 1.4 Hz, H_5), 7.67 (d, 1H, $^{4}J_{H_7-H_5}$ = 1.4 Hz, H_7), 7.40 (d, 1H, $^{4}J_{H_{2'}-H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.26 (dd, 1H, $^{3}J_{H_{6'}-H_{5'}}$ = 8.5 Hz , $^{4}J_{H_{6'}-H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.97 (d, 1H, $^{3}J_{H_{5'}-H_{6'}}$ = 8.5 Hz, $H_{5'}$), 4.87 (d, 2H, $^{3}J_{H_d-NH}$ = 5.5 Hz, H_d), 4.38 (q, 2H, $^{3}J_{H_b-H_c}$ = 7.1 Hz, H_b), 3.92 (s, 3H, H_e), 3.23 (q, 2H, $^{3}J_{H_f-H_g}$ = 7.5 Hz, H_f), 1.40 (t, 3H, $^{3}J_{H_c-H_b}$ = 7.1 Hz, H_c), 1.35(t, 3H, $^{3}J_{H_g-H_f}$ = 7.5 Hz, H_g). Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyanoquinoline-3-carboxylate (**3b1**). R_f (SiO_2, cyclohexane/ethyl acetate, 3/7, v/v): 0.20; Mp: 155-157 ℃; IR (cm^{-1}): 2226 ($\nu_{C\equiv N}$), 1671 ($\nu_{C=O}$), 1572 (δ_{N-H}), 1504 ($\nu_{C=C}$), 1280 (ν_{C-O}), 1185 (ν_{C-O}), 1064 (ν_{C-O}); ^{1}H NMR ($CDCl_3$,

400 MHz) δ 9.85 (t, 1H, $^{3}J_{NH\text{-}H_d}$ = 5.1 Hz, NH), 9.19 (s, 1H, H_2), 8.54 (d, 1H, $^{4}J_{H_5\text{-}H_7}$ = 1.5 Hz, H_5), 8.02 (d, 1H, $^{3}J_{H_8\text{-}H_7}$ = 8.7 Hz, H_8), 7.79 (dd, 1H, $^{3}J_{H_7\text{-}H_8}$ = 8.7 Hz, $^{4}J_{H_7\text{-}H_5}$ = 1.5 Hz, H_7), 7.41 (d, 1H, $^{4}J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz , $H_{2'}$), 7.27 (dd, 1H, $^{3}J_{H_{6'}\text{-}H_{5'}}$ = 8.5 Hz , $^{4}J_{H_{6'}\text{-}H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.98 (d, 1H, $^{3}J_{H_{5'}\text{-}H_{6'}}$ = 8.5 Hz, $H_{5'}$), 4.90 (d, 2H, $^{3}J_{H_d\text{-}NH}$ = 5.5 Hz, H_d), 4.38 (q, 2H, $^{3}J_{H_b\text{-}H_c}$ = 7.1 Hz, H_b), 3.92 (s, 3H, H_e), 1.41 (t, 3H, $^{3}J_{H_c\text{-}H_b}$ = 7.1 Hz, H_c).

4.1.2. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-ethyl-3-(hydroxymethyl)quinoline-6-carbonitrile (4b). An anhydrous 1.1 M solution of lithium tri-*tert*-butoxyaluminum hydride in tetrahydrofuran (10.5 mL, 11.55 mmol, 7.0 eq.) was added, under stirring and argon, to a solution of **3b** (700 mg, 1.65 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (30 mL). The resulting mixture was heated at 50 °C for 27 hours. After cooling to rt, methanol (2 mL) was added slowly. This mixture was then diluted with dichloromethane (100 mL) before addition of a 1.0 M aqueous sodium hydroxide solution (100 mL). After decantation, the aqueous layer was then extracted with dichloromethane (2 × 100 mL). The combined organic layers were washed successively with a 1.0 M aqueous sodium hydroxide solution (100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, ethyl acetate/cyclohexane, 4/6, v/v) to give the desired product **4b**[30] (500 mg, 1.31 mmol, 79%) as a yellow solid. R_f (SiO_2, cyclohexane/ethyl acetate, 1/9, v/v): 0.32; Mp: 177-179 ºC; IR (cm^{-1}): 3413 ($\nu_{N\text{-}H}$), 2922 (ν_{CH_3}), 2230 ($\nu_{C\equiv N}$), 1548 ($\delta_{N\text{-}H}$), 1504 ($\nu_{C=C}$), 1260 ($\nu_{C\text{-}O}$), 1068 ($\nu_{C\text{-}O}$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.79 (d, 1H, $^{4}J_{H_5\text{-}H_7}$ = 1.5 Hz, H_5), 8.49 (s, 1H, H_2), 7.75 (d, 1H, $^{4}J_{H_7\text{-}H_5}$ = 1.4 Hz, H_7), 7.44 (t, 1H, $^{3}J_{NH\text{-}H_b}$ = 6.7 Hz, NH), 7.39 (d, 1H, $^{4}J_{H_{2'}\text{-}H_{6'}}$ = 2.0 Hz, $H_{2'}$), 7.24 (dd, 1H, $^{3}J_{H_{6'}\text{-}H_{5'}}$ = 8.5 Hz, $^{4}J_{H_{6'}\text{-}H_{2'}}$ = 2.0 Hz, $H_{6'}$), 7.11 (d, 1H, $^{3}J_{H_{5'}\text{-}H_{6'}}$ = 8.5 Hz, $H_{5'}$), 5.38 (t, 1H, $^{3}J_{OH\text{-}H_a}$= 5.2 Hz, OH), 4.81 (d, 2H, $^{3}J_{H_b\text{-}NH}$ = 6.7 Hz, H_b), 4.44 (d, 2H, $^{3}J_{H_a\text{-}OH}$ = 5.2 Hz, H_a), 3.81 (s, 3H, H_c), 3.13 (q, 2H, $^{3}J_{H_d\text{-}H_e}$ = 7.5 Hz, H_d), 1.25 (t, 3H, $^{3}J_{H_e\text{-}H_d}$ = 7.5 Hz, H_e).

4.1.3. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-(fluoromethyl)quinoline-6-carbonitrile (5a). Diethylaminosulfur trifluoride (58 mg, 0.36 mmol, 2.0 eq.) was added dropwise, at -60 ºC and under stirring to a solution of **4a**[12] (70 mg, 0.18 mmol, 1.0 eq.) in anhydrous dichloromethane (20 mL). The temperature increased slowly to rt over 2 hours and the mixture was stirred at this temperature for additional 2 hours. Then, a saturated aqueous sodium hydrogen carbonate solution (30 mL) was added to the mixture cooled at -20 ºC (pH = 7-8). After return back to rt and decantation, the aqueous layer was extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford a highly unstable white solid **5a** (60 mg, 0.15 mmol, 83%). Mp:

121-123 ºC; IR (cm^{-1}): 3405 (ν_{N-H}), 2230 ($\nu_{C\equiv N}$), 1504 ($\nu_{C=C}$), 1260 (ν_{C-O}), 1068 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.65 (d, 1H, $^4J_{H_2\text{-}F}$ = 1.7 Hz, H_2), 8.23 (m, 1H, H_5), 7.36 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.1 Hz, $H_{2'}$), 7.27 (m, 1H, H_7), 7.20 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.1 Hz, $H_{6'}$), 6.94 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 5.46 (d, 2H, $^3J_{H_a\text{-}F}$ = 49.2 Hz, H_a), 4.78 (d, 2H, $^3J_{H_b\text{-}NH}$ = 5.7 Hz, H_b), 3.91 (s, 3H, H_c), 3.08 (m, 1H, H_d), 1.23 (m, 2H, H_e or $H_{e'}$), 0.83 (m, 2H, H_e or $H_{e'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.0 ($C_{4'}$), 154.6 (C_2), 152.2 (C_4), 150.2 (C_{8a}), 145.4 (C_8), 131.6 ($C_{1'}$), 129.4 ($C_{2'}$), 126.8 ($C_{6'}$), 125.8 (C_5), 124.7 (C_7), 123.2 ($C_{3'}$), 119.7 (C_{4a}), 119.4 (CN), 112.7 ($C_{5'}$), 111.5 (d, $^2J_{C_3\text{-}F}$ = 18 Hz, C_3), 108.4 (C_6), 81.4 (d, $^1J_{C_a\text{-}F}$ = 164 Hz, C_a), 56.4 (C_c), 50.9 (C_b), 11.2 (C_d), 10.1 (2C, C_e, $C_{e'}$); ESI-MS calculated for $C_{22}H_{19}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 396.12, found $C_{22}H_{19}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 396.12 (100%); $C_{22}H_{19}{}^{37}ClFN_3O$ $[M+H]^+$ m/z 398.09 (33%). If the crude product was purified over silica gel, alumina or C-18, (*Z*,*E*)-4-[((3-chloro-4-methoxyphenyl)methylene)amino]-8-cyclopropyl-3-methylquinoline-6-carbonitrile (**6a**) was obtained. R_f (SiO_2, ethyl acetate/cyclohexane, 3/7, v/v): 0.24; Mp: 216-218 ºC; IR (cm^{-1}): 2227 ($\nu_{C\equiv N}$), 1502 ($\nu_{C=C}$), 1276 ($\nu_{as\ C\text{-}O\text{-}C}$), 1187 ($\nu_{s\ C\text{-}O\text{-}C}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.91 (s, 1H, H_2), 8.20 (s, 1H, H_b) 8.09 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.0 Hz, $H_{2'}$), 7.95 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.7 Hz, H_5), 7.82 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.5 Hz) , $^4J_{H_{6'}\text{-}H_{2'}}$= 2.0 Hz, $H_{6'}$), 7.23 (m, 1H, H_7), 7.09 (d, 1H, $^4J_{H_{5'}\text{-}H_{6'}}$= 8.5 Hz, $H_{5'}$), 4.03 (s, 3H, H_c), 3.17 (m, 1H, H_d), 2.34 (s, 3H, H_a), 1.25 (m, 2H, H_e or $H_{e'}$), 0.86 (m, 2H, H_e or $H_{e'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.6 (C_b), 158.8 ($C_{4'}$), 155.6 (C_4), 154.6 (C_2), 147.4 (C_{8a}), 144.8 (C_8), 130.6 ($C_{2'}$), 129.9 ($C_{6'}$), 128.5 ($C_{1'}$), 127.0 (C_5), 124.0 (C_7), 123.4 ($C_{3'}$), 121.4 (C_{4a}), 119.4 (CN), 118.7 (C_3), 112.1 ($C_{5'}$), 109.6 (C_6), 56.7 (C_c), 15.2 (C_a), 11.0 (C_d), 10.1 (2C, C_e, $C_{e'}$); HRMS calculated for $C_{22}H_{18}{}^{35}ClN_3O$ $[M+H]^+$ m / z 376.1217, found $C_{22}H_{18}{}^{35}ClN_3O$ $[M+H]^+$ m/z 376.1214 (100%); $C_{22}H_{18}{}^{37}ClN_3O$ $[M+H]^+$ m/z 378.1169 (33%).

4.1.4. (E)-Diethyl 2-[((2-bromo-4-cyanophenyl)amino)methylene]succinate (9). 4-Amino-3-bromobenzonitrile (2.0 g, 10.2 mmol, 1.0 eq.) was added to a mixture of **8**[34] (5.16 g, 25.5 mmol, 2.0 eq.) in anhydrous toluene (24 mL) under stirring. The resulting mixture was refluxed for 8 hours. After cooling to rt, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (Al_2O_3, ethyl acetate/cyclohexane, 4/6, v/v) to give the desired product **9** (3.0 g, 7.87 mmol, 77%) as a white solid. R_f (SiO_2, cyclohexane/ethyl acetate, 6/4, v/v): 0.62; Mp: 138-140 ºC; IR (cm^{-1}): 2223 ($\nu_{C\equiv N}$), 1677 ($\nu_{C=O}$), 1625 ($\nu_{C=C}$), 1596 (δ_{N-H}), 1191 ($\nu_{as\ C\text{-}O\text{-}C}$), 1023 ($\nu_{s\ C\text{-}O\text{-}C}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.68 (d, 1H, $^3J_{NH\text{-}H_a}$ = 11.6 Hz, NH), 7.82 (d, 1H, $^4J_{H_3\text{-}H_5}$ = 1.8 Hz, H_3), 7.54 (dd, 1H, $^3J_{H_5\text{-}H_6}$ = 8.6 Hz, $^4J_{H_5\text{-}H_3}$ = 1.8 Hz, H_5), 7.22 (d, 1H, $^3J_{H_a\text{-}NH}$ = 11.6 Hz, H_a), 7.10 (d, 1H, $^3J_{H_6\text{-}H_5}$ = 8.6 Hz, H_6), 4.27 (q, 2H, $^3J_{H_e\text{-}H_f\ or\ H_{e'}\text{-}H_{f'}}$ = 7.1 Hz, H_e or $H_{e'}$), 4.17 (q, 2H, $^3J_{H_e\text{-}H_d\ or\ H_{e'}\text{-}H_{d'}}$ = 7.1 Hz, H_e or $H_{e'}$), 3.24 (s, 2H, H_c), 1.30 (t, 3H, $^3J_{H_f\text{-}H_e\ or\ H_{f'}\text{-}H_{e'}}$ = 7.1 Hz, H_e or $H_{e'}$), 1.25 (t, 3H, $^3J_{H_f\text{-}H_e\ or\ H_{f'}\text{-}H_{e'}}$ = 7.1 Hz, H_f or $H_{f'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8 (C_d), 168.5

($C_{d'}$), 142.5 (C_1), 139.1 (C_a), 136.7 (C_3), 132.5 (C_5), 117.8 (CN), 112.7 (C_6), 111.2 (C_2 or C_4), 104.9 (C_2 or C_4), 99.6 (C_b), 60.9 (C_e or $C_{e'}$), 60.6 (C_e or $C_{e'}$), 36.0 (C_c), 14.2 (2C, C_f, $C_{f'}$); HRMS calculated for $C_{16}H_{17}{}^{79}BrN_2O_4$ $[M+H]^+$ m/z 381.0450, found $C_{16}H_{17}{}^{79}BrN_2O_4$ $[M+H]^+$ m/z 381.0476 (100%); $C_{16}H_{17}{}^{81}BrN_2O_4$ $[M+H]^+$ m/z 383.0457 (98%).

4.1.5. Ethyl 2-(8-bromo-6-cyano-4-oxo-1,4-dihydroquinolin-3-yl)acetate (10). A suspension of **9** (510 mg, 1.34 mmol, 1.0 eq.) in DowTherm A (10 mL) was placed into a bath beforehand heated at 240-250 °C. The mixture was stirred at this temperature for 2 hours. After cooling to rt and decantation overnight, the formed precipitate was collected by filtration, washed with diethyl ether (30 mL) and dried to give the desired product **10** (230 mg, 0.69 mmol, 51%) as a brown solid. Mp: 134-136 ºC; IR (cm^{-1}): 2232 ($\nu_{C\equiv N}$), 1740 ($\nu_{C=O}$), 1548 (δ_{N-H}), 1505 ($\nu_{C=C}$), 1166 (ν_{C-O}); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.55 (d, 1H, $^3J_{NH-H_2}$ = 5.9 Hz, NH), 8.48 (d, 1H, $^3J_{H_5-H_7 \text{ or } H_7-H_5}$ = 1.8 Hz, H_5 or H_7), 8.46 (d, 1H, $^3J_{H_5-H_7 \text{ or } H_7-H_5}$ = 1.8 Hz, H_5 or H_7), 8.02 (d, 1H, $^3J_{H_2-NH}$ = 5.9 Hz, H_2), 4.06 (q, 2H, $^3J_{H_c-H_d}$= 7.1 Hz, H_c), 3.51 (s, 2H, H_a), 1.18 (t, 3H, $^3J_{H_d-H_c}$= 7.1 Hz, H_d); HRMS calculated for $C_{14}H_{11}{}^{79}BrN_2O_3$ $[M+H]^+$ m/z 335.0031, found $C_{14}H_{11}{}^{79}BrN_2O_3$ $[M+H]^+$ m/z 335.0034 (100%); $C_{14}H_{11}{}^{81}BrN_2O_3$ $[M+H]^+$ m/z 337.0004 (98%).

4.1.6. Ethyl 2-(8-bromo-4-chloro-6-cyanoquinolin-3-yl)acetate (11). A mixture of **10** (0.53 g, 1.58 mmol, 1.0 eq.) in phosphorous oxychloride (15 mL) was stirred at reflux for 5 hours. After cooling to rt, the solvent was evaporated. Then, ice (10 mL) and a saturated aqueous sodium carbonate solution (30 mL) were slowly added. The aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were washed with brine (80 mL), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The brown residue was purified by column chromatography (SiO_2, ethyl acetate/cyclohexane, 3/7, v/v) to give the desired product **11** as a brown solid (0.45 g, 1.27 mmol, 81%). R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.71; Mp: 179-181 ºC; IR (cm^{-1}): 2235 ($\nu_{C\equiv N}$), 1721 ($\nu_{C=O}$), 1213 ($\nu_{as\ C-O-C}$), 1151 ($\nu_{s\ C-O-C}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.99 (s, 1H, H_2), 8.60 (d, 1H, $^4J_{H_5-H_7}$ = 1.7 Hz, H_5), 8.20 (d, 1H, $^4J_{H_7-H_5}$ = 1.7 Hz, H_7), 4.20 (q, 2H, $^3J_{H_c-H_d}$ = 7.1 Hz, H_c), 4.02 (s, 2H, H_a), 1.26 (t, 3H, $^3J_{H_d-H_c}$ = 7.1 Hz, H_d); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.7 (C_b), 155.2 (C_2), 146.4 (C_{8a}), 143.0 (C_4), 133.9 (C_7), 130.2 (C_5), 128.6 (C_3), 126.9 (C_{4a} or C_8), 126.8 (C_{4a} or C_8), 116.9 (CN), 112.1 (C_6), 61.8 (C_c), 37.1 (C_a), 14.2 (C_d); HRMS calculated for $C_{14}H_{10}{}^{79}Br^{35}ClN_2O_2$ $[M+H]^+$ m / z 352.9692, found $C_{14}H_{10}{}^{79}Br^{35}ClN_2O_2$ $[M+H]^+$ m/z 352.9671 (77%); $C_{14}H_{10}{}^{81}Br^{35}ClN_2O_2$ or $C_{14}H_{10}{}^{79}Br^{37}ClN_2O_2$ $[M+H]^+$ m/z 354.9647 (100%); $C_{14}H_{10}{}^{81}Br^{37}ClN_2O_2$ $[M+H]^+$ m/z 356.9632 (24%).

4.1.7. Ethyl 2-(4-chloro-6-cyano-8-cyclopropylquinolin-3-yl)acetate (13). Cyclopropylboronic acid (73 mg, 0.85 mmol, 1.5 eq.), tetrakis(triphenylphosphine)palladium(0) (39 mg, 33.7 µmol, 0.06 eq.), caesium carbonate (460 mg, 1.41 mmol, 2.5 eq.) were added, under stirring and argon, to a solution of **11** (200 mg, 0.57 mmol, 1.0 eq.) in anhydrous toluene (10 mL). The resulting mixture was refluxed for 22 h. After cooling to rt, the reaction mixture was filtered over Celite® 545, washed with ethyl acetate (40 mL). The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, ethyl acetate/cyclohexane, 3/7, v/v) to give the desired product **13** (110 mg, 0.35 mmol, 62%) as a light yellow solid. R_f (Al_2O_3, cyclohexane/ethyl acetate, 8/2, v/v): 0.40; Mp: 126-128 ºC; IR (cm^{-1}): 2232 ($\nu_{C\equiv N}$), 1742 ($\nu_{C=O}$), 1482 ($\nu_{C=C}$), 1151 ($\nu_{as\ C\text{-}O\text{-}C}$), 1043 ($\nu_{s\ C\text{-}O\text{-}C}$); ^{1}H NMR (DMSO-$d_6$, 400 MHz) δ 8.93 (s, 1H, H_2), 8.45 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.6 Hz, H_5), 7.31 (d, 1H, $^4J_{H_7\text{-}H_5}$ = 1.6 Hz, H_7), 4.21 (q, 2H, $^3J_{H_c\text{-}H_d}$ = 7.1 Hz, H_c), 4.01 (s, 2H, H_a), 3.19 (m, 1H, H_e), 1.26 (m, 5H, H_d, H_f or $H_{f'}$), 0.89 (m, 2H, H_f or $H_{f'}$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.0 (C_b), 153.1 (C_2), 148.0 (C_{8a}), 145.8 (C_8), 142.4 (C_4), 127.2 (C_3), 127.0 (C_5), 125.5 (C_{4a}), 123.9 (C_7), 118.4 (CN), 111.2 (C_6), 61.4 (C_c), 36.8 (C_a), 14.0 (C_d), 10.6 (C_e), 10.5 (2C, C_f, $C_{f'}$); HRMS calculated for $C_{17}H_{15}{}^{35}ClN_2O_2$ $[M+H]^+$ m/z 315.0900, found $C_{17}H_{15}{}^{35}ClN_2O_2$ $[M+H]^+$ m/z 315.0915 (100%); $C_{17}H_{15}{}^{37}ClN_2O_2$ $[M+H]^+$ m/z 317.0922 (33%).

4.1.8. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-formylquinoline-6-carbonitrile (15a). Manganese oxide (331 mg, 3.81 mmol, 10 eq.) was added to a solution of **4a** [12] (150 mg, 0.38 mmol, 1.0 eq.) in dichloromethane (20 mL). The resulting mixture was stirred at rt for 5 hours, filtered on Celite® 545 and washed with dichloromethane (40 mL). The filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, ethanol/dichloromethane, 3/97, v/v) to give the desired product **15a** (140 mg, 0.36 mmol, 95%) as a light yellow solid. R_f (SiO_2, ethanol/dichloromethane, 1/9, v/v): 0.49; Mp: 209-211 ºC; IR (cm^{-1}): 2228 ($\nu_{C\equiv N}$), 1642 ($\nu_{C=O}$), 1571 ($\delta_{N\text{-}H}$), 1501 ($\nu_{C=C}$), 1181 ($\nu_{C\text{-}O}$), 1022 ($\nu_{C\text{-}O}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.56 (t, 1H, $^3J_{NH\text{-}H_b}$ = 5.5 Hz, NH), 9.91 (s, 1H, H_a), 8.74 (s, 1H, H_2), 8.34 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.7 Hz, H_5), 7.38 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.26 (m, 2H, H_7, $H_{6'}$), 6.95 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.5 Hz, $H_{5'}$), 4.92 (d, 2H, $^3J_{H_b\text{-}NH}$ = 5.5 Hz, H_b), 3.89 (s, 3H, H_c), 3.08 (m, 1H, H_d), 1.19 (m, 2H, H_e or $H_{e'}$), 0.78 (m, 2H, H_e or $H_{e'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.7 (C_a), 157.0 (C_2), 155.5 (C_4), 155.3 ($C_{4'}$), 151.8 (C_{8a}), 145.5 (C_8), 129.5 ($C_{1'}$), 129.5 (C_5), 129.4 ($C_{2'}$), 127.0 ($C_{6'}$), 126.9 (C_7), 123.4 ($C_{3'}$), 119.0 (CN), 118.0 (C_3), 112.7 ($C_{5'}$), 111.6 (C_{4a}), 107.7 (C_6), 56.3 (C_c), 51.1 (C_b), 11.4 (C_d), 10.1 (2C, C_e, $C_{e'}$); HRMS calculated for $C_{22}H_{18}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 392.1166, found $C_{22}H_{18}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 392.1151 (100%); $C_{22}H_{18}{}^{37}ClN_3O_2$ $[M+H]^+$ m/z 394.1126 (33%).

4.1.9. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-ethyl-3-formylquinoline-6-carbonitrile (15b). Manganese oxide (1.4 g, 16.1 mmol, 10.0 eq.) was added to a solution of **4b** (0.6 g, 1.57 mmol, 1.0 eq.) in dichloromethane (40 mL). The resulting mixture was stirred at rt for 20 hours before filtration on celite® 545 and washing with dichloromethane (2 × 40 mL). The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v) to give the desired product **15b** (0.27 g, 0.71 mmol, 45%) as a yellow solid. R_f (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v): 0.25; Mp: 168-170 ºC; IR (cm^{-1}): 2226 ($\nu_{C\equiv N}$), 1651 ($\nu_{C=O}$), 1576 (δ_{NH}), 1505 ($\nu_{C=C}$), 1266 (ν_{C-O}), 1069 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.57 (t, 1H, $^3J_{NH\text{-}H_b}$ = 5.5 Hz, NH), 9.91 (s, 1H, H_a), 8.72 (s, 1H, H_2), 8.43 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.7 Hz, H_5), 7.70 (m, 1H, H_7), 7.40 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.3 Hz, $H_{2'}$), 7.27 (dd, 1H, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.3 Hz, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.6 Hz, $H_{6'}$), 6.97 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.6 Hz, $H_{5'}$), 4.94 (d, 2H, $^3J_{H_b\text{-}NH}$ = 5.5 Hz, H_b), 3.90 (s, 3H, H_c), 3.20 (q, 2H, $^3J_{H_d\text{-}H_e}$ = 7.5 Hz, H_d), 1.33 (t, 3H, $^3J_{H_e\text{-}H_d}$ = 7.5 Hz, H_e); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.7 (C_a), 157.0 (C_2), 155.4 ($C_{4'}$), 155.3 (C_4), 151.2 (C_{8a}), 145.4 (C_8), 131.5 (C_7), 130.5 (C_5), 129.5 ($C_{1'}$), 129.4 ($C_{2'}$), 126.9 ($C_{6'}$), 123.4 ($C_{3'}$), 119.0 (CN), 118.0 (C_{4a}), 112.7 ($C_{5'}$), 111.5 (C_3), 107.6 (C_6), 56.3 (C_c), 51.1 (C_b), 25.3 (C_d), 14.5 (C_e); HRMS calculated for $C_{21}H_{18}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 380.1166, found $C_{21}H_{18}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 380.1159 (100%); $C_{21}H_{18}{}^{37}ClN_3O_2$ $[M+H]^+$ m/z 382.1113 (33%).

4.1.10. (*Z*, *E*)-4-[(3-Chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-(2-methoxyvinyl)quinoline-6-carbonitrile (16a). A 1.6 M solution of *n*-butyllithium (4.1 mL, 6.56 mmol, 9.9 eq.) in hexane was slowly added at -7 °C, under argon and stirring, to a suspension of (methoxymethyl)triphenylphosphonium chloride (2.28 g, 6.65 mmol, 10.0 eq.) in anhydrous tetrahydrofuran (20 mL). The resulting mixture was then stirred at rt for 20 minutes. After cooling to -15 °C, a solution of **15a** (260 mg, 0.66 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (20 mL) was slowly added. The resulting mixture was stirred at rt for 2 hours before addition of a saturated aqueous ammonium chloride solution (40 mL) at -5 °C. This aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue obtained was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v) to give the desired product **(*Z*, *E*)-16a** (0.19 g, 0.45 mmol, 68%) as a yellow solid (ratio *Z*/*E* = 1/0.4). R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.32, 0.23; Mp: 140-142 ºC; IR (cm^{-1}): 3405 ($\nu_{N\text{-}H}$), 2226 ($\nu_{C\equiv N}$), 1651 ($\nu_{C=C}$), 1498 ($\nu_{C=C}$), 1255 ($\nu_{C\text{-}O}$), 1099 ($\nu_{C\text{-}O}$), 1061 ($\nu_{C\text{-}O}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.96 (s, 1H, $H_{2(Z)}$), 8.69 (s, 1H, $H_{2(E)}$), 8.15 (d, 1H, $^4J_{H_{5(Z)}\text{-}H_{7(Z)}}$ = 1.6 Hz, $H_{5(Z)}$), 8.12 (d, 1H, $^4J_{H_{5(E)}\text{-}H_{7(E)}}$ = 1.6 Hz, $H_{5(E)}$), 7.32 (d, 1H, $^4J_{H_{2'(Z/E)}\text{-}H_{6'(Z/E)}}$ = 2.2 Hz, $H_{2'(E/Z)}$), 7.16 (m, 1H, $H_{7(Z/E)}$), 7.12 (dd, 1H, $^4J_{H_{6'(Z)}\text{-}H_{2'(Z)}}$ = 2.2 Hz, $^3J_{H_{6'(Z)}\text{-}H_{5'(Z)}}$ = 8.4 Hz, $H_{6'(Z)}$), 7.11 (dd, 1H, $^4J_{H_{6'(E)}\text{-}}$

$H_{2'(E)}$ = 2.2 Hz, $^3J_{H_{6'(E)}\text{-}H_{5'(E)}}$ = 8.4 Hz, $H_{6'(E)}$), 6.88 (d, 1H, $^3J_{H_{5'(Z/E)}\text{-}H_{6'(Z/E)}}$ = 8.4 Hz, $H_{5'(Z/E)}$), 6.85 (d, 1H, $^3J_{H_{b(E)}\text{-}H_{a(E)}}$ = 12.8 Hz, $H_{b(E)}$), 6.27 (d, 1H, $^3J_{H_{b(Z)}\text{-}H_{a(Z)}}$ = 6.8 Hz, $H_{b(Z)}$), 5.72 (d, 1H, $^3J_{H_{a(E)}\text{-}H_{b(E)}}$ = 12.8 Hz, $H_{a(E)}$), 5.28 (d, 1H, $^3J_{H_{a(Z)}\text{-}H_{b(Z)}}$ = 6.8 Hz, $H_{a(Z)}$), 4.58 (s, 2H, $H_{d(E)}$), 4.55 (s, 2H, $H_{d(Z)}$), 3.88 (s, 3H, $H_{e(Z/E)}$), 3.73 (s, 3H, $H_{c(Z)}$), 3.65 (s, 3H, $H_{c(E)}$), 3.10 (m, 1H, $H_{f(Z/E)}$), 1.19 (m, 2H, $H_{g(Z/E)}$ or $H_{g'(Z/E)}$), 0.81 (m, 2H, $H_{g(Z/E)}$ or $H_{g'(Z/E)}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.8 ($C_{4'(E)}$), 154.7 ($C_{4'(Z)}$), 154.0 ($C_{2(Z)}$), 152.0 ($C_{2(E)}$), 151.3 ($C_{b(E)}$), 149.1 (2C, $C_{b(Z)}$, $C_{4(Z/E)}$), 148.6 ($C_{8a(E)}$), 148.4 ($C_{8a(Z)}$), 145.3 ($C_{8(E)}$), 144.9 ($C_{8(Z)}$), 132.4 ($C_{1'(Z)}$), 132.3 ($C_{1'(E)}$), 129.5 ($C_{2'(Z)}$), 129.4 ($C_{2'(E)}$), 127.0 ($C_{6'(Z)}$), 126.9 ($C_{6'(E)}$), 126.6 ($C_{5(Z)}$), 125.8 ($C_{5(E)}$), 123.3 ($C_{7(Z)}$), 123.1 ($C_{7(E)}$), 123.0 ($C_{3'(E)}$), 122.9 ($C_{3'(E)}$), 121.0 ($C_{4a(Z)}$), 120.7 ($C_{4a(E)}$), 119.7 ($CN_{(Z)}$), 119.6 ($CN_{(E)}$), 117.4 ($C_{3(E)}$), 116.7 ($C_{3(Z)}$), 112.4 ($C_{5'(E)}$), 112.4 ($C_{5'(Z)}$), 108.4 ($C_{6(E)}$), 108.0 ($C_{6(Z)}$), 99.4 ($C_{a(Z)}$), 98.6 ($C_{a(E)}$), 60.8 ($C_{c(Z)}$), 56.9 ($C_{c(E)}$), 56.3 ($C_{e(Z/E)}$), 52.5 ($C_{d(Z)}$), 52.0 ($C_{d(E)}$), 11.2 ($C_{f(Z)}$), 11.1 ($C_{f(E)}$), 10.0 (2C, $C_{g(E)}$/$C_{g'(E)}$), 9.9 (2C, $C_{g(Z)}$/$C_{g'(Z)}$); HRMS calculated for $C_{24}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 420.1479, found $C_{24}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 420.1505 (100%); $C_{24}H_{22}{}^{37}ClN_3O_2$ $[M+H]^+$ m/z 422.1455 (33%).

4.1.11. (*Z*)-4-[(3-Chloro-4-methoxybenzyl)amino]-8-ethyl-3-(2-methoxyvinyl)quinoline-6-carbonitrile (16b). A solution of 1.6 M *n*-butyllithium (4.1 mL, 6.56 mmol, 9.2 eq) in hexane was added slowly to a suspension of (methoxymethyl)triphenylphosphonium chloride (2.4 g, 7.00 mmol, 9.9 eq) in anhydrous tetrahydrofuran (20 mL) under argon and stirring at -15 ^{o}C. The resulting mixture was then stirred at rt for 20 minutes before addition of a solution of **15b** (270 mg, 0.71 mmol, 1.0 eq) in anhydrous tetrahydrofuran (15 mL) at -10 ^{o}C over 15 minutes. The resulting mixture was stirred at rt for another 2 hours before addition of a saturated aqueous solution of ammonium chloride (40 mL) at a temperature between -5 ^{o}C and 0 ^{o}C. The resulting solution was extracted with ethyl acetate (3 × 30 mL) and the combined organic layers were washed with brine (100 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v) to give the desired product **(*Z*)-16b** (140 mg, 0.34 mmol, 48%) as a yellow solid. R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.24; Mp: 151-153 ºC; IR (cm^{-1}): 3405 ($\nu_{N\text{-}H}$), 2228 ($\nu_{C\equiv N}$), 1499 ($\delta_{N\text{-}H}$), 1390 (δ_{CH_3}), 1255 ($\nu_{C\text{-}O}$), 1099 ($\nu_{C\text{-}O}$), 1060 ($\nu_{C\text{-}O}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.88 (s, 1H, H_2), 8.14 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.8 Hz, H_5), 7.49 (m, 1H, H_7), 7.27 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.07 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.82 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 6.21 (d, 1H, $^3J_{H_b\text{-}H_a}$ = 6.9 Hz, H_b), 5.22 (d, 1H, $^3J_{H_a\text{-}H_b}$ = 6.9 Hz, H_a), 4.64 (t, 1H, $^3J_{NH\text{-}H_d}$ = 6.2 Hz, NH), 4.47 (d, 2H, $^3J_{H_d\text{-}NH}$ = 6.2 Hz, H_d), 3.82 (s, 3H, H_e), 3.67 (s, 3H, H_c), 3.17 (q, 2H, $^3J_{H_f\text{-}H_g}$ = 7.5 Hz, H_f), 1.29 (t, 3H, $^3J_{H_g\text{-}H_f}$ = 7.5 Hz, H_g); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.7 ($C_{4'}$), 154.2 (C_2), 149.0 (C_b),

148.9 (C_4), 147.9 (C_{8a}), 145.1 (C_8), 132.5 ($C_{1'}$), 129.5 ($C_{2'}$), 127.5 (C_7), 127.4 ($C_{6'}$), 127.0 (C_5), 122.9 ($C_{3'}$), 121.1 (C_{4a}), 119.8 (CN), 116.7 (C_3), 112.4 ($C_{5'}$), 107.9 (C_6), 99.5 (C_a), 60.7 (C_c), 56.3 (C_e), 52.6 (C_d), 24.9 (C_f), 14.6 (C_g); HRMS calculated for $C_{23}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 408.1479, found $C_{23}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 408.1461 (100%); $C_{23}H_{22}{}^{37}ClN_3O_2$ $[M+H]^+$ m/z 410.1417 (33%).

4.1.12. 1-(3-Chloro-4-methoxybenzyl)-6-cyclopropyl-1*H*-pyrrolo[3,2-*c*]quinoline-8-carbonitrile (17a). A 4 M aqueous hydrochloric acid solution (0.1 mL) was added to a solution of **16a** (20 mg, 47.6 µmol, 1.0 eq.) in tetrahydrofuran (0.3 mL). The resulting mixture was stirred at 50 °C for 4 hours. After cooling to rt, an aqueous saturated sodium bicarbonate solution (20 mL) was added. The resulting solution was extracted with ethyl acetate (2 × 15 mL) and the combined organic layers were washed with brine (30 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v) to give product **17a** as a white solid (10 mg, 25.8 µmol, 54%); R_f (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v): 0.53; Mp: 196-198 ºC; IR (cm^{-1}): 2230 ($\nu_{C\equiv N}$), 1502 ($\nu_{C=C}$), 1258 ($\nu_{as\ C-O}$), 1064 ($\nu_{s\ C-O}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.32 (s, 1H, H_4), 8.44 (d, 1H, $^4J_{H_9-H_7}$ = 1.6 Hz, H_9), 7.76 (d, 1H, $^3J_{H_2-H_3}$ = 3.1 Hz, H_2), 7.31 (d, 1H, $^4J_{H_7-H_9}$ = 1.6 Hz, H_7), 7.19 (d, 1H, $^4J_{H_{2'}-H_{6'}}$ = 2.1 Hz, $H_{2'}$), 7.04 (d, 1H, $^3J_{H_{5'}-H_{6'}}$ = 8.6 Hz, $H_{5'}$), 6.98 (d, 1H, $^3J_{H_3-H_2}$ = 3.1 Hz, H_3), 6.88 (dd, 1H, $^4J_{H_{6'}-H_{2'}}$ = 2.1 Hz, $^3J_{H_{6'}-H_{5'}}$ = 8.6 Hz, $H_{6'}$), 5.95 (s, 2H, H_a), 3.76 (s, 3H, H_b), 3.31 (m, 1H, H_c), 1.10 (m, 2H, H_d or $H_{d'}$), 0.88 (m, 2H, H_d or $H_{d'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.8 ($C_{4'}$), 147.7 (C_4), 144.9 (C_6), 143.8 (C_{5a}), 132.6 (C_2), 132.3 (C_{9b}), 130.3 ($C_{1'}$), 127.7 ($C_{2'}$), 126.0 ($C_{6'}$), 124.2 (C_9), 123.1 (C_{3a}), 121.4 ($C_{3'}$), 121.0 (C_7), 119.3 (CN), 117.1 (C_{9a}), 113.1 ($C_{5'}$), 107.7 (C_8), 102.7 (C_3), 56.1 (C_b), 51.1 (C_a), 11.2 (C_c), 10.3 (2C, C_d, $C_{d'}$); HRMS calculated for $C_{23}H_{18}{}^{35}ClN_3O$ $[M+H]^+$ m/z 388.1217, found $C_{23}H_{18}{}^{35}ClN_3O$ $[M+H]^+$ m/z 388.1197 (100%); $C_{23}H_{18}{}^{37}ClN_3O$ $[M+H]^+$ m/z 390.1178 (33%).

4.1.13. Allyl (3-chloro-4-methoxybenzyl)-[(6-cyano-8-cyclopropyl-3-(2-methoxyvinyl))quinolin-4-yl]carbamate (19a). Sodium hydride powder (moistened with oil, 55-65%, 91 mg, 2.26 mmol, 5.0 eq.) was added to a solution of **16a** (190 mg, 0.45 mmol, 1.0 eq.) in anhydrous *N,N*-dimethylformamide (10 mL). The resulting mixture was stirred at rt for 20 minutes before careful addition of allyl chloroformate (192 µL, 1.81 mmol, 4.0 eq.). The mixture was then stirred at rt for one hour before addition of water (20 mL). The resulting solution was extracted with ethyl acetate (3 × 30 mL) and the combined organic layers were washed with brine (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 6/4, v/v) to give the desired product **19a** as yellow oil corresponding to a mixture of *Z*/*E* isomers (ratio: *Z*/*E* = 1/0.7, 170 mg, 0.34 mmol, 76%). IR

(cm^{-1}): 2228 ($\nu_{C\equiv N}$), 1703 ($\nu_{C=O}$), 1635 ($\nu_{C=C}$), 1501 ($\nu_{C=C}$), 1257 (ν_{C-O}), 1220 (ν_{C-O}), 1118 (ν_{C-O}); HRMS calculated for $C_{28}H_{26}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 504.1690, found $C_{28}H_{26}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 504.1676 (100%); $C_{28}H_{26}{}^{37}ClN_3O_4$ $[M+H]^+$ m/z 506.1830 (33%). During the purification by column chromatography, a few fractions containing each pure isomer were obtained. The quantities isolated allowed to characterise the isomers *Z* and *E* by NMR analyses separately. Allyl (*E*)-(3-chloro-4-methoxybenzyl)-[(6-cyano-8-cyclopropyl-3-(2-methoxyvinyl))quinolin-4-yl]carbamate (**(*E*)-19a**). R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.62; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.09 (s, 1H, H_2), 7.61 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.6 Hz, H_5), 7.16 (d, 1H, $^3J_{H_b\text{-}H_a}$ = 13.1 Hz, H_b), 7.15 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.14 (d, 1H, $^4J_{H_7\text{-}H_5}$ = 1.6 Hz, H_7), 7.01 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.77 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 5.66 (m, 1H, H_g), 5.40 (d, 1H, $^3J_{H_a\text{-}H_b}$ = 13.1 Hz, H_a), 5.02 (m, 2H, H_h), 4.90 (d, 1H, $^2J_{H_d\text{-}H_{d'}}$ = 14.3 Hz, H_d), 4.58 (m, 1H, H_f), 4.53 (d, 1H, $^2J_{H_{d'}\text{-}H_d}$ = 14.3 Hz, $H_{d'}$), 4.46 (m, 1H, $H_{f'}$), 3.86 (s, 3H, H_i), 3.55 (s, 3H, H_c), 3.17 (m, 1H, H_j), 1.23 (m, 2H, H_k or $H_{k'}$), 0.87 (m, 2H, H_k or $H_{k'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5 (C_e), 155.2 ($C_{4'}$), 152.5 (C_b), 150.5 (C_2), 147.9 (C_{8a}), 145.8 (C_8), 139.6 (C_4), 132.1 (C_g), 131.6 ($C_{2'}$), 129.4 ($C_{6'}$), 129.3 ($C_{1'}$), 125.9 (C_5), 125.7 (C_{4a}), 122.9 (C_7), 122.5 ($C_{3'}$), 119.5 (C_3), 118.9 (CN), 118.2 (C_h), 112.0 ($C_{5'}$), 111.1 (C_6), 97.7 (C_a), 66.9 (C_f), 56.6 (C_c), 56.3 (C_i), 52.8 (C_d), 10.8 (C_j), 10.5 (2C, C_k, $C_{k'}$). Allyl (*Z*)-(3-chloro-4-methoxybenzyl)-[(6-cyano-8-cyclopropyl-3-(2-methoxyvinyl))quinolin-4-yl]carbamate (**(*Z*)-19a**). R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.55; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.77 (s, 1H, H_2), 7.48 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.6 Hz, H_5), 7.11 (d, 1H, $^4J_{H_7\text{-}H_5}$ = 1.6 Hz, H_7), 7.06 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.1 Hz, $H_{6'}$), 7.02 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.1 Hz, $H_{2'}$), 6.76 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 6.26 (d, 1H, $^3J_{H_b\text{-}H_a}$ = 7.1 Hz, H_b), 5.64 (m, 1H, H_g), 5.04 (d, 1H, $^3J_{H_a\text{-}H_b}$ = 7.1 Hz, H_a), 4.97 (m, 2H, H_h), 4.81 (d, 1H, $^2J_{H_d\text{-}H_{d'}}$ = 14.2 Hz, H_d), 4.56 (d, 1H, $^2J_{H_{d'}\text{-}H_d}$ = 14.2 Hz, $H_{d'}$), 4.48 (m, 2H, H_f), 3.84 (s, 3H, H_i), 3.82 (s, 3H, H_c), 3.16 (m, 1H, H_j), 1.20 (m, 2H, H_k or $H_{k'}$), 0.84 (m, 2H, H_k or $H_{k'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.1 (2C, C_e, $C_{4'}$), 154.8 (C_2), 151.5 (C_b), 147.4 (C_{8a}), 145.4 (C_8), 140.0 (C_4), 132.1 (C_g), 131.6 ($C_{2'}$), 129.4 ($C_{6'}$), 129.3 ($C_{1'}$), 128.7 (C_3), 126.5 (C_5), 125.7 (C_{4a}), 122.9 (C_7), 122.3 ($C_{3'}$), 118.9 (CN), 117.8 (C_h), 112.0 ($C_{5'}$), 110.4 (C_6), 97.3 (C_a), 66.7 (C_f), 61.2 (C_c), 56.3 (C_i), 52.9 (C_d), 10.8 (C_j), 10.2 (2C, C_k, $C_{k'}$).

4.1.14. (*Z*)-Allyl 3-chloro-4-methoxybenzyl[6-cyano-8-ethyl-3-(2-methoxyvinyl)quinoline-4-yl]carbamate (19b). Sodium hydride powder (moistened with oil, 55-65%, 113 mg, 2.82 mmol, 5.0 eq.) was added to a solution of **16b** (230 mg, 0.56 mmol, 1.0 eq.) in anhydrous *N,N*-dimethylformamide (10 mL). The resulting mixture was stirred at rt for 20 minutes before careful addition of allyl chloroformate (240 µL, 2.26 mmol, 4.0 eq.). The mixture was stirred for 2 hours before addition of water (20 mL). The resulting solution was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with

brine (50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v) to give the desired product **19b** as yellow oil (170 mg, 0.35 mmol, 63%). R_f (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v): 0.31; IR (cm^{-1}): 2228 ($\nu_{C\equiv N}$), 1707 ($\nu_{C=O}$), 1645 ($\nu_{C=C}$), 1502 ($\nu_{C=C}$), 1281 (ν_{C-O}), 1258 (ν_{C-O}), 1102 (ν_{C-O}), 1065 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.75 (s, 1H, H_2), 7.53 (m, 2H, H_5 & H_7), 7.08 (dd, 1H, $^3J_{H6'-H5'}$ = 8.4 Hz, $^4J_{H6'-H2'}$ = 2.1 Hz, $H_{6'}$), 7.01 (d, 1H, $^4J_{H2'-H6'}$ = 2.1 Hz, $H_{2'}$), 6.77 (d, 1H, $^3J_{H5'-H6'}$ = 8.4 Hz, $H_{5'}$), 6.26 (d, 1H, $^3J_{Hb-Ha}$ = 7.1 Hz, H_b), 5.64 (m, 1H, H_g), 5.04 (d, 1H, $^3J_{Ha-Hb}$ = 7.1 Hz, H_a), 4.98 (m, 2H, H_f), 4.82 (d, 1H, $^2J_{Hd-Hd'}$ = 14.3 Hz, H_d), 4.54 (d, 1H, $^2J_{Hd'-Hd}$ = 14.3 Hz, $H_{d'}$), 4.47 (m, 2H, H_h), 3.85 (s, 3H, H_i), 3.81 (s, 3H, H_c), 3.27 (q, 2H, $^3J_{Hj-Hk}$ = 7.5 Hz, H_j), 1.36 (t, 3H, $^3J_{Hk-Hj}$ = 7.5 Hz, H_k); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.1 ($C_{4'}$ or C_e), 155.0 ($C_{4'}$ or C_e), 154.6 (C_2), 151.5 (C_b), 146.7 (C_{8a}), 145.1 (C_8), 139.9 (C_4), 132.1 (C_g), 131.6 ($C_{2'}$), 129.4 ($C_{6'}$), 129.3 ($C_{1'}$), 128.6 (C_3), 127.5 (C_5 or C_7), 127.4 (C_5 or C_7), 125.7 (C_{4a}), 122.2 ($C_{3'}$), 118.9 (CN), 117.8 (C_h), 112.0 ($C_{5'}$), 110.3 (C_6), 97.2 (C_a), 66.7 (C_f), 61.3 (C_c), 56.3 (C_i), 53.0 (C_d), 24.6 (C_j), 14.7 (C_k); HRMS calculated for $C_{27}H_{26}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 492.1690, found $C_{27}H_{26}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 492.1658 (100%); $C_{27}H_{26}{}^{37}ClN_3O_4$ $[M+H]^+$ m/z 494.1739 (33%).

4.1.15. Allyl 3-chloro-4-methoxybenzyl[6-cyano-8-cyclopropyl-3-(2-oxoethyl)quinoline-4-yl]carbamate (20a). A mixture of **19a** (170 mg, 0.34 mmol, 1.0 eq.) in formic acid (10 mL) was stirred at 50 °C for 20 hours. After cooling to rt, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) before addition of a saturated aqueous sodium carbonate solution (25 mL). After decantation, the aqueous layer was then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (60 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 7/3, v/v) to give the desired product **20a** (100 mg, 0.20 mmol, 60%) as yellow oil. R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.33; IR (cm^{-1}): 2228 ($\nu_{C\equiv N}$), 1702 ($\nu_{C=O}$), 1501 ($\nu_{C=C}$), 1387 (δ_{CH_3}), 1257 (ν_{C-O}), 1064 (ν_{C-O}), 1020 (ν_{C-O}); Analyses of NMR spectra revealed that this product was a mixture of two rotamers, named A and B with a ratio A/B = 1/0.3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.48 (s, 1H, $H_{b(A/B)}$), 8.96 (s, 1H, $H_{2(B)}$), 8.93 (s, 1H, $H_{2(A)}$), 7.80 (m, 1H, $H_{5(B)}$), 7.72 (d, 1H, $^4J_{H5(A)-H7(A)}$ = 1.6 Hz, $H_{5(A)}$), 7.26 (m, 1H, $H_{7(A/B)}$), 7.09 (d, 1H, $^4J_{H2'(A/B)-H6'(A/B)}$ = 2.1 Hz, $H_{2'(A/B)}$), 7.03 (dd, 1H, $^3J_{H6'(A)-H5'(A)}$ = 8.4 Hz, $^4J_{H6'(A)-H2'(A)}$ = 2.1 Hz, $H_{6'(A)}$), 6.89 (m, 1H, $H_{6'(B)}$), 6.80 (d, 1H, $^3J_{H5'(A)-H6'(A)}$ = 8.4 Hz, $H_{5'(A)}$), 6.78 (d, 1H, $^3J_{H5'(B)-H6'(B)}$ = 8.4 Hz, $H_{5'(B)}$), 6.11 (m, 1H, $H_{f(B)}$), 5.65 (m, 1H, $H_{f(A)}$), 5.46 (m, 2H, $H_{g(B)}$), 5.03 (m, 3H, $H_{g(A)}$, $H_{c(A/B)}$), 4.80 (m, 2H, $H_{e(B)}$), 4.49 (m, 2H, $H_{e(A)}$), 4.39 (d, 1H, $^2J_{Hc'(B)-Hc(B)}$ = 14.7 Hz, $H_{c'(B)}$), 4.38 (d, 1H, $^2J_{Hc'(A)-Hc(A)}$ = 14.7 Hz, $H_{c'(A)}$), 3.87 (s, 3H, $H_{h(A/B)}$), 3.48 (m, 2H, $H_{a(A/B)}$), 3.23 (m,

1H, $H_{i(A/B)}$), 1.26 (m, 2H, $H_{j(A/B)}$ or $H_{j'(A/B)}$), 0.91 (m, 2H, $H_{j(A/B)}$ or $H_{j'(A/B)}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.0 ($C_{b(B)}$), 196.3 ($C_{b(A)}$), 155.3 ($C_{4'(A/B)}$), 154.7 ($C_{d(A/B)}$), 154.6 ($C_{2(A/B)}$), 149.3 ($C_{8a(A/B)}$), 146.5 ($C_{8(A/B)}$), 144.5 ($C_{4(A/B)}$), 131.9 ($C_{f(B)}$), 131.7 ($C_{f(A)}$), 131.4 ($C_{2'(A)}$), 131.3 ($C_{2'(B)}$), 129.3 ($C_{6'(A/B)}$), 129.0 ($C_{1'(A)}$), 128.9 ($C_{1'(B)}$), 126.0 ($C_{3(A/B)}$), 125.9 ($C_{5(A/B)}$), 125.1 ($C_{4a(A/B)}$), 124.0 ($C_{7(A/B)}$), 122.7 ($C_{3'(A/B)}$), 119.9 ($C_{g(B)}$), 119.0 ($C_{g(A)}$), 118.7 ($CN_{(A/B)}$), 112.2 ($C_{5'(A/B)}$), 111.4 ($C_{6(A/B)}$), 67.8 ($C_{e(B)}$), 67.3 ($C_{e(A)}$), 56.3 ($C_{h(A/B)}$), 53.5 ($C_{c(B)}$), 53.3 ($C_{c(A)}$), 43.8 ($C_{a(B)}$), 43.5 ($C_{a(A)}$), 11.0 ($C_{i(B)}$), 10.9 ($C_{i(A)}$), 10.7 ($C_{j(A)}$ or $C_{j'(A)}$), 10.5 ($C_{j(B)}$ or $C_{j'(B)}$); ESI-MS calculated for $C_{27}H_{24}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 490.15, found $C_{27}H_{24}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 490.14 (100%); $C_{27}H_{24}{}^{37}ClN_3O_4$ $[M+H]^+$ m/z 492.10 (33%).

4.1.16. Allyl 3-chloro-4-methoxybenzyl[6-cyano-8-ethyl-3-(2-oxoethyl)quinoline-4-yl]carbamate (20b). A mixture of **19b** (170 mg, 0.35 mmol, 1.0 eq.) in formic acid (10 mL) was stirred at 50 °C for 20 hours. After cooling to rt, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) before addition of a saturated aqueous sodium carbonate solution (30 mL). After decantation, the aqueous layer was extracted ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, cyclohexane/ethyl acetate, 6/4, v/v) to give the product **20b** (0.11 g, 0.23 mmol, 67%) as yellow oil. R_f (SiO_2, cyclohexane/ethyl acetate, 6/4, v / v): 0.24; IR (cm^{-1}): 2224 ($\nu_{C\equiv N}$), 1705 ($\nu_{C=O}$), 1260 (ν_{C-O}), 1065 (ν_{C-O}); Analyses of NMR spectra revealed that this product was a mixture of two rotamers, named A and B with a ratio A/B = 1/0.3. ^1H NMR ($CDCl_3$, 400 MHz) δ 9.48 (s, 1H, $H_{b(A/B)}$), 8.92 (s, 1H, $H_{2(B)}$), 8.89 (s, 1H, $H_{2(A)}$), 7.85 (m, 1H, $H_{5(B)}$), 7.78 (d, 1H, $^4J_{H5(A)\text{-}H7(A)}$ = 1.7 Hz, $H_{5(A)}$), 7.68 (m, 1H, $H_{7(A/B)}$), 7.08 (d, 1H, $^4J_{H2'(A/B)\text{-}H6'(A/B)}$ = 2.1 Hz, $H_{2'(A/B)}$), 7.03 (dd, 1H, $^3J_{H6'(A)\text{-}H5'(A)}$ = 8.4 Hz, $^4J_{H6'(A)\text{-}H2'(A)}$ = 2.1 Hz, $H_{6'(A)}$), 6.89 (m, 1H, $H_{6'(B)}$), 6.80 (d, 1H, $^3J_{H5'(A)\text{-}H6'(A)}$ = 8.4 Hz, $H_{5'(A)}$), 6.78 (d, 1H, $^3J_{H5'(B)\text{-}H6'(B)}$ = 8.4 Hz, $H_{5'(B)}$), 6.10 (m, 1H, $H_{f(B)}$), 5.65 (m, 1H, $H_{f(A)}$), 5.50 (d, 1H, $^3J_{Hg(B)\text{-}Hf(B)}$ = 17.2 Hz, $H_{g(B)}$), 5.42 (d, 1H, $^3J_{Hg'(B)\text{-}Hf(B)}$ = 10.3 Hz, $H_{g'(B)}$), 5.02 (m, 3H, $H_{g(A)}$, $H_{c(A/B)}$), 4.81 (m, 2H, $H_{e(B)}$), 4.48 (m, 2H, $H_{e(A)}$), 4.40 (d, 1H, $^2J_{Hc'(B)\text{-}Hc(B)}$ = 14.7 Hz, $H_{c'(B)}$), 4.38 (d, 1H, $^2J_{Hc'(A)\text{-}Hc(A)}$ = 14.7 Hz, $H_{c'(A)}$), 3.86 (s, 3H, $H_{h(A/B)}$), 3.48 (m, 2H, $H_{a(A/B)}$), 3.32 (q, 2H, $^3J_{Hi(A/B)\text{-}Hj(A/B)}$ = 7.5 Hz, $H_{i(A/B)}$), 1.39 (t, 3H, $^3J_{Hj(A/B)\text{-}Hi(A/B)}$ = 7.5 Hz, $H_{j(A/B)}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.0 ($C_{b(B)}$), 196.4 ($C_{b(A)}$), 155.3 ($C_{4'(A/B)}$), 154.8 ($C_{d(A/B)}$), 154.6 ($C_{2(A/B)}$), 148.6 ($C_{8a(A/B)}$), 146.1 ($C_{8(A/B)}$), 144.5 ($C_{4(A/B)}$), 131.7 ($C_{f(A/B)}$), 131.4 ($C_{2'(A/B)}$), 129.3 ($C_{6'(A/B)}$), 129.0 ($C_{1'(A/B)}$), 128.8 ($C_{5(A/B)}$), 127.1 ($C_{7(A/B)}$), 125.9 ($C_{3(A/B)}$), 125.2 ($C_{4a(A/B)}$), 122.7 ($C_{3'(A/B)}$), 119.9 ($C_{g(B)}$), 119.0 ($C_{g(A)}$), 118.7 ($CN_{(A/B)}$), 112.2 ($C_{5'(A/B)}$), 111.3 ($C_{6(A/B)}$), 67.8 ($C_{e(B)}$), 67.3 ($C_{e(A)}$), 56.3 ($C_{h(A/B)}$), 53.5 ($C_{c(B)}$), 53.4 ($C_{c(A)}$), 43.8 ($C_{a(B)}$), 43.5 ($C_{a(A)}$), 24.7 ($C_{i(B)}$), 24.6 ($C_{i(A)}$), 14.8 ($C_{j(B)}$), 14.6 ($C_{j(A)}$); ESI-MS calculated for $C_{26}H_{24}{}^{35}ClN_3O_4$

$[M+H]^+$ m/z 478.15, found $C_{26}H_{24}{}^{35}ClN_3O_4$ $[M+H]^+$ m/z 478.05 (100%); $C_{26}H_{24}{}^{37}ClN_3O_4$ $[M+H]^+$ m/z 480.11 (33%).

4.1.17. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-(2-hydroxyethyl)quinoline-6-carbonitrile (21a). A 1.1 M solution of lithium tri-*tert*-butoxyaluminum hydride in tetrahydrofuran (1.7 mL, 1.87 mmol, 3.0 eq.) was added, under argon and stirring, to a solution of **20a** (300 mg, 0.61 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (30 mL). The resulting mixture was stirred at 60 °C for 20 hours. After cooling to rt, water (50 mL) was slowly added under stirring. This aqueous layer was then extracted with dichloromethane (100 mL) using a Soxhlet extractor for 20 hours. The organic layer was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, ethanol/dichloromethane, 1/9, v/v) to give the desired product **21a** (130 mg, 0.32 mmol, 52%) as a yellow oil. R_f (SiO_2, dichloromethane/ethanol, 9/1, v/v): 0.20; IR (cm^{-1}): 2226 ($\nu_{C\equiv N}$), 1500 ($\nu_{C=C}$), 1257 (ν_{C-O}), 1062 (ν_{C-O}), 1020 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.56 (s, 1H, H_2), 8.20 (d, 1H, $^4J_{H_5-H_7}$ = 1.7 Hz, H_5), 7.29 (d, 1H, $^4J_{H_{2'}-H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.17 (d, 1H, $^4J_{H_7-H_5}$ = 1.7 Hz, H_7), 7.09 (dd, 1H, $^3J_{H_{6'}-H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}-H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.86 (d, 1H, $^3J_{H_{5'}-H_{6'}}$ = 8.4 Hz, $H_{5'}$), 4.48 (s, 2H, H_c), 3.86 (s, 3H, H_d), 3.83 (t, 2H, $^3J_{H_b-H_a}$ = 5.8 Hz, H_b), 2.92 (m, 1H, H_e), 2.80 (t, 2H, $^3J_{H_a-H_b}$ = 5.8 Hz, H_a), 1.15 (m, 2H, H_f or $H_{f'}$), 0.77 (m, 2H, H_f or $H_{f'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.7 ($C_{4'}$), 153.4 (C_2), 153.0 (C_4), 149.0 (C_{8a}), 144.4 (C_8), 132.1 ($C_{1'}$), 129.4 ($C_{2'}$), 127.8 (C_5), 127.0 ($C_{6'}$), 123.8 (C_7), 122.8 ($C_{3'}$), 121.0 (C_{4a}), 120.8 (C_3), 119.4 (CN), 112.4 ($C_{5'}$), 107.8 (C_6), 63.2 (C_b), 56.3 (C_d), 53.0 (C_c), 32.8 (C_a), 11.3 (C_e), 9.6 (2C, C_f, $C_{f'}$); HRMS calculated for $C_{23}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 408.1479, found $C_{23}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 408.1478 (100%); $C_{23}H_{22}{}^{37}ClN_3O_2$ $[M+H]^+$ m/z 410.1430 (33%).

4.1.18. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-ethyl-3-(2-hydroxyethyl)quinoline-6-carbonitrile (21b). A 1.1 M solution of lithium tri-*tert*-butoxyaluminum hydride in tetrahydrofuran (0.63 mL, 0.69 mmol, 3.0 eq.) was added, under argon and stirring, to a solution of **20b** (110 mg, 0.23 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (15 mL). The resulting mixture was stirred at 60 °C for 20 hours. After cooling to rt, water (3 mL) was added slowly and the mixture was stirred at this temperature for 4 hours. The solid formed was filtered and washed with ethyl acetate (20 mL). The filtrate was concentrated under reduced pressure. The residue obtained was purified by column chromatography (SiO_2, ethanol/dichloromethane, 1/9, v/v) to give the desired product **21b** (40 mg, 0.10 mmol, 44%) as a yellow solid. R_f (SiO_2, ethanol/dichloromethane, 1/9, v/v): 0.20; IR (cm^{-1}): 2226 ($\nu_{C\equiv N}$), 1504 (δ_{N-H}), 1279 (ν_{C-O}), 1261 (ν_{C-O}), 1048 (ν_{C-O}), 1024 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.55 (s, 1H, H_2), 8.28 (d, 1H, $^4J_{H_5-H_7}$ = 1.7 Hz, H_5), 7.54 (m, 1H, H_7), 7.30 (d, 1H, $^4J_{H_{2'}-H_{6'}}$ = 2.1 Hz, $H_{2'}$), 7.12 (dd, 1H, $^3J_{H_{6'}-H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}-H_{2'}}$ = 2.1 Hz, $H_{6'}$), 6.88 (d, 1H, $^3J_{H_{5'}-H_{6'}}$ = 8.4 Hz,

$H_{5'}$), 4.49 (s, 2H, H_c), 3.88 (s, 3H, H_d), 3.86 (t, 2H, $^3J_{H_b\text{-}H_a}$ = 5.8 Hz, H_b), 3.19 (q, 2H, $^3J_{H_e\text{-}H_f}$ = 7.5 Hz, H_e), 2.82 (t, 2H, $^3J_{H_a\text{-}H_b}$ = 5.8 Hz, H_a), 1.33 (t, 3H, $^3J_{H_f\text{-}H_e}$ = 7.5 Hz, H_f); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.7 ($C_{4'}$), 153.8 (C_2), 152.7 (C_4), 148.6 (C_{8a}), 144.8 (C_8), 132.3 ($C_{1'}$), 129.5 ($C_{2'}$), 128.5 (C_5), 127.6 (C_7), 127.0 ($C_{6'}$), 122.8 ($C_{3'}$), 121.4 (C_{4a}), 120.9 (C_3), 119.6 (CN), 112.4 ($C_{5'}$), 107.8 (C_6), 63.4 (C_b), 56.3 (C_d), 53.2 (C_c), 32.8 (C_a), 24.8 (C_e), 14.5 (C_f); HRMS calculated for $C_{22}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 396.1479, found $C_{22}H_{22}{}^{35}ClN_3O_2$ $[M+H]^+$ m/z 396.1512 (100%); $C_{22}H_{22}{}^{37}ClN_3O_2$ $[M+H]^+$ m/z 398.1501 (33%).

4.1.19. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-(2-fluoroethyl)quinoline-6-carbonitrile (22a). Diethylaminosulfur trifluoride (42 µL, 0.32 mmol, 2.0 eq.) was added at -78 °C, under argon and stirring, to a solution of **21a** (65 mg, 0.16 mmol, 1.0 eq.) in anhydrous dichloromethane (10 mL). The temperature was slowly increased to rt over 3 hours. The mixture was then cooled to -10 °C before addition of a saturated sodium bicarbonate solution (20 mL). After decantation, the aqueous layer was extracted with dichloromethane (4 × 15 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate, filtered, concentrated under reduced pressure. The mixture was purified by column chromatography (SiO_2, dichloromethane/ethanol, 9/1, v/v) to give by order of elution: the desired product **22a** (30 mg, 73.2 µmol, 46%) as yellow oil and the by-product 1-(3-chloro-4-methoxybenzyl)-6-cyclopropyl-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carbonitrile **23a** (10 mg, 25.6 µmol, 15%) as yellow oil. 4-[(3-chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-(2-fluoroethyl)quinoline-6-carbonitrile (**22a**). R_f (SiO_2, dichloromethane/ethanol, 9/1, v/v): 0.56; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.68 (s, 1H, H_2), 8.25 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.6 Hz, H_5), 7.31 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.23 (d, 1H, $^4J_{H_7\text{-}H_5}$ = 1.6 Hz, H_7), 7.13 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.91 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 4.64 (dt, 2H, $^2J_{H_b\text{-}F}$ = 47.0 Hz, $^3J_{H_b\text{-}H_a}$ = 5.8 Hz, H_b), 4.52 (d, 2H, $^3J_{H_c\text{-}NH}$ = 5.9 Hz, H_c), 3.91 (s, 3H, H_d), 3.10 (m, 1H, H_e), 3.02 (dt, 2H, $^3J_{H_a\text{-}F}$ = 26.8 Hz, $^3J_{H_a\text{-}H_b}$ = 5.8 Hz, H_a), 1.22 (m, 2H, H_f or $H_{f'}$), 0.84 (m, 2H, H_f or $H_{f'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.0 ($C_{4'}$), 154.1 (C_2), 152.2 (C_4), 149.3 (C_{8a}), 145.2 (C_8), 131.9 ($C_{1'}$), 129.6 ($C_{2'}$), 127.3 (C_5), 127.1 ($C_{6'}$), 123.7 (C_7), 123.1 ($C_{3'}$), 121.1 (C_3), 119.5 (CN), 118.4 (C_{4a}), 112.5 ($C_{5'}$), 108.4 (C_6), 84.1 (d, $^2J_{C_b\text{-}F}$ = 168 Hz, C_b), 56.4 (C_d), 53.4 (C_c), 30.9 (d, $^3J_{C_a\text{-}F}$ = 20 Hz, C_a), 11.2 (C_e), 10.0 (2C, C_f, $C_{f'}$); HRMS calculated for $C_{23}H_{21}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 410.1435, found $C_{23}H_{21}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 410.1420 (100%); $C_{23}H_{21}{}^{37}ClFN_3O$ $[M+H]^+$ m/z 412.1215 (33%). 1-(3-chloro-4-methoxybenzyl)-6-cyclopropyl-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carbonitrile (**23a**). R_f (SiO_2, dichloromethane/ethanol, 9/1, v/v): 0.12; IR (cm^{-1}): 2222 ($\nu_{C\equiv N}$), 1501 ($\nu_{C=C}$), 1294 ($\nu_{as\ C\text{-}O\text{-}C}$), 1252 ($\nu_{s\ C\text{-}O\text{-}C}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54 (s, 1H, H_2), 7.94 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.7 Hz, H_5), 7.31 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.15 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.5 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.2 Hz,

$H_{6'}$), 7.09 (d, 1H, $^4J_{H_7\text{-}H_5}$ = 1.7 Hz, H_7), 6.91 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.5 Hz, $H_{5'}$), 4.78 (s, 2H, H_c), 3.85 (m, 5H, H_d, H_b), 3.24 (t, 2H, $^3J_{H_a\text{-}H_b}$ = 9.4 Hz, H_a), 2.92 (m, 1H, H_e), 1.14 (m, 2H, H_f or $H_{f'}$), 0.73 (m, 2H, H_f or $H_{f'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3 ($C_{4'}$), 154.7 (C_4), 149.3 (C_{8a}), 145.8 (C_2), 144.3 (C_8), 129.3 ($C_{1'}$), 128.2 ($C_{2'}$), 126.5 (C_5 or $C_{6'}$), 125.8 (C_5 or $C_{6'}$), 123.8 (C_7), 123.3 ($C_{3'}$), 121.2 (C_3), 119.4 (CN), 115.0 (C_{4a}), 112.7 ($C_{5'}$), 106.6 (C_6), 56.2 (C_d), 55.8 (C_b), 53.7 (C_c), 25.5 (C_a), 11.8 (C_e), 9.3 (2C, C_f, $C_{f'}$); HRMS calculated for $C_{23}H_{20}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 390.1373, found $C_{23}H_{20}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 390.1374 (100%); $C_{23}H_{20}{}^{37}ClFN_3O$ $[M+H]^+$ m/z 392.1176 (33%).

4.1.20. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-ethyl-3-(2-fluoroethyl)quinoline-6-carbonitrile (22b). Diethylaminosulfur trifluoride (27 µL, 0.20 mmol, 2.0 eq.) was added at -78 °C, under argon and stirring, to a solution of **21b** (40 mg, 0.10 mmol, 1.0 eq.) in anhydrous dichloromethane (10 mL). After return back to rt over 1.5 hours, a saturated sodium hydrogen carbonate solution (15 mL) was added slowly at -10 °C. After decantation, the aqueous layer was extracted with dichloromethane (4 × 15 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, dichloromethane/ethanol, 9/1, v/v) to give, by order of elution: the desired product **22b** (6.0 mg, 15.1 µmol, 15%) as a yellow oil and the by-product 1-(3-chloro-4-methoxybenzyl)-6-ethyl-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carbonitrile **23b** (25 mg, 66.2 µmol, 68%) as yellow oil. 4-[(3-chloro-4-methoxybenzyl)amino]-8-ethyl-3-(2-fluoroethyl)quinoline-6-carbonitrile (**22b**); R_f (SiO_2, dichloromethane/ethanol, 93/7, v/v): 0.51; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.64 (s, 1H, H_2), 8.31 (d, 1H, $^4J_{H_5\text{-}H_7}$ = 1.7 Hz, H_5), 7.63 (m, 1H, H_7), 7.32 (d, 1H, $^4J_{H_{2'}\text{-}H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.15 (dd, 1H, $^3J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz, $^4J_{H_{6'}\text{-}H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.93 (d, 1H, $^3J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 4.65 (dt, 2H, $^2J_{H_b\text{-}F}$ = 47.0 Hz, $^3J_{H_b\text{-}H_a}$ = 5.8 Hz, H_b), 4.52 (d, 2H, $^3J_{H_c\text{-}NH}$ = 5.7 Hz, H_c), 3.92 (s, 3H, H_d), 3.27 (q, 2H, $^3J_{H_e\text{-}H_f}$ = 7.4 Hz, H_e), 3.02 (dt, 2H, $^3J_{H_a\text{-}F}$ = 27.0 Hz, $^3J_{H_a\text{-}H_b}$ = 5.8 Hz, H_a), 1.38 (t, 3H, $^3J_{H_f\text{-}H_e}$ = 7.4 Hz, H_f); HRMS calculated for $C_{22}H_{21}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 398.1435, found $C_{22}H_{21}{}^{35}ClFN_3O$ $[M+H]^+$ m/z 398.1451 (100%); $C_{22}H_{21}{}^{37}ClFN_3O$ $[M+H]^+$ m/z 400.1242 (33%). 1-(3-chloro-4-methoxybenzyl)-6-ethyl-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carbonitrile (**23b).** R_f (SiO_2, dichloromethane/ethanol, 93/7, v/v): 0.20; IR (cm^{-1}): 2219 ($\nu_{C\equiv N}$), 1501 ($\nu_{C=C}$), 1254 ($\nu_{as\ C\text{-}O}$), 1061 ($\nu_{s\ C\text{-}O}$); Analyses of NMR spectra revealed that this product was a mixture of two rotamers, named A and B with a ratio A/B = 1/0.2; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1H, $H_{2(B)}$), 8.52 (s, 1H, $H_{2(A)}$), 8.31 (d, 1H, $^4J_{H_{5(B)}\text{-}H_{7(B)}}$ = 1.8 Hz, $H_{5(B)}$), 8.04 (d, 1H, $^4J_{H_{5(A)}\text{-}H_{7(A)}}$ = 1.8 Hz, $H_{5(A)}$), 7.58 (m, 1H, $H_{7(B)}$), 7.47 (m, 1H, $H_{7(A)}$), 7.36 (d, 1H, $^4J_{H_{2'(A)}\text{-}H_{6'(A)}}$ = 2.2 Hz, $H_{2'(A)}$), 7.32 (d, 1H, $^4J_{H_{2'(B)}\text{-}H_{6'(B)}}$ = 2.2 Hz, $H_{2'(B)}$), 7.19 (dd, 1H, $^3J_{H_{6'(A)}\text{-}H_{5'(A)}}$ = 8.4 Hz, $^4J_{H_{6'(A)}\text{-}H_{2'(A)}}$ = 2.2 Hz, $H_{6'(A)}$), 7.13 (dd, 1H, $^3J_{H_{6'(B)}\text{-}H_{5'(B)}}$

= 8.4 Hz, $^{4}J_{H_{6'(B)}\text{-}H_{2'(B)}}$ = 2.2 Hz, $H_{6'(B)}$), 6.95 (d, 1H, $^{3}J_{H_{5'(A)}\text{-}H_{6'(A)}}$ = 8.4 Hz, $H_{5'(A)}$), 6.89 (d, 1H, $^{3}J_{H_{5'(B)}\text{-}H_{6'(B)}}$ = 8.4 Hz, $H_{5'(B)}$), 4.80 (s, 2H, $H_{c(A)}$), 4.49 (s, 2H, $H_{c(B)}$), 3.91 (s, 3H, $H_{d(A)}$), 3.90 (s, 3H, $H_{d(B)}$), 3.86 (t, 2H, $^{3}J_{H_{b(A/B)}\text{-}H_{a(A/B)}}$ = 9.5 Hz, $H_{b(A/B)}$), 3.27 (t, 2H, $^{3}J_{H_{a(A/B)}\text{-}H_{b(A/B)}}$ = 9.5 Hz, $H_{a(A/B)}$), 3.19 (q, 2H, $^{3}J_{H_{e(A/B)}\text{-}H_{f(A/B)}}$ = 7.5 Hz, $H_{e(A/B)}$), 1.36 (t, 3H, $^{3}J_{H_{f(B)}\text{-}H_{e(B)}}$ = 8.4 Hz, $H_{f(B)}$), 1.34 (t, 3H, $^{3}J_{H_{f(A)}\text{-}H_{e(A)}}$ = 8.4 Hz, $H_{f(A)}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2 (C_4), 154.9 ($C_{4'}$), 149.4 (C_{8a}), 146.9 (C_2), 145.1 (C_8), 129.9 ($C_{1'}$), 128.5 ($C_{2'}$), 127.4 (C_5 or C_7), 127.3 (C_5 or C_7), 126.0 ($C_{6'}$), 123.5 ($C_{3'}$), 121.1 (C_3), 119.8 (CN), 115.6 (C_{4a}), 112.9 ($C_{5'}$), 107.7 (C_6), 56.5 (C_d), 55.9 (C_b), 54.1 (C_c), 25.9 (C_a), 25.6 (C_e), 14.4 (C_f); HRMS calculated for $C_{22}H_{20}{}^{35}ClN_3O$ $[M+H]^+$ m/z 378.1373, found $C_{22}H_{20}{}^{35}ClN_3O$ $[M+H]^+$ m/z 378.1392 (100%); $C_{22}H_{20}{}^{37}ClN_3O$ $[M+H]^+$ m/z 380.1329 (33%).

4.1.21. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-[(2-fluoroethoxy)methyl]quinoline-6-carbonitrile (24a). A solution of **4a** [12] (150 mg, 0.38 mmol, 1.0 eq.) in thionyl chloride (4 mL) was stirred at rt, under argon, for 30 minutes. The solvent was then removed under reduced pressure for at least 3 hours. The residue was dissolved in anhydrous *N,N*-dimethylformamide (5 mL) under argon before addition of 2-fluoroethanol (0.15 mL, 2.55 mmol, 6.7 eq.) at rt and the mixture was stirred at 80 °C for 27 hours. After cooling to rt, a saturated sodium hydrogen carbonate solution (25 mL) was added. This mixture was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (2 × 40 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, ethyl acetate/cyclohexane, 2/8 to 5/5, v/v) to give the desired product **24a** (60 mg, 0.14 mmol, 37%) as a white solid. R_f (SiO_2, cyclohexane/ethyl acetate, 5/5, v/v): 0.50; Mp: 152-154 °C; IR (cm^{-1}): 2226 ($\nu_{C\equiv N}$), 1506 ($\nu_{C=C}$), 1261 ($\nu_{C\text{-}O}$), 1188 ($\nu_{C\text{-}O}$), 1068 ($\nu_{C\text{-}O}$), 1021 ($\nu_{C\text{-}O}$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.56 (s, 1H, H_2), 8.25 (d, 1H, $^{4}J_{H_5\text{-}H_7}$ = 1.5 Hz, H_5), 7.32 (d, 1H, $^{4}J_{H_{2'}\text{-}H_{6'}}$ = 2.1 Hz, $H_{2'}$), 7.19 (d, 1H, $^{4}J_{H_7\text{-}H_5}$ = 1.5 Hz, H_7), 7.15 (dd, 1H, $^{3}J_{H_{6'}\text{-}H_{5'}}$ = 8.4 Hz , $^{4}J_{H_{6'}\text{-}H_{2'}}$ = 2.1 Hz, $H_{6'}$), 6.89 (d, 1H, $^{3}J_{H_{5'}\text{-}H_{6'}}$ = 8.4 Hz, $H_{5'}$), 5.75 (m, 1H, NH), 4.70 (d, 2H, $^{3}J_{H_d\text{-}NH}$ = 5.6 Hz, H_d), 4.62 (s, 2H, H_a), 4.51 (m, 2H, H_c), 3.88 (s, 3H, H_e), 3.67 (m, 2H, H_b), 3.09 (m, 1H, H_f), 1.18 (m, 2H, H_g or $H_{g'}$), 0.80 (m, 2H, H_g or $H_{g'}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.8 ($C_{4'}$), 153.1 (C_4), 152.8 (C_2), 150.7 (C_{8a}), 145.3 (C_8), 132.1 ($C_{1'}$), 129.4 ($C_{2'}$), 127.4 (C_5), 126.9 ($C_{6'}$), 123.7 (C_7), 123.0 ($C_{3'}$), 120.0 (C_{4a}), 119.5 (CN), 115.1 (C_3), 112.5 ($C_{5'}$), 107.6 (C_6), 82.7 (d, $^{1}J_{C_c\text{-}F}$ = 169 Hz, C_c), 70.1 (C_a), 69.0 (d, $^{2}J_{C_b\text{-}F}$ = 19 Hz, C_b), 56.3 (C_e), 52.1 (C_d), 11.2 (C_f), 9.9 (2C, C_g, $C_{g'}$); HRMS calculated for $C_{24}H_{23}{}^{35}ClFN_3O_2$ $[M+H]^+$ m/z 440.1541, found $C_{24}H_{23}{}^{35}ClFN_3O_2$ $[M+H]^+$ m/z 440.1505 (100%); $C_{24}H_{23}{}^{37}ClFN_3O_2$ $[M+H]^+$ m/z 442.1481 (33%).

4.1.22. 4-[(3-Chloro-4-methoxybenzyl)amino]-8-ethyl-3-[(2-fluoroethoxy)methyl]quinoline-6-carbonitrile (24b). A solution of **4b** (250 mg, 0.65 mmol, 1.0 eq.) in thionyl chloride (4 mL) was stirred at rt, under argon, for 30 minutes. The solvent was then removed under reduced pressure for at least three hours. The residue was dissolved in anhydrous *N,N*-dimethylformamide (15 mL) before addition of 2-fluoroethanol (0.38 mL, 6.47 mmol, 10.0 eq.) under stirring and argon at rt. The mixture was stirred at 80 °C for 30 hours. After cooling to rt, a saturated aqueous sodium hydrogen carbonate solution (30 mL) was added. The mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (2 × 40 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, ethyl acetate/cyclohexane, 2/8 to 5/5, v/v) to give the desired product **24b** (80 mg, 0.19 mmol, 29%) as a yellow solid. R_f (SiO_2, cyclohexane/ethyl acetate, 6/4, v/v): 0.51; Mp: 149-151 °C; IR (cm^{-1}): 2229 ($\nu_{C\equiv N}$), 1505 ($\nu_{C=C}$), 1260 (ν_{C-O}), 1104 (ν_{C-O}), 1067 (ν_{C-O}), 1013 (ν_{C-O}); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (s, 1H, H_2), 8.32 (d, 1H, $^4J_{H_5-H_7}$ = 1.7 Hz, H_5), 7.59 (m, 1H, H_7), 7.34 (d, 1H, $^4J_{H_{2'}-H_{6'}}$ = 2.2 Hz, $H_{2'}$), 7.17 (dd, 1H, $^3J_{H_{6'}-H_{5'}}$ = 8.4 Hz , $^4J_{H_{6'}-H_{2'}}$ = 2.2 Hz, $H_{6'}$), 6.91 (d, 1H, $^3J_{H_{5'}-H_{6'}}$ = 8.4 Hz, $H_{5'}$), 5.78 (brs, 1H, NH), 4.71 (d, 2H, $^3J_{H_d-NH}$ = 5.1 Hz, H_d), 4.62 (s, 2H, H_a), 4.52 (m, 2H, H_c), 3.89 (s, 3H, H_e), 3.68 (m, 2H, H_b), 3.22 (q, 2H, $^3J_{H_f-H_g}$ = 7.5 Hz, H_f), 1.35 (t, 3H, $^3J_{H_g-H_f}$ = 7.5 Hz, H_g); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.8 ($C_{4'}$), 153.1 (C_4), 152.5 (C_2), 149.8 (C_{8a}), 145.0 (C_8), 132.0 ($C_{1'}$), 129.3 ($C_{2'}$), 128.2 (C_5), 128.1 (C_7), 126.8 ($C_{6'}$), 123.0 ($C_{3'}$), 119.9 (C_{4a} or CN), 119.5 (C_{4a} or CN), 114.8 (C_3), 112.4 ($C_{5'}$), 107.5 (C_6), 82.7 (d, $^1J_{C_c-F}$ = 168 Hz, C_c), 70.1 (C_a), 69.0 (d, $^2J_{C_b-F}$ = 19 Hz, C_b), 56.2 (C_e), 52.0 (C_d), 24.9 (C_f), 14.4 (C_g); HRMS calculated for $C_{23}H_{23}{}^{35}ClFN_3O_2$ $[M+H]^+$ m/z 428.1541, found $C_{23}H_{23}{}^{35}ClFN_3O_2$ $[M+H]^+$ m/z 428.1492 (100%); $C_{23}H_{23}{}^{37}ClFN_3O_2$ $[M+H]^+$ m/z 430.1510 (33%).

Similar experimental protocols used for the synthesis of compounds **26**-**32** bearing a trifluoromethyl substituent at position C-6 are given in electronic supplementary information.

4.2. Biology.

All tests were performed at the SB Drug Discovery company (Glasgow, UK). The phosphodiesterase assays were performed using recombinant human PDE enzymes expressed in a baculoviral system and selected for their similarity to PDE enzymes taken from human tissue using known inhibitor standards. The radiometric assay method was a modification of the two-step method of Thompson and Appleman,[37] adapted for 96 well plate format.

Compounds were tested at a concentration of 1 μM against human PDE2A3, PDE3A, PDE4A1, PDE4C2, PDE6AB, PDE9A1, PDE10A1 and PDE11A1 and at a concentration of 100 nM against human PDE5A1. The percentage of inhibition of the compounds and

standard inhibitors were determined and compared to historical assay data to ensure that they fell within acceptable ranges. For IC_{50} values measurements, compounds were tested at concentrations of 1000, 100, 10, 1, 0.5, 0.25, and 0.1 nM against PDE5A1. Data generated were analyzed using Prism software (GraphPad Inc)

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