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Design and development of photoswitchable DFG-Out RET kinase inhibitors

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ABSTRACT

REarranged during Transfection (RET) is a transmembrane receptor tyrosine kinase that is required for development of multiple human tissues, but which is also an important contributor to human cancers. RET activation through rearrangement or point mutations occurs in thyroid and lung cancers. Furthermore, activation of wild type RET is an increasingly recognized mechanism promoting tumor growth and dissemination of a much broader group of cancers. RET is therefore an attractive therapeutic target for small-molecule kinase inhibitors. Non-invasive control of RET signaling with light offers the promise of unveiling its complex spatiotemporal dynamics *in vivo*. In this work, photoswitchable DFG-out RET kinase inhibitors based on heterocycle-derived azobenzenes were developed, enabling photonic control of RET activity. Based on the binding mode of DFG-out kinase inhibitors and using RET kinase as the test model, we developed a photoswitchable inhibitor with a quinoline "head" constituting the azoheteroarene. This azo compound was further modified by three different strategies to increase the difference in biological activity between the *E*-isomer and the light enriched *Z*-isomer. Stilbene-based derivatives were used as model compounds to guide in the selection of substituents that could eventually be introduced to the corresponding azo compounds. The most promising quinoline-based compound showed more than a 15-fold difference in bioactivity between the two isomers in a biochemical assay. However, the same compound showed a decreased *Z*/*E* (IC_{50}) ratio in the cellular assay, tentatively assigned to stability issues. The corresponding stilbene compound gave a *Z*/*E* (IC_{50}) ratio well above 100, consistent with that measured in the biochemical assay. Ultimately, a 7-azaindole based photoswitchable DFG-out kinase inhibitor was shown to display more than a 10-fold difference in bioactivity between the two isomers, in both a biochemical and a cell-based assay, as well as excellent stability even under reducing conditions.

1. Introduction

Modern medical treatments are largely based on the ability of pharmacological drugs to interact with a molecular target, e.g. enzymes and receptors, in order to evoke a disease-relevant physiological response. However, many pharmacological targets are constitutively expressed throughout the organism in both healthy and diseased tissues and the intended mechanism of action of a drug is never fully orthogonal to all other bio-relevant processes. This results in undesired side effects, and remains a critical consideration when developing drugs.

The problems caused by poor selectivity originate in uncontrolled drug activity in time and space; i.e. the drug is active at times and sites within the intended target tissue and elsewhere in man where drug activity is not beneficial. Consequently, there is a need for developing novel molecular approaches for local drug

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activation/inactivation by external control. Light can be used to control biological systems with high spatial and temporal resolution [1]. It has the advantages of being non-invasive and allows remote action, reversibility, speed, and facile modulation of the energies involved. Unlike optogenetics, which relies on the introduction of photoreceptors at the genetic level, light-regulated small molecules can photocontrol endogenous targets without genetic manipulation. These compounds can be tested and validated using drug discovery/development procedures and can be applied directly to wildtype model organisms and humans. This concept is today referred to as photopharmacology [2–4]. Irreversible control can be achieved by the introduction of a photocleavable protecting group, which will result in a pro-drug that can be light-activated [5]. Reversible photocontrol over drug activity can be achieved through the introduction of a photoswitchable functional group into the structure of the drug. As for the molecular photoswitches, the azobenzene derivatives are still enjoying clear preference, but alternatives from other photochromic families such as spiropyrans and diarylethenes are also being frequently used in this context [2,4,6]. The *E*- and *Z*-isomers of azobenzenes can be reversibly interconverted by the application of light at different wavelengths. The reversible photoisomerization reactions are accompanied by marked changes in shape and physicochemical properties of the molecule, and these differences can be employed to design molecules with photoswitchable biological activity.

As the dysregulation and mutations of protein kinases play causal roles in human disease, this family of enzymes has become one of the most important drug targets [7]. To date, there are 71 small-molecule kinase inhibitors approved by the United States Food and Drug Administration [8]. X-ray crystal structures of kinase-inhibitor complexes have revealed details about the binding behaviors of these inhibitors. Inhibitors bind in different modes depending on specific binding locations and conformational aspects of the target kinase and can been classified based on their binding modes [9]. Type I inhibitors occupy the ATP-bound pocket of the kinase in the 'Aspartic acid-Phenylalanine-Glycine-in' (DFG-in) conformation, while Type II inhibitors bind on kinase in the 'Aspartic acid-Phenylalanine-Glycine-out' (DFG-out) inactive states. Type III and Type IV inhibitors are allosteric inhibitors. Type V inhibitors bind to two different regions of the protein kinase domain and are therefore bivalent inhibitors. Type VI inhibitors bind covalently to their target enzyme.

Despite the fact that small molecule inhibitors provide a powerful approach of studying the *in vivo* or cellular roles of kinase signaling, and are of high clinical interest, only a few photoswitchable kinase inhibitors have been reported. Azobenzene-based photoswitchable kinase inhibitors have been developed to target RET [10], vascular endothelial growth factor receptor 2 [11], p38/casein kinase 1δ [12], v-raf murine sarcoma viral oncogene homolog $B1^{V600E}$ [13] and casein kinases I [14]. All these compounds are Type I inhibitors. In addition, the Trauner group recently published a photoswitchable covalent c-Jun NH_2-terminal kinase 3 inhibitor (Type VI) [15].

Here we describe the design, synthesis and evaluation of DFG-out RET inhibitors (Type II) with photocontrolled activity. Structure and ligand-based design were used to integrate an azaheteroarene moiety into the pharmacophore of DFG-out inhibitors. We also prepared the corresponding stilbene derivatives as model compounds, as these are reported be more thermally stable and less sensitive to light compared to the aza-derivatives [16]. All compounds were spectroscopically characterized and the activities of both their respective *E*- and *Z*-isomers were determined in biochemical and whole cell assays.

2. Results and discussion

To design a photoswitchable compound targeting the DFG-out conformations of RET, we used the crystal structures of several 'DFG-out' bound inhibitors to different targets (Fig. 1) as the starting point. Although not developed to target RET, these type II inhibitors have proven to be highly potent inhibitors of this kinase. Examples are sorafenib (IC_{50} = 47 nM) [17], regorafenib (IC_{50} = 1.5 nM) [18], AD80 (IC_{50} = 1.3 nM) [19], ponatinib (IC_{50} = 2 nM) [20], agerafenib (IC_{50} = 2 nM) [21] and carbozantinib (IC_{50} = 5.2 nM) [22] (Fig. 1). All type II inhibitors shown in Fig. 1 share common structural features that define the pharmacophore of this type of kinase inhibitors.

All the inhibitors contain a heterocyclic 'head' group (green, Fig. 1) that binds to the hinge and occupies the region normally occupied by the adenine part of ATP. This heterocyclic head can be modified with substituents to tune the activity and selectivity. Next to the heterocyclic head there is a hydrophobic 'linker' moiety (cyan, Fig. 1) passing through the space beside the kinase gatekeeper residue. Finally, the hydrophobic 'tail' portion occupies the pocket created by the flipping of the DFG motif (orange, Fig. 1). Between the 'linker' and 'tail' moieties is a small unit (usually an amide or urea) that can form a H-bond with the αC-Helix glutamic acid residue and aspartic acid residue of the DFG motif [23]. Based on the type II kinase inhibitors shown in Fig. 1, we designed a potential photoswitchable DFG-out RET kinase inhibitor (Fig. 1B). By incorporating an *E*-form azo moiety into the 'linker' moiety, the key interactions with the adenine-binding pocket and the geometry for triggering DFG-out conformation should be preserved. Furthermore, we hypothesized that the RET kinase adenine-binding pocket would not tolerate the inhibitor in the *Z*-form, thus weakening or even losing the inhibition effect. Initially, it was decided to use a quinolone moiety as the 'head' group, as previous studies showed that the quinoline-based azoheteroarene exhibited good photophysical properties [24]. Furthermore, the quinoline moiety features in a number of kinase inhibitors, such as bosutinib [25].

A problem common to the majority of all similar studies using azo compounds is the potential thermal instability of the *Z*-isomer. Fast $Z \rightarrow E$ thermal isomerization makes it difficult to isolate and characterize the spectroscopic and biologic properties of the *Z*-isomer. Therefore, we prepared also the corresponding stilbene derivatives (**13*E*** and **13*Z***, Scheme 1) as model compounds, as these are reported to display a better thermal stability and are less sensitive to light compared to the aza-derivatives [16]. The bioactivity differences between the stilbene *E*- and *Z*-isomers would serve as a guide in the selection of substituents that could eventually be introduced to the corresponding azo compounds.

In order to prepare the target azo compound, key building block **2** was synthesized by Ullmann-type coupling reaction from **1** (Scheme 1). Azo compound **8** was prepared from 3-bromo- quinoline (**3**) by a four-step synthesis. Compound **3** was reacted with **2** under Buchwald–Hartwig reaction conditions to provide **4** in excellent yield. A 'one pot, two steps' dehydrogenation strategy, involving thermal removal of the Boc-protecting group followed by oxidation to form the azo moiety, furnished **5**. The ethyl ester of **5** was hydrolysed by LiOH, and the resulting carboxylic acid was coupled with previously reported 'tail' moiety **7** [26] to give the target product **8** (Scheme 1). The salt form **8·HCl** was prepared by stirring **8** with 4 N HCl in 1,4-dioxane and then removing the solvent.

The synthesis of the stilbene model compounds started with a Sonagashira reaction between **3** and building block **9n** (which was prepared by a literature procedure) [27] to produce the key

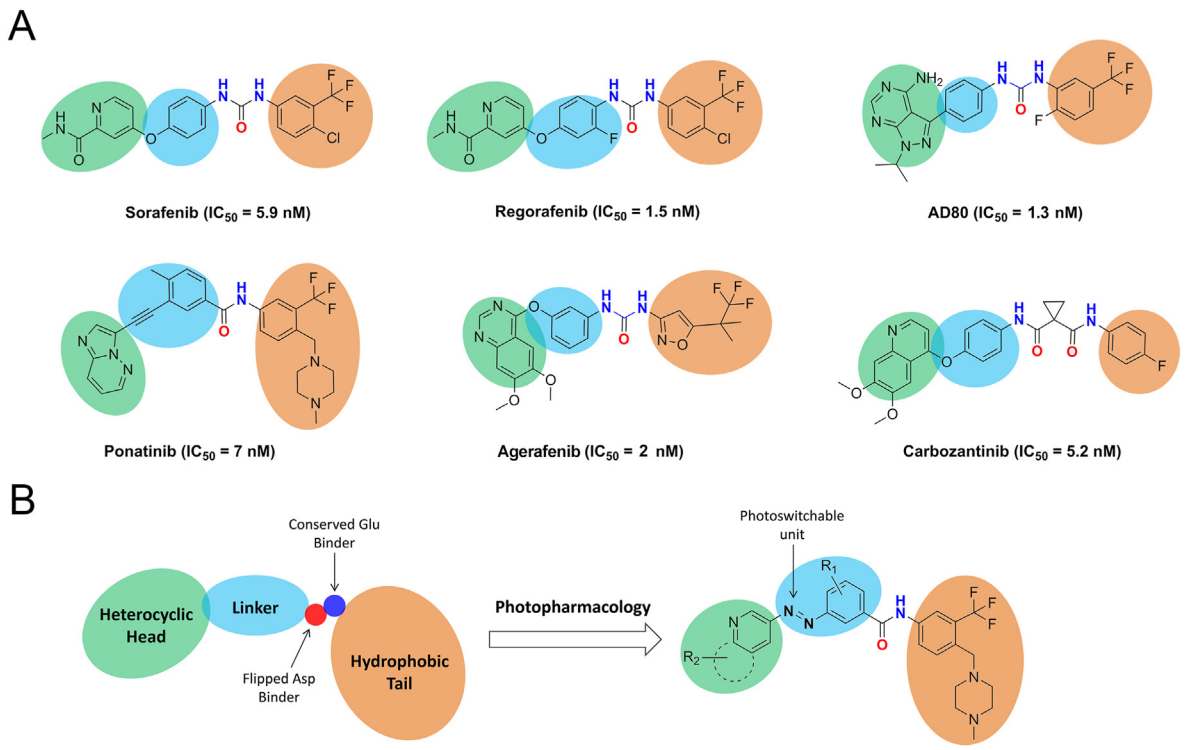
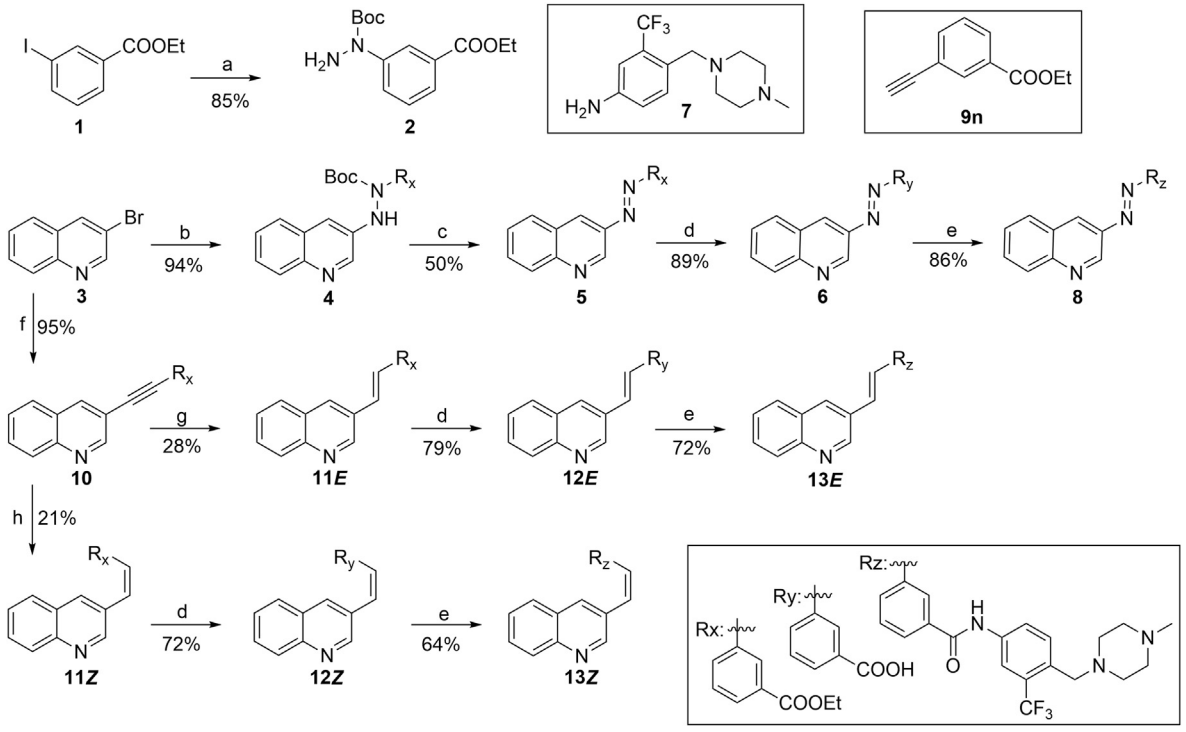


Fig. 1. (A) Examples of known type II kinase inhibitors and their structure moiety labelled by different colours (green: hinge binder with heterocyclic head; cyan: linker; orange: hydrophobic tail), as well as their enzymatic inhibition activities (IC_{50}) over RET kinase; (B) Schematic representation of the design of the herein-reported photoswitchable DFG-out RET kinase inhibitors (right) inspired by the general pharmacophore of type II kinase inhibitors (left).



Scheme 1. Synthesis of azo compound 8 and corresponding stilbene model compounds 12*E* and 12*Z* [aa]Reagents and conditions: (a) Boc-hydrazine, CuI, Cs_2CO_3, DMSO, 50 °C. (b) **2**, $Pd(OAc)_2$, $tBu_3P{\cdot}HBF_4$, Cs_2CO_3, 1,4-dioxane, MW, 110 °C, 2 h. (c) 1) DMF, 180 °C, 45 min; 2) O_2, 100 °C, 2 h. (d) LiOH, THF/H_2O/MeOH, 50 °C, 3 h. (e) **7**, EDC·HCl, HOBt, Et_3N, DCM, rt, 36 h. (f) **9n**, $Pd(MeCN)_2Cl_2$, RuPhos, Cs_2CO_3, MeCN, 80 °C, 6 h. (g) $RuCl_2(PPh_3)_3$, CuI, Zn, H_2O, 1,4-dioxane, 100 °C, 8 h. (h) $Pd(OAc)_2$, KOH, DMF, 150 °C, 6 h.

intermediate **10**. Subsequently, **10** was submitted to two different metal-catalysed stereoselective reduction methods to provide the *E*-isomer (**11*E***) and the *Z*-isomer (**11*Z***) of stilbene intermediates, respectively [28]. They were then hydrolysed with LiOH, and the resulting carboxylic acids were coupled with 'tail' moiety **7** to provide target stilbene compounds **13*E*** and **13*Z*** (Scheme 1).

After **8** and its HCl salt forms were produced, their photophysical properties were characterized based on their UV−vis absorption spectra (Table 1, Fig. 2A). Compound **8** has a $\pi \rightarrow \pi^*$ absorption maximum at 340 nm in a thermally adapted DMSO solution. Once irradiated by UV light at 365 nm, a photostationary distribution (PSS) containing 90% *Z*-isomer was obtained.

Additional exposure of this sample to 460 nm light yielded the *E*-isomer with a PSS with 87%. The thermal stability (half-life) of the *Z*-isomer in DMSO was determined to be $\tau_{½} = 208$ h (Table 1). The aqueous solution was prepared by dissolving **8·HCl** in H_2O. Both $\pi \rightarrow \pi^*$ (*E*-isomer) and $n \rightarrow \pi^*$ (*Z*-isomer) absorption bands showed a small hypsochromic shift compared to the DMSO solution. The thermal stability of **8** in aqueous solution was determined to be $\tau_{½} = 217$ h (Table 1). Furthermore, compound **8** showed good fatigue resistance both in DMSO and aqueous solutions, as no significant degradation was observed after 10 cycles of 365 nm and 460 nm irradiation-induced photoswitching.

After assessing the photophysical properties of **8**, the kinase inhibition activity of **8** and stilbene compounds **13*E*** and **13*Z*** was determined by an ADP-Glo™ human RET kinase assay. In this assay, purified RET kinase was used, the *E*-isomer of azo compounds were thermally adapted before serial dilution, and all the experiments involving azo and stilbene compounds were performed under red light. With IGF1Rtide as the substrate, the enzymatic activity is quantified by luminescence which is proportional to the amount of consumed ATP during kinase reaction [29]. Considering that DFG-out kinase inhibitors tend to be slow binders (low k_{on} and even lower k_{off}) [30], an additional 30 min pre-incubation procedure was added to the manufacturer's protocol. With the modified procedure, the activities of the *E*-isomer and UV-enriched *Z*-isomer of **8** were analysed. However, the IC_{50} value of the *E*-isomer was only 1.6-fold higher than the *Z*-isomer (Fig. 2B), although both were very potent in the low nM range. The IC_{50} of **13*Z*** was determined to be 900 nM, 115-fold higher than the IC_{50} of **13*E*** (IC_{50} = 7.8 nM) (Fig. 2B).

In order to better understand the difference in activity between *Z*-isomers of azo compound **8** and **13*Z***, computational modelling was performed. Compounds **8*E*** and **8*Z*** were docked into the DFG-out RET kinase structure created by homology modelling with the c-Kit crystal structure (PDB code 4U0I) as a template (Fig. 2C) [31]. Per our design, **8*E*** adopts a canonical type II binding mode: the quinoline 'head' forms an H-bond at the hinge with Ala108, the phenyl azo 'linker' traversed the area beside the gatekeeper Val105, and the amide bond forms two H-bonds with Glu76 of the αC-Helix and Asp108 of the DFG motif (out). Furthermore, an additional H-bond forms between the amide and Ser192 residue, which also contributes to the binding affinity of **8*E***. Moreover, the 'tail' moiety locates in the hydrophobic pocket adjacent to the catalytic region. However, even though **8*Z*** adopts a similar pose to the *E*-isomer, no H-bonds forms with the key amino acids on the hinge (conserved Glu76 and Asp193) while these binding features mainly contributes to the activity of DFG-out RET inhibitors (although an H-bond formed between the 'tail' and His173 of HRD motif). The **13*E***-isomer adopts almost the same binding model with **8*E*** (Fig. 2D). Due to the steric strain, the *Z*-isomer lost H-bonds with the hinge and the DFG motif and only maintained the bond with Glu76. The modelling implies that compounds **8*Z*** and **13*Z*** would indeed be expected to bind with substantially lower affinity to the RET kinase compared to the corresponding *E*-isomers, in contrast to the experimentally observed differences in the IC_{50} of only a factor 1.6.

Strategies to increase the isomerization induced biological activity difference include enhancing the associated geometry changes, thus weakening the binding affinity of the *Z*-isomer while maintaining the affinity of the *E*-isomer. We decided to adopt to this strategy, and our initial efforts concerned the introduction of substituents to the quinoline 'head'. We speculated that the activity of the *E*-isomer can be regulated by adjusting the binding affinity of the 'head' moiety on the hinge or creating contact between the substituents and amino acid residues in the adenosine binding pocket. More importantly, the bulkier 'head' moiety and the increased geometric distortion of the *Z*-isomer make it more difficult to bind into the adenosine binding pocket compared to the *E*-isomer, thus enlarging the binding affinity difference between them.

To evaluate the effect of substituents on the geometry and on the biological activity of the designed photoswitchable kinase inhibitors, we prepared eight pairs of stilbene-type model compounds with substituents on the C4 and C8 positions of the quinoline 'head' (Scheme 2). Among them, four pairs of stilbene compounds were substituted with alkyls (methyl, ethyl propyl and isopropyl) on the C8 position of the quinoline ring (**20a-d**). The other four pairs were substituted with methoxyl, dimethylamine, pyrrolidyl or aniline on the C4 position of the quinoline ring (**26a-d**). The synthesis of C8-substituted stilbene compounds started with a Kumada coupling reaction on 8-bromoquinoline (**14**) to introduce alkyl groups (8-methylquinoline is commercially available) [32]. Ethyl and propyl groups were introduced on C8 successfully with decent yields. A mixture of isopropyl and propyl substituted products was obtained when *i*-PrMgBr was used to attempt to install the isopropyl group on the C8 position. In this reaction, the propyl substitution side reaction resulted from β-H elimination and subsequent olefin insertion in the mechanism after the isopropyl group coordinated on the catalyst complex [33]. Compound **15d** was very difficult to isolate from **15c** since they have almost the same polarity and completely overlapped on thin layer chromatography (TLC) plates. An alternative method to prepare **15d** is to firstly introduce the isopropenyl group by Kumada coupling to provide 8-isopropenylquinoline, then convert isopropenyl to isopropyl by hydrogenation [34]. These C8 substituted substrates were then reacted with NIS in AcOH to provide C3 iodinated **16a-d** with low to medium yield [35]. Subsequently, Sonagashira coupling reactions were performed to install the 'linker' precursor **9n**/**9m** [27], providing the key intermediates **17a-d**. These diarylacetylene intermediates were then treated with two

Table 1
Photophysical properties of the azo compounds.

Compound	λ_{max} *E* (nm) $\pi \rightarrow \pi^*$ (DMSO)	λ_{max} Z (nm) PSS (DMSO)	Ratio of cis at PSS	Thermal stability in DMSO	λ_{max} *E* (nm) $\pi \rightarrow \pi^*$ (H_2O)	λ_{max} Z (nm) PSS (H_2O)	Thermal stability in H_2O
8	340	440	90%	$\tau = 208$ h	334	431	$\tau = 217$ h
30	334	446	93%	$\tau = 302$ h	330	435	$\tau = 402$ h
36	335	432	86%	$\tau = 779$ h	331	423	$\tau = 38$ h
37 [46]	358	438	97%	$\tau = 377$ h	349	429	$\tau = 629$ h

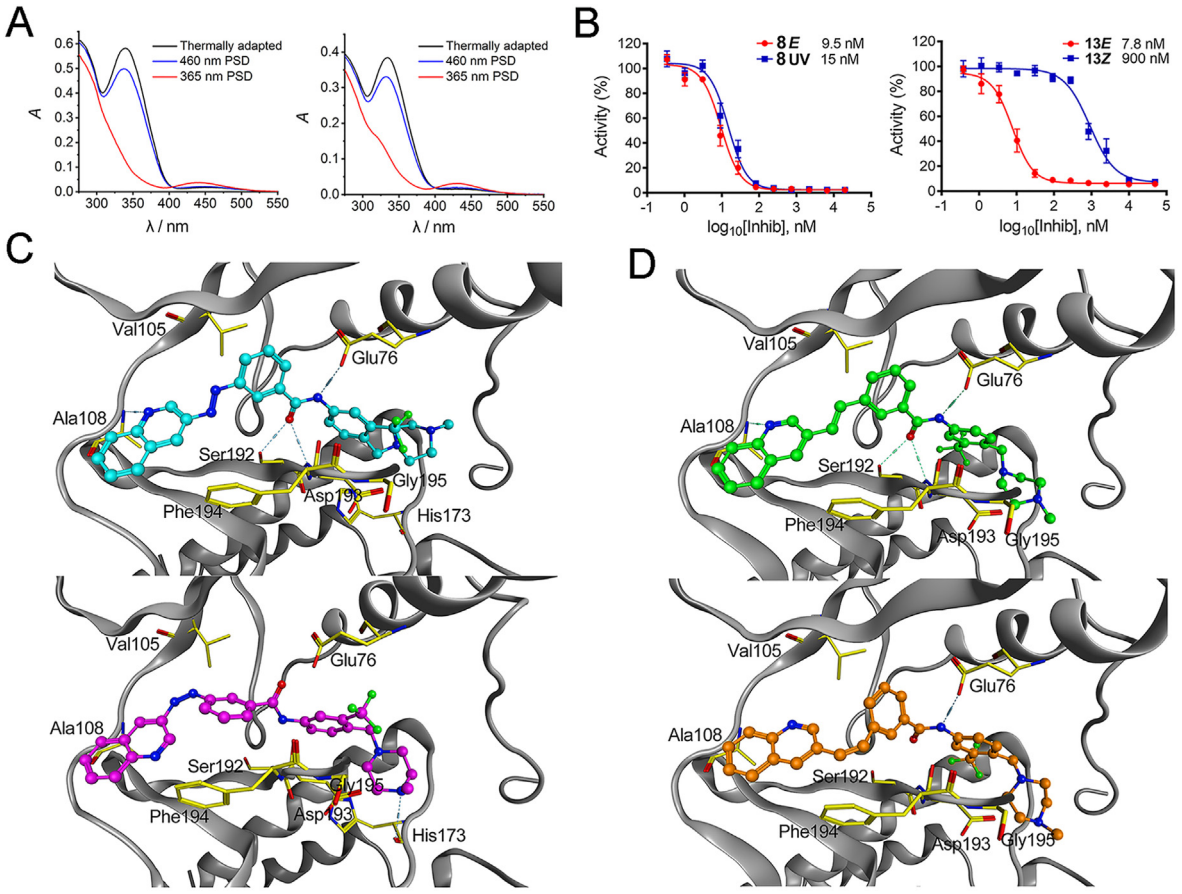


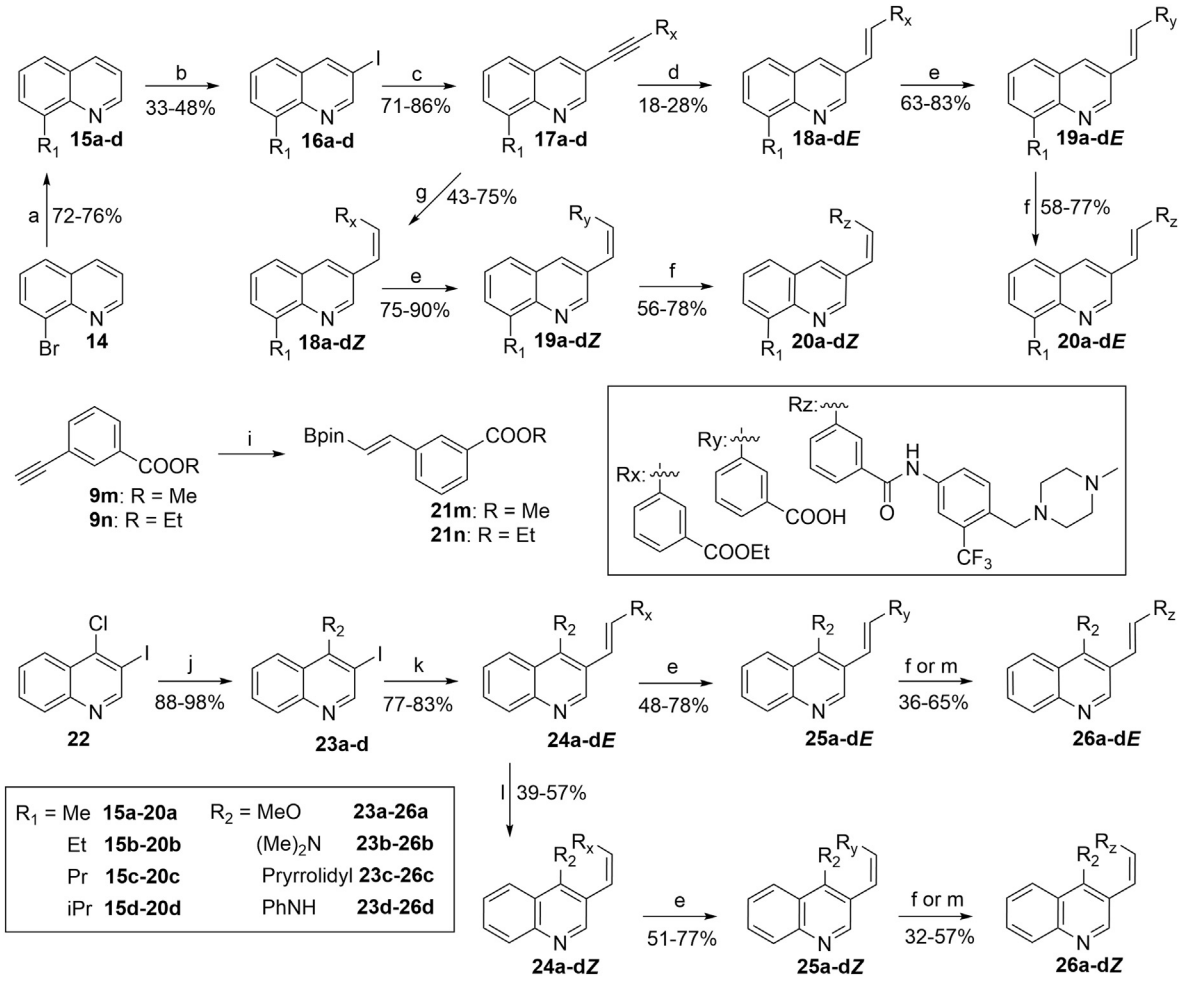
Fig. 2. (A) Photophysical characterization of **8**: UV−vis isomerization absorption spectrum in DMSO (left) and in H_2O (right); (B) Concentration-response curves and IC_{50} for compound **8** *E*-isomer and UV-enriched *Z*-isomer (left), **13*E*** and **13*Z*** (right), derived from an ADP-Glo™ RET kinase assay. (C) Docked poses of compound **8*E*** (cyan) and **8*Z*** (magenta) in the DFG-out RET kinase. Compound **8*Z*** does not form any H-bonds with Ala108, Glu76 and Ser192-Asp193. (D) Docked poses of compound **13*E*** (green) and **13*Z*** (orange) in the DFG-out RET kinase. Compound **8*Z*** does not form any H-bonds with Ala108 and Ser192-Asp193. The DFG-out model was obtained by homology modelling, which applied the RET protein sequence (NCBI database GeneID: 547807) on a template of KIT crystal structure (PDB code 4U0I).

different metal-catalysed stereoselective reduction conditions: a $RuCl_2(PPh_3)_3$/CuI/Zn mixture was used to catalyse the reduction, producing the *E*-isomers of stilbene intermediates **18a-d*E*** in moderate yields [36]. A Lindlar catalyst was applied to generate the *Z*-isomer of stilbene intermediates **18a-d*Z*** with medium yields [37]. These stilbene intermediates were then hydrolysed by LiOH, and the resulting carboxylic acids were coupled with the 'tail' moiety, to provide target stilbene inhibitors **20a-d*E*** and **20a-d*Z*** (Scheme 2).

The synthesis of the C4-substituted stilbene compounds started with nucleophilic substitution on commercially available 4-chloro-3-iodoquinoline (**22**) and produced substituted substrates **23a-d** [38,39]. The solvents and temperatures used in this reaction depended on different nucleophiles. The subsequent synthesis step followed the same strategy as C8-substituted stilbene compounds. However, no reaction was observed when the C4-substituted diarylacetylene intermediates were treated with the two metal-catalysed stereoselective reduction conditions. Therefore, we utilised a strategy of initially preparing the *E*-isomer of stilbene intermediates by coupling between **23a-d** and (*E*)-phenyl alkenyl building blocks (**21m** or **21n**) and then converting it to the *Z*-isomer by light-induced isomerization (Scheme 2). Building blocks **21m** and **21n** were prepared via a benzoic acid-catalysed hydroboration of **9m** and **9n**, respectively; the solvent of the reaction mixture was removed and the residue used for the next step [40]. Subsequently, a Suzuki coupling reaction was run between the crude products of **21m** or **21n**, and **23a-d** in high yields to provide **24a-d*E***, which were then irradiated by 365 nm UV light for 2 h to generate a mixture of *E*/*Z*-isomers; the *Z*-isomers **24a-d*Z*** were isolated by PLC plates. The intermediates **24a-d*E*** and **24a-d*Z*** were hydrolysed with LiOH, and the resulting carboxylic acids formed amide bonds with the 'tail' to provide final C4 substituted stilbene compounds **26a-d*E*** and **26a-d*Z***, respectively.

The substituted stilbene compounds were analysed in the same kinase assay as for the aza-compounds to determine their IC_{50} values (Table 2). As predicted, the introduction of alkyl groups on the C8 position largely weakened the activity of *Z*-isomer, especially **20c** and **20d**. However, the corresponding *E*-isomers lost more activity (reflected in the high IC_{50} values) in all four pairs of C8-substituted compounds, likely due to that the substituents pushed the quinoline nitrogen away from the hinge, preventing it from forming the H-bond. Such relative shift of the biological activities led to smaller IC_{50} differences between the *Z*- and *E*-isomers. The results were similar for the C4-substituted compounds, except for methoxyl-substituted **26 a*E*** and **26a*Z***. A *Z*/*E* IC_{50} ratio of 142 was achieved for this pair of compounds, while the potency of the *E*-isomer was preserved (IC_{50} = 12.4 nM).

The effect of the methoxy group on the bioactivity of stilbene compounds was also confirmed by molecular docking (see Supporting Material). Compound **26 a*E*** can dock in the DFG-out RET



Scheme 2. Synthesis of eight pairs of *E*/*Z*-isomers of substituted stilbene compounds[aa]Reagents and conditions: (a) RMgBr, $Ni(dppp)Cl_2$, THF, rt, 4 h. (b) NIS, AcOH, 90 °C, 16 h. (c) **9m/9n**, $Pd(MeCN)_2Cl_2$, RuPhos, Cs_2CO_3, MeCN, 80 °C, 6 h. (d) $RuCl_2(PPh_3)_3$, CuI, Zn, H_2O, 1,4-dioxane, 100 °C, 8 h. (e) LiOH, THF/H_2O/MeOH, 50 °C, 3 h. (f) **7**, EDC·HCl, HOBt, Et_3N, DCM, rt, 36 h. (g) Lindlar cat., H-cube, EtOH, 100 bar, 1.5 mL/min, 80 °C. (i) HBpin, benzoic aicd, heptane, 100 °C, 16 h. (j) Nucleophile solution, MW. (k) **21m/21n**, $Pd(OAc)_2$, RuPhos, K_3PO_4, H_2O, 1,4-dioxane, 80 °C, 16 h. (l) UV 365 nm, MeCN, rt, 2 h. (m) **7**, DIPEA, HATU/TBTU, DMF, rt, 18 h.

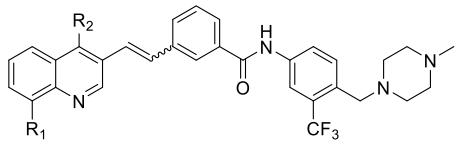
kinase structure by a proximate pose as **8*E*** or **13*E***. It can also form H-bonds with key amino acid residues, whereas the *Z*-isomer **26a*Z*** did not H-bond with the kinase structure. The successful methoxy modification of the stilbene compounds encouraged us to develop the corresponding azo compounds with a methoxy group on the C4 position of the quinoline ring.

Using the same strategy as for **8**, the synthesis of these substituted azo compounds started with a Buchwald–Hartwig coupling reaction between **2** and **23a** to provide **27** (Scheme 3). This intermediate was subjected to MW heating and subsequent O_2 oxidation to form the azo intermediate **28**, which was then hydrolysed by LiOH. The resulting carboxylic acid was coupled with the 'tail' to produce the target azo product **30**.Inspired by the work of the Hecht group [41,42], we introduced two fluorine atoms onto *ortho*-positions of the 'linker' phenyl ring of **8**. By this introduction, we hoped to achieve higher *Z*-isomer thermal stability, thus maintaining the purity of the *Z*-isomer during biological assays. An additional benefit with this strategy is that the introduction of strong electron-withdrawing groups, e.g. fluorine, can separate the $n \rightarrow \pi^*$ bands of *E*/*Z*-isomers, allowing their use to trigger forward and reverse isomerizations with visible light [41].

The synthesis of the *ortho*-difluoride photoswitchable RET kinase inhibitor is shown in Scheme 3. Compound **31** was prepared from 1,5-difluoroaniline by a literature procedure [43]. Oxidation was achieved by Oxone to provide nitroso product **32**, which was then reacted with **33** in an alkaline Mills reaction to provide **34** [43]. Subsequently, we attempted the carboxylation condition based on lithiation and CO_2; [44] however, only a dehalogenated product was detected. Instead, a palladium-catalysed carboxylation was conducted with Ac_2O/HCOOH (CO produced in situ) to prepare the key intermediate **35** [45]. This intermediate was then coupled with the 'tail' moiety **7** to provide the final product **36**.

Photophysical properties of the azo-type RET kinase inhibitors **30** and **36** were characterized based on UV–vis absorption spectra, and the main parameters are listed in Table 1. We also included compound **37** that has been reported in a separate publication [46]. Measurements were done both in DMSO and in aqueous solutions. All the azo compounds showed a hypsochromic shift by approximately 10 nm in going from DMSO to aqueous solutions. The introduction of the methoxy group on the quinoline ring not only enhanced the bioactivity difference of the *E*/*Z*-isomers but also improved their photophysical properties. Compound **30**, as well as **37** [46] showed higher *Z*-isomer enrichment (93% and 97%, respectively) than the original azo compound **8** after UV (365 nm) irradiation. They also demonstrated better thermal stability in DMSO and aqueous solutions, e.g. the half-life of **37*Z*** in H_2O is almost 3 times longer than that of **8*Z***. However, **36** did not display improved photophysical properties compared to the non-

Table 2
IC_{50} of substituted stilbene compounds.[a].



Inhibitors	R_1	R_2	IC_{50}	*Z*/*E* (IC_{50}) ratio
20a*E*	Methyl	H	0.257 ± 0.05 μM	49
20a*Z*	Methyl	H	12.6 ± 4.0 μM	
20bE	Ethyl	H	1.7 ± 0.3 μM	14.7
20b*Z*	Ethyl	H	25 ± 7.5 μM	
20c*E*		H	5.6 ± 3.2 μM	–
20c*Z*		H	>500 μM	
20d*E*		H	9.8 ± 1.5 μM	–
20d*Z*		H	>0.5 mM	
26a*E*	H	methoxy	0.0124 ± 0.0027 μM	142
26a*Z*	H	methoxy	1.8 ± 0.3 μM	
26bE	H		0.098 ± 0.016 μM	24.4
26b*Z*	H		2.4 ± 0.4 μM	
26c*E*	H		0.066 ± 0.017 μM	33.4
26c*Z*	H		2.2 ± 0.5 μM	
26d*E*	H		2.3 ± 1.2 μM	21.3
26d*Z*	H		49 ± 10 μM	

[a] IC_{50} values were determined using the ADP-Glo™ RET kinase assay. Data represent the mean and standard deviation of three experimental replicates.

fluorinated version. The thermal stability of the *ortho*-difluoride azo compound **36*Z*** in aqueous solution was only 38 h, indicating that a significant fraction of **36*Z*** could isomerize to the corresponding *E*-isomer during the biological assays that run for hours. Moreover, the *Z*-isomer was only enriched to 86% in the UV-induced (365 nm) PSS. Notably, these three azo compounds (**30**, **36**, and **37**[46]) showed good fatigue resistance in DMSO and aqueous solutions after ten cycles of 365 nm and 460 nm irradiation-induced photoswitching.

Having investigated the photophysical properties, we proceeded to evaluate the biological activity in biochemical and cell assays. 1,4-Dithiothreitol (DTT) was used in the ADP-Glo™ kinase assay to maintain the proteins in a reduced state according to the manufacturer's protocol. Although glutathione (GSH) is a natural antioxidant in mammalian cells, DTT and GSH have been reported to be potential reducing factors for azo compounds in biological assays [47]. We excluded the factor of DTT/GSH reduction on the biological assay results by testing the four azo compounds (**8**, **30**, **36**, **37**[46]) in DTT/GSH assays and monitoring the reactions by LC-MS to determine if reduction products were generated. Azo compounds **8**, **36**, and **37** [46] were stable during the incubation with 2 mM DTT or 10 mM GSH for 20 h, as no reduction products were detected by LC-MS. However, reduction products were observed in the assay with **30**, especially when treated with 10 mM GSH (Figs. S15 and S16). Additional UV–vis cyclic isomerization experiment of **30** in aqueous solution containing 1 mM GSH also showed degradation after 2.5 h incubation, judging by the decrease in absorbance over time (Fig. 3A).

Before the ADP-Glo™ kinase assay, the azo compounds were thermally adapted to (nearly 100%) *E*-isomers by heating the stock solution for 30 min at 60 °C. The *Z*-isomer samples were prepared by irradiating the serially diluted *E*-isomer samples on 96-well plates with a 365 nm light emitting diode (LED) array (2.3 mW, 15 min). Using the same protocol as used for **8**, the IC_{50} of **30*E*** was determined to be 24 nM, and UV-enriched **30*Z*** showed a 15-fold decrease in activity with an IC_{50} of 363 nM (Fig. 3B). The IC_{50} of **37*E*** was determined to be 3 nM, which is 17-fold more potent than the UV-enriched sample of **37** [46]. However, the assay with *ortho*-difluoride compound **36** showed only a 1.5-fold difference in activity between the *E*-isomer and UV-enriched *Z*-isomer (Fig. 3C). Both isomers were potent with IC_{50} values of 11 nM and 17 nM, respectively. The narrow activity difference could be attributed to the low enrichment of **36*Z*** after UV irradiation and subsequent thermal isomerization $Z \rightarrow E$; as a result, the *E*-isomer of **36** activity dominated in the assay.

Encouraged by the results from the biochemical assay,

compound **30** was further tested in a NanoBRET™ intracellular RET kinase assay. This assay allows evaluating compounds that bind to RET fused to NanoLuc luciferase in a competitive format by using a cell-permeable fluorescent NanoBRET tracer in HEK293 cells (human embryonic kidney cells). In this assay, the *E*-isomer of azo compounds were thermally adapted before serial dilution, and all the operations involving azo and stilbene compounds were performed under red light. Initially, the assay was run with 90 min incubation at 37 °C. The reference compound ponatinib gave an IC_{50} value of 13 nM (see Supporting Material). The IC_{50} value for **30*E*** was determined to 0.92 μM (Fig. 3E). We also evaluated the cellular activity of the *Z*-isomers by stepwise in situ irradiation. The *E*-isomer was initially incubated then gradually irradiated during the assay to maximise the UV-induced PSS of the *Z*-isomer throughout the incubation. We employed such a strategy after considering the relatively long residence time of the *E*-isomer bound in the kinase pocket [48], as it is generally assumed that the photoswitching of the azo-type inhibitor only occurs when the ligand is not bound to its target [3]. In addition, UV-induced isomerization kinetics in cell assay medium showed that 35 s of UV exposure was sufficient to achieve the *Z*-isomer enriched PSS (Fig. 3D). Therefore, a total of 35 s of UV light was delivered to the cells in three steps: 1) after incubation of **30*E*** at 37 °C for 80 min, the cells were irradiated for 15 s using a 365 nm LED array (2.3 mW); 2) after an additional 10 min incubation, the cells were irradiated for another 10 s; 3) after cooling to room temperature, the cells were irradiated for 10 s then incubated at ambient conditions for 10 min before reading the luminescence signals. The IC_{50} of UV-enriched **30*Z*** was determined to 17.2 μM (Fig. 3E), i.e., four-times higher than that observed for the pure *E*-isomer. The corresponding stilbene compounds **26 a*E*** and **26a*Z*** gave IC_{50} values of 0.15 μM and 17.2 μM, respectively (Fig. 3F), resulting in a *Z*/*E* (IC_{50}) ratio consistent with that measured in the biochemical assay. We tentatively rationalise the relatively high potency of UV-enriched **30*Z*** and decreased *Z*/*E* (IC_{50}) ratio in the cellular assay by GSH reduction of the azo bond because the resulting hydrazine products were reported to be more potent than the original azo compound [12].

3. Conclusions

Based on the binding model of known DFG-out kinase inhibitors, we have developed a series of photoswitchable azoheteroarene versions using three approaches: 1) installing substituents on the quinoline 'head' moiety, 2) introducing fluorine atoms on the ortho-position of the azoheteroarene phenyl ring, and 3) changing the heterocyclic head. Among all the modified azo derivatives, compound **30** showed excellent photophysical properties in terms of a highly *Z*-isomer enriched photostationary distribution after UV irradiation and excellent thermal stability. However, repeated photoisomerization under GSH reduction stress resulted in significant degradation of the compound. The corresponding stilbene version gave a *Z*/*E* (IC_{50}) ratio well above 100, consistent with that measured in the biochemical assay. Compound **37** did not show any instability under reducing conditions and showed more than a 10-fold difference in bioactivity between the two isomers, both in a biochemical and a cell-based assay. These results set the stage for further developments of photoswitchable DFG-out inhibitors, also targeting other kinases.

4. Experimental section

Chemical synthesis. General reagent and solvents for the synthesis of compounds were purchased from commercial sources and used as supplied, unless otherwise stated. THF and toluene were distilled from Na/benzophenone. Reactions were monitored by Thin Layer Chromatography (TLC, Merck, silica gel 60 F254) and visualized under UV (254 nm/365 nm) or by potassium permanganate solution ($KMnO_4$) staining. Those reactions that employed microwave irradiation were conducted using a Biotage InitiatoRTM reactor with fixed hold time. Purification by flash column chromatography was performed by manual flash chromatography (wet-packed silica, 0.04–0.063 mm) or by automated flash chromatography on a Biotage SP-X or Isolera instrument using prefabricated silica columns. ^{1}H and ^{13}C NMR spectra were obtained at 400 MHz, using a Varian 400/54 spectrometer, or 700 MHz Bruker spectrometer for some ^{19}F-, ^{1}H and ^{13}C NMR spectra. The HRMS data was determined by CMSI (Chalmers Mass Spectrometry Infrastructure) at Chalmers University of Technology. The photostationary distributions were determined by 500 MHz ^{1}H NMR with DMSO-d_6 as the solvent. All reactions where azo bond were involved were carried out avoiding direct light, i.e. covering reaction vessels and columns with aluminum foil and working with the fume hood lamp turned off (ceiling lamp was left on). UV–Vis absorption spectra were recorded using a Varian CaryBio 50 spectrophotometer. In the ns transient absorption experiments, sample excitation was provided by the third harmonic of an Nd:YAG laser (Quantel, BrilliantB) that delivered 10 ns pulses at 355 nm, or 410 nm after the OPO. Analyzing light was provided by a 150 W Xe lamp in a flash photolysis spectrometer (Applied Photophysics LKS.60) and detected by a P928 five stage photomultiplier tube (PMT). The PMT signal was digitized using an Agilent Technologies Infiniium digital oscilloscope (600 MHz). All spectra were recorded in 10 × 10 mm quartz cuvettes. The light sources for isomerization purposes were LEDs (LED Engin) centered at around 365 nm (LZ1-10UV00, fwhm = 12 nm), 405 nm (LZ1-10UB00–00U8, fwhm = 19 nm), 460 nm (LZ1-10B200, fwhm = 21 nm).

Purity Analysis. All target compounds used were >95% pure by RP-HPLC.

General Procedure IA. To a solution of ethyl ester (1 equiv) in THF/H_2O/MeOH (1:1:0.2) was added LiOH (4 equiv) and the mixture was then stirred at 50 °C for 3 h. After cooling down, the organic solvent was blown away by airflow, and pH was adjusted to 5 by dropwise addition of 1 M HCl. After additional 5 min of stirring, the reaction mixture was filtered, the solid was rinsed by water once, and air-dried. The crude product was used directly in the next step without any additional purification.

General Procedure IB. To a solution of ethyl ester (1 equiv) in THF/H_2O/MeOH (1:1:0.2) was added LiOH (4 equiv) and the mixture was then stirred at 50 °C for 3 h. After cooling down, 1 M HCl was added dropwise to adjust the pH to 5. After additional 5 min of stirring, the mixture was extracted with EtOAc/*i*-PrOH (3:1, 10 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The desired product was used in the next step without any additional purification.

General Procedure IIA. To a microwave vial was added a carboxylic acid derivative (1 equiv), EDC·HCl (4 equiv), HOBt (2 equiv) and Et_3N (8 equiv), the vial was then sealed, evacuated and backfilled with N_2. A solution of **8** (1.2 equiv) in anhydrous DCM was added and the reaction mixture was stirred at rt for 36 h. The reaction mixture was diluted with H_2O (5 mL) and the aqueous layer was extracted with DCM (10 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash column chromatography (6–10% MeOH in DCM) afforded the target compound.

General Procedure IIB. To a solution of a carboxylic acid derivative (1 equiv) and HATU/HBTU/TBTU (1.1 equiv) in DMF was added DIPEA (3 equiv) under N_2. The reaction mixture was

stirred for 30 min at rt before **8** (1.2 equiv) was added. The vial was purged with N_2 and stirred at rt overnight. Excess solvent was removed under reduced pressure. Purification by flash column chromatography (6–10% MeOH in DCM) afforded the target compound.

General Procedure III. To an oven-dried microwave vial was added alkyne **2** (1.2 equiv), RuPhos (0.09 equiv), $Pd(MeCN)_2Cl_2$ (0.03 equiv) and Cs_2CO_3 (2 equiv), the vial was then sealed, evacuated and backfilled with N_2 three times. 3-Iodo-8-alkylquinoline (**16**, equiv) was then added in anhydrous MeCN, the flask was again evacuated, and backfilled with N_2 three times. The reaction mixture was stirred at 80 °C overnight. After cooling to rt, the reaction mixture was filtered through a celite pad with EtOAc (50 mL) as the washing solvent. The solution was concentrated under reduced pressure. Purification by flash column chromatography afford the target product.

General Procedure IV. To an oven-dried microwave vial was added C4 substituted 3-iodo-quoniline (**24**, 1 equiv), pinacolborane derivative (1.2 equiv), $Pd(OAc)_2$ (4 mol%), RuPhos (8 mol%), and K_3PO_4 (3 equiv). The vial was capped and purged with N_2 before 1,4-dioxane and H_2O (5 equiv) were added. The reaction mixture was stirred at 80 °C for 4 h. After cooling to rt, the reaction mixture was filtered through a celite pad with EtOAc (30 mL) as the washing solvent. Excess solvent was removed. Purification by flash column chromatography afforded the target product.

General Procedure V. An oven-dried microwave vial was charged with Zn (3 equiv), CuI (0.1 equiv) and $RuCl_2(PPh_3)_3$ (0.05 equiv) under N_2 atmosphere. Water (8 equiv) and alkyne **17** (1 equiv) in 1,4-dioxane was added and the vial was evacuated and backfilled with N_2 three times. The reaction was stirred at 100 °C for 24 h. After cooling to rt, the reaction mixture was filtered through a celite pad with EtOAc (50 mL) as the washing solvent. The solution was concentrated under reduced pressure. Purification by flash column chromatography to afford the target product.

General Procedure VI. A solution of alkyne in MeOH or EtOH (0.05 M) was cycled repeatedly through the H-cube using a Lindlar catalyst ($Pd/Ca_2CO_3/Pb$) cartridge. Flow rate: 1.5 mL/min, pressure: 100 bars, temperature: 80 °C. Upon completion, (determined by TLC or LC-MS) the solution was collected and evaporated. Purification by flash column chromatography with afforded the desired product.

General Procedure VII. An oven-dried microwave vial was charged with 8-bromoquinoline (1 equiv) and $Ni(dppp)Cl_2$ (0.1 equiv). The vial was then sealed, evacuated and backfilled with N_2 three times. Anhydrous THF was added to the vial via a syringe, the mixture was again evacuated and backfilled with N_2 three times. Alkylmagnesium halide (2 equiv) was added dropwise to the mixture and the reaction mixture was stirred at rt until full conversion was observed by TLC. The reaction was quenched by dropwise addition of 1 M HCl solution until the mixture become clear. The aqueous layer was extracted with Et_2O (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash column chromatography afforded the target product.

General Procedure VIII. To a round bottom flask, containing 8-alkylquionoline (**15**, 1 equiv) was added HOAc and the mixture was stirred for 10 min. Then *N*-iodosuccinimide (1.5 equiv) was added and the reaction was stirred at 90 °C for 24 h Na_2SO_3 (3 equiv) was then added and the reaction was let to stir at 90 °C for 1 h. After cooling to rt, the pH was adjusted to 8 using 5 M NaOH solution. The aqueous layer was extracted with DCM (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash column chromatography to afforded the target product.

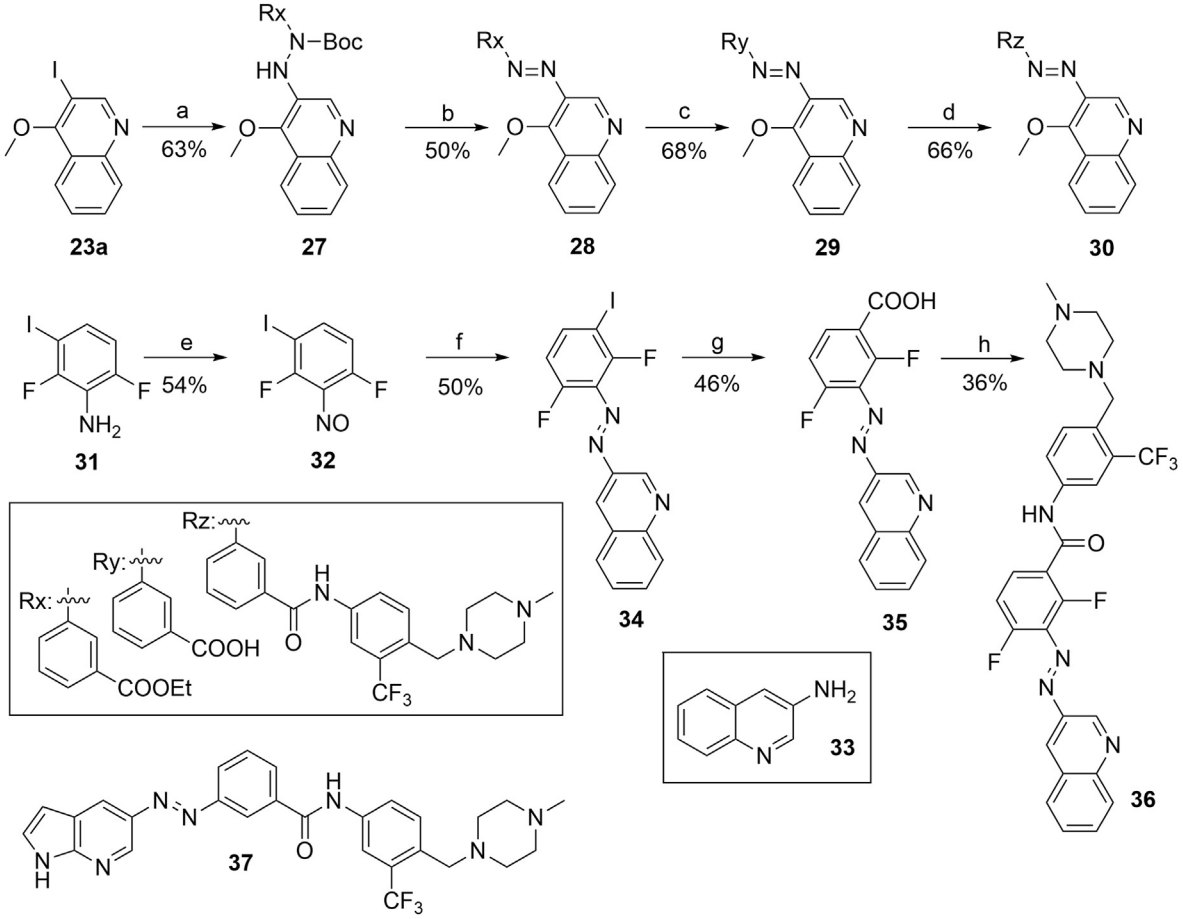
General Procedure IX. A solution of a (*E*)-stilbene compound in distilled MeCN was submitted to UV LED (365 nm) irradiation (3.0 mW/cm^2) for 2 h under stirring. Excess solvent was removed. Purification by PLC silica gel plate chromatography afforded the target compound.

***tert*-Butyl 1-(3-(methoxycarbonyl)phenyl)hydrazine-1-carboxylate (2).** To an oven-dried microwave vial was added *N*-Boc hydrazine (475 mg, 3.60 mmol), Cs_2CO_3 (1.46 g, 4.50 mmol), CuI (28.5 mg, 0.15 mmol). The vial was then capped and backfilled with N_2 3 times before 3-Iodobenoic acid ethyl ester (830 mg, 500 μL, 3.00 mmol) and DMSO (3 mL) was added. The reaction mixture was stirred at 50 °C for 5 h. After cooling to rt, saturated NH_4Cl solution (5 mL) was added to dilute the reaction mixture, which was then extracted by EtOAc (3 × 10 mL). The combined organic phase was washed by water (5 × 30 mL), followed by brine (20 mL), dried over Na_2SO_4, filtered and excess solvent was removed. Purification by flash column chromatography (5% EtOAc in pentane) afforded the target compound (716 mg, 2.55 mmol, 85%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (t, J = 1.9 Hz, 1H), 7.77 (ddd, J = 7.7, 1.6, 1.1 Hz, 1H), 7.68 (ddd, J = 8.1, 2.3, 1.0 Hz, 1H), 7.35 (t, J = 7.7 Hz, 1H), 4.45 (s, 2H), 4.36 (q, J = 7.1 Hz, 1H), 1.50 (s, 9H), 1.38 (t, J = 7.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 154.9, 143.3, 130.5, 128.1, 127.3, 125.4, 124.2, 82.2, 81.0, 28.3, 14.3.

***tert*-Butyl 1-(3-(methoxycarbonyl)phenyl)-2-(quinolin-3-yl) hydrazine-1-carboxylate (4).** To an oven-dried microwave vial was added **2** (203 mg, 0.72 mmol), 3-bromoquinoline (125 mg, 0.60 mmol), $Pd(OAc)_2$ (14 mg, 0.062 mmol), tBu$_3$P·HBF$_4$ (28 mg, 0.097 mmol) and Cs_2CO_3 (390 mg, 1.20 mmol). The vial was then capped and backfilled with N_2 3 times before 1,4-dioxane (5 mL) was added. The resulting reaction mixture was heated using microwave irradiation at 110 °C for 2 h. After cooling to rt, the reaction mixture was filter filtered through a Celite pad with ethyl acetate as the washing solvent. Excess of solvent was removed. Purification by flash column chromatography (50% EtOAc in pentane) afforded the target compound (230 mg, 0.56 mmol, 94%) as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (t, J = 2.8 Hz, 1H), 8.33 (s, 1H), 8.00 (t, J = 8.3 Hz, 1H), 7.83 (dd, J = 8.0, 1.9 Hz, 2H), 7.64 (d, J = 7.2 Hz, 1H), 7.52 (ddd, J = 8.4, 6.9, 1.6 Hz, 1H), 7.48–7.43 (m, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.33 (d, J = 2.7 Hz, 1H), 6.92 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 1.39 (s, 9H), 1.37 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 153.4, 144.3, 142.5, 141.2, 141.0, 131.1, 129.1, 128.7, 128.6, 127.2, 126.7, 126.6, 125.9, 125.8, 122.7, 114.3, 83.4, 61.2, 14.3. HRMS (ESI-TOF) m/z calcd for $C_{23}H_{26}N_3O_4^+$ $[M + H]^+$: 408.1918, found: 408.1938.

Ethyl (*E*)-3-(quinolin-3-yl-diazenyl)benzoate (5). A solution of **4** (200 mg, 0.49 mmol) in DMF (5 mL) was heated using microwave irradiation at 180 °C for 45 min. After cooling to rt, pure oxygen was backfilled into the vial 2 times. The reaction mixture was heated at 100 °C for 2 h. The reaction mixture was cooled to rt and excess solvent removed. Purification by flash column chromatography (17% EtOAc in pentane) afforded the target compound (84 mg, 0.28 mmol, 56%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.50 (d, J = 2.3 Hz, 1H), 8.64–8.59 (m, 2H), 8.22–8.12 (m, 3H), 7.98 (d, J = 7.8 Hz, 1H), 7.78 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 4.44 (q, J = 7.2 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 152.5, 149.2, 145.6, 144.6, 132.3, 131.8, 131.0, 130.2, 129.54, 129.46, 129.3, 127.8, 127.5, 126.8, 124.3, 61.4, 14.4. HRMS (ESI-TOF) m/z calcd for $C_{18}H_{16}N_3O_2^+$ $[M + H]^+$: 306.1237, found: 306.1244.

(*E*)-3-(Quinolin-3-yl-diazenyl)benzoic acid (6). A solution of **5** (80 mg, 0.26 mmol) and LiOH (42 mg, 1.75 mmol) in THF/H_2O/



Scheme 3. The synthesis of the optimized azo type RET kinase inhibitos 36 and the structure of 7-Azaindole based inhibitor 37 [aa]Reagents and conditions: (a) **2**, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, Toluene, MW, 110 °C, 2 h; (b) DMF, 180 °C, 45 min; then O_2, 100 °C, 2 h; (c) LiOH, THF/H_2O/MeOH, 50 °C, 3 h. (d) **7**, EDC·HCl, HOBt, Et_3N, DCM, rt 36 h. (e) OXONE, H_2O/DCM, rt, 25 h. (f) **33**, 40% NaOH/toluene (1:1), 80 °C, 3 h. (g) $Pd(OAc)_2$, Xanthphos, Ac_2O, HCOOH, propanol, Et_3N, toluene, 80 °C, 12 h.

MeOH (2.7 mL, 1:1:0.2) was reacted according to General Procedure IA. The target compound (65 mg, 0.23 mmol, yield 89%) was obtained as a red solid. ^{1}H NMR (700 MHz, DMSO-d_6) δ 9.47 (d, J = 2.4 Hz, 1H), 8.95 (d, J = 2.3 Hz, 1H), 8.49 (t, J = 1.9 Hz, 1H), 8.26 (m, 2H), 8.18 (dt, J = 7.6, 1.4 Hz, 1H), 8.15 (dd, J = 8.4, 1.1 Hz, 1H), 7.92 (ddd, J = 8.3, 6.8, 1.4 Hz, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.76 (ddd, J = 8.0, 6.8, 1.2 Hz, 1H); ^{13}C NMR (176 MHz, DMSO-d_6) δ 167.1, 152.4, 149.2, 145.2, 144.6, 132.82, 132.76, 132.0, 131.1, 130.6, 130.5, 129.4, 128.3, 128.1, 128.0, 122.9. HRMS (ESI-TOF) m/z calcd for $C_{16}H_{12}N_3O_2^+$ $[M + H]^+$: 278.0924, found: 278.0934.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(quinolin-3-yldiazenyl)benzamide (8). To an oven-dried microwave vial was added **6** (63 mg, 0.23 mmol), **7** (76 mg, 0.28 mmol), EDC·HCl (176 mg, 0.92 mmol), HOBt (62 mg, 0.46 mmol), Et_3N (189 mg, 260 μL, 1.87 mmol), DCM (2.5 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in $CHCl_3$) provided the target compound (104 mg, 0.20 mmol, 86%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.50 (d, J = 2.3 Hz, 1H), 8.62 (d, J = 1.7 Hz, 1H), 8.42 (t, J = 1.7 Hz, 1H), 8.34 (s, 1H), 8.17 (m, 2H), 8.07 (ddd, J = 7.8 1.9, 1.2 Hz, 1H), 8.02–7.97 (m, 1H), 7.97–7.91 (m, 2H), 7.83–7.77 (m, 2H), 7.68 (t, J = 7.8 Hz, 1H), 7.63 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 3.65 (s, 2H), 2.71–2.41 (m, 8H), 2.34 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.1, 152.4, 149.3, 145.4, 144.5, 136.6, 135.6, 133.9, 131.5, 131.2, 130.5, 130.3, 130.0, 129.51, 129.47, 129.3 (q, J_{CF} = 30.6 Hz), 127.8, 127.6, 127.0, 125.4 (q, J_{CF} = 272.7 Hz), 123.4, 120.6, 117.7 (q, J_{CF} = 6.0 Hz), 57.8, 55.1, 52.9, 45.9. m.p. 197–200 °C. HRMS (ESI-TOF) m/z calcd for $C_{29}H_{28}F_3N_6O^+$ $[M + H]^+$: 533.2271, found: 533.2279.

Ethyl 3-(quinolin-3-ylethynyl)benzoate (10). To an oven-dried microwave vial was added **9n** (200 mg, 1.15 mmol), $Pd(MeCN)_2Cl_2$ (8.8 mg, 0.034 mmol), RuPhos (44 mg, 0.094 mmol), Cs_2CO_3 (672 mg, 2.06 mmol), **3** (206 mg, 140 μL, 1.03 mmol), MeCN (8.0 mL), and the reaction was carried out according to General Procedure III. Purification by flash column chromatography (15% EtOAc in pentane) afforded the target compound (295.0 mg, 0.98 mmol, 95%) as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (d, J = 2.1 Hz, 1H), 8.30 (d, J = 2.2 Hz, 1H), 8.25 (t, J = 1.8 Hz, 1H), 8.09 (d, J = 8.5 Hz, 1H), 8.03 (dt, J = 7.8, 1.5 Hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.74 (dt, J = 6.2, 1.4 Hz, 1H), 7.71 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.56 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 152.0, 146.9, 138.4, 135.6, 132.8, 130.9, 130.2, 129.7, 129.4, 128.6, 127.6, 127.3, 127.2, 123.0, 117.0, 91.5, 87.4, 61.3, 14.3. HRMS (ESI-TOF) m/z calcd for $C_{20}H_{16}NO_2^+$ $[M + H]^+$: 302.1176, found: 302.1180.

Ethyl (*E*)-3-(2-(quinolin-3-yl)vinyl)benzoate (11*E*). An oven-dried microwave vial was charged with Zn (314.4 mg, 4.8 mmol), CuI (45.6 mg, 0.24 mmol) and $RuCl_2(PPh_3)_3$ (115.2 mg, 0.12 mmol) under N_2. Then water (360 μL, 360 mg, 20 mmol) and alkyne **10** (720 mg, 2.4 mmol) in anhydrous 1,4-dioxane was added and the vial was evacuated and backfilled with N_2 three times. The reaction mixture was stirred at 100 °C for 24 h. After cooling to rt, the reaction mixture was filtered through a Celite pad with ethyl acetate

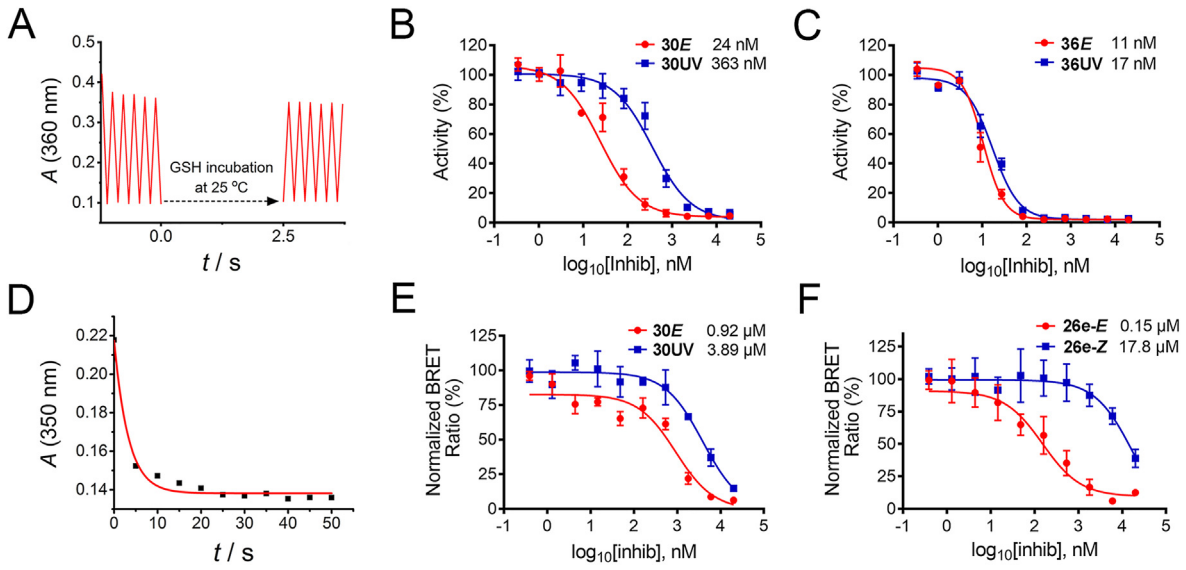


Fig. 3. (A) UV−vis cyclic isomerization and incubation of **30** in 33% DMSO/PBS containing 1 mM GSH. (B) and (C) Dose-response curves and IC_{50} for **30*E*** and **36*E***, and the corresponding UV-enriched *Z*-isomers measured in the ADP-Glo™ RET kinase assay. (D) Using LED assay (2.3 mW, 12.0 V) irradiation to induce the *E*→*Z* isomerization of **30** in Opti-MEM medium (20 μM). The red line shows the fit using mono-exponential (first order) kinetics. (E) Dose-response curves and IC_{50} for **30*E*** and the corresponding UV-enriched *Z*-isomers measured in the NanoBRET™ TE Intracellular RET kinase assay. (F) Dose-response curves and IC_{50} for **26e-*E*** and **26e-*Z*** measured in the NanoBRET™ TE Intracellular RET kinase assay.

as the washing solvent. Excess solvent was removed. Purification by flash column chromatography (25% EtOAc in pentane) afforded the target compound (144 mg, 0.48 mmol, 20%) as a light brown powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (t, J = 2.1 Hz, 1H), 8.25 (d, J = 1.6 Hz, 1H), 8.17 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.97 (ddd, J = 7.7, 2.7, 1.1 Hz, 1H), 7.81 (dt, J = 8.1, 1.6 Hz, 1H), 7.72 (ddd, J = 7.8, 3.1, 1.5 Hz, 1H), 7.68 (ddt, J = 8.4, 6.9, 1.5 Hz, 1H), 7.54 (ddt, J = 8.1, 6.8, 1.3 Hz, 1H), 7.46 (dt, J = 7.8, 1.4 Hz, 1H), 7.34 (d, J = 16.5 Hz, 1H), 7.29 (d, J = 16.5 Hz, 1H), 4.42 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 149.3, 147.6, 137.0, 132.6, 131.1, 130.8, 129.9, 129.7, 129.4, 129.3, 129.1, 128.8, 128.0, 127.9, 127.6, 127.1, 126.4, 61.2, 14.4. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{18}NO_2^+$ [M + H]$^+$: 304.1332, found: 304.1340.

(*E*)-3-(2-(Quinolin-3-yl)vinyl)benzoic acid (12*E*). A solution of **11*E*** (110 mg, 0.36 mmol) and LiOH (45 mg, 1.88 mmol) in THF/H_2O/MeOH (3.0 mL, 1:1:0.2) was reacted according to General Procedure IA. The target compound (75 mg, 0.31 mmol, 75%) was obtained as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (d, J = 2.1 Hz, 1H), 9.16 (s, 1H), 8.27 (d, J = 9.5 Hz, 1H), 8.25−8.18 (m, 2H), 7.99 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 7.89 (dd, J = 7.8, 1.7 Hz, 1H), 7.88−7.83 (m, 1H) 7.81 (d, J = 16.5 Hz, 1H), 7.62 (d, J = 16.5 Hz, 1H), 7.56 (t, J = 7.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5, 145.6, 139.6, 139.3, 137.0, 133.3, 132.7, 131.9, 131.7, 131.4, 129.82, 129.77, 129.74, 129.3, 128.8, 128.1, 124.6, 123.0. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{14}NO_2^+$ [M + H]$^+$: 276.1019, found: 276.1022.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(quinolin-3-yl)vinyl)benzamide (13*E*). To an oven-dried microwave vial was added **12*E*** (50 mg, 0.18 mmol), **7** (55 mg, 0.20 mmol), EDC·HCl (140 mg, 0.73 mmol), HOBt (50 mg, 0.37 mmol), Et_3N (150 mg, 200 μL, 1.43 mmol) and DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (8% MeOH in $CHCl_3$) afforded the target compound (56 mg, 0.11 mmol, 58%) as a light brown solid. m.p. 75−79 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (d, J = 2.2 Hz, 1H), 8.55 (s, 1H), 8.09−8.00 (m, 3H), 7.96−7.88 (m, 2H), 7.80−7.73 (m, 3H), 7.69−7.62 (m, 2H), 7.52 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.44 (t, J = 7.7 Hz, 1H) 7.23 (d, J = 16.5 Hz, 1H), 7.18 (d, J = 16.5 Hz, 1H), 3.62 (s, 2H), 2.66−2.33 (m, 8H), 2.28 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 149.1, 147.5, 137.4, 136.7, 135.1, 133.8, 132.8, 131.3, 130.1, 129.7, 129.5, 129.4, 129.24, 129.16 (q, J_{CF} = 30.6 Hz), 129.1, 128.0, 127.9, 127.2, 126.6, 126.5, 125.3, 122.7 (q, J_{CF} = 272.7 Hz) 117.7 (q, J_{CF} = 6.0 Hz), 57.8, 55.2, 53.1, 46.1. HRMS (ESI-TOF) *m/z* calcd for $C_{31}H_{30}F_3N_4O^+$ [M + H]$^+$: 531.2366, found: 531.2377.

Ethyl (*Z*)-3-(2-(Quinolin-3-yl)vinyl)benzoate (11*Z*). To an oven-dried microwave vial was added **10** (295.0 mg, 0.98 mmol), $Pd(OAc)_2$ (4.2 mg, 0.019 mmol) and KOH (80 mg, 1.45 mmol). The vial was sealed and purged with N_2 for 3 times before anhydrous DMF (2 mL) was added. The reaction mixture was stirred at 145 °C for 24 h. After cooling down, water (5 mL) and EtOAc (10 mL) was added. The aqueous phase was extracted with EtOAc (2 × 10 mL), the combined organic phase was washed with brine (15 mL), then dried over Na_2SO_4 and filtered. Excess solvent was removed. Purification by flash column chromatography (25% EtOAc in pentane) afforded the target compound (50.0 mg, 0.16 mmol, 17%) as a light brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 2.2 Hz, 1H), 8.02 (m, 1H), 7.97 (s, 1H), 7.95 (s, 1H), 7.90 (dt, J = 7.8, 1.2 Hz, 1H), 7.66 (m, 2H), 7.49 (ddd, J = 8.2, 6.7, 1.2 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.27 (t, J = 7.8 Hz, 1H), 6.84 (d, J = 12.2 Hz, 1H), 6.78 (d, J = 12.2 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 151.1, 147.0, 136.8, 135.1, 132.8, 131.7, 131.0, 130.0, 129.4, 129.2, 128.7, 128.6, 127.8, 127.7, 127.6, 126.8, 61.0, 14.1. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{18}NO_2^+$ [M + H]$^+$: 304.1332, found: 304.1344.

(*Z*)-3-(2-(Quinolin-3-yl)vinyl)benzoic acid (12*Z*). A solution of **11*Z*** (106 mg, 0.35 mmol) and LiOH (42 mg, 1.75 mmol) in THF/H_2O/MeOH (3.0 mL, 1:1:0.2) was reacted according to General Procedure IA. The target compound (84 mg, 0.31 mmol, 87%) was obtained as a yellow powder. ^{1}H NMR (700 MHz, DMSO-d_6) δ 8.63 (d, J = 2.2 Hz, 1H), 8.22 (d, J = 2.2 Hz, 1H), 7.96 (dd, J = 8.4, 1.1 Hz, 1H), 7.88 (dd, J = 8.2, 1.4 Hz, 1H), 7.85 (t, J = 1.8 Hz, 1H), 7.83 (dt, J = 7.7, 1.6 Hz, 1H) 7.73 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.59 (ddd, J = 8.0, 6.8, 1.2 Hz, 1H), 7.47 (dt, J = 7.6, 1.2 Hz, 1H), 7.41 (t, J = 7.7 Hz, 1H), 6.96 (d, J = 12.2 Hz, 1H), 6.91 (d, J = 12.2 Hz, 1H); ^{13}C NMR (176 MHz, DMSO-d_6) δ 167.4, 150.9, 146.8, 137.3, 135.4, 133.2, 131.9, 131.7, 130.3, 130.1, 129.9, 129.4, 129.1, 128.9, 128.5, 128.1, 127.9, 127.4. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{14}NO_2^+$ [M + H]$^+$: 276.1019, found: 276.1026.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(quinolin-3-yl)vinyl)benzamide (13*Z*). To an oven-dried microwave vial was added **12*Z*** (38 mg, 0.14 mmol), **7** (40 mg, 0.15 mmol), EDC·HCl (40 mg, 0.21 mmol) and HOBt (38 mg, 0.28 mmol), DIPEA (37 mg, 50 μL, 0.28 mmol), DCM (1.5 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (8% MeOH in $CHCl_3$) afforded the target compound (45 mg, 0.085 mmol, 62%) as a light brown solid. m.p. 60–64 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, J = 2.2 Hz, 1H), 8.02 (dd, J = 1.2 Hz, 1H), 7.98 (d, J = 2.1 Hz, 1H), 7.93 (s, 1H), 7.74–7.62 (m, 7H), 7.50 (ddd, J = 8.0, 7.0, 1.2 Hz, 1H), 7.39 (dt, J = 7.8, 1.4 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 6.82 (d, J = 12.2 Hz, 1H), 6.78 (d, J = 12.2 Hz, 1H), 3.59 (s, 2H), 2.59–2.33 (m, 8H), 2.29 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 150.9, 146.9, 137.2, 136.5, 135.3, 135.0, 133.6, 132.3, 131.5, 131.2, 129.9, 129.7, 129.2, 129.1, 128.9 (q, J_{CF} = 30.7 Hz), 127.9, 127.8, 127.3, 127.1, 126.4, 124.1 (q, J_{CF} = 272.3 Hz), 123.2, 117.5 (q, J_{CF} = 6.0 Hz), 57.7, 55.2, 53.0, 46.0. HRMS (ESI-TOF) *m/z* calcd for $C_{31}H_{30}F_3N_4O^+$ $[M + H]^+$: 531.2366, found: 531.2367.

8-Ethylquinoline (15b). The reaction was carried out according to General Procedure VII using **14** (1.02 g, 4.92 mmol), Ni(dppp)Cl_2 (128.0 mg, 0.24 mmol), EtMgBr (3.2 mL, 3 M in Et_2O) and anhydrous THF (24 mL). Purification by flash column chromatography (2% EtOAc in pentane) afforded the target compound (580 mg, 3.70 mmol, 75%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (dd, J = 4.2, 1.8 Hz, 1H), 8.14 (dd, J = 8.2, 1.8 Hz, 1H), 7.66 (dd, J = 8.1, 1.5 Hz, 1H), 7.58 (dd, J = 7.1, 1.5 Hz, 1H), 7.48 (dd, J = 8.1, 7.1 Hz, 1H), 7.39 (dd, J = 8.2, 4.2 Hz, 1H), 3.32 (q, J = 7.5 Hz, 2H), 1.40 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.4, 146.9, 143.1, 136.5, 128.5, 128.0, 126.6, 125.9, 120.9, 24.7, 15.2.

8-Propylquinoline (15c). The reaction was carried out according to General Procedure VII using **14** (1.04 g, 5.0 mmol), Ni(dppp)Cl_2 (130.0 mg, 0.25 mmol), *n*-PrMgCl (5 mL, 2 M in Et_2O) and anhydrous THF (25 mL). Purification by flash column chromatography (2% EtOAc in pentane) afforded the target compound (592 mg, 3.50 mmol, 70%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (dd, J = 4.2, 1.8 Hz, 1H), 8.12 (dd, J = 8.2, 1.8 Hz, 1H), 7.66 (dd, J = 8.1, 1.2 Hz, 1H), 7.56 (dd, J = 7.1, 1.2 Hz, 1H), 7.46 (dd, J = 8.1, 7.1 Hz, 1H), 7.37 (dd, J = 8.2, 4.2 Hz, 1H), 3.26 (t, J = 7.4 Hz, 2H), 1.84 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.3, 147.0, 141.5, 136.4, 128.9, 128.5, 126.4, 126.0, 120.9, 33.6, 23.8, 14.4.

8-Isopropylquinoline (15d). $Pd(PPh_3)_4$ (275 mg, 0.21 mmol) was added to a solution of **14** (1.24 g, 6.0 mmol) in THF (25 mL). After evacuating and backfilling with N_2, isopropenylmagnesium bromide (0.75 M in THF, 16.0 mL, 12.0 mmol) was added dropwise at 0 °C. The resulted reaction mixture was at 45 °C for 2.5 h. After cooling down, the reaction mixture was quenched by 1 M HCl (1 mL), the aqueous phase was extracted by Et_2O (50 mL × 3), the combined organic phase was dried over Na_2SO_4 and filtered. Excess solvent was removed. Purification by flash column chromatography (3% EtOAc in pentane) afforded **8-isopropenylquinoline** (720 mg, 4.25 mmol, 72%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (dd, J = 4.1, 1.9 Hz, 1H), 8.11 (dd, J = 8.3, 1.9 Hz, 1H), 7.71 (dd, J = 8.1, 1.5 Hz, 1H), 7.57 (dd, J = 7.1, 1.6 Hz, 1H), 7.47 (dd, J = 8.2, 7.2 Hz, 1H), 7.36 (dd, J = 8.3, 4.3 Hz, 1H), 5.39 (s, 1H), 5.17 (s, 1H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.7, 146.1, 145.9, 143.5, 136.2, 128.4, 128.3, 127.2, 126.3, 120.8, 115.7, 24.7.

To a solution of **8-isopropenylquinoline** (720 mg, 4.2 mmol) in MeOH (18 mL) was added 10% Pd/C (112 mg), the flask was evacuated and backfilled with H_2 three times, the resulted mixture was allowed to stir for 3 h at rt under H_2 balloon. The reaction mixture was filtered through a Celite pad with ethyl acetate as the washing solvent (60 mL), the filtrate was washed by sat. aq. NH_4Cl and brine, dried over Na_2SO_4 and filtered. Excess solvent was removed. Purification by flash column chromatography (3% EtOAc in pentane) afforded the target product (352 mg, 2.2 mmol, 52%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (dd, J = 4.3, 1.9 Hz, 1H), 8.14 (dd, J = 8.3, 1.9 Hz, 1H), 7.66 (dd, J = 8.0, 1.5 Hz, 1H), 7.62 (dd, J = 7.2, 1.4 Hz, 1H), 7.52 (dd, J = 8.0, 7.2 Hz, 1H), 7.39 (dd, J = 8.3, 4.2 Hz, 1H), 4.36 (m, 1H), 1.40 (d, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.1, 147.3, 146.1, 136.4, 128.3, 126.5, 125.6, 125.1, 120.7, 27.2, 23.5.

3-Iodo-8-methylquinoline (16a). The reaction was carried out according to General Procedure VIII using **15a** (510 mg, 3.56 mmol), *N*-iodosuccinimide (1.19 g, 5.28 mmol) and HOAc (15 mL). Purification by flash column chromatography (15% DCM in pentane) afforded the target compound (560 mg, 2.08 mmol, 58%) as a light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (d, J = 2.1 Hz, 1H), 8.50 (d, J = 2.1 Hz, 1H), 7.57 (dd, J = 7.0, 1.4 Hz, 1H), 7.54 (dd, J = 8.3, 1.4 Hz, 1H), 7.44 (dd, J = 8.3, 7.0 Hz, 1H), 2.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.5, 145.6, 144.0, 137.6, 130.3, 130.2, 127.4, 125.0, 90.0, 18.0.

3-Iodo-8-Ethylquinoline (16b). The reaction was carried out according to General Procedure VIII **15b** (512 mg, 3.18 mmol), *N*-iodosuccinimide (1.12 g, 4.98 mmol) and HOAc (15 mL). Purification by flash column chromatography (15% DCM in pentane) afforded the target compound (426 mg, 1.51 mmol, 47%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (d, J = 2.2 Hz, 1H), 8.48 (d, J = 2.2 Hz, 1H), 7.57 (dd, J = 6.8, 1.7 Hz, 1H), 7.52 (dd, J = 8.2, 1.7 Hz, 1H), 7.47 (dd, J = 8.2, 6.8 Hz, 1H), 3.26 (q, J = 7.5 Hz, 2H), 1.37 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 145.0, 144.0, 143.4, 130.2, 128.6, 127.5, 124.9, 90.0, 24.6, 15.2.

3-Iodo-8-Propylquinoline (16c). The reaction was carried out according to General Procedure VIII **15c** (559 mg, 3.28 mmol), *N*-iodosuccinimide (1.08 g, 4.80 mmol) and HOAc (16 mL). Purification by flash column chromatography (15% DCM in pentane) afforded the target compound (451 mg, 1.45 mmol, 46%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (d, J = 2.2 Hz, 1H), 8.49 (d, J = 2.2 Hz, 1H), 7.57–7.52 (m, 2H), 7.47 (dd, J = 8.3, 6.8 Hz, 1H), 3.19 (t, J = 7.5 Hz, 2 H), 1.79 (m, J = 7.4 Hz, 2H), 1.01 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 145.2, 144.00, 141.9, 130.3, 129.5, 127.4, 125.0, 90.0, 33.5, 23.9, 14.3.

3-Iodo-8-Isopropylquinoline (16d). The reaction was carried out according to General Procedure VIII using **15d** (528 mg, 3.1 mmol), *N*-iodosuccinimide (1.0 g, 4.53 mmol) and HOAc (16 mL). Purification by flash column chromatography (15% DCM in pentane) afforded the target compound (484 mg, 1.64 mmol, 52%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, J = 2.3 Hz, 1H), 8.50 (d, J = 2.2 Hz, 1H), 7.64–7.59 (m, 1H), 7.51 (s, 1H), 7.50 (d, J = 1.8 Hz, 1H), 4.27 (m, 1H), 1.37 (d, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.2, 147.7, 144.2, 143.9, 130.1, 127.4, 125.7, 124.5, 89.9, 27.2, 23.4.

Ethyl 3-((8-methylquinolin-3-yl)ethynyl)benzoate (17a). To an oven-dried microwave vial was added **9n** (313 mg, 1.80 mmol), RuPhos (75 mg, 0.15 mmol), Pd(MeCN)$_2$$Cl_2$ (13 mg, 0.05 mmol), Cs_2CO_3 (1.0 g, 3.05 mmol) and **16a** (404 mg, 1.50 mmol), MeCN (10 mL), and the reaction was carried out according to General Procedure III. Purification by flash column chromatography (5% EtOAc in pentane) afforded the target compound (405 mg, 1.29 mmol, 86%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, J = 2.1 Hz, 1H), 8.36 (d, J = 2.1 Hz, 1H), 8.28 (ddd, J = 1.7, 0.5 Hz, 1H), 8.06 (ddd, J = 7.9, 1.7, 1.4 Hz, 1H), 7.77 (ddd, J. = 7.9, 1.7, 1.4 Hz, 1H), 7.68 (d, J = 7.7 Hz, 1H), 7.61 (d, J = 7.7 Hz, 1H), 7.53–7.49 (dd, J = 7.7 Hz, 1H), 7.49–7.44 (ddd, J = 7.9, 0.5 Hz, 1H), 4.42 (q, J = 7.1 Hz, 2H), 2.85 (s, 3H, 1.43 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 150.9, 146.2, 138.9, 137.4, 135.8, 132.9, 131.1, 130.7, 129.8, 128.7, 127.3, 127.3, 125.9, 123.3, 116.9, 91.5, 87.8, 61.4, 18.2, 14.5.

Ethyl 3-((8-ethylquinolin-3-yl)ethynyl)benzoate (17b). To an oven-dried microwave vial was added **9n** (313 mg, 1.80 mmol), RuPhos (75 mg, 0.15 mmol), Pd(MeCN)$_2$$Cl_2$ (13 mg, 0.05 mmol),

Cs_2CO_3 (1.0 g, 3.05 mmol), **16b** (425 mg, 1.50 mmol), MeCN (10 mL), and the reaction was carried out according to General Procedure III. Purification by flash column chromatography (5% EtOAc in pentane) to afford the target compound (352 mg, 1.07 mmol, 71%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, J = 2.1 Hz, 1H), 8.31 (d, J = 2.1 Hz, 1H), 8.27 (ddd, J = 1.7, 0.6 Hz, 1H), 8.05 (ddd, J = 7.9, 1.7, 1.4 Hz, 1H), 7.76 (ddd, J = 7.6, 1.7, 1.4 Hz, 1H), 7.65 (dd, J = 7.9, 1.2 Hz, 1H), 7.59 (dd, J = 7, 1.2 Hz, 1H), 7.51 (dd, J = 7.9, 7 Hz, 1H), 7.47 (ddd, J = 7.9, 7.6, 0.6 Hz, 1H), 4.42 (q, J = 7.2 Hz, 2H), 3.31 (q, J = 7.5 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H) 1.40 (t, J = 7.5 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 150.9, 145.6, 143.2, 139.0, 135.8, 133.0, 131.1, 129.8, 129.0, 128.7, 127.4, 127.4, 125.8, 123.3, 116.8, 91.4, 87.8, 61.4, 24.7, 15.2, 14.5.

Ethyl 3-((8-propylquinoline-3-yl)ethynyl)benzoate (17c). To an oven-dried microwave vial was added **9n** (313 mg, 1.80 mmol), RuPhos (75 mg, 0.15 mmol), $Pd(MeCN)_2Cl_2$ (13 mg, 0.05 mmol), Cs_2CO_3 (1.0 g, 3.05 mmol), **16c** (446 mg, 1.50 mmol), MeCN (10 mL), and the reaction was carried out according to General Procedure III. Purification by flash column chromatography (4% EtOAc in pentane) afforded the target compound (401 mg, 1.17 mmol, 78%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, J = 2.2 Hz, 1H), 8.30 (d, J = 2.2, Hz, 1H), 8.27 (ddd, J = 1.7, 0.6 Hz, 1H), 8.05 (ddd, J = 7.8, 1.7, 1.2 Hz, 1H), 7.76 (ddd, J = 7.8, 1.7, 1.2 Hz, 1H), 7.65 (dd, J = 8.0, 1.5 Hz, 1H), 7.57 (dd, J = 7.1, 1.5 Hz, 1H), 7.52−7.49 (m, 1H), 7.50−7.42 (m, 1H). 4.42 (q, J = 7.1 Hz, 2H), 3.25 (t, J = 7.2 Hz, 2H), 1.82 (m, 2H), 1.43 (t, J = 7.1 Hz, 3H), 1.04 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 150.7, 145.6, 141.5, 138.9, 135.7, 132.8, 130.9, 129.7, 129.6, 128.6, 127.3, 127.1, 125.7, 123.1, 116.6, 91.3, 87.7, 61.3, 33.4, 23.8, 14.3, 14.2.

Ethyl 3-((8-isopropylquinoline-3-yl)ethynyl)benzoate (17d). To an oven-dried microwave vial was added, **9n** (313 mg, 1.80 mmol), RuPhos (75 mg, 0.15 mmol), $Pd(MeCN)_2Cl_2$ (13 mg, 0.05 mmol), Cs_2CO_3 (1.0 g, 3.05 mmol), **16d** (446 mg, 1.50 mmol), MeCN (10 mL), and the reaction was carried out according to General Procedure III. Purification by flash column chromatography (4% EtOAc in pentane) afforded the target compound (429 mg, 1.25 mmol, 83%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (d, J = 2.2 Hz, 1H), 8.29 (d, J = 2.2 Hz, 1H), 8.27 (t, J = 1.7 Hz, 1H), 8.04 (ddd, J = 1.3, 1.7, 7.9 Hz, 1H), 7.75 (ddd, J = 1.2, 1.7, 7.7 Hz, 1H), 7.64 (s, 1H), 7.62 (s, 1H), 7.53 (dd, J = 7.1, 8.2 Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 4.41 (q, J = 7.2, 2H), 4.33 (m, 1H), 1.42 (t, J = 7.3, 3H), 1.39 (d, J = 6.8, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 150.6, 147.5, 144.8, 138.9, 135.7, 132.8, 130.9, 129.6, 128.6, 127.30, 127.26, 126.1, 125.4, 123.1, 116.6, 91.3, 87.7, 61.3, 27.2, 23.5, 14.3.

Ethyl (*E*)-3-(2-(8-methylquinolin-3-yl)vinyl)benzoate (18 a*E*). The reaction was carried out according to General Procedure V using **17a** (158 mg, 0.50 mmol), $RuCl_2(PPh_3)_3$ (28 mg, 0.03 mmol), CuI (10 mg, 0.05 mmol), Zn (92 mg, 1.5 mmol), water (75 μL, 4.0 mmol) and 1,4-dioxane (6 mL). Purification by flash column chromatography (8% EtOAc in pentane) afforded the target compound (90 mg, 0.28 mmol, 58%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (d, J = 2.1 Hz, 1H), 8.25 (d, J = 1.7 Hz, 1H), 8.17 (d, J = 2.1 Hz, 1H), 7.98 (ddd, J = 7.7, 1.7, 0.9 Hz, 1H), 7.74 (ddd, J = 7.9, 1.7, 0.9 Hz, 1H), 7.68 (dd, J = 7.6, 1.0 Hz, 1H), 7.54 (dd, J = 7.0, 1.0 Hz, 1H), 7.49−7.46 (m, 1H), 7.45−7.42 (m, 1H), 7.33 (d, J = 16 Hz, 2H), 4.42 (q, J = 7.1 Hz, 2H), 2.83 (s, 3H), 1.44 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 148.1, 146.6, 137.1, 137.0, 132.9, 131.1, 130.8, 129.7, 129.6, 129.0, 128.8, 128.0, 127.6, 126.9, 126.5, 126.0, 61.2, 18.10, 14.4.

Ethyl (*E*)-3-(2-(8-ethylquinoline-3-yl)vinyl)benzoate (18bE). The reaction was carried out according to General Procedure V using **17b** (165 mg, 0.50 mmol), $RuCl_2(PPh_3)_3$ (28 mg, 0.03 mmol), CuI (10 mg, 0.05 mmol), Zn (92 mg, 1.5 mmol), water (75 μL, 4.0 mmol) and 1,4-dioxane (6 mL). Purification by flash column chromatography (8% EtOAc in pentane) afforded the target compound (86 mg, 0.26 mmol, 52%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (d, J = 2.3 Hz, 1H), 8.26 (dd, J = 1.7, 0.8 Hz, 1H), 8.18 (d, J = 2.3 Hz, 1H), 7.98 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.75 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.68 (dd, J = 8.1, 1.2 Hz, 1H), 7.55 (dd, J = 7.1, 1.2 Hz, 1H), 7.50−7.48 (m, 1H), 7.48−7.45 (m, 1H), 7.34 (d, J = 15 Hz, 2H), 4.43 (q, J = 7.1 Hz, 2H), 3.32 (q, J = 7.5 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H), 1.40 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 148.1, 146.1, 142.9, 137.2, 132.9, 131.1, 130.8, 129.51, 129.49, 129.0, 128.8, 128.1, 128.0, 127.6, 127.0, 126.6, 125.9, 61.2, 24.6, 15.0, 14.4.

Ethyl (*E*)-3-(2-(8-propylquinoline-3-yl)vinyl)benzoate (18 c*E*). The reaction was carried out according to General Procedure V using **17c** (172 mg, 0.50 mmol), $RuCl_2(PPh_3)_3$ (28 mg, 0.03 mmol), CuI (10 mg, 0.05 mmol), Zn (92 mg, 1.5 mmol), water (75 μL, 4.0 mmol) and 1,4-dioxane (6 mL). Purification by flash column chromatography (6% EtOAc in pentane) afforded the target compound (84 mg, 0.24 mmol, 49%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (d, J = 2.3 Hz, 1H), 8.26 (d, J = 1.5 Hz, 1H), 8.16 (d, J = 2.3 Hz, 1H), 7.98 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.77−7.71 (m, 1H), 7.67 (dd, J = 8.0, 1.2 Hz, 1H), 7.53 (dd, J = 7.0, 1.2 Hz, 1H), 7.49−7.45 (m, 2H), 7.33 (s, 2H), 4.43 (q, J = 7.1 Hz, 2H), 3.25 (t, J = 5 Hz, 2H), 1.83 (m, 2H), 1.44 (t, J = 7.1 Hz, 3H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 148.1, 146.2, 141.4, 137.2, 132.9, 131.1, 130.8, 129.5, 129.4, 129.4, 129.0, 128.9, 128.8, 128.2, 127.6, 126.8, 126.6, 125.9, 61.2, 33.5, 23.8, 14.4, 14.2.

Ethyl (*E*)-3-(2-(8-isopropylquinoline-3-yl)vinyl)benzoate (18 d*E*). The reaction was carried out according to General Procedure V using **17d** (172 mg, 0.50 mmol), $RuCl_2(PPh_3)_3$ (28 mg, 0.03 mmol), CuI (10 mg, 0.05 mmol), Zn (92 mg, 1.5 mmol), water (75 μL, 4.0 mmol) and 1,4-dioxane (6 mL). Purification by flash column chromatography (5% EtOAc in pentane) afforded the target compound (69 mg, 0.20 mmol, 40%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (d, J = 2.3 Hz, 1H), 8.26 (t, J = 1.6 Hz, 1H), 8.16 (d, J = 2.3 Hz, 1H), 7.97 (ddd, J = 7.8, 1.4, 1.1 Hz, 1H), 7.73 (dt, J = 8.0, 1.5 Hz, 1H), 7.66 (dd, J = 8.0, 1.3 Hz, 1H), 7.59 (dd, J = 7.2, 1.6 Hz, 1H), 7.54−7.49 (m, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 16.9 Hz, 1H), 7.30 (d, J = 16.9 Hz, 1H), 4.42 (q, J = 7.4 Hz, 2H), 4.35 (m, 1H), 1.43 (t, J = 7.3 Hz, 3H), 1.41 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 148.0, 147.3, 145.4, 137.1, 133.1, 131.1, 130.8, 129.42, 129.40, 129.0, 128.8, 128.1, 127.6, 127.0, 126.6, 125.7, 125.2, 61.2, 27.3, 23.5, 14.4.

(*E*)-3-(2-(8-Methylquinoline-3-yl)vinyl)benzoic acid (19 a*E*). A solution of **18a** (90 mg, 0.28 mmol) and LiOH (48.0 mg, 1.12 mmol) in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) was reacted according to General Procedure IA. The target compound (67 mg, 0.23 mmol, 83%) was obtained as a light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.07 (s, 1H), 9.25 (d, J = 2.3 Hz, 1H), 8.50 (d, J = 2.3 Hz, 1H), 8.24 (d, J = 1.8 Hz, 1H), 7.93 (dd, J = 7.8, 2.9 Hz, 1H), 7.88 (dd, J = 7.7, 2.9 Hz, 1H), 7.79 (dd, J = 8.2, 1.7 Hz, 1H), 7.66 (d, J = 16.6 Hz, 1 H), 7.61−7.54 (m, 3H), 7.50 (dd, J = 8.2, 7.0 Hz, 1H), 2.73 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 149.0, 146.4, 137.6, 136.8, 133.1, 131.9, 131.1, 130.2, 130.1, 129.9, 129.6, 129.2, 128.1, 127.9, 127.3, 126.8, 126.6, 18.1.

(*E*)-3-(2-(8-Ethylquinoline-3-yl)vinyl)benzoic acid (19bE). A solution of **18b** (86 mg, 0.26 mmol) and LiOH (43 mg, 1.04 mmol) in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) was reacted according to General Procedure I. The target compound (58 mg, 0.19 mmol, 72%) was obtained as a light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.06 (s, 1H), 9.24 (d, J = 2.2 Hz, 1H), 8.48 (d, J = 2.2 Hz, 1H), 8.22 (d, J = 1.7 Hz, 1H), 7.91 (dd, J = 7.8, 1.4 Hz, 1H), 7.87 (dd, J = 7.7, 1.4 Hz, 1H), 7.78 (dd, J = 8.1, 1.7 Hz, 1H), 7.65 (d, J = 16.6 Hz, 1H), 7.59−7.49 (m, 4H), 3.21 (q, J = 7.5 Hz, 2H), 1.29 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 149.0, 145.7, 142.6, 137.6, 133.3, 131.8, 131.1, 130.2, 130.0, 129.6, 129.2, 128.4, 128.2, 127.9, 127.4, 126.8, 126.6, 24.4, 15.8.

(*E*)-3-(2-(8-Propylquinoline-3-yl)vinyl)benzoic acid (19 c*E*). A solution of **18c** (84 mg, 0.24 mmol) and LiOH (40 mg, 0.96 mmol) in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) was reacted according to General Procedure IA. The target compound (53 mg, 0.17 mmol, 68%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, 1H), 9.23 (d, J = 2.3 Hz, 1H), 8.46 (d, J = 2.2 Hz, 1H), 8.21 (s, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.77 (dd, J = 7.8, 1.7 Hz, 1H), 7.64 (d, J = 16.4 Hz, 1H), 7.57–7.45 (m, 4H), 3.15 (t, J = 7.3 Hz, 2H), 1.71 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 149.0, 145.9, 140.9, 137.6, 133.3, 131.8, 131.1, 130.1, 130.0, 129.6, 129.23, 129.16, 128.2, 127.9, 127.2, 126.8, 126.7, 33.4, 24.1, 14.5.

(*E*)-3-(2-(8-Isopropylquinoline-3-yl)vinyl)benzoic acid (19 d*E*). A solution of **18d** (69 mg, 0.20 mmol) and LiOH (33 mg, 0.80 mmol) in THF/H_2O/MeOH (2.6 mL, 1:1:0.2) was reacted according to General Procedure IA. The target compound (57 mg, 0.18 mmol, 90%) as obtained as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.45 (s, 1H), 8.25 (s, 1H), 7.89 (d, J = 6.7 Hz, 1H), 7.85 (d, J = 6.7 Hz, 1H), 7.76 (d, J = 7.3, 1H), 7.70–7.42 (m, 5H), 4.25 (m, 1H), 1.31 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 148.8, 146.8, 145.0, 137.6, 133.4, 131.9, 131.0, 130.05, 129.99, 129.5, 129.2, 128.1, 127.9, 127.4, 126.8, 126.4, 125.5, 27.1, 23.8.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-methylquinolin-3-yl)vinyl)benzamide (20 a*E*). To an oven-dried microwave vial was added **19a** (45 mg, 0.15 mmol), EDC·HCl (120 mg, 0.63 mmol), HOBt (41 mg, 0.31 mmol), Et_3N (123 mg, 180 μL, 1.24 mmol), **7** (50 mg, 0.18 mmol), DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (6% MeOH in DCM) afforded the target compound (49 mg, 0.09 mmol, 58%) as a yellow solid. m.p. 74–78 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.11 (d, J = 2.3 Hz, 1H), 8.17 (br s, 1H), 8.12 (d, J = 2.3 Hz, 1H), 8.08 (t, J = 1.7 Hz, 1H), 7.92 (dd, J = 8.4, 2.2 Hz, 1H), 7.89 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.75 (ddd, J = 7.8, 1.7, 1.0 Hz, 1H), 7.72 (ddd, J = 7.8, 1.7, 1.0 Hz, 1H), 7.68–7.64 (m, 1H), 7.53 (ddd, J = 7.1, 1.4, 1.0 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.46 (dd, J = 8.1, 7.0 Hz, 1H), 7.37 (s, 2H), 3.68 (s, 2H), 2.83 (s, 3H), 2.70–2.36 (m, 8H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 148.1, 146.7, 137.7, 137.0, 136.6, 135.0, 133.8, 133.0, 131.4, 130.1, 129.8, 129.4 (q, $^2J_{C\text{-}F}$ = 30.3 Hz), 129.34, 129.27, 129.2, 128.0, 127.0, 126.9, 126.2, 126.0, 125.4, 123.3, 122.7 (q, $^1J_{C\text{-}F}$ = 277.8 Hz), 117.6 (q [3]$J_{C\text{-}F}$ = 6.2 Hz), 57.8, 55.2, 53.0, 46.0, 18.1. HRMS (ESI-TOF) *m/z* calcd for $C_{32}H_{31}F_3N_4O^+$ [M + H]$^+$: 545.2523, found: 545.2529.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-ethylquinolin-3-yl)vinyl)benzamide (20bE). To an oven-dried microwave vial was added **19b** (50 mg, 0.17 mmol), EDC·HCl (127 mg, 0.66 mmol), HOBt (45 mg, 0.33 mmol), Et_3N (133 mg, 200 μL, 1.32 mmol), **7** (55 mg, 0.20 mmol), DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (6% MeOH in DCM) afforded the target compound (60 mg, 0.11 mmol, 63%) as a yellow solid. m.p. 70–74 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (d, J = 2.3 Hz, 1H), 8.15 (d, J = 2.4 Hz, 1H), 8.09 (t, J = 1.7 Hz, 1H), 8.04 (br s, 1H), 7.92 (dd, J = 8.4, 2.2 Hz, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.78–7.73 (m, 2H), 7.67 (dd, J = 8.1, 1.2 Hz, 1H), 7.55 (ddd, J = 7.2, 1.6, 0.8 Hz, 1H), 7.53–7.46 (m, 2H), 7.33 (s, 2H), 3.64 (s, 2H), 3.31 (q, J = 7.6 Hz, 2H), 2.62–2.37 (m, 8H), 2.31 (s, 3H), 1.40 (t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 148.0, 146.1, 142.9, 137.8, 136.5, 135.1, 133.9, 133.1, 131.4, 130.1, 129.30, 129.29, 129.2 (q, $^2J_{C\text{-}F}$ = 30.3 Hz), 129.1, 128.10, 128.08, 127.1, 127.0, 126.1, 125.9, 125.4, 123.3, 122.9 (q, $^1J_{C\text{-}F}$ = 274.8 Hz), 117.6 (q [3]$J_{C\text{-}F}$ = 6.2 Hz), 57.8, 55.2, 53.1, 46.0, 24.6, 15.0. HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{33}F_3N_4O^+$ [M + H]$^+$: 559.2679, found: 559.2692.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-propylquinoline-3-yl)vinyl)benzamide (20 c*E*). To an oven-dried microwave vial was added **19c** (50 mg, 0.16 mmol), EDC·HCl (121 mg, 0.63 mmol), HOBt (42 mg, 0.32 mmol), Et_3N (130 mg, 180 μL, 1.28 mmol), **7** (52 mg, 0.19 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in DCM) afforded the target compound (64 mg, 0.11 mmol, 72%) as a yellow solid. m.p. 74–78 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, J = 2.3 Hz, 1H), 8.46 (br s, 1H), 8.03 (t, J = 1.7 Hz, 1H), 8.02 (d, J = 2.3 Hz, 1H), 7.91 (dd, J = 8.4, 2.0 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.72 (ddd, J = 7.9, 1.7, 1.0 Hz, 1H), 7.65 (dt, J = 7.9, 1.3 Hz, 1H), 7.60 (dd, J = 8.1, 1.7 Hz, 1H), 7.49 (dd, J = 7.2, 1.6 Hz, 1H), 7.46–7.38 (m, 2H), 7.20 (s, 2H), 3.61 (s, 2H), 3.21 (m, 2H), 2.65–2.34 (m, 8H), 2.28 (s, 3H), 1.80 (m, 2H), 1.01 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 147.9, 146.2, 141.3, 137.6, 136.6, 135.0, 133.8, 133.0, 131.3, 130.1, 129.3 (q, $^2J_{C\text{-}F}$ = 30.3 Hz), 129.2, 129.0, 128.9, 128.1, 126.9, 126.8, 126.3, 126.0, 125.3, 123.4, 122.7 (q, $^1J_{C\text{-}F}$ = 274.8 Hz), 117.7 (q [3]$J_{C\text{-}F}$ = 6.2 Hz), 57.8, 55.2, 53.0, 46.0, 33.5, 23.8, 14.2. HRMS (ESI-TOF) *m/z* calcd for $C_{34}H_{35}F_3N_4O^+$ [M + H]$^+$: 573.2836, found: 573.2847.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-isopropylquinoline-3-yl)vinyl) benzamide (20 d*E*). To an oven-dried microwave vial was added **19d** (50 mg, 0.16 mmol), EDC·HCl (121 mg, 0.63 mmol), HOBt (42 mg, 0.32 mmol), Et_3N (130 mg, 180 μL, 1.28 mmol) **7** (52 mg, 0.19 mmol), anhydrous DCM (2 mL) and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in DCM) afforded the target compound (62 mg, 0.11 mmol, 70%) as a yellow solid. m.p. 75–79 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (d, J = 2.4 Hz, 1 H), 8.55 (br s, 1H), 8.12 (t, J = 1.8 Hz, 1H), 8.09 (d, J = 2.3 Hz, 1H), 7.98–7.92 (m, 2H), 7.79 (dt, J = 7.7, 1.3 Hz, 1H), 7.71–7.65 (m, 2H), 7.62 (dd, J = 8.1, 1.6 Hz, 1H), 7.57 (dd, J = 7.3, 1.5 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.31 (d, J = 16.6 Hz, 1H), 7.27 (d, J = 16.6 Hz, 1H), 4.31 (m, 1 H), 3.62 (s, 2H), 2.81–2.45 (m, 8H), 2.38 (s, 3H), 1.38 (d, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 147.9, 147.3, 145.4, 137.6, 137.0, 134.9, 133.2, 133.1, 131.4, 130.1, 129.4 (q, $^2J_{C\text{-}F}$ = 30.8 Hz), 129.2, 129.0, 128.0, 127.1, 127.0, 126.4, 125.7, 125.4, 125.3, 123.5, 122.7 (q, $^1J_{C\text{-}F}$ = 273.6 Hz), 117.9 (q [3]$J_{C\text{-}F}$ = 6.1 Hz), 57.7, 54.8, 52.1, 45.3, 27.3, 23.5. HRMS (ESI-TOF) *m/z* calcd for $C_{34}H_{35}F_3N_4O^+$ [M + H]$^+$: 573.2836, found: 573.2841.

Ethyl (*Z*)-3-(2-(8-methylquinolin-3-yl)vinyl)benzoate (18a*Z*) VI. The reaction was carried out according to General Procedure VI using **17a** (127 mg, 0.40 mmol) and MeOH (9 mL). Purification by flash column chromatography (4% EtOAc in pentane) afforded the target compound (101 mg, 0.32 mmol, 79%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, J = 2.2 Hz, 1H), 7.96 (d, J = 3.4 Hz, 2H), 7.90 (dt, J = 7.8, 1.5 Hz, 1H), 7.51 (dt, J = 7.6, 0.9 Hz, 2H), 7.43–7.36 (m, 2H), 7.32–7.23 (m, 1H), 6.84 (d, J = 12.2 Hz, 1H), 6.79 (d, J = 12.2, Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 2.76 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 149.8, 145.9, 136.9, 136.8, 135.6, 132.9, 131.6, 131.0, 130.0, 129.8, 129.7, 128.7, 128.6, 127.7, 127.7, 126.6, 125.9, 61.0, 18.1, 14.1.

Ethyl (*Z*)-3-(2-(8-ethylquinolin-3-yl)vinyl)benzoate (18b*Z*). The reaction was carried out according to General Procedure VI using **17b** (181 mg, 0.55 mmol) and EtOH (11 mL). Purification by flash column chromatography (4% EtOAc in pentane) afforded the target compound (107 mg, 0.32 mmol, 59%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (d, J = 2.2 Hz, 1H), 7.97–7.94 (m, 2H), 7.90 (dt, J = 7.7, 1.5 Hz, 1H), 7.54–7.48 (m, 2H), 7.45–7.40 (m, 2H), 7.28 (d, J = 7.8 Hz, 1H), 6.84 (d, J = 12.1 Hz, 1H), 6.79 (d, J = 12.1 Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 3.25 (q, J = 7.5 Hz, 2H), 1.36 (t, J = 7.5 Hz, 3H), 1.25 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 149.9, 145.4, 142.7, 137.0, 135.5, 132.9, 131.5, 130.9, 130.0, 129.6, 128.7, 128.6, 128.0, 127.8, 127.8, 126.7, 125.8, 60.9, 24.5, 15.0, 14.1.

Ethyl (*Z*)-3-(2-(8-propylquinolin-3-yl)vinyl)benzoate (18c*Z*). The reaction was carried out according to General Procedure VI using **17c** (200 mg, 0.58 mmol) and EtOH (12 mL). Purification by flash column chromatography (4% EtOAc in pentane) afforded the target compound (104 mg, 0.30 mmol, 52%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, J = 2.3 Hz, 1H), 7.97–7.92 (m, 2H), 7.90 (dt, J = 7.8, 1.5 Hz, 1H), 7.52–7.47 (m, 2H), 7.43–7.38 (m, 2H), 7.27 (t, J = 7.7 Hz, 1H), 6.82 (d, J = 12.3 Hz, 1H), 6.77 (d, J = 12.3 Hz, 1H), 4.29 (q, J = 7.2 Hz, 2H), 3.23–3.14 (m, 2H), 1.84–1.73 (m, 1H), 1.25 (t, J = 7.2 Hz, 3H), 1.00 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 149.8, 145.6, 141.1, 137.0, 135.4, 132.9, 131.4, 130.9, 130.0, 129.5, 128.8, 128.7, 128.6, 127.83, 127.79, 126.5, 125.8, 60.9, 33.4, 23.7, 14.2, 14.1.

Ethyl (*Z*)-3-(2-(8-isopropylquinolin-3-yl)vinyl)benzoate (18d*Z*). The reaction was carried out according to General Procedure VI using **17d** (200 mg, 0.58 mmol) and EtOH (12 mL). Purification by flash column chromatography (4% EtOAc in pentane) afforded the target compound (86 mg, 0.25 mmol, 43%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, J = 2.3 Hz, 1H), 7.98–7.94 (m, 2H), 7.90 (dt, J = 7.8, 1.5 Hz, 1H), 7.57 (dd, J = 6.8, 1.8 Hz, 1H), 7.52–7.45 (m, 2H), 7.45–7.40 (m, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.84 (d, J = 12.3 Hz, 1H), 6.78 (d, J = 12.3 Hz, 1H), 4.33–4.20 (m, 3H), 1.37 (d, J = 6.9 Hz, 6H), 1.25 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 149.8, 147.1, 144.8, 137.0, 135.5, 132.9, 131.4, 130.9, 130.0, 129.5, 128.7, 128.6, 127.80, 127.78, 126.7, 126.6, 125.2, 60.9, 27.2, 23.5, 14.1.

(*Z*)-3-(2-(8-Methylquinolin-3-yl)vinyl)benzoic acid (19a*Z*). A solution of **18a*Z*** (90 mg, 0.28 mmol) and LiOH (48.0 mg, 1.12 mmol) in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) reacted according to General Procedure IA. The target product (59 mg, 0.20 mmol, 73%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.62 (d, J = 2.2 Hz, 1H), 8.15 (d, J = 2.2 Hz, 1H), 7.84 (d, J = 1.8 Hz, 1H), 7.81 (dd, J = 7.5, 1.6 Hz, 1 H), 7.66 (dd, J = 8.2, 2.1 Hz, 1H), 7.56 (dd, J = 7.0, 2.5 Hz, 1H), 7.48–7.40 (m, 2H), 7.38 (t, J = 7.6 Hz, 1H), 6.93 (d, J = 12.2 Hz, 1H), 6.88 (d, J = 12.2 Hz, 1H), 2.64 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.4, 149.8, 145.7, 137.3, 136.7, 135.7, 133.2, 131.8, 131.6, 130.1, 130.1, 129.9, 129.4, 128.9, 128.2, 127.8, 127.2, 126.5, 18.0.

(*Z*)-3-(2-(8-Ethylquinolin-3-yl)vinyl)benzoic acid (19b*Z*). A solution of **18b*Z*** (90 mg, 0.27 mmol) and LiOH (45 mg, 1.09 mmol) in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) was reacted according to General Procedure IA. The target product (65 mg, 0.22 mmol, 80%) was obtained as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.90 (s, 1H), 8.63 (d, J = 2.4 Hz, 1H), 8.15 (d, J = 2.4 Hz, 1H), 7.84 (dd, J = 1.6, 0.8 Hz, 1H), 7.81 (dt, J = 7.7, 1.5 Hz, 1H), 7.66 (dd, J = 8.1, 1.5 Hz, 1H), 7.56 (dd, J = 7.1, 1.5 Hz, 1H), 7.49 (dd, J = 8.2, 7.1 Hz, 1H), 7.45 (dt, J = 7.7, 1.3 Hz, 1H), 7.39 (t, J = 7.7 Hz, 1H), 6.93 (d, J = 12.2 Hz, 1H), 6.88 (d, J = 12.2 Hz, 1H), 3.14 (q, J = 7.5 Hz, 2H), 1.24 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.4, 149.8, 145.1, 142.5, 137.3, 135.8, 133.2, 131.8, 131.6, 130.0, 129.8, 129.4, 128.9, 128.6, 128.2, 127.9, 127.3, 126.5, 24.3, 15.7.

(*Z*)-3-(2-(8-Propylquinolin-3-yl)vinyl)benzoic acid (19c*Z*). A solution of **18c*Z*** (90 mg, 0.26 mmol) and LiOH (42 mg, 0.10 mmol) dissolved in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) was reacted according to General Procedure IA. The target product (71 mg, 0.22 mmol, 86%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 2.3 Hz, 1H), 8.12 (d, J = 2.3 Hz, 1H), 7.84 (t, J = 1.8 Hz, 1H), 7.81 (dt, J = 7.8, 1.7 Hz, 1H), 7.64 (dd, J = 8.1, 1.7 Hz, 1H), 7.51 (dd, J = 7.2, 1.7 Hz, 1H), 7.47–7.41 (m, 2H), 7.37 (t, J = 7.5 Hz, 1H), 6.91 (d, J = 12.1 Hz, 1H), 6.85 (d, J = 12.1 Hz, 1H), 3.07 (m, 2H), 1.64 (m, 2H), 0.89 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.4, 149.8, 145.3, 140.8, 137.3, 135.7, 133.2, 131.7, 129.9, 129.8, 129.4, 129.3, 128.9, 128.1, 127.9, 127.1, 126.5, 33.2, 24.0, 14.5.

(*Z*)-3-(2-(8-Isopropylquinolin-3-yl)vinyl)benzoic acid (19d*Z*). A solution of **18d*Z*** (86 mg, 0.24 mmol) and LiOH (41 mg, 0.97 mmol) in THF/H_2O/MeOH (3.3 mL, 1:1:0.2) was reacted according to General Procedure IA. The target product (62 mg, 0.19 mmol, 81%) was obtained as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, J = 2.3 Hz, 1H), 8.08–8.01 (m, 2H), 7.95 (dt, J = 7.8, 1.5 Hz, 1H), 7.62 (dd, J = 6.5, 2.4 Hz, 1H), 7.55–7.47 (m, 2H), 7.45 (dt, J = 7.8, 1.5 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 6.88 (d, J = 12.1 Hz, 1H), 6.78 (d, J = 12.1 Hz, 1H), 4.25 (m, 1H), 1.35 (d, d, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 149.1, 146.1, 142.9, 137.3, 136.9, 133.4, 132.0, 130.5, 130.3, 129.6, 129.5, 128.8, 128.1, 127.4, 127.1, 126.3, 125.8, 27.3, 23.4.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-methylquinolin-3-yl)vinyl)benzamide (20a*Z*). To an oven-dried microwave vial was added **19a** (50 mg, 0.17 mmol), EDC·HCl (133 mg, 0.69 mmol), HOBt (45 mg, 0.34 mmol), Et_3N (135 mg, 200 μL, 1.36 mmol) **7** (56 mg, 0.20 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (6% MeOH in DCM) provided the taget compound (3 mg, 0.07 mmol, 42%) as a yellow solid. m.p. 66–70 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (d, J = 2.1 Hz, 1H), 7.97 (d, J = 2.1 Hz, 1H), 7.75 (m, 1H), 7.72–7.66 (m, 4H), 7.59 (dd, J = 8.5, 2.3 Hz, 1H), 7.55–7.49 (m, 2H), 7.43–7.37 (m, 2H), 7.35–7.29 (m, 1H), 6.83 (d, J = 12.6 Hz, 1H), 6.80 (d, J = 12.4 Hz, 1H), 3.59 (s, 2H), 2.73 (s, 3H), 2.58–2.37 (m, 8H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.6, 149.7, 146.0, 137.4, 136.9, 136.5, 135.6, 134.9, 133.6, 132.2, 131.3, 131.2, 130.0, 129.6, 129.1, 129.0 (q, $^2J_{C\text{-}F}$ = 30.4 Hz), 128.0, 127.7, 127.3, 126.8, 126.4, 125.8, 123.2, 122.9 (q, $^1J_{C\text{-}F}$ = 275.3 Hz), 117.5 (q $^3J_{C\text{-}F}$ = 6.1 Hz), 57.7, 55.2, 53.1, 46.0, 18.0. HRMS (ESI-TOF) *m/z* calcd for $C_{32}H_{31}F_3N_4O^+$ $[M + H]^+$: 545.2523, found: 545.2543.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-ethylquinolin-3-yl)vinyl)benzamide (20b*Z*). To an oven-dried microwave vial was added **19b*Z*** (50 mg, 0.17 mmol), EDC·HCl (127 mg, 0.66 mmol), HOBt (45 mg, 0.33 mmol), Et_3N (133 mg, 200 μL, 1.32 mmol), **7** (55 mg, 0.20 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (6% MeOH in DCM) afforded the target compound (55 mg, 0.10 mmol, 56%) as a yellow solid. M.M. 69–73 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (d, J = 2.3 Hz, 1H), 7.98 (d, J = 2.3 Hz, 1H), 7.73–7.65 (m, 5H), 7.57–7.51 (m, 3H), 7.45 (d, J = 7.4 Hz, 1H), 7.42 (dt, J = 8.4, 1.5 Hz, 1H), 7.32 (dt, J = 7.2, 1.3 Hz, 1H), 6.83 (d, J = 12.3 Hz, 1H), 6.80 (d, J = 12.3 Hz, 1H), 3.59 (s, 2H), 3.22 (q, J = 7.5 Hz, 2H), 2.58–2.36 (m, 8H), 2.29 (s, 3H), 1.32 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.5, 149.8, 145.4, 142.8, 137.4, 136.4, 135.6, 134.9, 133.7, 132.3, 131.22, 131.20, 129.5, 129.2, 129.0 (q, $^2J_{C\text{-}F}$ = 31.4 Hz), 128.2, 128.1, 127.8, 127.2, 127.0, 126.4, 125.7, 123.1, 122.9 (q, $^1J_{C\text{-}F}$ = 274.9 Hz), 117.4 (q $^3J_{C\text{-}F}$ = 6.1 Hz), 57.4, 55.2, 53.1, 46.0, 24.4, 14.9. HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{33}F_3N_4O^+$ $[M + H]^+$: 559.2679, found: 559.2701.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-propylquinolin-3-yl)vinyl)benzamide (20c*Z*). To an oven-dried microwave vial was added **19c*Z*** (50 mg, 0.16 mmol), EDC·HCl (121 mg, 0.63 mmol), HOBt (42 mg, 0.32 mmol), Et_3N (130 mg, 180 μL, 1.28 mmol) **7** (52 mg, 0.19 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure II. Purification by flash column chromatography (7% MeOH in DCM) afforded the target compound (65 mg, 0.12 mmol, 66%) as a yellow solid. m.p. 60–65 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 2.3 Hz, 1H), 7.95 (d, J = 2.3 Hz, 1H), 7.93 (br s, 1H), 7.73–7.63 (m, 4H), 7.56 (dd, J = 8.5, 2.2 Hz, 1H), 7.53–7.47 (m, 2H), 7.42 (d, J = 7.5 Hz, 1H), 7.41–7.37 (m, 1H), 7.27 (t, J = 8.2 Hz, 1H), 6.77 (s, 2H), 3.58 (s, 2H), 3.14 (m, 2H), 2.48 (m, 8H), 2.29 (s, 3H), 1.74 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 149.7, 145.5, 141.2, 137.4, 136.5, 135.6, 134.9, 133.6, 132.2, 131.18, 131.16, 129.5, 129.14, 129.06, 128.9 (q, $^2J_{C\text{-}F}$ = 30.4 Hz),

128.1, 127.8, 127.3, 126.8, 126.4, 125.8, 123.2, 122.6 (q, $^{1}J_{C\text{-}F}$ = 274.9 Hz), 117.6 (q $^{3}J_{C\text{-}F}$ = 6.1 Hz), 57.7, 55.2, 53.0, 46.0, 33.3, 23.6, 14.2. HRMS (ESI-TOF) *m/z* calcd for $C_{34}H_{35}F_3N_4O^+$ $[M + H]^+$: 573.2836, found: 573.2840.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(8-isopropylquinolin-3-yl)vinyl) benzamide (20d*Z*). To an oven-dried microwave vial was added **19d*Z*** (50 mg, 0.16 mmol), EDC·HCl (121 mg, 0.63 mmol), HOBt (42 mg, 0.32 mmol), Et_3N (130 mg, 180 μL, 1.28 mmol), **7** (52 mg, 0.19 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure II. Purification by flash column chromatography (7% MeOH in DCM) afforded the target compound (74 mg, 0.14 mmol, 75%) as a yellow solid. m.p. 65–69 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, *J* = 2.3 Hz, 1H), 7.98 (d, *J* = 2.3 Hz, 1H), 7.84 (br s, 1H), 7.76–7.69 (m, 3H), 7.64 (d, *J* = 8.3 Hz, 1H), 7.57 (dd, *J* = 6.8, 1.9 Hz, 1H), 7.52 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.50 (d, *J* = 7.4 Hz, 1H), 7.48 (dd, *J* = 8.2, 6.8 Hz, 1H), 7.41 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 12.2 Hz, 1H), 6.78 (d, *J* = 12.2 Hz, 1H), 4.21 (m, 1H), 3.59 (s, 2H), 2.62–2.41 (m, 8H), 2.33 (s, 3H), 1.31 (d, *J* = 7.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 149.7, 147.2, 144.7, 137.4, 136.5, 135.7, 134.8, 133.4, 132.3, 131.21, 131.20, 129.5, 129.2, 129.0 (q, $^{2}J_{C\text{-}F}$ = 30.6 Hz), 128.1, 127.8, 127.3, 127.0, 126.4, 125.54, 125.46, 123.2, 122.6 (q, $^{1}J_{C\text{-}F}$ = 275.3 Hz), 117.5 (q $^{3}J_{C\text{-}F}$ = 6.1 Hz), 57.7, 55.1, 52.7, 45.8, 27.2, 23.4. HRMS (ESI-TOF) *m/z* calcd for $C_{34}H_{35}F_3N_4O^+$ $[M + H]^+$: 573.2836, found: 573.2844.

(*E*)-methyl 3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) vinyl) benzoate (21a) A dry sealed vial was charged with **9m** (400 mg, 2.31 mmol), benzoic acid (15 mg, 0.1 mmol), HBPin (1.4 mL, 9.4 mmol) and heptane (4.5 mL). The vial was backfilled with N_2 and left to stir at 100 °C for 12 h. The solvent was removed under reduced pressure. Remove the solvent, the residue as light-yellow oil was used for next step without further purification.

(*E*)-ethyl 3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) vinyl) benzoate (21b) A dry sealed vial was charged with **9n** (400 mg, 2.29 mmol), benzoic acid (14 mg, 0.1 mmol), HBPin (1.3 mL, 9.2 mmol) and heptane (4.5 mL). The vial was backfilled with N_2 and left to stir at 100 °C for 12 h. The solvent was removed under reduced pressure. Remove the solvent, the residue as light-yellow oil was used for next step without further purification.

3-Iodo-4-methoxyquinoline (23a). To a solution of **22** (520 mg, 1.80 mmol) in MeOH (10 mL) was added NaOMe (580 mg, 10.80 mmol) under N_2. After evacuating and backfilling with N_2, the reaction mixture was allowed to stir at 75 °C for 3 h. Then H_2O (10 mL) was added to dilute the reaction mixture which was then extracted by EtOAc (15 mL × 2), washed by H_2O (20 mL) and brine (30 mL), dried over Na_2SO_4 and filtered. Excess solvent was removed. Purification by flash column chromatography (16% EtOAc in pentane) provided the target compound (461 mg, 1.62 mmol, 90%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.11–8.05 (m, 2H), 7.73 (ddd, *J* = 8.5, 6.9, 1.5 Hz, 1H), 7.56 (ddd, *J* = 8.2, 6.9, 1.5 Hz, 1H), 4.06 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 157.6, 149.5, 130.2, 129.6, 127.1, 124.8, 121.9, 84.5, 62.0.

3-Iodo-*N*,*N*-dimethylquinolin-4-amine (23b). To a microwave vial was added **22** (600 mg, 2.07 mmol) and 33% Me_2NH in EtOH solution (12 mL, 22.2 mmol). After evacuating and backfilling with N_2, the reaction mixture was allowed to stir at 100 °C overnight. Excess solvent was removed. Purification by flash column chromatography (16% EtOAc in pentane) afforded the target compound (590 mg, 1.98 mmol, 96%) as a light brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.07–8.00 (m, 2H), 7.65 (ddd, *J* = 8.5, 6.9, 1.5 Hz, 1H), 7.49 (ddd, *J* = 8.2, 6.9, 1.5 Hz, 1H), 3.12 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7, 158.4, 149.0, 129.9, 129.4, 129.1, 126.2, 124.3, 90.0, 43.4.

3-Iodo-4-(pyrrolidin-1-yl)quinoline (23c). To a microwave vial was added **22** (600 mg, 2.07 mmol) and neat pyrrolidine (4 mL). After evacuating and backfilling with N_2, the reaction mixture was submitted to microwave reactor at 110 °C for 45 min. Excess of pyrrolidine was removed. Purification by flash column chromatography (16% EtOAc in pentane) afforded the target compound (660 mg, 2.03 mmol, 98%) as a light brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.05 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.98 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.66 (ddd, *J* = 8.3, 6.7, 1.4 Hz, 1H), 7.48 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 3.52 (m, 4H), 2.15 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 155.4, 149.0, 129.9, 129.7, 129.5, 126.3, 124.0, 92.7, 51.1, 26.6.

3-Iodo-*N*-phenylquinolin-4-amine (23d). To a microwave vial was added **22** (520 mg, 1.80 mmol) and neat aniline (3.5 mL). After evacuating and backfilling with N_2, the reaction mixture was submitted to microwave reactor at 150 °C for 15 min. Excess aniline was removed. Purification by flash column chromatography (40% DCM in pentane) afforded the target compound (545 mg, 1.57 mmol, 88%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.05 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.67 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.64 (ddd, *J* = 8.3, 6.8, 1.4 Hz, 1H), 7.29 (ddd, *J* = 8.4, 6.8, 1.3 Hz, 1H), 7.27–7.21 (m, 2H), 7.07–7.01 (m, 1H), 6.89–6.83 (2H), 6.38 (s, 1H), 3.52 (m, 4H), 2.15 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.6, 148.8, 147.5, 143.3, 129.8, 129.7, 129.4, 125.8, 124.8, 123.0, 122.9, 119.2, 86.9.

Methyl (*E*)-3-(2-(4-methoxyquinolin-3-yl)vinyl)benzoate (24a*E*). The reaction was carried out according to General Procedure IV using **23a** (430 mg, 1.50 mmol), **21a** (520 mg, 1.80 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), RuPhos (58 mg, 0.12 mmol), K_3PO_4 (960 mg, 4.52 mmol), H_2O (140 μL, 7.58 mmol) and 1,4-dioxane (10 mL). Purification by flash column chromatography (40% EtOAc in pentane) afforded the target compound (395 mg, 1.24 mmol, 82%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (br s, 1H), 8.27 (t, *J* = 1.8 Hz, 1H), 8.14 (dt, *J* = 8.3, 0.9 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.97 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.77 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.69 (ddd, *J* = 8.3, 6.9, 1.5 Hz, 1H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 16.7 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 16.6, Hz, 1H), 4.04 (s, 3H), 3.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 160.5, 150.1, 149.3, 137.4, 133.7, 130.9, 130.7, 130.3, 129.6, 129.1, 128.9, 127.7, 126.8, 123.6, 122.2, 121.8, 121.3, 62.6, 52.3.

Methyl (*E*)-3-(2-(4-(dimethylamino)quinolin-3-yl)vinyl)benzoate (24b*E*). The reaction was carried out according to General Procedure IV using **23b** (460 mg, 1.52 mmol), **21a** (520 mg, 1.80 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), RuPhos (58 mg, 0.12 mmol), K_3PO_4 (960 mg, 4.52 mmol), H_2O (140 μL, 7.77 mmol) and 1,4-dioxane (10 mL). Purification by flash column chromatography (33% EtOAc in pentane) afforded the target compound (410 mg, 1.18 mmol, 77%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.24 (t, *J* = 1.8 Hz, 1H), 8.08 (dd, *J* = 8.4, 1.5 Hz, 1H), 8.05 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.96 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.74 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.62 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.52–7.40 (m, 3H), 7.08 (d, *J* = 16.5, Hz, 1H), 3.96 (s, 3H), 3.21 (s, 6H), ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 154.2, 150.7, 149.4, 137.8, 130.7, 129.9, 128.88, 128.84, 128.80, 128.7, 127.5, 126.1, 126.0, 125.7, 124.8, 124.7, 52.3, 44.7.

Ethyl (*E*)-3-(2-(4-(Pyrrolidin-1-yl)quinolin-3-yl)vinyl)benzoate (24c*E*). The reaction was carried out according to General Procedure IV using **23c** (480 mg, 1.48 mmol), **21b** (540 mg, 1.80 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), RuPhos (58 mg, 0.12 mmol), K_3PO_4 (960 mg, 4.52 mmol), H_2O (140 μL, 7.77 mmol) and 1,4-dioxane (10 mL). Purification by flash column chromatography (50% EtOAc in pentane) afforded the target compound (440 mg, 1.18 mmol, 80%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.22 (t, *J* = 1.7 Hz, 1H), 8.07–8.02 (m, 2H), 7.95 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.70 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.61 (ddd, *J* = 8.3, 6.8, 1.5 Hz, 1H), 7.46 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 16.5, Hz, 1H), 7.09 (d, *J* = 16.5, Hz, 1H), 4.41 (q, *J* = 7.1, Hz, 2H), 3.60 (m, 4H), 2.14 (m, 4H), 1.42 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5, 151.3, 150.5, 149.1, 137.8, 131.0, 130.6,

129.8, 128.82, 128.80, 128.76, 128.7, 127.4, 126.6, 125.9, 125.6, 125.2, 124.6, 61.1, 53.0, 26.4, 14.3.

Ethyl (*E*)-3-(2-(4-(Phenylamino)quinolin-3-yl)vinyl)benzoate (24d*E*). The reaction was carried out according to General Procedure IV using **23d** (520 mg, 1.50 mmol), **21b** (540 mg, 1.80 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), RuPhos (58 mg, 0.12 mmol), K_3PO_4 (960 mg, 4.52 mmol), H_2O (140 μL, 7.77 mmol) and 1,4-dioxane (10 mL). Purification by flash column chromatography (42% EtOAc in pentane) afforded the target compound (490 mg, 1.24 mmol, 83%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.08 (dd, *J* = 8.5, 1.2 Hz, 1H) 7.98 (t, *J* = 1.7 Hz, 1H), 7.91(dd, *J* = 8.4, 1.2 Hz, 1H), 7.88 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.63 (ddd, *J* = 8.4, 7.0, 1.4 Hz, 1H), 7.45 (dt, *J* = 7.9, 1.6 Hz, 1H), 7.41 (ddd, *J* = 8.3, 6.9, 1.2 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.25–7.16 (m, 3H), 7.12 (d, *J* = 16.5, Hz, 1H), 6.93 (tt, *J* = 8.5, 1.1 Hz, 1H), 6.87–6.82 (m, 2H), 6.54 (br s, 1H), 4.36 (q, *J* = 7.2, Hz, 2H), 1.39 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 149.8, 148.6, 144.2, 142.4, 137.3, 131.0, 130.5, 130.2, 129.9, 129.34, 129.32, 128.9, 128.7, 127.7, 126.4, 123.9, 123.6, 123.2, 122.0, 121.4, 117.4, 61.1, 14.3.

(*E*)-3-(2-(4-Methoxyquinolin-3-yl)vinyl)benzoic acid (25a*E*). A solution of **24a*E*** (160 mg, 0.50 mmol) and LiOH (60 mg, 2.51 mmol) dissolved in THF/H_2O/MeOH (1:1:0.2, 3.3 mL) was reacted according to General Procedure IA. The target compound (114 mg, 0.37 mmol, 75%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.19 (t, *J* = 1.7 Hz, 1H), 8.14 (ddd, *J* = 8.4, 1.5, 0.7 Hz, 1H), 8.04 (dt, *J* = 8.3, 0.8 Hz, 1H), 7.97 (dt, *J* = 7.9, 1.5 Hz, 1H), 7.88 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.76 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.70 (d, *J* = 16.6, Hz, 1H), 7.65 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.57 (d, *J* = 16.5 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 4.05 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 160.1, 150.8, 149.1, 137.8, 131.8, 131.0, 130.9, 130.2, 129.60, 129.58, 129.3, 128.2, 127.5, 123.4, 122.6, 121.50, 121.48, 63.2.

(*E*)-3-(2-(4-(Dimethylamino)quinolin-3-yl)vinyl)benzoic acid (25b*E*). A solution of **24b*E*** (150 mg, 0.43 mmol) and LiOH (52 mg, 2.17 mmol) in THF/H_2O/MeOH (1:1:0.2, 3.3 mL) was reacted according to General Procedure IA. The target compound (67 mg, 0.21 mmol, 48%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.16 (t, *J* = 1.8 Hz, 1H), 8.10 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.96 (dt, *J* = 8.0, 1.5 Hz, 1H), 7.93 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.85 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.65 (ddd, *J* = 8.3, 6.7, 1.4 Hz, 1H), 7.57–7.48 (m, 3H), 7.34 (d, *J* = 16.7, Hz, 1H), 3.16 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 153.8, 151.0, 149.2, 138.1, 131.9, 130.7, 129.9, 129.5, 129.3, 129.2, 129.0, 128.0, 126.3, 125.91, 125.88, 125.4, 124.6, 44.8.

(*E*)-3-(2-(4-(Pyrrolidin-1-yl)quinolin-3-yl)vinyl)benzoic acid (25c*E*). A solution of **24c*E*** (120 mg, 0.32 mmol) and LiOH (40 mg, 1.68 mmol) in THF/H_2O/MeOH (1:1:0.2, 3.3 mL) was reacted according to General Procedure IB. The target compound (86 mg, 0.25 mmol, 78%) was obtained as a yellow solid. ^{1}H NMR (700 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.22 (t, *J* = 1.8 Hz, 1H), 8.16 (dd, *J* = 8.5, 1.4 Hz, 1H), 8.02 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.73 (ddd, *J* = 8.4, 6.7, 1.4 Hz, 1H), 7.61–7.59 (ddd, *J* = 8.3, 6.8, 1.3 Hz, 1 H), 7.60–7.58 (t, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 16.7 Hz, 1H), 7.42 (d, *J* = 16.7 Hz, 1H), 3.62 (m, 4H), 2.16 (m, 4H). ^{13}C NMR (351 MHz, DMSO-d_6) δ 168.0, 150.9, 149.0, 138.0, 132.0, 130.4, 129.8, 129.5, 129.4, 129.3, 129.0, 127.9, 126.2, 126.1, 125.7, 125.2, 124.9.53.1, 26.3.

(*E*)-3-(2-(4-(Phenylamino)quinolin-3-yl)vinyl)benzoic acid (25d*E*). A solution of **24d*E*** (140 mg, 0.35 mmol) and LiOH (43 mg, 1.80 mmol) in THF/H_2O/MeOH (1:1:0.2, 3.3 mL) was reacted according to General Procedure IA. The target compound (94 mg, 0.26 mmol, 73%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H), 8.04 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.74 (t, *J* = 1.8 Hz, 1H), 7.53 (dt, *J* = 7.5, 1.3 Hz, 1H), 7.34 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.19/7.17/7.15 (ddd, *J* = 8.3, 6.6, 1.3 Hz, 1H), 7.18/7.14 (d, *J* = 16.3 Hz, 1H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.97–6.90 (m, 2H), 6.87 (dt, *J* = 7.9, 1.5 Hz, 1H), 6.81 (ddd, *J* = 8.3, 6.6, 1.4 Hz, 1H), 6.75 (d, *J* = 16.3 Hz, 1H), 6.55–6.49 (m, 2 H), 6.41 (tt, *J* = 7.3, 1.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.4, 151.4, 142.6, 140.9, 138.0, 137.3, 133.4, 131.6, 130.6, 129,9, 129.5, 129.2, 129.1, 127.9, 127.6, 126.1, 125.2, 123.9, 123.4, 121.0, 119.9, 115.6.

(*E*)-3-(2-(4-Methoxyquinolin-3-yl)vinyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl) phenyl)benzamide (26a*E*). To an oven-dried microwave vial was added **25a*E*** (50 mg, 0.16 mmol), EDC·HCl (126 mg, 0.65 mmol), HOBt (45 mg, 0.33 mmol), Et_3N (138 mg, 190 μL, 1.33 mmol), **7** (54 mg, 0.20 mmol) anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in DCM) provided the target compound (45 mg, 0.08 mmol, 49%) as a yellow solid. m.p. 85–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.44 (s, 1H), 8.14 (m, 3H), 7.95–7.89 (m, 2H), 7.79–7.74 (m, 2H), 7.72 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.67 (ddd, *J* = 8.3, 6.8, 1.5 Hz, 1H), 7.55 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.50/7.46 (d, *J* = 16.7 Hz, 1H), 7.49/7.47/7.45 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 16.7 Hz, 1H), 4.02 (s, 3H), 3.63 (s, 2H), 2.64–2.36 (m, 8H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 160.6, 150.0, 149.2, 137.8, 136.7, 135.1, 133.7, 131.4, 130.1, 130.0, 129.7, 129.4, 129.2, 129.1 (q, $^2J_{C\text{-}F}$ = 30.5 Hz), 126.9, 126.4, 125.43, 125.40/122.7 (q, $^1J_{C\text{-}F}$ = 274.3 Hz), 123.5, 123.4, 122.2, 122.1, 121.1, 117.7 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 62.7, 57.8, 55.1, 53.0, 45.9. HRMS (ESI-TOF) *m/z* calcd for $C_{32}H_{32}F_3N_4O_2^+$ $[M + H]^+$: 561.2472, found: 561.2480.

(*E*)-3-(2-(4-(Dimethylamino)quinolin-3-yl)vinyl)-*N*-(4-((4-methylpiperazin-1-yl) methyl)-3-(trifluoromethyl)phenyl) benzamide (26b*E*). To an oven-dried microwave vial was added **25b*E*** (60 mg, 0.19 mmol), EDC·HCl (150 mg, 0.78 mmol), HOBt (52 mg, 0.38 mmol), Et_3N (152 mg, 210 μL, 1.51 mmol), **7** (62 mg, 0.23 mmol), anhydrous DCM (2.5 mL) and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in DCM) afforded the target compound (41 mg, 0.07 mmol, 38%) as a yellow solid. m.p. 78–82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.52 (s, 1H), 8.10 (t, *J* = 1.8 Hz, 1H), 8.05 (dd, *J* = 8.7, 1.5 Hz, 1H), 8.02 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.94–7.90 (m, 2 H), 7.79–7.73 (m, 2H), 7.70 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.61 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.50/7.48/7.45 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.49/7.47/7.45 (t, *J* = 7.7 Hz, 1H), 7.42 (d, *J* = 16.5 Hz, 1H), 7.02 (d, *J* = 16.5 Hz, 1H), 3.63 (s, 2H), 3.18 (s, 6H), 2.67–2.35 (m, 8H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 154.4, 150.5, 149.3, 138.3, 136.8, 135.2, 133.7, 131.4, 129.9, 129.74, 129.67, 129.2, 129.1 (q, $^2J_{C\text{-}F}$ = 30.7 Hz), 128.9, 128.6, 126.4, 126.05, 125.98, 125.8, 125.3, 124.9, 124.5, 123.4, 122.7 (q, $^1J_{C\text{-}F}$ = 274.5 Hz), 117.7 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 57.8, 55.1, 53.0, 45.9, 44.7. HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{35}F_3N_5O^+$ $[M + H]^+$: 574.2788, found: 574.2791.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-(pyrrolidin-1-yl)quinolin-3-yl)vinyl)benzamide (26c*E*). To an oven-dried microwave vial was added **25c*E*** (52 mg, 0.15 mmol), HBTU (60 mg, 0.16 mmol), DIPEA (60 mg, 80 μL, 0.46 mmol), **7** (50 mg, 0.18 mmol), anhydrous DMF (2 mL), and the reaction was carried out according to General Procedure IIB. Purification by flash column chromatography (10% MeOH in DCM) afforded the target compound (33 mg, 0.06 mmol, 36%) as a yellow solid. m.p. 76–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.74 (s, 1H), 8.12 (t, *J* = 1.8 Hz, 1H), 8.05 (dd, *J* = 8.7, 1.5 Hz, 1H), 8.02 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.94–7.90 (m, 2H), 8.02/8.00 (d, *J* = 7.8 Hz, 1H), 8.01–7.99 (d, *J* = 7.8 Hz, 1H), 7.97–7.92 (m, 2H), 7.78 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.59 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.49–7.41 (m, 2H), 7.36 (d, *J* = 16.5 Hz, 1H), 6.96 (d, *J* = 16.5 Hz, 1H), 3.63 (s, 2H), 3.58 (m, 4H), 2.67–2.36 (m, 8H), 2.31 (s, 3H), 2.07 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 151.8, 149.8, 148.4, 138.1, 136.9, 135.1, 133.5, 131.3, 129.8, 129.3 (q, $^2J_{C\text{-}F}$ = 30.7 Hz), 129.2, 129.07, 129,04, 128.6, 126.4, 126.2, 125.7, 125.5, 125.2, 124.8, 124.0, 123.5, 122.7 (q,

$^{1}J_{C\text{-}F}$ = 274.5 Hz), 117.7 (q $^{3}J_{C\text{-}F}$ = 6.2 Hz), 57.8, 55.1, 53.2, 52.9, 45.9, 26.3. HRMS (ESI-TOF) *m/z* calcd for $C_{35}H_{37}F_3N_5O^+$ [M + H]$^+$: 600.2945, found: 600.2949.

(*E*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-(phenylamino)quinolin-3-yl)vinyl)benzamide (26d*E*). To an oven-dried microwave vial was added **25d*E*** (55 mg, 0.15 mmol), EDC·HCl (94 mg, 0.61 mmol), HOBt (41 mg, 0.30 mmol), Et_3N (123 mg, 170 μL, 1.22 mmol), **7** (52 mg, 0.18 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in DCM) afforded the target compound (61 mg, 0.10 mmol, 65%) as a yellow solid. m.p. 103−108 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.75 (s, 1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.94−7.87 (m, 2H), 7.84 (dd, d, *J* = 8.4, 1.4 Hz, 1H), 7.81 (s, 1H), 7.70 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.58 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.39−7.32 (m, 2H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 16.6 Hz, 1H), 7.18−7.11 (m, 2H), 6.97 (d, *J* = 16.6 Hz, 1H), 6.90−6.84 (m, 2H), 6.84−6.78 (m, 2H), 3.56 (s, 2H), 2.61−2.37 (m, 8H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 149.3, 148.4, 144.3, 142.8, 137.6, 137.0, 134.7, 133.1, 131.3, 130.2, 129.5, 129.4, 129.2, 129.1, 128.9 (q, $^{2}J_{C\text{-}F}$ = 30.4 Hz), 126.8, 126.3, 124.7, 124.4, 123.60, 123.55, 123.4, 122.7 (q, $^{1}J_{C\text{-}F}$ = 274.3 Hz), 121.5, 121.3, 118.0 (q $^{3}J_{C\text{-}F}$ = 6.2 Hz), 117.6, 57.7, 55.0, 52.4, 45.5. HRMS (ESI-TOF) *m/z* calcd for $C_{37}H_{35}F_3N_5O^+$ [M + H]$^+$: 622.2788, found: 622.2794.

Methyl (*Z*)-3-(2-(4-methoxyquinolin-3-yl)vinyl)benzoate (24a*Z*). The reaction was carried out according to General Procedure IX using **24a*E*** (70 mg, 0.22 mmol) and distilled MeCN (18 mL). The *E/Z* isomers was separated by PLC silica gel plate with pentane/EtOAc (2:1) affording the target compound (38 mg, 0.12 mmol, 54%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.16 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.99 (dt, *J* = 8.5, 0.8 Hz, 1H), 7.92 (t, *J* = 2.1 Hz, 1H), 7.85 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.67 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.53 (ddd, *J* = 8.3, 6.8, 1.3 Hz, 1H), 7.36 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.23 (t, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 12.1 Hz, 1H), 6.85 (d, *J* = 12.2, Hz, 1H), 4.11 (s, 3 H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 161.1, 152.6, 148.9, 136.7, 132.7, 131.4, 130.5, 130.3, 129.7, 129.2, 128.7, 128.6, 126.4, 124.2, 123.4, 122.1, 119.1, 61.7, 52.1.

Methyl (*Z*)-3-(2-(4-(dimethylamino)quinolin-3-yl)vinyl)benzoate (24b*Z*). The reaction was carried out according to General Procedure IX using **24b*E*** (80 mg, 0.24 mmol) in distilled MeCN (20 mL). The *E/Z* isomers was separated by PLC silica gel plate with pentane/EtOAc (2:1) affording the target compound (41 mg, 0.12 mmol, 51%) as alight yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.08 (ddd, *J* = 8.4, 1.4, 0.6 Hz, 1H), 7.96 (ddd, *J* = 8.4, 1.4, 0.6 Hz, 1H), 7.88 (td, *J* = 1.8, 0.6 Hz, 1H), 7.81 (ddd, *J* = 7.8, 1.8, 1.3 Hz, 1H), 7.61 (ddd, *J* = 8.3, 6.8, 1.4 Hz, 1H), 7.47 (ddd, *J* = 8.3, 6.8, 1.4 Hz, 1H), 7.34 (ddd, *J* = 7.8, 1.8, 1.3 Hz, 1H), 7.18 (td, *J* = 7.7, 0.6 Hz, 1H), 6.76 (s, 2H), 3.79 (s, 3H), 3.12 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 154.9, 152.6, 149.0, 136.9, 132.8, 130.38, 130.36, 129.7, 129.2, 128.8, 128.5, 128.4, 128.2, 125.37, 125.35, 124.8, 122.8, 52.0, 44.2.

Ethyl (*Z*)-3-(2-(4-(pyrrolidin-1-yl)quinolin-3-yl)vinyl)benzoate (24c*Z*). The reaction was carried out according to General Procedure IX using **24c*E*** (80 mg, 0.21 mmol) and distilled MeCN (20 mL). The *E/Z* isomers was separated by PLC silica gel plate with pentane/EtOAc (1:1) affording the target compound (31 mg, 0.08 mmol, 39%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.10 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.94 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.86 (t, *J* = 1.8 Hz, 1H), 7.81 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.59 (ddd, *J* = 8.3, 6.8, 1.4 Hz, 1H), 7.42 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.35 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 6.77 (d, *J* = 11.9 Hz, 1H), 6.72 (d, *J* = 11.9 Hz, 1H), 4.22 (q, *J* = 7.3 Hz, 2H), 3.63 (m, 4H), 1.98 (m, 4H), 1.18 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 152.6, 151.8, 148.9, 136.9, 132.9, 130.6, 130.2, 129.5, 129.0, 128.7, 128.6, 128.34, 128.33, 125.3, 124.84, 124.82, 121.6, 60.9, 52.5, 26.0, 14.0.

Ethyl (*Z*)-3-(2-(4-(phenylamino)quinolin-3-yl)vinyl) benzoate (24d*Z*). The reaction was carried out according to General Procedure IX using **24d*E*** (90 mg, 0.23 mmol) and distilled MeCN (20 mL). The *E/Z* isomers was separated by PLC silica gel plate with pentane/EtOAc (2:1) affording the target compound (51 mg, 0.12 mmol, 57%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.01 (dd, *J* = 8.5, 1.3 Hz, 1H), 7.85 (t, *J* = 1.8 Hz, 1H), 7.82 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.78 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.60 (ddd, *J* = 8.5, 6.9, 1.6 Hz, 1H), 7.36−7.29 (m, 2H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.18−7.14 (m, 2H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.79−6.73 (m, 2H), 6.71 (d, *J* = 12.0 Hz, 1H), 6.57 (d, *J* = 12.0 Hz, 1H), 6.36 (s, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 151.7, 148.5, 143.7, 143.5, 136.4, 132.5, 132.1, 130.8, 129.9, 129.8, 129.2, 129.1, 128.8, 128.6, 125.6, 125.0, 123.8, 122.6, 121.9, 120.7, 118.3, 60.9, 14.0.

(Z)*-3-(2-(4-Methoxyquinolin-3-yl)vinyl)benzoic acid (25a*Z*).** A solution of **24a*Z (105 mg, 0.33 mmol) and LiOH (45 mg, 1.88 mmol) in THF/H_2O/MeOH (1:1:0.2, 2.6 mL) was reacted according to General Procedure IA. The target compound (74 mg, 0.24 mmol, 74%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.9 (br s, 1H), 8.36 (s, 1H), 8.14 (ddd, *J* = 8.4, 1.6, 0.7 Hz, 1H), 8.04 (ddd, *J* = 8.5, 1.3, 0.7 Hz, 1H), 7.82 (t, *J* = 1.8 Hz, 1H), 7.77 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.73 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.60 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.41 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 6.987 (d, *J* = 12.2, Hz, 1H), 6.94 (d, *J* = 12.2 Hz, 1H), 4.09 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.3, 160.8, 153.6, 152.6, 148.7, 137.1, 133.2, 131.7, 131.5, 131.1, 130.5, 130.3, 130.1, 129.5, 129.4, 129.2, 128.9, 127.2, 127.1, 124.8, 123.2, 122.8, 122.5, 119.9, 63.2.

(*Z*)-3-(2-(4-(Dimethylamino)quinolin-3-yl)vinyl)benzoic acid (25b*Z*). A solution of **24b*Z*** (98 mg, 0.28 mmol) and LiOH (35 mg, 1.46 mmol) in THF/H_2O/MeOH (1:1:0.2, 2.6 mL) was reacted according to General Procedure IB. The target compound (69 mg, 0.22 mmol, 77%) was obtained as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.09 (ddd, *J* = 8.5, 1.5, 0.6 Hz, 1H), 7.83 (ddd, *J* = 8.3, 1.4, 0.5 Hz, 1H), 7.80 (t, *J* = 1.6 Hz, 1H), 7.73 (dt, *J* = 7.7, 1.6 Hz, 1H), 7.63 (ddd, *J* = 8.3, 6.7, 1.4 Hz, 1H), 7.51 (ddd, *J* = 8.3, 6.7, 1.4 Hz, 1H), 7.39 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.31 (t, *J* = 7.7 Hz, 1H), 6.85 (s, 2H), 3.07 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.4, 154.7, 152.3, 148.8, 137.3, 133.3, 131.4, 130.1, 129.7, 129.3, 129.2, 129.1, 128.8, 128.6, 125.9, 125.3, 125.1, 122.7, 44.3.

(*Z*)-3-(2-(4-(Pyrrolidin-1-yl)quinolin-3-yl)vinyl)benzoic acid (25c*Z*). A solution of **24c*Z*** (150 mg, 0.40 mmol) and LiOH (50 mg, 2.09 mmol) in THF/H_2O/MeOH (1:1:0.2, 3.3 mL) was reacted according to General Procedure IB. The target compound (71 mg, 0.21 mmol, 51%) was obtained as a yellow solid. ^{1}H NMR (700 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.20 (dd, *J* = 8.6, 1.3 Hz, 1H), 8.03 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.85 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.80 (dt, *J* = 7.6, 1.7 Hz, 1H), 7.65 (t, *J* = 1.8 Hz, 1H), 7.54 (ddd, *J* = 8.5, 6.9, 1.4 Hz, 1H), 7.40 (dt, *J* = 7.8, 1.7 Hz, 1H), 7.31 (t, *J* = 7.7 Hz, 1H), 6.96 (d, *J* = 12.1 Hz, 1H), 6.82 (d, *J* = 12.0 Hz, 1H), 3.61 (m, 4H), 1.76 (m, 4H). ^{13}C NMR (351 MHz, DMSO-d_6) δ 167.3, 156.4, 142.4, 138.7, 136.9, 132.8, 132.4, 131.4, 129.08, 129.04, 128.8, 128.7, 127.3, 125.0, 119.5, 118.0, 110.6, 56.0, 25.4.

(*Z*)-3-(2-(4-(Phenylamino)quinolin-3-yl)vinyl)benzoic acid (25d*Z*). A solution of **24d*Z*** (128 mg, 0.32 mmol) and LiOH (39 mg, 1.62 mmol) in THF/H_2O/MeOH (1:1:0.2, 3.3 mL) was reacted according to General Procedure IA. The target compound (88 mg, 0.24 mmol, 74%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.35 (s, 1H), 8.10 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.87 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.82 (t, *J* = 1.8 Hz, 1H), 7.77 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.67 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.49 (ddd, *J* = 8.4, 6.8, 1.3 Hz, 1H), 7.46 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.18−7.11 (m, 2H), 6.87−6.79 (m, 3H), 6.60 (d, *J* = 12.1 Hz, 1H), 6.41 (d, *J* = 12.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.3, 150.8, 147.0, 145.6, 144.3, 137.2, 132.9, 131.4, 130.2, 129.8, 129.4, 129.2, 129.0, 128.6, 128.5, 126.8, 126.3, 124.2, 123.1, 121.5, 119.7, 118.7.

(*Z*)-3-(2-(4-Methoxyquinolin-3-yl)vinyl)-*N*-(4-((4-

methylpiperazin-1-yl)methyl)-3-(trifluoromethyl) phenyl)benzamide (26a*Z*). To an oven-dried microwave vial was added **25a*Z*** (52 mg, 0.17 mmol), HATU (72 mg, 0.19 mmol), DIPEA (72 mg, 90 μL, 0.52 mmol), **7** (56 mg, 0.20 mmol), anhydrous DMF (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (6% MeOH in DCM) afforded the target compound (39 mg, 0.07 mmol, 41%) as a yellow solid. m.p. 71–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.16 (dd, J = 8.3, 1.1 Hz, 1H), 8.03 (s, 1H), 7.98 (dt, J = 8.5, 0.9 Hz, 1H), 7.73–7.60 (m, 6H), 7.53 (ddd, J = 8.5, 6.8, 1.2 Hz, 1H), 7.35 (dt, J = 7.8, 1.5 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 6.90 (d, J = 12.1 Hz, 1H), 6.83 (d, J = 12.1 Hz, 1H), 3.12 (s, 3H), 2.14 (s, 2H), 2.63–2.33 (m, 8H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 161.2, 152.6, 148.8, 137.0, 136.6, 134.9, 133.4, 132.2, 131.3, 131.2, 129.9, 129.1, 129.0, 128.9 (q, $^2J_{C\text{-}F}$ = 30.4 Hz), 127.5, 126.7, 126.4, 124.5, 123.4, 123.3, 122.6 (q, $^1J_{C\text{-}F}$ = 274.6 Hz), 122.2, 119.7, 117.6 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 61.8, 57.7, 55.1, 52.9, 45.9. HRMS (ESI-TOF) *m/z* calcd for $C_{32}H_{31}F_3N_4O_2^+$ $[M + H]^+$: 561.2472, found: 561.2477.

(*Z*)-3-(2-(4-(Dimethylamino)quinolin-3-yl)vinyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)benzamide (26b*Z*). To an oven-dried microwave vial was added **25b*Z*** (50 mg, 0.16 mmol), EDC·HCl (121 mg, 0.63 mmol), HOBt (43 mg, 0.32 mmol), Et_3N (130 mg, 180 μL, 1.29 mmol), **7** (52 mg, 0.19 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7.5% MeOH in DCM) afforded the target compound (37 mg, 0.06 mmol, 41%) as a yellow solid. m.p. 69–73 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.07 (dd, J = 8.5, 1.5 Hz, 1H), 7.96 (dd, J = 8.5, 1.3 Hz, 1H), 7.76 (s, 1H), 7.73 (d, J = 2.3 Hz, 1H), 7.68–7.63 (m, 3H), 7.61 (ddd, J = 8.3, 6.8, 1.5 Hz, 1H), 7.53 (dd, J = 8.3, 2.3 Hz, 1H), 7.50 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.36 (dt, J = 7.8, 1.5 Hz, 1H), 7.28–7.22 (m, 1H), 6.81 (d, J = 12.1 Hz, 1H), 6.76 (d, J = 12.1 Hz, 1H), 3.59 (s, 2H), 3.16 (s, 6H), 2.61–2.36 (m, 8H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 155.0, 152.6, 148.9, 137.2, 136.5, 134.8, 133.5, 132.4, 131.1, 129.6, 129.04, 128.99,128.92 (q, $^2J_{C\text{-}F}$ = 30.4 Hz), 128.5, 127.3, 126.2, 125.6, 125.3, 124.9, 123.1, 122.7, 122.6 (q, $^1J_{C\text{-}F}$ = 274.2 Hz), 117.4 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 57.7, 55.2, 53.1, 46.0, 44.3. HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{35}F_3N_5O^+$ $[M + H]^+$: 574.2788, found: 574.2788.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-(pyrrolidin-1-yl)quinolin-3-yl)vinyl)benzamide (26c*Z*). To an oven-dried microwave vial was added **25c*Z*** (60 mg, 0.17 mmol), HATU (75 mg, 0.20 mmol) and DIPEA (75 mg, 100 μL, 0.58 mmol), **7** (56 mg, 0.20 mmol), anhydrous DMF (2 mL), and the reaction was carried out according to General Procedure IIB. Purification by flash column chromatography (10% MeOH in DCM) afforded the target compound (33 mg, 0.06 mmol, 32%) as a yellow solid. m.p. 69–73 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.09 (dd, J = 8.6, 1.4 Hz, 1H), 7.95 (dd, J = 8.4, 1.3 Hz, 1H), 7.75 (s, 1 H), 7.73 (d, J = 2.4 Hz, 1H), 7.67–7.61 (m, 3H), 7.58 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.49 (dd, J = 8.5, 2.4 Hz, 1H), 7.40 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.36 (dt, J = 7.8, 1.4 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 6.80 (d, J = 11.8 Hz, 1H), 6.71 (d, J = 11.8 Hz, 1H), 3.65 (m, 4H), 3.59 (s, 2H), 2.59–2.39 (m, 8H), 2.31 (s, 3H), 2.00 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 152.3, 152.1, 148.5, 137.2, 136.5, 134.7, 133.4, 132.4, 131.1, 129.2, 129.01, 128.98, 128.90, 128.64, 128.60 (q, $^2J_{C\text{-}F}$ = 30.7 Hz), 127.1, 126.2, 125.0, 124.93, 124.88, 123.1, 122.6 (q, $^1J_{C\text{-}F}$ = 274.7 Hz), 120.8, 117.5 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 57.7, 55.2, 52.9, 52.8, 45.9, 26.0. HRMS (ESI-TOF) *m/z* calcd for $C_{35}H_{37}F_3N_5O^+$ $[M + H]^+$: 600.2945, found: 600.2953.

(*Z*)-*N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-(phenylamino)quinolin-3-yl)vinyl)benzamide (26d*Z*). To an oven-dried microwave vial was added **25d*Z*** (55 mg, 0.15 mmol), EDC·HCl (94 mg, 0.61 mmol), HOBt (41 mg, 0.30 mmol), Et_3N (123 mg, 170 μL, 1.22 mmol), **7** (52 mg, 0.18 mmol), anhydrous DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (6% MeOH in DCM) afforded the target compound (53 mg, 0.09 mmol, 57%) as a yellow solid. m.p. 95–99 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.00 (d, J = 8.3 Hz, 1H), 7.81–7.74 (m, 2H), 7.69 (d, J = 2.0 Hz, 1H), 7.67–7.54 (m, 5H), 7.36–7.29 (m, 2H), 7.26–7.20 (m, 1H), 7.20–7.13 (m, 2H), 6.94 (t, J = 7.4 Hz, 1H), 6.80–6.74 (m, 2H), 6.70 (d, J = 12.2 Hz, 1H), 6.59 (d, J = 12.2 Hz, 1H), 6.34 (br s, 1H), 3.57 (s, 2H), 2.59–2.33 (m, 8H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 151.7, 148.4, 143.7, 143.5, 136.8, 136.5, 134.9, 133.5, 132.0, 131.9, 131.1, 129.7, 129.5, 129.1, 128.9 (q, $^2J_{C\text{-}F}$ = 30.4 Hz), 127.1, 126.5, 125.9, 125.2, 123.8, 123.1, 122.6 (q, $^1J_{C\text{-}F}$ = 274.3 Hz), 122.4, 122.1, 120.4, 118.4, 117.4 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 57.7, 55.1, 52.9, 45.9. HRMS (ESI-TOF) *m/z* calcd for $C_{37}H_{35}F_3N_5O^+$ $[M + H]^+$: 622.2788, found: 622.2783.

***tert*-Butyl 1-(3-(ethoxycarbonyl)phenyl)-2-(4-methoxyquinolin-3-yl)hydrazine-1-carboxylate (27).** To an oven-dried microwave vial was added **23e** (216 mg, 0.76 mmol), **2** (255 mg, 0.91 mmol), $Pd(OAc)_2$ (21 mg, 0.091 mmol), Xantphos (90 mg, 0.16 mmol) and Cs_2CO_3 (420 mg, 1.29 mmol). The vial was then capped and backfilled with N_2 3 times before toluene (6 mL) was added. The mixture was stirred at 110 °C for 18 h (TLC showed complete reaction). After cooling to rt, the reaction mixture was filter over Celite with EtOAc as eluent. Excess solvent was removed. Purification by flash column chromatography (pentane-EtOAc, 30% EtOAc) afforded the target compound (210 mg, 0.48 mmol, yield 63%) as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.32 (t, J = 2.1 Hz, 1 H), 8.04–7.96 (m, 2H), 7.87–7.81 (m, 2H), 7.57–7.49 (m, 2H), 7.43 (t, J = 7.9 Hz, 1H), 7.06 (s, 1H), 4.37 (t, J = 6.9 Hz, 2H), 4.07 (s, 3H), 1.39 (s, 9H), 1.38 (t, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 153.5, 146.8, 145.8, 143.0, 140.5, 133.3, 131.1, 129.7, 128.7, 127.0, 126.8, 126.5, 126.1, 123.3, 123.2, 120.6, 83.3, 61.3, 61.1, 28.1, 14.3.

Ethyl (*E*)-3-((4-methoxyquinolin-3-yl)diazenyl)benzoate (28). To a microwave vial was added **27** (210 mg, 0.48 mmol) and DMF (5 mL), the resulting mixture was then submitted to microwave reactor and stirred at 175 °C for 40 min. After cooling to rt, pure oxygen was backfilled into the vial 2 times, and the reaction was stirred at 100 °C and for 2 h. The reaction mixture was cooled to rt and excess solvent removed. Purification by flash column chromatography (pentane-EtOAc, 30% EtOAc) afforded the target compound (81 mg, 0.24 mmol, 50%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.55 (t, J = 1.9 Hz, 1H), 8.37 (ddd, J = 8.3, 1.5, 0.6 Hz, 1H), 8.16 (dt, J = 7.8, 1.4 Hz, 1H), 8.06 (ddd, J = 8.0, 2.0, 1.2 Hz, 1H), 8.04 (dt, J = 7.3, 1.0 Hz, 1H), 7.74 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 7.57 (ddd, J = 8.2, 6.9, 1.1 Hz, 1H), 4.63 (s, 3H), 4.42 (q, J = 7.2 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 159.9, 152.7, 149.7, 142.3, 132.7, 131.8, 131.6, 131.1, 129.3, 129.2, 126.6, 124.1, 123.8, 123.2, 64.5, 61.4, 14.3.

(*E*)-3-((4-Methoxyquinolin-3-yl)diazenyl)benzoic acid (29). A solution of **28** (81 mg, 0.24 mmol) and LiOH (30 mg, 1.25 mmol) in THF/H_2O/MeOH (1:1:0.2, 2.2 mL) was reacted according to General Procedure IA. The target compound (50 mg, 0.16 mmol, 68%) was obtained as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (br s, 1H), 9.14 (s, 1H), 8.40 (t, J = 1.8 Hz, 1H), 8.34 (dd, J = 8.6, 1.4 Hz, 1H), 8.14 (ddd, J = 8.1, 2.1, 1.1 Hz, 1H), 8.10 (dt, J = 7.7, 1.4 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.83 (ddd, J = 8.4, 6.8, 1.5 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.69–7.64 (m, 1H), 4.58 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 159.8, 152.6, 149.5, 142.1, 132.74, 132.68, 132.2, 132.0, 130.5, 129.3, 127.6, 126.8, 124.07, 124.04, 123.0, 65.2.

(*E*)-3-((4-Methoxyquinolin-3-yl)diazenyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)-phenyl)benzamide (30). To an oven-dried microwave vial was added **29** (50 mg, 0.16 mmol), EDC·HCl (126 mg, 0.65 mmol), HOBt (45 mg, 0.33 mmol), Et_3N (138 mg, 190 μL, 1.33 mmol) **7**, (48 mg, 0.18 mmol),

anhydrous DCM (2 mL) and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography (7% MeOH in DCM), followed by additional purification by PLC plate (DCM/MeOH (8:1)) provided the target compound (60 mg, 0.11 mmol, 66%) as a red solid. m.p. 71–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 9.03 (s, 1H), 8.14 (m, 3H), 8.28–8.24 (m, 2H), 7.97–7.90 (m, 4H), 7.89 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.53/7.51/7.49 (t, J = 7.8 Hz, 1H), 7.50 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 4.48 (s, 3H), 3.61 (s, 2H), 2.60–2.37 (m, 8H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 159.9, 152.5, 149.3, 142.1, 136.8, 135.8, 133.8, 132.5, 131.3, 131.2, 129.61, 129.56, 129.3 (q, $^2J_{C\text{-}F}$ = 30.5 Hz), 128.7, 126.7, 125.5, 123.9, 123.5, 123.1, 125.4/122.7 (q, $^1J_{C\text{-}F}$ = 274.3 Hz), 121.7, 117.8 (q $^3J_{C\text{-}F}$ = 6.2 Hz), 64.5, 57.8, 55.2, 53.0, 46.0. HRMS (ESI-TOF) *m/z* calcd for $C_{30}H_{30}F_3N_6O_2^+$ [M + H]$^+$: 563.2377, found: 563.2380.

2,6-Difluoro-3-iodoaniline (32). To a solution of 2,6-difluoroacetanilide (600 mg, 3.51 mmol) in *conc.* H_2SO_4 (18 mL) was added *N*-iodosuccinimide (720 mg, 3.51 mmol) portion-wise at room temperature. The resulting mixture was stirred at rt for 24 h and then poured into crushed ice (500 g). The aqueous layer was extracted with EtOAc (3 × 80 mL), the combined organic phase was washed with H_2O (200 mL), sat. aq. $NaHCO_3$ (200 mL), sat. aq. $Na_2S_2O_3$ (200 mL), brine (100 mL), dried over Na_2SO_4 and filtered. The solvent was evaporated. The residue was dissolved in MeOH (3 mL) and *conc*. HCl (2 mL) was added. The reaction mixture was stirred at 60 °C for 12 h. The reaction mixture was allowed to reach rt, H_2O (100 mL) was added, the solution was neutralized with solid $NaHCO_3$ and extracted with EtOAc (3 × 80 mL). The combined organic phase was washed by brine, dried over Na_2SO_4, filtered and excess solvent was removed. Purification by flash column chromatography (pentane-EtOAc, 15% EtOAc) provided the target compound (581 mg, 2.28 mmol, 85%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (ddd, J = 8.8, 6.8, 5.8 Hz, 1H), 6.63 (ddd, J = 10.3, 8.8, 1.8 Hz, 1H), 3.82 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.3 (dd, J_{CF} = 241.7, 6.3 Hz), 149.8 (dd, J_{CF} = 238.2, 7.5 Hz), 125.2 (m), 124.5 (m), 112.6 (m), 74.9 (m).

(*E*)-3-((2,6-Difluoro-3-iodophenyl)diazenyl)quinolone (34). To a solution of **32** (500 mg, 1.96 mmol) in DCM (4 mL) was added dropwise a solution of Oxone (1.25 g, 1.98 mmol) in water (4 mL) during stirring. The reaction mixture was stirred for 18 h at rt. The aqueous layer was extracted with DCM (3 × 10 mL), the combined organic phase was washed by brine, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and a solution of **33** (146 mg, 1.03 mmol) in aq. 40% NaOH/toluene (1:1, 4 mL) was added, under N_2 atmosphere. The reaction mixture was stirred at 80 °C for 2 h. After cooling down, the aqueous phase was extracted with EtOAc (3 × 10 mL), the combined organic phase was washed by brine, dried over Na_2SO_4 and filtered. Excess solvent was removed. Purification by flash column chromatography (pentane-DCM, 25% Pentane) afforded the target compound (252 mg, 0.64 mmol, 33%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (d, J = 2.4 Hz, 1H), 8.63 (d, J = 1.5 Hz, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.81 (ddd, J = 8.4, 7.0, 1.5 Hz, 1H), 7.76 (ddd, J = 9.0, 6.6, 5.7 Hz, 1H), 7.63 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 6.94 (ddd, J = 10.1, 9.0, 1.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2 (dd, J_{CF} = 262.5, 3.5 Hz), 154.5 (dd, J_{CF} = 257.0, 4.3 Hz), 149.6, 145.2, 145.1, 139.4 (dd, J_{CF} = 9.5, 3.3 Hz), 131.51, 131.46 (t, J_{CF} = 1.8 Hz), 130.7, 129.7, 129.6, 127.7, 114.4 (dd, J_{CF} = 20.9, 4.1 Hz), 76.6 (dd, J_{CF} = 25.5, 4.4 Hz). ^{19}F NMR (659 MHz, DMSO-d_6) −100.6 (d, $^4J_{FF}$ = 2.8 Hz), −121.3 (d, $^4J_{FF}$ = 2.8 Hz). HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_9F_2IN_3^+$ [M + H]$^+$: 395.9804, found: 395.9813.

(*E*)-2,4-Difluoro-3-(quinolin-3-yldiazenyl)benzoic acid (35). To an oven-dried MW reaction vial was added $Pd(OAc)_2$ (3.8 mg, 0.017 mmol and Xantphos (10 mg, 0.017 mmol) under N_2. A solution of **34** (150 mg, 0.38 mmol), and 1-propanol (121 mg, 150 μL, 2.0 mmol) dissolved in toluene (1.0 mL) were added to the reaction vial. After backfilling with N_2, a pre-stirred mixture of formic acid (37 mg, 30 μL, 0.80 mmol) and acetic anhydride (82 mg, 75 μL, 0.80 mmol), was added dropwise to the reaction vial. Then Et_3N (203 mg, 280 μL, 2.0 mmol) was added. The mixture was stirred for 12 h at 80 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc (10 mL) and extracted by 3 M aq. NaOH (2 × 10 mL). The combined aqueous layer was acidified with 3 M HCl till pH 4 and extracted with EtOAc (3 × 15 mL). The combined organic phase was washed with brine, dried over Na_2SO_4 and filtered. Excess solvent was removed. Purification by flash column chromatography ($CHCl_3$–MeOH, 15% MeOH) afforded the target compound (60 mg, 0.19 mmol, yield 50%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (d, J = 2.4 Hz, 1H), 8.88 (d, J = 2.4 Hz, 1H), 8.25 (d, J = 7.4 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.98 (dt, J = 8.5, 6.4 Hz, 1H), 7.90 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.72 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.38 (t, J = 9.4 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.1 (m), 157.4–154.7 (m), 156.2–153.5 (m), 149.4, 145.2, 144.4, 134.4 (d, J_{CF} = 10 Hz), 132.4, 131.5, 131.1 (t, J_{CF} = 10.7 Hz), 130.7, 129.4, 128.4, 127.8, 120.8, 113.0 (d, J_{CF} = 17.2 Hz). ^{19}F NMR (659 MHz, DMSO-d_6) −115.9 (s), −118.5 (s). HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{10}F_2N_3O_2^+$ [M + H]$^+$:314.0736, found: 314.0739.

(*E*)-2,4-Difluoro-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(quinolin-3-yldiazenyl)benzamide (36). To an oven-dried microwave vial was added **35** (52 mg, 0.17 mmol), **7** (52 mg, 0.19 mmol), EDC·HCl (160 mg, 0.83 mmol), HOBt (56 mg, 0.41 mmol), Et_3N (145 mg, 200 μL, 1.43 mmol), DCM (2 mL), and the reaction was carried out according to General Procedure IIA. Purification by flash column chromatography ($CHCl_3$–MeOH, 10% MeOH) afforded the target compound (33 mg, 0.058 mmol, 35%) as a red solid. m.p. 64–68 °C. ^{1}H NMR (700 MHz, DMSO-d_6) δ 10.92 (s, 1H), 9.40 (d, J = 2.4 Hz, 1H), 8.96 (d, J = 14.2 Hz, 1H), 8.31 (dd, J = 8.3, 1.4 Hz, 1H), 8.18 (d, J = 2.6 Hz, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.98–7.91 (m, 3H), 7.78 (ddd, J = 8.1, 6.8, 1.1 Hz, 1H), 7.74 (d, J = 8.6 Hz, 1H), 7.56 (t, J = 9.5 Hz, 1H), 3.58 (s, 2H), 2.54 (m, 8H), 2.18 (s, 3H); ^{13}C NMR (176 MHz, DMSO-d_6) δ 162.1, 156.9 (d, J_{CF} = 271.8 Hz), 153.6 (d, J_{CF} = 271.8 Hz), 149.3, 145.3, 144.4, 138.2, 133.0, 132.8 (d, J_{CF} = 9.0 Hz), 132.6, 132.0, 131.8, 130.8, 130.7 (t, J_{CF} = 9.9 Hz), 129.5, 128.5, 128.1 (q, J_{CF} = 30.4 Hz), 127.9, 125.5 (q, J_{CF} = 272.8 Hz), 123.6, 122.9 (d, J_{CF} = 13.1 Hz), 117.2 (d, J_{CF} = 6.5 Hz), 113.8 (d, J_{CF} = 20.3 Hz), 57.7, 55.1, 52.8, 45.8. ^{19}F NMR (659 MHz, DMSO-d_6) −58.0 (s), −117.3 (s), −122.0 (s). HRMS (ESI-TOF) *m/z* calcd for $C_{29}H_{26}F_5N_6O^+$ [M + H]$^+$: 569.2083, found: 569.2087.

UV–vis absorption spectrum. Stock solutions (3 mM) of azo compounds in DMSO or the HCL-salt forms of the azo compounds in H_2O, were thermally adapted and diluted with DMSO and H_2O, respectively to 30 μM solutions. PSS was reached upon 365 nm irradiation. The samples was then exposed to visible light irradiation (460 nm light was used for azo compounds **8** and **30**, 405 nm light was used for compound **36**) until PSS was reached.

Determination of thermal stability. The *E*-isomers of the azo compounds were dissolved in DMSO at rt; the aqueous solution of each azo compound (the HCl salt form of **8**, **36** was dissolved in H_2O, respectively, **30** was dissolved in 20% DMSO/H_2O) was prepared at rt. The PSS was reached upon 365 nm irradiation, after which the samples were left in the dark and their absorption spectra was recorded once every day. The time-based absorption (at 360 nm for DMSO solution and 350 nm for aqueous solution) was fitted using mono-exponential (first order) kinetics.

PSS determination by ^{1}H-NMR. Azo compounds were dissolved in DMSO-d_6 (5 mM, 25 °C) and irradiated by LEDs (365nm/460 nm) to achieve the respective PSS (as judged by monitoring the UV–vis absorption spectra). 0.6 mL of the irradiated samples was submitted to ^{1}H NMR measurement (400 MHz, 128 scans).

GSH reduction assay. UV/vis cyclic isomerization GSH reduction assay: diluting DMSO stock solution of azo compounds (3 mM) with 1 mM GSH solution in H_2O to achieve a final concentration of 30 μM. Five cycles of 365 nm/460 nm light irradiation were performed, during the six circle, 365 nm UV light enriched *Z*-isomer sample was incubated at 25 °C for 2.5 h, then extended five circles of 460 nm/365 nm light irradiation were performed.

ADP-GloTM RET kinase assay. The assays were performed under red light. Azo compounds were dissolved in DMSO (1 mM) and heated at 60 °C for 30 min prior to use to ensure that it consisted of pure *E*-isomers. Serially diluted inhibitors (both *E* and *Z*-isomers of the DFG-out inhibitors, as well as staurosporine and ponantinib as reference compounds, using concentration gradients ranging from 1 mM to 17 nM) were pipetted into white 96-well V bottom plate. The concentration gradient of the *Z*-isomers were prepared by irradiating the serially diluted samples of the *E*-isomers on the 96-well V bottom plate, with a 96 array UV365 LED (AMUZA INC, LEDA-365, 11V, 20 min). The ADP-Glo™ RET Kinase Assay was carried out according to the manufacturer's protocol (Promega protocol TM313), with white 384-well low volume plate, 5 μL total reaction volume, and in triplicate replicates for each inhibitor and condition. Kinase reactions were run in kinase buffer (40 mM Tris (pH 7.5), 20 mM $MgCl_2$, 0.1 mg/mL BSA, 60 μM DTT) with 10 μM ATP, 4.0 ng human RET kinase, and 0.4 mg/mL of substrate peptide (IGF1Rtide). All the serially diluted samples (in DMSO) were first diluted (1:10) by kinase buffer to prepare 5 × inhibitor stock solutions. Then 1 μL of each 5 × inhibitor stock solution (including DMSO control solution for each inhibitor) were added into separate wells of the 384-well plate, followed by addition of RET kinase (2 μL) and then the mixture was incubated at rt for 40 min. The kinase reaction was initiated by adding the RET substrate working solution (2 μL) and the mixture was incubated at rt for 60 min. The reaction was terminated with 5 μL of the ADP-Glo™ Reagent and incubated at rt for 40 min, before 10 μL Detection reagent was added to each well and the mixture incubated at rt for 40 min. The plate was kept with a lid on and enclosed under humid conditions, during all incubations to prevent evaporation. The luminescence signal was recorded by a plate reader (Molecular Devices SpectraMax iD5). Kinase activities were calculated as the percent of activity observed for DMSO controls and plotted against the logarithm of inhibitor concentration. Data points were means of triplicates with standard deviations as error bars. Sigmoidal dose-response fitting (log_{10}(inhibitor) vs. Activity—variable slope) were performed by GraphPad Prism 7.0 to give the IC_{50} values.

NanoBRET™ TE Intracellular Kinase Assays. Full-length RET ORF (Promega, V804 M) cloned in frame with a C-terminal NanoLuc-fusion were transfected into HEK293 cells following the protocol from the supplier (TM589, Promega). The RET-transfected cells (2×10^4 cells/mL) were added to 96 well white plates (Corning 3610; 100 μl/well) in Opti-MEM medium without phenol red. Twenty-four hours after transfection, the serially diluted inhibitors (both *E* and *Z*-isomers of the DFG-out inhibitors (the *E*-isomer of azo compounds had been thermally adapted), as well as staurosporine and ponantinib as reference compounds) were added to the cells (0.39 nM - 20 mM). Thereafter, the NanoBRET Kinase Tracer K10 (Promega, N2642) was added to achieve a final concentration of 0.13 μM. The system was allowed to equilibrate for 90 min at 37 °C/5% CO_2. Prior to BRET measurements, some set of cells were exposed to UV-radiation at 365 nm using a UV365 LED array (AMUZA INC, 12.0 V, 2.3 mV) to convert *E*-isomer of azo compounds to *Z*-isomer. The UV-irradiation was carried out in three rounds: First for 15 s, then 10 min in the incubator, followed by a 10 s irradiation before the cells was left for 15 min at rt and further 10 s after equilibrium at rt. To measure BRET, a stock solution containing Extracellular NanoLuc Inhibitor (Promega, N2161) at 60 μM and NanoBRET NanoGlo Substrate was added according to the manufacturer's protocol, and filtered luminescence was measured on a SpectraMax iD5 (Molecular Devices) equipped with a 447 nm filter (donor) and a 610 nm filter (acceptor). Competitive displacement data were then graphed using GraphPad Prism 7 software applying sigmoidal dose-response fitting (log_{10}(inhibitor) vs. Activity—variable slope). All the experiments involving azo and stilbene compounds should be performed under red light.

Docking study. The structure of RET DFG-out conformation was obtained by homology modelling. The protein sequences of RET were obtained from NCBI database (GeneID: 547807). The crystal structure of human KIT (PDB code 4U0I) was used as the template. The sequence similarity between these two proteins is 46%. The homology model was built using the Structure Prediction Wizard in the Schrödinger Suite (Schrödinger, LLC, New York, NY). The energy-based model-building method is used. The ClustalW method was used to align the target and template sequences in Prime. With the kinase structure obtained above, the docking simulation was then performed by MOE (2016.0802 win64) in forcefield MMFF94X. The Site Finder tool was used to define the binding site, using a method of Triangle Matcher Placement and Rigid Receptor Refinement.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2022.114226.

Abbreviations used

RET	REarranged during Transfection (RET)
DFG-in	Aspartic acid-Phenylalanine-Glycine-in
DFG-out	Aspartic acid-Phenylalanine-Glycine-out
TLC	thin layer chromatography
Boc	tert-butyloxycarbonyl
DMSO	Dimethyl sulfoxide
MW	Microwave heating
DMF	Dimethylformamide
THF	Tetrahydrofuran
EDC·HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBt	Hydroxybenzotriazole
DCM	Dichloromethane
MeCN	Acetonitrile
RuPhos	2-Dicyclohexylphosphino-2,6-diisopropoxybiphenyl
DIPEA	*N*,*N*-Diisopropylethylamine
HATU	2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
PLC	Preparative layer chromatography
DTT	1,4-Dithiothreitol

GSH	Glutathione
LED	light emitting diode
rt	room temperature

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