Journal Pre-proof

Potent antiproliferative activity of bradykinin B2 receptor selective agonist FR-190997 and analogue structures thereof: A Paradox Resolved?

Gerasimos Rassias, Sofia Leonardi, Dionisia Rigopoulou, Eleanna Vachlioti, Konstantinos Afratis, Zoi Piperigkou, Christos Koutsakis, Nikos K. Karamanos, Haralambos Gavras, Dionissios Papaioannou

PII: S0223-5234(20)30920-X

DOI: https://doi.org/10.1016/j.ejmech.2020.112948

Reference: EJMECH 112948

To appear in: *European Journal of Medicinal Chemistry*

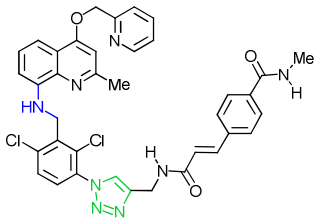
Received Date: 27 August 2020

Revised Date: 24 September 2020

Accepted Date: 14 October 2020

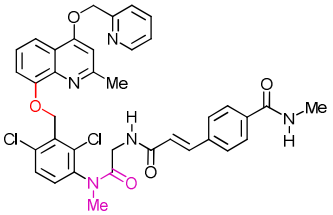
Please cite this article as: G. Rassias, S. Leonardi, D. Rigopoulou, E. Vachlioti, K. Afratis, Z. Piperigkou, C. Koutsakis, N.K. Karamanos, H. Gavras, D. Papaioannou, Potent antiproliferative activity of bradykinin B2 receptor selective agonist FR-190997 and analogue structures thereof: A Paradox Resolved?, *European Journal of Medicinal Chemistry*, https://doi.org/10.1016/j.ejmech.2020.112948.

Graphical abstract



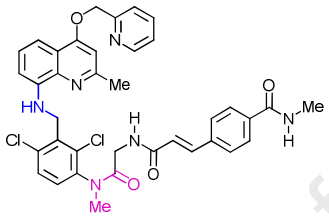
9

MCF-7 IC_{50} 0.73 μM
MDA-MB-231 IC_{50} 0.43 μM



FR-190997

2.14 μM
0.08 μM



10

1.21 μM
5.9×10^{-5} μM

Potent antiproliferative activity of bradykinin B2 receptor selective agonist FR-190997 and analogue structures thereof: A Paradox Resolved?

Gerasimos Rassias[a*], Sofia Leonardi[a], Dionisia Rigopoulou[a], Eleanna Vachlioti[a], Konstantinos Afratis[a,b], Zoi Piperigkou[c,d], Christos Koutsakis[c], Nikos K. Karamanos[c,d], Haralambos Gavras[e*], Dionissios Papaioannou[a,*]

[a]*Laboratory of Synthetic Organic Chemistry, Department of Chemistry, University of Patras, 26504 Patras, Greece*
[b]*Current address: Organic Chemistry Research Laboratory, University of Oxford, 12 Mansfield Rd, OX1 3TA, Oxford, UK*
[c]*Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, 26504 Patras, Greece*
[d]*Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece*
[e]*Hypertension and Atherosclerosis Section, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts 02118, USA*

* Corresponding author.
E-mail addresses: rassiasg@upatras.gr (G. Rassias), dapapaio@upatras.gr (D. Papaioannou).

Highlights

- Significant antiproliferative activity is demonstrated for the first time with a B2R agonist (FR-190997)
- SAR on FR-190997 led to novel analogue **10** demonstrating subnanomolar inhibition in the MDA-MB-231 assay.
- The antiproliferative performance of analogue **10** in MDA-MB-231 cells is superior to that exhibited by FDA-approved breast cancer drugs
- The mechanism probably involves agonism-induced B2R internalisation/degradation and inhibition of endosomal B2R signalling

1. Introduction

Kinins exert their biological effects by activating two types of G protein-coupled receptors (GPCRs), traditionally known as B1 (B1R) and B2 (B2R) which are coupled to $Gaq_{/11}$ and Gai/o G proteins. These receptors have been shown to modulate diverse cellular functions leading to pro-inflammatory activity, vasodilatation, vascular permeability, nociception, chemotaxis and sodium and chloride excretion by the renal tubules [1-3].

In general, B1 and B2 activation triggers essentially the same signalling cascades nevertheless the observed responses stemming from B2R are acute and of short duration whereas those from B1R are more sustained and of lower intensity. The B2R is constitutively expressed in a variety of cell types whereas the physiological expression of B1R is low but up-regulated significantly in cases of tissue injury, cytokine stimulation, inflammation and cancer.

Accumulating data with bradykinin (BK) and its active metabolite des-Arg9-BK (DABK), selective activators of B2 and B1 receptors respectively, suggests that activation of these receptors enhance proliferation and invasion of gliomas, colorectal, breast, and cervical cancer cells and that these trends are reversed by B1/2 receptor antagonists [4-11].

In contrast, the protective effects of B1 agonism with des-Arg9-BK in mice with tumors derived from TM5 melanoma cells has also been demostrated [12]. Equally one cannot ignore the reduced metastatic potential in B16F10 murine melanoma model following B1 agonism with DABK reported recently [13].

In the long and significant contribution of the Marceau group in this area, compound B-9870 (CU201) was found to inhibit tumour cell growth both *in vitro* and *in vivo*. In particular it activated c-Jun kinase and caspase-3 while antagonizing BK-induced calcium signalling causing a debate as to whether this compound is a B2R antagonist or biased agonist [14].

It is yet unclear whether these controversies originate from different cell lines, cancer type and microenvironment or there is a subtle yet important factor in determining the ultimate cell response upon B1/2R stimulation.

In an attempt to shed light into this, we decided to explore the effect of B2 agonism in the context of cancer using the highly selective and potent non-peptidic partial agonist FR-190997 (Figure 1) developed by Fujisawa (legacy company of Astellas) [15]. We reasoned that this approach would complement the established

data which almost invariably has been generated using either the natural peptidic substrates of B1/2 receptor or synthetic peptidic and non-peptidic antagonists. To the best of our knowledge, a B2 selective non-peptidic small molecule partial agonist has not been utilized in antineoplasmatic studies.

2. Results and Discussion

2.1 *Scaffold selection and SAR design*

Chronic stimulation of B2R has been proposed as a promising approach for the prevention and treatment of cardiovascular disease [1-3,16,17]. Nevertheless, BK suffers from rapid breakdown *in vivo*, thus a research team from Fujisawa developed a series of novel and stable non-peptidic B2R agonists based on a quinoline warhead. In that work it was established that the quinoline substitution at position 4 is crucial for exhibiting either selective antagonism (no substitution or amino-substitution) or agonism (alkoxy substitution) at B2R [15, 18-20]. Optimised agonists of this class include FR-190997 and **1A** (IC_{50} 0.41 and 0.36 nm, respectively) while the other warhead that imparts agonistic profile is an appropriately substituted benzimidazole such as the one in full agonist **1B** (IC_{50} 0.68 nm). Changes in the terminal amide part of the molecule only fine tune solubility and the magnitude of the functional profile determined by the warhead. Fujisawa's FR-173657 [21,22] (Figure 1) is a B2R antagonist due to the absence of the agonist-imparting substituent at the quinoline warhead despite sharing the same terminal amide as agonist **1B**.

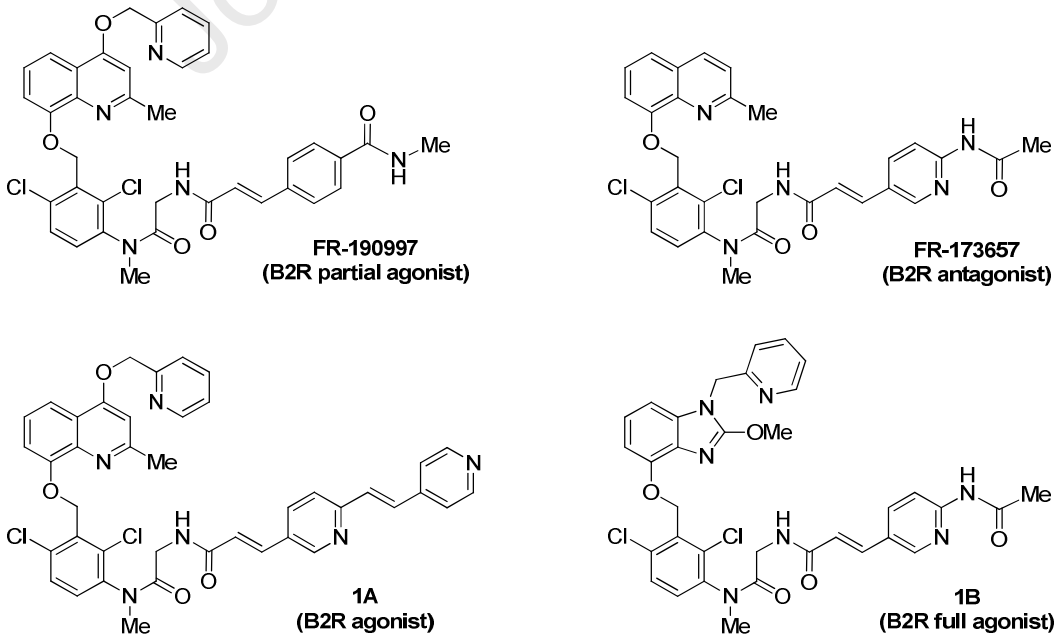


Figure 1. Structures of B2R ligands **FR-190997**, **FR-173657**, **1A** and **1B.**

The best characterized member of this class of non-peptide B2R agonists is FR-190997 (Figure 1); a highly potent and subtype selective partial agonist of B2R (in terms of inositol phosphates, prostagladin production and calcium mobilization in comparison with bradykinin). FR-190997 was originally developed and progressed through rigorous preclinical studies for cardiovascular indications although it was subsequently repurposed to the treatment of glaucoma/ocular hypertension [23]. We selected FR-190997 to interrogate the significance of B2 agonism in the context of cancer primarily because of its high B2R potency and complete lack of affinity for the B1 receptor or other targets for that matter, hence potential antiproliferative activity would not be associated with off-target effects. Another reason for choosing FR-190997 for the present study was the modular nature of its structure allowing for a focused SAR on its respective fragments which in turn are amenable to independent modification.

FR-190997 consists of four modules, namely a 2-methyl-4-(2-pyridylmethoxy)-substituted quinoline, a 4-substituted cinnamic acid, a 2,4-dichloro-3-hydroxymethyl-aniline and glycine. The latter two domains are connected through an N-methylated amide bond which has been shown to adopt preferentially the *cis*-conformation, with the planes of the amide bond and the phenyl ring almost perpendicular to each other [17,20]. This central part of the structure seems to play a crucial role in defining the active conformation of the molecule. In addition, the structure of the acid component in the terminal amide appears to fine-tune the magnitude of activity and solubility properties in these series [15, 18-22]. Inspection of these structural components led us to recognize two privileged scaffolds in cancer drug discovery embedded within FR-190997, namely quinoline [24,25] and cinnamic acid [26,27], and reasoned that FR-190997 could act perhaps as a quinoline-cinnamic acid conjugate.

In terms of cancer cell lines we decided to investigate the response elicited by FR-190997 initially in the MCF-7 breast cancer cells as it has been reported that bradykinin agonism at B2R induces proliferation in this and related breast cancer assays by activation of the oncogenic pathway PKCδ/ε, PKB/Akt, and ERK1/2 [28-30]. We also chose to examine the response elicited by the B2R agonist FR-190997 in the triple negative breast cancer (TNBC) cell line MDA-MB-231 because of recent work [31] demonstrating benefits with the B2R antagonist FR-173657, a close analogue of FR-190997 (Figure 1) also from the Fujisawa collection. Our *in vitro* tests using FR-190997 in these two breast cancer lines, MCF-7 (low invasive

potential) and MDA-MB-231 (high invasive potential), presented substantial antiproliferative activity with IC_{50} values of 2.14 μM and 0.08 μM respectively for the two cell lines.

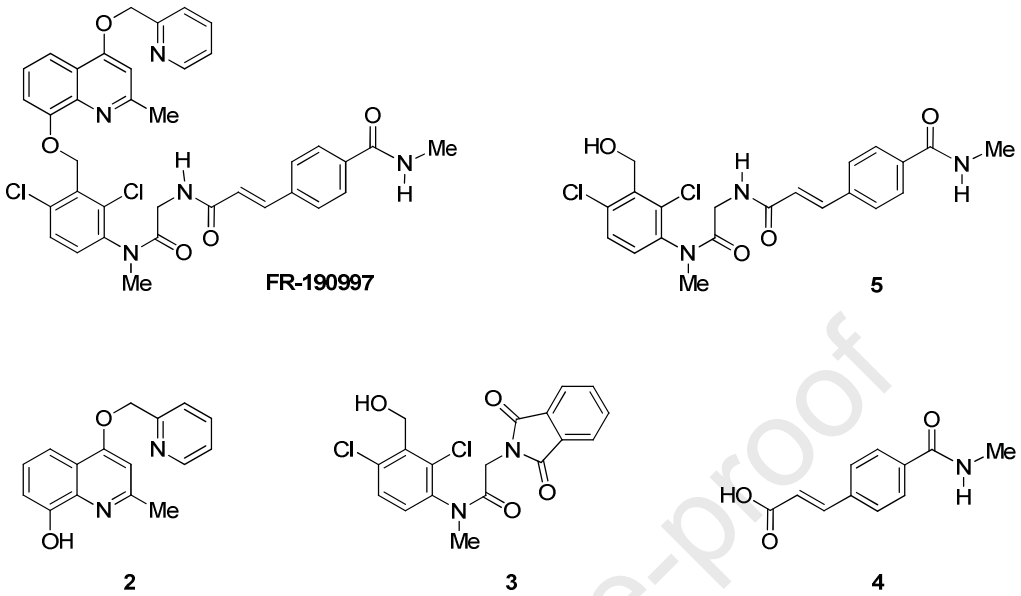


Figure 2. Structures of B2R selective agonist FR-190997 and its fragments **2-5**.

We therefore decided to (a) identify which parts of the molecule were responsible for the observed biological effect (see compounds **2-5**, Figure 2) and (b) synthesize a series of novel analogs of FR-190997 and key-fragments thereof (Figure 3) in order to compare their potential antiproliferative activity to that of the prototype compound FR-190997 with the view of simplifying the active structure. For the latter we considered modifications in (i) the linker connecting the quinoline with the 2,6-dichlorophenyl ring (compounds **9**-**11**), (ii) the N-methylated amide bond (compounds **8** and **9**), which is crucial for the overall shape of the molecule, and (iii) the acid residue in the terminal amide (**6** and **7**). The compounds thus conceived, synthesized and evaluated as potential antiproliferative agents are depicted in Figure 3. It should be noted that compound **12** was designed to assess the impact of the hydrogen bond donating/accepting capacity of the bis-amide terminal domain in FR-190997, whereas compound **7**, an FR-190997 hybrid with *all-trans*-retinoic acid (atRA), was designed to mimic the extended conjugated system of analogue **1A**. Additionally, atRA is itself an anticancer drug for acute promyelotic leukemia (APL). Furthermore, compounds **13** and **14** are analogues of the FR-190997-fragments **2** and **3**, respectively.

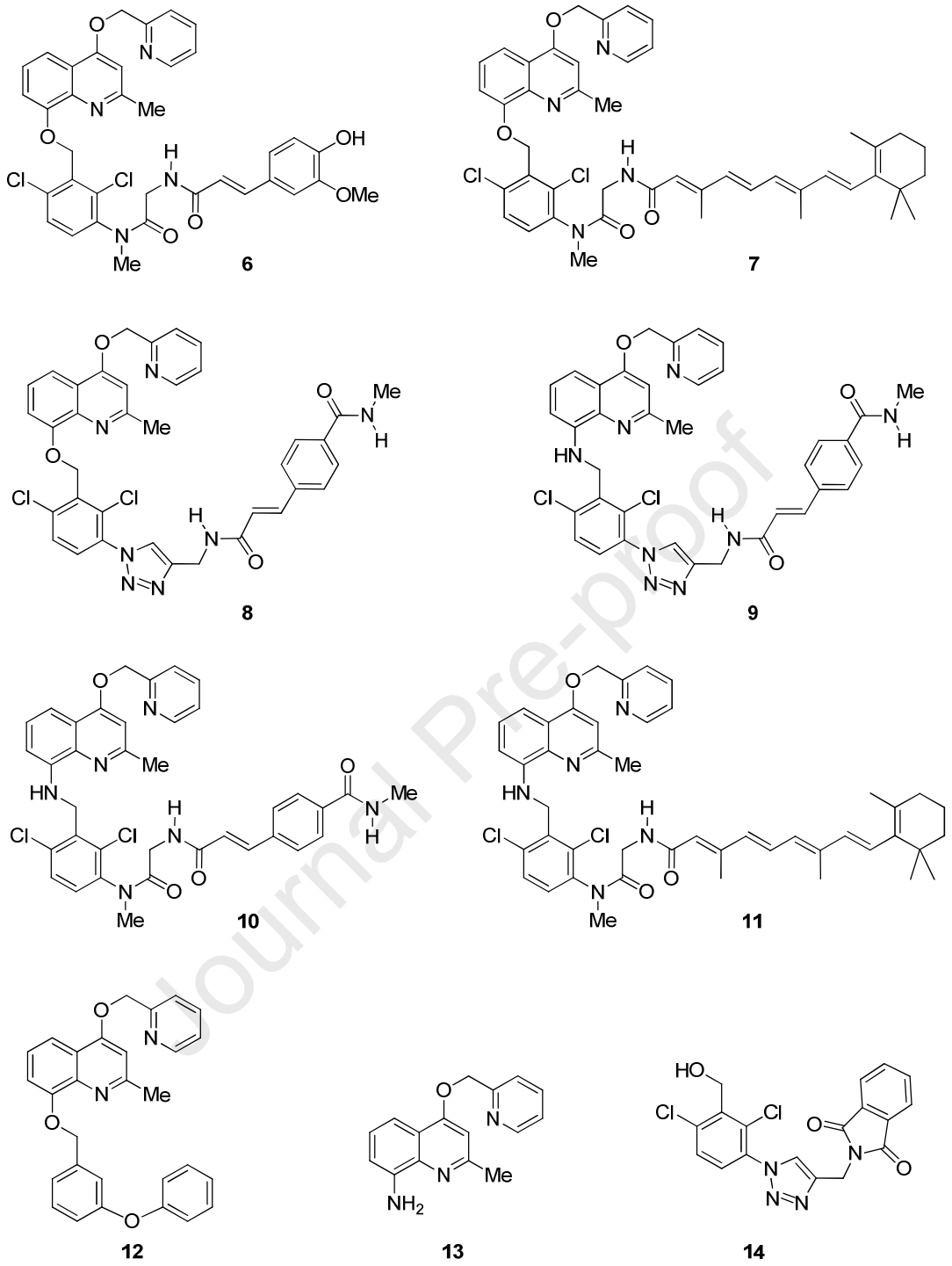
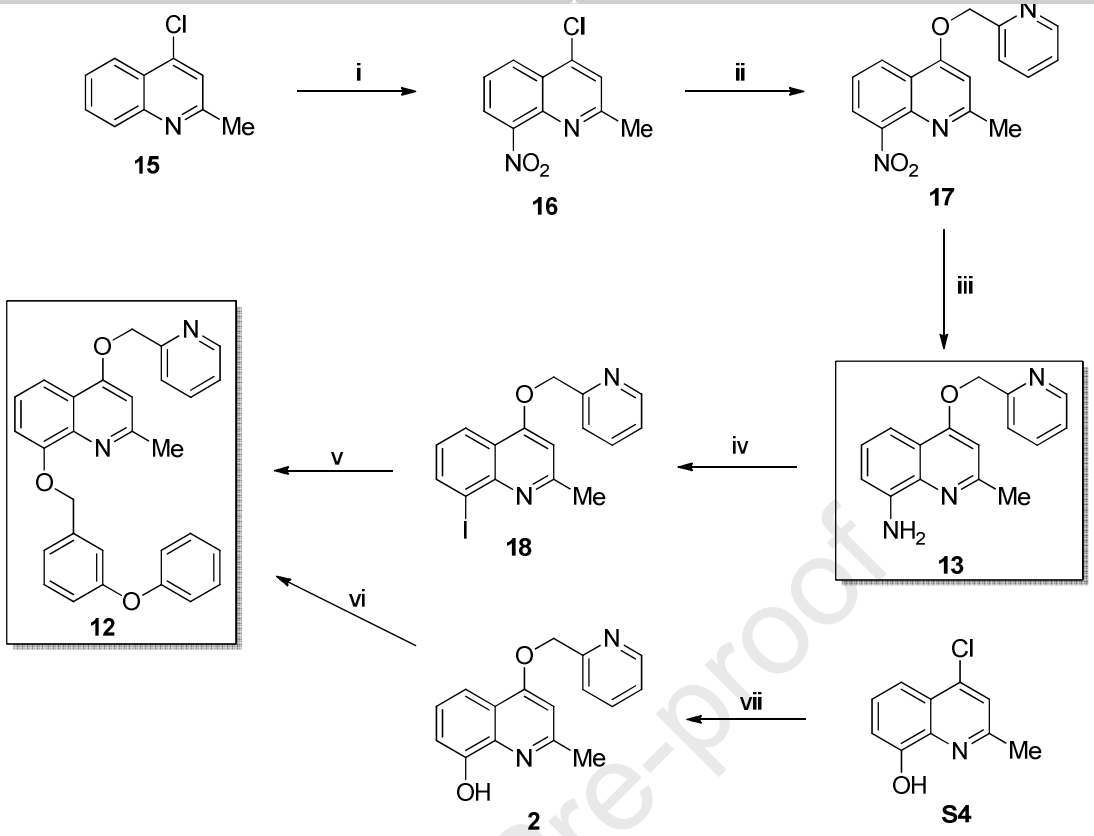


Figure 3. Structures of new analogues of FR-190997 (**6-11**) and fragments (**12-14**) thereof synthesized in the context of the present work.

2.2 Chemistry

The synthesis of fragments **2**-**5** *en route* to the synthesis of the target molecule FR-190997 have been described by Fujisawa's researchers in a series of articles [15, 18-22] and summarized in reference 17. For the needs of the present study, we synthesized these compounds based on the Fujisawa routes and implemented some minor modifications (see Supplementary Material (SM), Figures S1-S4 and related experimental procedures). Incorporation of the quinoline substructure in the ether-type analogs **6**-**8** and **12** was effected using 8-hydroxyquinoline derivative **2**, whereas for the amino-type analogs **9**-**11** and, alternatively, for fragment analog **12**, 8-aminoquinoline derivative **13** was used as the key precursor. This compound was synthesized from the commercially available 4-chloro-2-methylquinoline (**15**) and (pyridin-2-yl)methanol using the three-step procedure outlined in Scheme 1.

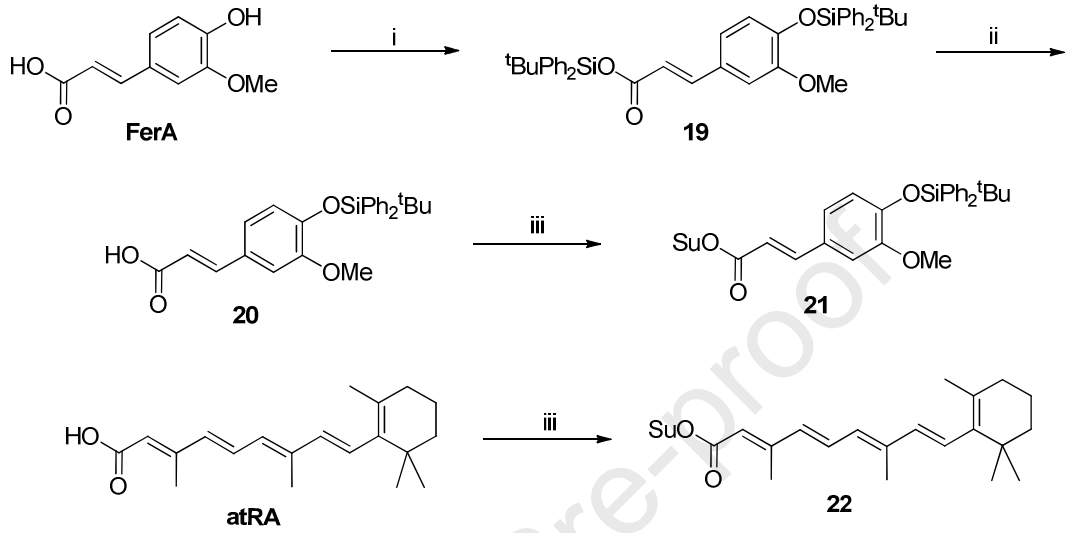
Selective nitration of **15** produced the 8-nitro derivative **16** [32] in 49% yield. Nucleophilic aromatic substitution by (pyridin-2-yl)methanol in the presence of ^{t}BuOK was accomplished, as expected, under much milder conditions than the corresponding reaction with the hydroxyl group at position 8 of the quinoline group (see Scheme S1), and gave intermediate **17** in 43% yield. Finally, the nitro group was reduced with hydrazine monohydrate, in the presence of a catalytic quantity of $FeCl_3 \cdot 6H_20$, to provide fragment **13** in 72% yield. On the other hand, diazotization of **13** followed by KI treatment produced the iodide **18** in 74% yield. Initially, this compound was considered as a key-intermediate for the assembly of the FR-190997 molecule *via* the Ullmann reaction. Attempted condensation of iodide **18** with alcohol **5** in the presence of Cs_2CO_3, CuI and 1,10-phenanthroline failed however to produce the target molecule FR-190997. Interestingly, Ullmann reaction of iodide **18** with the simpler 3-phenoxybenzyl alcohol under identical reaction conditions, produced fragment **12** in low yield (18%) suggesting that the reason for the failure of the Ullmann coupling of **5** is not entirely steric. In both reactions the alcohol partner was consumed and reduction of iodide **18** was also observed indicating that the stability of the substrates may be responsible for the poor performance of the Ullmann reactions. Alternatively, fragment **12** could be obtained in a much better yield (60%) through the Mitsunobu reaction of fragment **2** with *m*-phenoxybenzyl alcohol.



Scheme 1. Synthesis of fragments **12** and **13**. *Reagents and conditions*: (i) KNO_3, 96% H_2SO_4, -10 °C for 50 min then 25 °C,12 h, 49%; (ii) (Pyridin-2-yl)methanol, ^tBuOK, DMI, 0 °C then -10 °C for 50 min and finally 25 °C, 12 h, 43%; (iii) $N_2H_4 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$ (cat.), activated C, 80% aq. MeOH, 75 °C, 3 h, 72%; (iv) $NaNO_2$, 37% HCl,H_2O, 5 °C for 50 min, then 3M aq. KI, 5 °C for 10 min then 25 °C, 1 h, 74%; (v) CuI, 1,10-phenathroline, Cs_2CO_3, *m*-phenoxybenzyl alcohol, 1,3-dimethyl-2-imidazolidinone (DMI), 110 °C, 12 h, 18%; (vi) *m*-phenoxybenzyl alcohol, TPP, DIAD, dry THF, 0 °C for 30 min then 25 °C, 48 h, 60%; (vii) (Pyridin-2-yl)methanol, NaH, DMI, 0 °C for 1 h then 25 °C and finally at 100 ºC, 12 h, 83%.

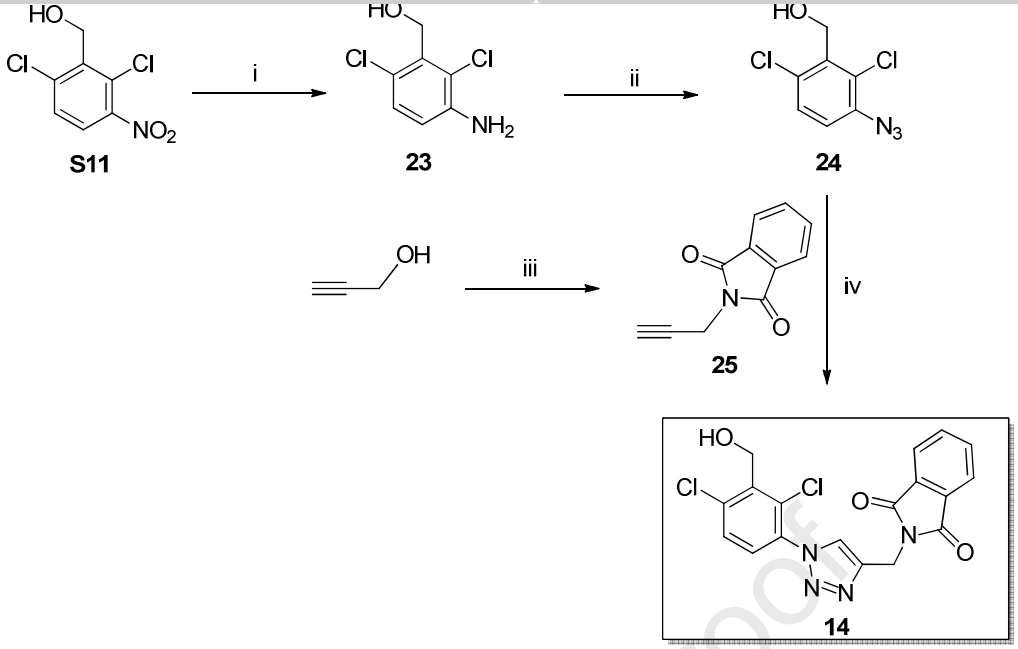
We envisaged that replacing the cinnamic acid moiety of FR-190997 with hydroxycinnamic acid (HCA) ferulic acid (FerA) or the retinoid acid atRA would require the availability of suitable activated esters, e.g. the succinimidyl esters. Isolable succinimidyl esters of *α,β*-unsaturated carboxylic acids are known to selectively acylate primary amino groups in the presence of secondary ones [33,34]. We therefore selected the succinimidyl 'active' ester (**21**) of *O*-(*tert*-butyl)diphenylsilylferulic acid (**20**) and the known isolable succinimidyl 'active' ester (**22**) of atRA [34] for generating analogues **6** and **11**, respectively. The synthesis of the former was realized in 42% overall yield by fully protecting FerA with the (*tert*-butyl)diphenylsilyl (TBDPS) group to give intermediate **19**, followed by selective

carboxyl group deprotection providing acid **20**, and finally activation of the latter with *N*-hydroxysuccinimide (HOSu) and *N*,*N*'-dicyclohexylcarbodiimide (DCC) (Scheme 2). On the other hand, the also isolable crystalline succinimidyl 'active' ester **22** of atRA was prepared as described in the literature [34], using the same system (HOSu/DCC) for activation.



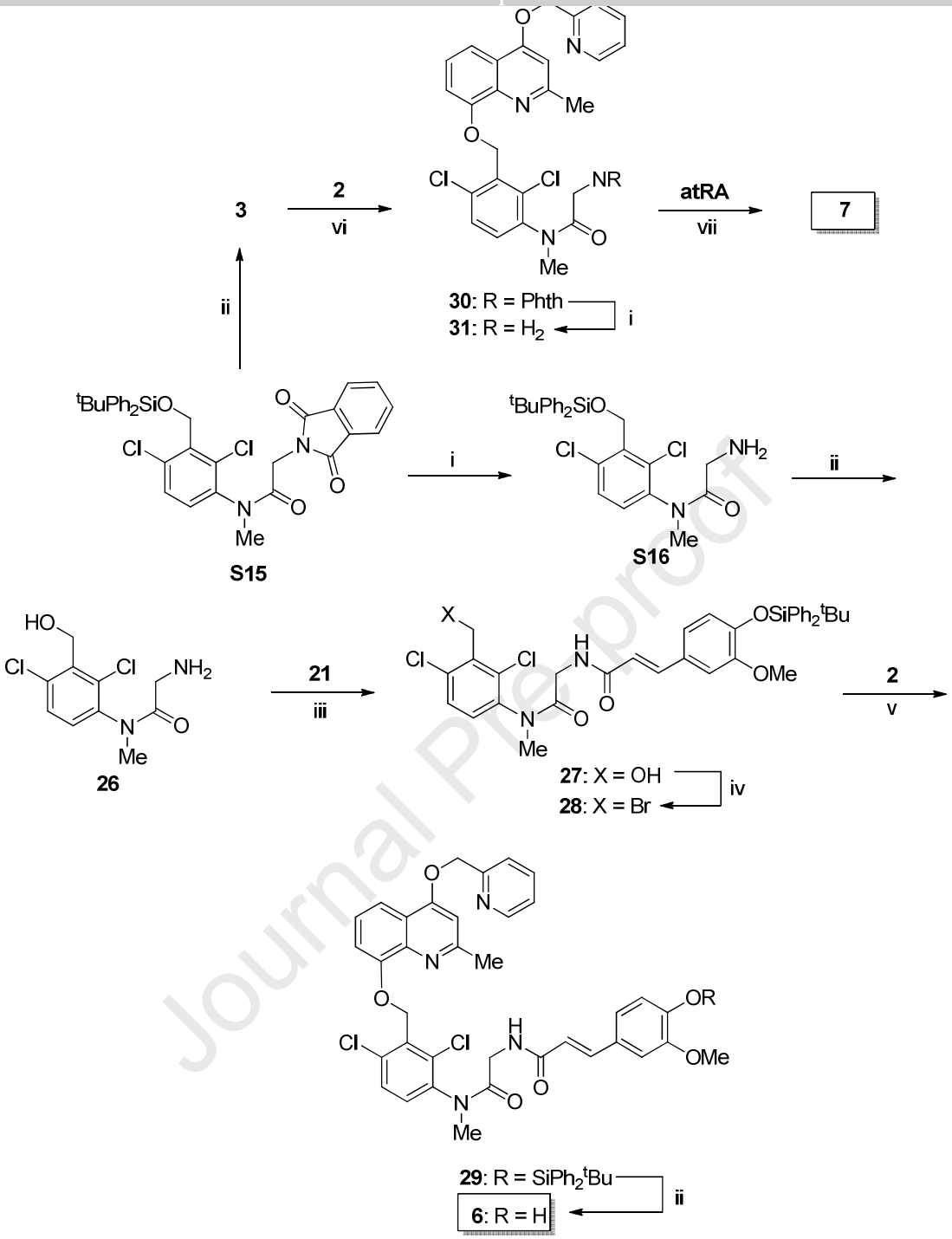
Scheme 2. Synthesis of isolable 'active' esters **21** and **22**. *Reagents and conditions*: (i) TBDPSCl, imidazole, dry DMF, 25 °C, 3 h, 81%; (ii) 10% aq. K_2CO_3, MeOH, THF, 0 ^{o}C then 25 °C, 1 h, 61%; (iii) HOSu, DCC, dry THF, 0 °C for 30 min then 25 °C 12 h, 85% (**21**), 73% (**22**).

The synthesis of FR-190997 analogs **8** and **9** required the prior synthesis of compound **14**, which is an analog of fragment **3**, in which the *cis*-amide bond functionality has been replaced with a triazole ring. Compound **14** was synthesized as depicted in Scheme 3 and involved the reduction of intermediate **S11** (see Scheme S3 of SM) with hydrazine monohydrate and a catalytic quantity of $FeCl_3{\cdot}6H_2O$ to produce aniline derivative **23** in 81% yield, followed by diazotization and reaction with sodium azide to provide aryl azide **24** in 79% yield. The latter was subjected to a Huisgen 1,3-dipolar cycloaddition reaction with the N-phthalylated propargylamine **25**, to give the desired triazole derivative **14** in 70% yield.



Scheme 3. Synthesis of fragment **14**. *Reagents and conditions*:(i) $N_2H_4 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$ (cat.), activated C, 80% aq. MeOH, 75 °C, 4 h, 81%; (ii) $NaNO_2$, CF_3CO_2H (TFA), 0 °C, 45 min, then NaN_3, 0 °C, 5 min then 25 °C for 30 min, 79%; (iii) Phthalimide, Ph_3P (TPP), $^{i}PrO_2CN{=}NCO_2{}^{i}Pr$ (DIAD), dry THF, 0 °C then 25 °C, 2 h, 60%; (iv) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O, DCM, 25 °C, 3 h, 70%.

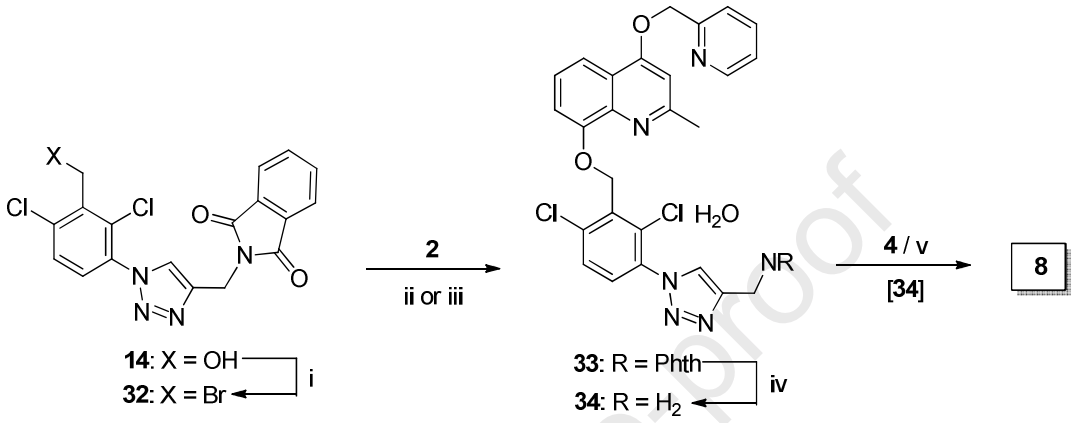
Having established the availability of all required intermediates, the synthesis of FR-190997 analogs **6**-**11** was realized as follows. The key-intermediate **S15** was subjected to hydrazinolysis to provide amine **S16** (Scheme S4, SM), which was then treated with tetrabutylammonium fluoride (TBAF) giving the aminoalcohol **26** (Scheme 4) in 48% yield for the two steps. Selective N-acylation of intermediate **26** with 'active' ester **21** gave alcohol **27** in 85% yield. Alcohol **27** was first converted to the corresponding bromide **28** in 72% yield using Appel reaction, and this was further used to alkylate fragment **2**, providing intermediate **29** in 44% yield. From compound **29**, the projected FR-190997 analog **6** was finally obtained in 64% yield after treatment with TBAF (Scheme 4). On the other hand, selective *O*-deprotection of key-intermediate **S15** gave fragment **3** in 55% yield (see also Scheme S3 of SM) which was condensed with fragment **2**, under Mitsunobu reaction conditions, to give the new intermediate **30** in 70% yield. The latter, upon hydrazinolysis and coupling of the thus obtained, in 40% yield, amine **31** with atRA, in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), provided the FR-190997 analog **7** in 50% yield.



Scheme 4. Synthesis of FR-190997 analogs **6** and **7**. *Reagents and conditions*:(i) $N_2H_4 \cdot H_2O$, EtOH, reflux, 1-2 h, 88% (**S16**), 40% (**31**); (ii) TBAF·$3H_2O$, THF, 25 °C, 3 h, 57% (**3**), 56% (**26**),64% (**6**); (iii) dry Et_3N, dry DMF, 25 °C, 1 h, 85%; (iv) CBr_4, TPP, dry THF, 0 °C then 25 °C, 3 h, 72%; (v) K_2CO_3, dry DMF, 25 °C, 3 h, 44%; (vi) TPP, DIAD, dry DMF, 25 ^{o}C, 1 h then 65-70 ^{o}C, 12 h, 70%; (vii) HBTU, dry Et_3N, dry DMF, 25 °C, 1 h, 50%.

For the synthesis of analog **8**, fragment **2** was first condensed with fragment **14** under Mitsunobu reaction conditions. However, the reaction was sluggish and gave

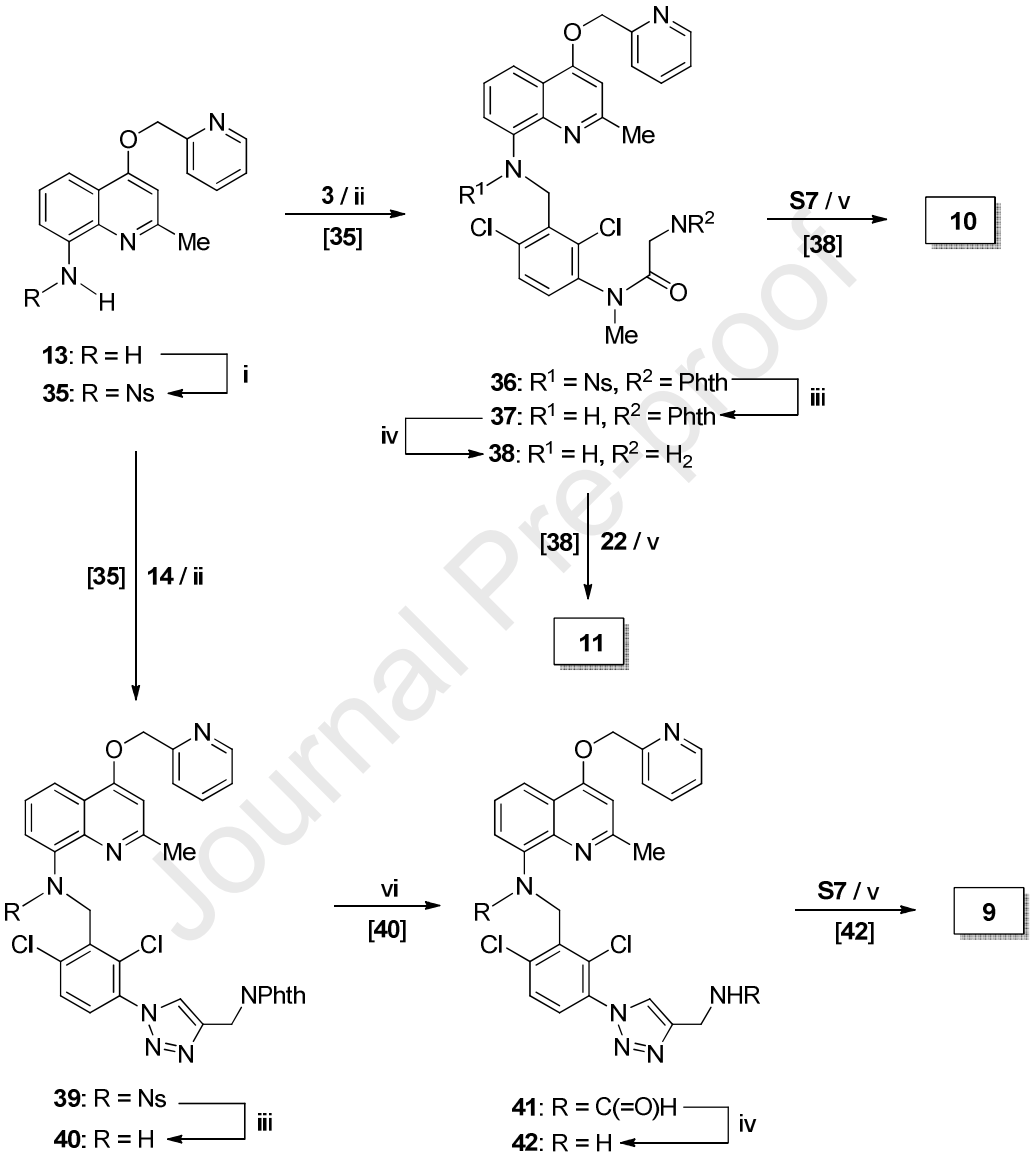
the product **33** in low yield (15%). Alternatively, fragment **14** was first converted to the corresponding bromide **32** in 47% yield, under Appel conditions, and this was then used to alkylate fragment **2** in the presence of K_2CO_3 (Scheme 5). Product **33** was then received in 84% yield. Hydrozinolysis of intermediate **33** gave the free amine **34** in 45% yield, which was then coupled with fragment **4**, in the presence of the coupling agent HBTU and Et_3N, to give analog **8** in 76% yield.



Scheme 5. Synthesis of FR-190997 analog **8**. *Reagents and conditions*: (i) CBr_4, TPP, dry THF, 0 ^oC then 25 °C, 3 h, 47%; (ii) TPP, DIAD, dry DMF, 25 ^oC to 60-70 ^oC,48 h, 15%; (iii) K_2CO_3, dry DMF, 25 °C, 3 h, 84%; (iv) $N_2H_4 \cdot H_2O$, DMF, MeOH, 70–80 °C, 1 h, 45%; (v) HBTU, dry Et_3N, dry DMF, 25 °C, 1 h, 76%.

Fragment **13** was envisaged as the key-intermediate for the synthesis of analogs **9-11** and was initially converted to the N-nosylated derivative **35** in 91% yield (Scheme 6). The nosylamide was subsequently coupled, under Mitsunobu reaction conditions [35], with alcohol **3** to give intermediate **36**. From this intermediate, the Ns group was removed with PhSH in the presence of Na_2CO_3 to give intermediate **37** in 52% yield for the two steps. Hydrazinolysis of compound **37** produced the diamine **38** in 97% yield, which was finally coupled with 'active' ester **S7** (Scheme S2 of SM) to give FR-190997 analog **10** in 68% yield. Similarly, diamine **38** was coupled with 'active' ester **22** giving the FR-190997 analog **11** in 43% yield. In an analogous manner, compound **35** was alkylated with alcohol **14**, under Mitsunobu reaction conditions, to produce intermediate **39**. The nosyl group was then removed from compound **39** to provide the intermediate **40** in 43% yield for the two steps. Finally, removal of the Phth protecting group with hydrazinolysis initially produced the N-formylated derivative **41** which, upon deformylation with hydrazine in refluxing

EtOH and coupling of the thus obtained diamine **42** with 'active' ester **S7**, produced the desired FR-190997 analog **9** in 37% yield (combined for the last three reactions). Compound **41** was formed as an intermediate in the hydrazinolysis reaction because DMF had to be used as cosolvent in the first step due to the insolubility of compound **40** in EtOH.



Scheme 6. Synthesis of FR-190997 analogs **9**-**11**. *Reagents and conditions*: (i) NsCl, dry Et_3N, dry DCM, 0 °C for 50 min then 25 °C for 48 h, 91%; (ii) TPP, DIAD, THF dry, 0 °C then 40-50°C, 4 h (**36**) or 0 °C to 40-50 °C for 3.5 h, then 25 °C, 12 h (**39**); (iii) PhSH, Na_2CO_3, dry DMF, 40–50 °C, 2 h, 52% (**37**) or 25 °C, 2 h then 40–50 °C for 1 h and then 25 °C, 48 h, 43% (**40**); (iv) $N_2H_4 \cdot H_2O$, EtOH, reflux, 1h, 97% (**38)** or 36 h 72% (**42)**; (v) dry Et_3N, dry DMF, 25 °C, 2 h, 51%(**9**) or 1.5 h,68% (**10**) or 12 h, 42% (**11**);(vi) $N_2H_4 \cdot H_2O$, DMF/EtOH, 80 °C, 12h.

2.3 Structure-Antiproliferative activity of FR-190997 and analogue structures

All synthesized compounds were tested for their antiproliferative potential using two breast cancer cell lines: the ERα-positive, epithelial MCF-7 cells with low metastatic potential and the ERβ-positive, mesenchymal MDA-MB-231 cells with high metastatic potential. For comparison purposes, the commercially available compounds FerA and atRA were also included in this assessment. The IC_{50} values of the tested compounds are summarized in Table 1.

Table 1. IC_{50} (μM) values of the synthesized compounds in MCF-7 and MDA-MB-231 breast cancer cells.

LIGAND	MCF-7	MDA-MB-231
FR-190997	2.14 ± 0.034	0.08 ± 0.089
2	0.16 ± 0.06	450 ± 12.169
3	53 ± 4.026	0.78 ± 0.039
4	2.12 ± 0.017	86 ± 1.049
5	0.93 ± 0.062	650 ± 12.427
6	0.33 ± 0.05	5.9 ± 0.028
7	65 ± 2.031	193 ± 9.048
8	91 ± 7.061	200 ± 10.012
9	0.73 ± 0.043	0.43 ± 0.046
10	1.21 ± 0.071	$5.94*10^{-5}$ ± 0.00005
11	64 ± 5.031	$5*10^{-3}$ ± 0.0014
12	407 ± 12.073	6.97 ± 0.103
13	0.86 ± 0.076	22 ± 0.57
14	0.07 ± 1.311	520 ± 10.227
FerA	0.36 ± 0.017	15 ± 1.156
atRA	6.14 ± 0.38	16 ± 1.106

In contrast to the hypothesis made earlier in the literature [4] that B2R agonists may only promote tumor proliferation, we found that B2R agonist FR-190997 inhibited cell proliferation of the MCF-7 cell line significantly with an IC_{50} of 2.14 μM whereas the antiproliferative effect in the MDA-MB-231 cell line was even more pronounced with an IC_{50} of 0.08 μM. The SAR results with FR-190997 analogue structures and sub-domains are discussed separately for each cell line.

MCF-7 cell line

Careful inspection of Table 1 reveals that the efficacy of FR-190997 in the MCF-7 cell line stems from the quinoline (compound **2**) and cinnamic acid anilide (compound **5**) domains which independently produce better antiproliferative activity

(IC_{50} 0.16 and 0.93 μM respectively). Connecting these two privileged structures into a single molecular entity (FR-190997) does not however produce an additive effect.

Interestingly, examination of the individual components of amide **5** reveals that the *p*-substituted cinnamic acid **4** alone exhibits essentially the same activity as FR-190997 whereas the N-Phth-protected dichloroanilino glycinamide core **3** possesses much reduced activity (IC_{50} 53 μM). This suggests that the acid component in the terminal amide of FR-190997 imparts significant activity to the glycinamide core hence we tested FR-190997 analogues with other carboxylic acids of established antitumor potential such as ferulic acid (FerA) and *all-trans*-retinoic acid (atRA). Indeed, FerA derivative **6** exhibited a 7-fold increase antiproliferative activity with respect to FR-190997 in the MCF-7 assay whereas atRA derivative **7** was less active by 30-fold. This implies that extended conjugation in the terminal acid does not necessarily impart significant antiproliferative activity in the combined structure and that probably cinnamate derivatives possessing a hydrogen bond donor/acceptor at the 4-position (CONH for FR-190997 and OH for **6**) is a much more important feature in this context.

Next, we interrogated modification at the N-methylanilide region of FR-190997, by replacing the *cis*-N-methylglycinamide moiety with a triazole ring (compound **8**) in an attempt to restrict the conformational freedom in this part of the molecule. The incorporation of the triazole ring in the structure of FR-190997 had a detrimental effect on antiproliferative activity (IC_{50} 91 μM) although when this modification was applied to the dichloroaryl core alone (compound **14**), a direct analogue of **3**, produced the most active derivative of the present series with nanomolar activity in the MCF-7 cell line (IC_{50} 0.07 μM).

Further modification of **8** by switching the ether to a secondary amine linker (compound **9**), joining the quinoline core with the 2,6-dichloro-3-triazolobenzyl domain of **8**, imparted nanomolar antiproliferative activity in the FR-190997 daughter triazole scaffold. Applying this modification directly on FR-190997 produced analogue **10** which showed 2-fold improved activity over FR-190997 yet slightly reduced activity with respect to its triazole counterpart **9**.

Consistent with the trend observed in the O-linked compounds, replacement of the substituted cinnamic acid residue in **10** with atRA (compound **11**), led to 50-fold reduction of the antiproliferative potency in the MCF-7 assay thus corroborating further the negative impact of the atRA-derived amides.

As mentioned above, the 8-hydroxy quinoline fragment (compound **2**) possesses significant activity against the MCF-7 cell line (IC_{50} 0.16 μM) which is retained to a large degree in its anilino-analogue **13 (**IC_{50} of 0.86 μM) whereas the corresponding quinoline ether derivative **12** lacking the hydrogen bond donor capacity of **2** and **13**, was essentially inactive in the MCF-7 cell line.

Overall, in the context of the MCF-7 cell line, these results support that the choice of the terminal carboxylic acid residue is of prime importance for antiproliferative activity with cinnamate derivatives possessing a hydrogen bond donor/acceptor at the 4-position being particularly active (compounds FR-190997, **6**, **8**, **9**, **10** *versus* **7** and **11**). Second in importance is the presence of a hydrogen bond donor in the linker region as derivatives with an NH linker are more active than their O-linked counterparts (compounds **9** and **10** *versus* **8** and FR-190997 respectively). A subtle yet noticeable feature that further increases the antiproliferative activity of the NH-linked FR-190997 analogue structures, involves the regidification around the amine component in the terminal amide domain by the use of 4-aminomethyltriazole in place of the conformationaly more labile glycine skeleton (compound **9** *versus* **10**). Based on the above, it is projected that the FerA analogue structures of **9** and **10** should be even more potent in the MCF-7 cell line.

MDA-MB-231 cell line

In contrast to the MCF-7 assay, the high cellular efficacy of FR-190997 in the MDA-MB-231 cell line appears to emanate largely from the 3-glycinamido-2,6-dichlorobenzyl core (compound **3)** rather than the quinoline domain (compound **2**) or terminal acid residue (compound **4**) (IC_{50} 0.78 versus 450 and 86 μM respectively in this assay, Table 1). Connecting the cinnamic acid in the form of terminal amide on core fragment **3** produces the essentially inactive derivative **5** (IC_{50} 650 μM). Interestingly, attaching the inactive quinoline domain **2** through an ether linker to the also inactive **5,** forms FR-190997 which possess nanomolar activity in the MDA-MB-231 assay. This unpredicted positive synergy of domains **2**, and **5** (in turn formed by **3** and **4**) is in stark contrast with what was observed in the MCF-7 assay where the same fragments exhibited significant antiproliferative activity yet their union into FR-190997 had a negative impact. Switching the terminal carboxylic acid residue from the 4-substituted cinnamate in FR-190997 to FerA and atRA in analogue structures **6** and **7** led to inferior potency in the MDA-MB-231 cell line, particularly so in the latter case.

Restricting the conformational freedom of the *cis*-N-methylglycinamide moiety in FR-190997 by replacing this with a triazole ring (compound **8**) also causes a sharp drop in the antiproliferative activity in the MDA-MB-231assay. The negative impact of the triazole modification is also consistent in the respective fragments **3** and **14** (IC_{50} 0.78 *versus* 520 μM respectively).

Finally, applying the O to NH switch in the linker region of FR-190997 produced analogue **10** which proved the most active compound in the present series with subnanomolar antiproliferative activity in the MDA-MB-231 cell line (IC_{50} $5.94*10^{-5}$ μM). In compound **9**, the O to NH replacement at the linker of the inactive FR-190997 triazole analogue **8** also rescues the antiproliferative activity precipitating nanomolar potency (IC_{50} 0.43 μM). The profound impact of this modification is clearly seen in analogue **11** where one of the consistently inactive atRA analogues is rendered a potent antiproliferative agent with low nanomolar potency (IC_{50} $5*10^{-3}$ μM). The evidently key 8-aminoquinoline fragment **13**, does possess antiproliferative capacity (IC_{50} 22 μM) but this is several orders of magnitude lower than those achieved by structures encompassing all three domains such as FR-190997 and analogues **9**, **10** and **11** which further corroborates the beneficial synergy among the quinoline, the 2,6-dichloro-3-glycinamido-benzyl and the terminal acid domains.

It is thus established for the MDA-MB-231 cell line that the NH linker in the FR-190997 analogue structures is of paramount importance for antiproliferative activity followed by the conformational flexibility of the 3-glycinamide side chain in the 2,6-dichlorobenzyl core while several carboxylic acids with extended conjugated systems may be tolerated in the terminal amide domain.

Overall, it is worth noticing that although the SARs for the MCF-7 and MDA-MB-231 cell lines follow opposite trends with respect to the triazole and terminal acid modifications, the O to N switch in the linker region is beneficial across both assays and that respective optimum potencies converge to analogue **10**.

It must be also emphasised that whatever the antiproliferative activity of each of fragments **2**, **3**, **4**, **5**, **12**, **13** and **14**, it is not anticipated to stem from B2R agonism alone, if at all, since molecules of this size tend to be promiscuous ligands and the origin of their activity could be attributed to association with more than one targets. In contrast, compounds **6**, **7**, **8**, **9**, **10** and **11** can be safely claimed as B2R agonists or partial agonists not simply due to their extensive structural resemblance to FR-190997 but mostly because of Fujisawa's extensive SAR and optimisation studies

which have shown that the agonist/antagonist profile is determined solely by the substitution at the 4-position of the quinoline domain [15, 18-22] (see also Figure 1). The same work established that the dichloro benzyl core is important for overall activity whereas the glycinamide and terminal acid portions modulate only the magnitude of the agonistic or antagonistic effect and does not change the functional aspect of the molecule.

2.4 Proposed mechanism of action based on the established knowledge on B2R

As mentioned above, for the two cancer lines examined, bradykinin activation of B2R is reported to promote cancer proliferation in the MCF-7 assay [28] and B2R antagonist FR-173657 showed antiproliferative effects in the MDA-MB-231 assay [31] therefore our antiproliferative results with a B2R agonist may appear to create a paradox. This apparent controversy may be resolved if certain important points are taken into consideration.

First, all work supporting the oncogenic fate of B2 agonism is based on stimulation with bradykinin [28-30, 36-40]. B2R stimulation with other types of agonists does not share the same characteristics with bradykinin simulation hence the oncogenicity of the latter may not apply to all types of agonists. Synthetic peptidic and non-peptidic B2R agonists do not actually mirror the functional aspects/selectivity of the natural hormone bradykinin post B2 stimulation neither in terms of type nor magnitude. For example, the highly potent and full agonist **1B** (Figure 1) stimulates phosphorylation of ERK (the oncogenic pathway) quicker and with much reduced efficacy in comparison to bradykinin (1 μM *vs* 10 nM, respectively) [41,42].

Second, bradykinin is rapidly degraded under physiological conditions whereas FR-190997 belongs to a class of stable and inactivation-resistant B2R ligands [41]. Third, the fate of B2 receptor itself varies after ligand binding/stimulation and this fate is dependent on ligand type [43]. Agonist binding to B2R causes internalization of the receptor *via* association with β-arrestin$_{1/2}$. This is a reversible process that with the short lived bradykinin the receptor is quickly released and reinstated at the cell membrane leading to a vicious circle of potent bursts of ERK activation. Conversely, as elegantly shown by Marceau, when degradation resistant (long lived) synthetic peptidic or non-peptidic agonists such as **1B** (and FR-190997) are bound to B2R, the internalization process is predominately followed by receptor degradation [44].

Fujisawa in collaboration with the Gobeil group have shown this is also the case for FR-190997 (from the same Fujisawa series as **1B**) as it induced desensitization of B2R [45] (by default driven by receptor internalization).

It is precisely this phenomenon that we believe contributes to the observed antiproliferative properties of FR-190997. Based on this well-established process for GPCRs, prolonged engagement of B2 receptor with a high affinity (partial) agonist would lead to receptor internalization/downregulation thus attenuation of the membrane-bound B2R oncogenic signalling initiated by over-expressed extracellular bradykinin.

This agonist-induced inhibitory effect mirrors the mechanism of action of fingolimod. Phoshorylated fingolimod (fingolimod is actually a prodrug) binds to S1P1 on the T cell surface and causes receptor internalization and degradation thereby rendering the cell unresponsive to the egress signal of membrane S1P thus allowing the lymph node signal to be retained [46-48]. Like fingolimode, FR-190997, related B2R agonists and derivatives thereof may also be designated as functional antagonists by virtue of their biased agonism towards β-arrestin$_{1/2}$ which perturbs B2 receptor homeostasis, leading to downregulation of B2R [49-51].

Furthermore, internalized B2Rs escaping degradation or recycling have been shown to be subject of further endosomal trafficking leading to nuclear translocation. Takano has shown that the B2 receptor is transported in the nucleus by forming heterodimers with lamin C where it is suggested to function as a transcriptional regulator of specific genes [52,53].

More importantly, the group of Gobeil have found functional B2 receptors in the nuclei of rat hepatocytes corroborating earlier reports of B2Rs residing in the nucleus. Bradykinin stimulation of isolated nuclei induced concentration-dependent mobilization of nucleoplasmic calcium activation/phosphorylation of Akt, acetylation of histone H3 and pro-inflammatory iNOS gene induction [54]. This intracrine B2R-mediated signalling was blocked by direct exposure of the nuclei to the peptidic B2R antagonist HOE-140 but not by the B1R antagonist R715.

The Gobeil team followed this with a landmark article describing for the first time nuclearly expressed B2R in the human triple-negative breast cancer (TNBC) cell line MDA-MB-231 (cell line of our study) including human TNBC specimens. Treatment with cell-permeable peptidic or small molecule B2R antagonists that could reach endosomal domains elicited far superior anticancer effects to those observed with

non-permeable ones that could only act at membrane-bound B2R [31]. The antiproliferating and apoptosis-promoting effects on MDA-MB-231 cells by these agents was not manifested in B2R shRNA-knockdown or non-B2R expressing (COS-1) cells. Since GPCR antagonists do not cause GPCR internalization, Gobeil's work has established that the most efficient antiproliferative mechanism for B2R antagonists is to permeate through the cell and inhibit the intracellular/nuclear B2R signalling rather than the cascade originating at membrane-bound B2R.

The classical definitions of GPCR agonists/antagonists are associated to specific signal transduction originating from extracellular ligand interactions with membrane-bound GPCRs. It is therefore apparent that the usual agonist/antagonist designations are not valid for a ligand which permeates the cell, binds to an intracellular GPCR and inhibits its endosomal signalling (different from the membrane-originating cascade).

The apparent paradox of having both B2R agonists and antagonists exhibiting antiproliferative efficacy is potentially resolved by this assertion because it disentangles membrane-bound from intracellular GPCR signalling as these do not necessarily involve the same receptor organisation, ligand interaction, and effectors/cascades [55-58]. It is fairer to say that permeable ligands capable of binding to intracellular GPCR and disrupting its endosomal signalling are best viewed as "interceptors" or "sequestrators" and this property is independent of their agonistic/antagonistic capacity at the same GCPR residing on the membrane.

As evidenced by Gobeil's work, cell permeability and high GPCR affinity such as those associated with the B2R antagonist used in that work, FR-173657 (IC_{50} 1.4 nm [21,22], ALogP 5.57, CXLogD (pH 7.4) 4.0 [59]) apparently suffice to precipitate inhibition of intracellular B2R signalling by entering the cell and sequestering intracellular B2Rs. The established FR-173657 type of action may be also manifested by the high affinity B2R ligand FR-190997 (IC_{50} 0.41 nm) which is orally active in several animal species [22,45] attribute for which cell permeability is a prerequisite and further supported by its logP value(s) (ALogP 5.95, CXLogD (pH 7.4) 4.07 [60]). Based on the B2R affinity and permeability profiles of FR-173657 and FR-190997 one would expect the two Fujisawa molecules to exert similar potencies *via* the sequestration mechanism. Nevertheless, in the triple-negative breast cancer (TNBC) cell line MDA-MB-231, agonist FR-190997 exhibits far superior antiproliferative activity than FR-173657 (IC_{50} 0.08 *versus* 20 μm respectively)

implying that agonist induced B2R internalisation/degradation mechanism (only available to agonists) is much more efficient than the sequestration mechanism (available to both agonists and antagonists of appropriate profile) in suppressing tumorous cell proliferation, at least in this assay.

To put things into perspective a comparison of the antiproliferative activity of optimised B2R ligands, approved breast cancer drugs and established anticancer agents against the (TNBC) cell line MDA-MB-231 is presented in Table 2.

Table 2: Antiproliferative activity of B2R antagonists, agonists and established chemotherapeutic agents against MDA-MB-231 cells

Ligand	Mode of Action	IC_{50} / μM	Ref.
FR-173657	B2R antagonist	20	31
NG68	B2R antagonist	45	
FR-190997	B2R agonist	0.08	This work
Compound 10	B2R agonist*	$5.94*10^{-5}$	
Compound 11	B2R agonist*	0.005	
Approved breast cancer drugs			
Alpesilib	PI3Kα	62.9	61
Cyclophosphamide	Alkylating agent	195.5	
Docetaxel	Microtubule stabiliser	0.01-9.6	
Epirubicin	DNA intercalator	0.15	
5-Fluorouracil	Antimetabolite	66.6	
Fulvestrant	ESR	196.3	
Gemcitabine	Pyrimidine antimetabolite	2.1	
Lapatinib	EGFR, ERBB2	10.6	
Olaparib	PARP1/2	62.5	
Paclitaxel	Microtubule stabiliser	0.9	
Paldociclib	CDK4/6	14.6	
Ribociclib	CDK4/6	27.3	
Tamoxifen	ESR1	69.7	
Vinblastine	Microtubule destabiliser	0.024	
Other established anticancer agents			
Bortezomib	Proteasome inhibitor	0.003	61
Rapamycin	PI3K, mTOR	0.9	
Cisplatin	DNA crosslinker	36.2	
Staurosporin	Broad spectrum kinase inhibitor	0.01	
Doxorubicin	DNA intercalator	0.1-1.3	
*Based on Fujisawa's Structure-Activity/Functional-Relationship work			17-22

The significant antiproliferative activity of FR-190997 arguably stems from both mechanisms (B2 receptor internalisation/degradation and sequestration of intracellular/nuclear B2 receptors) thus rendered potentially a multimodal functional inhibitor of B2R-mediated signalling. Cognizant of the fact that we have not confirmed B2R agonism or off-target effects for the best analogue structure **10** we

posit that its exceptional (subnanomolar) antiproliferative performance is due to the same multimodal capacity as of FR-190997 since these are structurally identical bar one atom and is consistent with the agonist profile established in Fujisawa's extensive Structure-Activity/Functional-Relationship work [15, 18-22]. Having gained insight into the structural elements of FR-190997 responsible for imparting high antiproliferative potency, our ongoing efforts are focused on preparing additional analogues that may achieve broader spectrum antitumorigenic properties. In addition, we intend to gather further evidence to support that the established agonism-induced B2 receptor internalization and degradation applies also to partial agonist FR-190997 and that the latter is capable of sequestering intracellular/nuclear B2 receptors. Unraveling the significance of subcellular GPCR trafficking and endosomal signalling is one of the most exciting topics in current biology research as it holds promise for novel therapeutic interventions [55-58].

3. Conclusions

We have demonstrated for the first time that the non-peptidic, cell-permeable, B2R partial agonist FR-190997 and related analogue structures exhibit exceptional antiproliferating activities particularly in the TNBC cell line MDA-MB-231 where our analogue **10** with subnanomolar activity, surpasses popular anticancer agents and approved breast cancer drugs. This work represents the latest piece in the B2R-cancer puzzle which we believe reconciles existing controversies by considering how the action of B2R agonists/antagonists in the classical sense, transcends into the context of endosomal B2R signalling.

4. Materials and methods

4.1 Chemistry

4.1.1 General

Melting points were determined with an Electrothermal apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained at 600 and 150 MHz, respectively, on Bruker AvanceIII HD spectrometer. Chemical shifts (δ) are reported for $CDCl_3$ solutions in parts per million (ppm) downfield from tetramethylsilane (TMS), used as internal standard, unless otherwise stated. Electron-spray ionization (ESI) mass spectra were recorded at 30 eV, on a Waters Micromass ZQ spectrometer using MeOH as solvent. Elemental analyses were determined on a Carlo Erba EA 1108 CHNS elemental analyzer.

Flash column chromatography (FCC) was performed on Acros Organics silica gel 0.035-0.070 mm, 60 Å and TLC on Merck silica gel 60 F_{254} films (0.2 mm) precoated on aluminium foil. Spots were visualized with UV light at 254 nm. The solvents or solvent systems used for the development of TLC or FCC are the following: (A) $CHCl_3$/MeOH (96:4), (B) $CHCl_3$/MeOH (95:5), (C) $CHCl_3$/MeOH (9:1), (D) $CHCl_3$/MeOH(8:2), (E) CH_2Cl_2/MeOH (9:1), (F) PhMe, (G) EtOAc, (I) PhMe/EtOAc (9:1), (J) PhMe/EtOAc (8:2), (K) PhMe/EtOAc (6:4), (L) PhMe/EtOAc (1:1), (M) PhMe/EtOAc (2:8), (N) PhMe/EtOAc (3:7), (O) Hexane/EtOAc (9:1), (P) Hexane/EtOAc (1:1), (R)Hexane/EtOAc (8:2), (S) PhMe/EtOAc (7:3), (T) $CHCl_3$/MeOH (98:2), (U) $CHCl_3$/MeOH (97:3), (V) $CHCl_3$/MeOH (92:8), (W) $CHCl_3$/MeOH (93:7).

All solvents were dried and/or purified according to standard procedures prior to use. Solvents were routinely removed at ca. 40 ºC under reduced pressure on Büchi Rotavapor RE 111 apparatus. Air-sensitive reagents were handled under inert atmosphere (Ar). All reagents employed in the present work were purchased from either Sigma-Aldrich or Alfa Aesar or Merck or Acros Organics or Fluorochem and were used without further purification. For the needs of the present work, 'active' ester **22** was prepared according to a published procedure [15].

*4.1.2 4-Chloro-2-methyl-8-nitroquinoline (**16**)*

To a cooled to -10 °C solution of 4-chloro-2-methylquinoline (2.5 g, 14.1 mmol) in 96% H_2SO_4 (8.9 mL) was added KNO_3 (2.2 g, 21.8 mmol) portion-wise over 50 min

and the mixture was stirred at room temperature (rt) overnight. The reaction mixture was poured carefully on ice (40 g), then adjusted to pH 7-8 with a saturated aq. solution of NaOH and finally extracted thrice with EtOAc. The organic phases were combined and washed twice with brine, dried over Na_2SO_4 and finally evaporated to dryness under reduced pressure. The residue was subjected to silica gel flash column chromatography (FCC), using PhMe as eluant, to afford pure compound **16**.

Yield: 1.54 g (49%); yellow solid, m.p. 113-114 °C, lit. [13] m.p. 111-113 ^{o}C; R_f (F) 0.26; MS (ESI) *m/z*: 245.24 $(M+Na)^+$, 223.13 $(M+H)^+$; ^{1}H-NMR: δ 8.38 (dd, J = 8.4 and 0.9 Hz, 1H), 7.98 (dd, J = 8.4 and 0.9 Hz, 1H), 7.63 (t, J = 8.4 Hz, 1H), 7.52 (s, 1H), 2.74 (s, 3H) ppm; ^{13}C-NMR: δ 161.9, 148.3, 142.5, 140.0, 127.7, 125.7, 125.2, 123.9, 123.7, 25.6 ppm.

*4.1.3 2-Methyl-8-nitro-4-(pyridyl-2-ylmethoxy)quinoline (**17**)*

To an ice-cold solution of (pyridin-2-yl)methanol (3.8 mL, 39.3 mmol) in DMI (3.8 mL) was added ^{t}BuOK (0.71 g, 6.3 mmol) and, following its dissolution, the temperature was lowered to-10 °C. Then, compound **16** (1.4 g, 6.3 mmol) was added portion-wise over 50 minutes and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with water (50 mL) and the thus resulting suspension was extracted thrice with EtOAc. The organic phases were combined and washed twice with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system L as eluant, to give pure compound **17**.

Yield: 0.8 g (43%); orange solid, m.p. 159.5–160.5 °C; R_f(L) 0.25; MS (ESI) *m/z*: 334.42 $(M+K)^+$, 318.42 $(M+Na)^+$, 296.50 $(M+H)^+$; ^{1}H-NMR: δ 8.65 (d, J = 4.3 Hz, 1H), 8.43 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.76 (t, J = 8.4 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.30 (dd, J = 7.8 and 4.3 Hz, 1H), 6.80 (s, 1H), 5.43 (s, 2H), 2.67 (s, 3H) ppm; ^{13}C-NMR: δ 163.4, 160.6, 155.4, 149.6, 147.9, 140.3, 137.1, 125.8, 123.7, 123.4, 123.3, 121.4, 121.2, 103.4, 71.2, 26.4 ppm; Anal. Calcd for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.38; H, 4.23; N, 14.02.

*4.1.4 2-Methyl-4-(pyridyl-2-ylmethoxy)quinoline-8-amine (**13**)*

To a solution of compound **17** (0.8 g, 2.71 mmol) in aq. MeOH (80%) (10.7 mL) were added $FeCl_3 \cdot 6H_2O$ (32.4 mg) and activated carbon (32.4 mg) and the resulting

mixture was heated to reflux. Then, $N_2H_4 \cdot H_2O$ (0.33 mL, 6.8 mmol) was added dropwise and the mixture was stirred at the same temperature for 2 hours. TLC analysis indicated that the reaction was not complete. An additional amount of $N_2H_4 \cdot H_2O$ (0.33 mL, 6.8 mmol) was then added and 1 h after the last addition, the reaction mixture was left to attain rt and diluted with EtOAc (30 mL). The organic phase was washed once with an aq. solution of 5% $NaHCO_3$ and twice with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system K as eluant, to afford pure compound **13**.

Yield: 0.52 g (72%); brown solid, m.p. 129.5-130 °C; R_f(L) 0.23; MS (ESI) *m/*z: 266.40 $(M+H)^+$; ^{1}H NMR: δ8.63 (d, *J* = 4.8 Hz, 1H), 7.74 (td, *J* = 8.4 and 1.2 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.57 (dd, *J* = 8.4 and 1.2 Hz, 1H), 7.28-7.22 (m, 2H), 6.91(dd, *J* = 8.4 and 1.2 Hz, 1H), 6.66 (s, 1H) 5.39 (s, 2H), 4.93 (br.s, 2H), 2.63 (s, 3H) ppm; ^{13}C NMR:δ 157.2, 156.4, 149.3, 143.2, 137.0, 129.0, 128.2, 125.6, 122.9, 121.1, 120.2, 110.9, 109.9, 102.0, 70.6, 25.8 ppm; Anal. Calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.13; H, 5.88; N, 16.12.

*4.1.5 8-Iodo-2-Methyl-4-(pyridyl-2-ylmethoxy)quinoline (**18**)*

To a suspension of **13** (0.13 g, 0.5 mmol) in H_2O (1.5 mL) and 37% aq. HCl (0.2 mL, 2.4 mmol) at 5 °C was added an aq. solution of 1.6 M $NaNO_2$ (0.34 mL, 0.55 mmol) dropwise. After 30 min of stirring at 5 °C, TLC analysis confirmedthe complete conversion of compound **13** to its corresponding diazonium salt and thus an ice cold aq. solution of 3 M KI (0.35 mL, 1.05 mmol) was added dropwise. The reaction mixture was stirred at 5 °C for 10 min and at 25 °C for 1 h. Then, it was adjusted to pH 9 with a saturated aq. solution of $NaHCO_3$ and extracted thrice with EtOAc. The organic phases were combined and washed once with a saturated aq. solution of $Na_2S_2O_3$ and twice with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system L, to afford pure compound **18**.

Yield: 0.14 g (74%); brown solid; mp 95.5-97.5 °C; R_f (L) 0.35; MS (ESI) *m/z*:377.31 $(M+H)^+$, 399.29 $(M+Na)^+$; ^{1}H-NMR: δ 8.64 (ddd, *J* = 4.8, 1.8 and 1.2 Hz, 1H), 8.29 (dd, *J* = 7.5 and 1.2 Hz, 1H), 8.26 (dd, *J* = 7.5 and 1.2 Hz, 1H), 7.75 (td, *J* = 7.8 & 1.8 Hz, 1H), 7.55 (unresolv. dt, *J* = 7.8 Hz, 1H), 7.28 (ddt, *J* = 7.8, 5.4 and 0.6 Hz, 1H), 7.17 (dd, *J* = 8.4 and 7.8 Hz, 1H), 6.74 (s, 1H), 5.41 (s, 2H), 2.72 (s, 3H)

ppm; ^{13}C-NMR: δ 161.4, 160.9, 155.9, 149.4, 147.6, 140.4, 137.1, 126.0, 123.1, 122.5, 121.3, 120.3, 102.5,102.4,71.0, 26.2 ppm; Anal. Calcd for $C_{16}H_{13}IN_2O$: C, 51.08; H, 3.48; N, 7.45. Found: C, 51.32; H, 3.20; N, 7.32.

*4.1.6 2-Methyl-8-(3-phenoxybenzyloxy)-4-(pyridyl-2-ylmethoxy)quinoline (**12**)*

Method A (Ullmann reaction). To a solution of CuI (3.6 mg, 0.019 mmol), 1,10-phenathroline (6.8 mg, 0.038 mmol) and Cs_2CO_3 (124.0 mg, 0.38 mmol) in DMI (0.1 mL) were added **18** (71 mg, 0.19 mmol) and *m*-phenoxybenzyl alcohol (66 μL, 0.38 mmol) and the reaction mixture was stirred at 110 °Covernight. The mixture was then directly subjected to FCC, using the solvent system L as eluant, to afford pure compound **12**.

Yield: 15 mg (18%); beige solid; mp 120-123.5 °C; R_f(L) 0.15; MS (ESI) *m/*z: 449.29 $(M+H)^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.64 (d, J = 4.2 Hz, 1H),7.84 (dd, J = 8.4 and 0.6 Hz, 1H), 7.75 (td, J = 7.8 and 1.8 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.33-7.24 (m, 6H), 7.18 (unresolv. t, 1H), 7.07 (unresolv. tt, J = 7.2 Hz, 1H), 7.01 (d, J = 7.2 Hz, 1H), 6.99-6.96 (m, 2H), 6.92 (dd, J = 7.8 and 1.8 Hz, 1H), 6.73 (s, 1H), 5.41 (s, 4H), 2.71 (s, 3H) ppm.^{13}C-NMR: 160.9, 160.8, 159.2, 157.5, 157.1, 156.2, 153.6, 149.4, 139.5, 137.0, 129.9, 129.7 (2C), 124.7, 123.2, 123.0, 121.6, 121.2, 121.1, 118.9 (2C), 118.0, 117.3, 114.0, 111.3, 102.5, 70.7, 70.6, 26.3 ppm; Anal. Calcd for $C_{29}H_{24}N_2O_3$: C, 77.66; H, 5.39; N, 6.25. Found: C, 77.48; H, 5.60; N, 6.45.

Method B (Mitsunobu reaction). To a solution of quinolinol **2** (100 mg, 0.38 mmol) and *m*-phenoxybenzyl alcohol (73 μL, 0.42 mmol) in dry THF (0.5 mL) was added TPP (121 mg, 0.46 mmol) under argon and the mixture was cooled to 0 °C. Then, DIAD (91 μL, 0.46 mmol) was added and the reaction mixture was stirred at rt for 1.5 h. TLC analysis indicated that the reaction was not complete. Therefore, additional *m*-phenoxybenzyl alcohol (73 μL, 0.42 mmol), TPP (121 mg, 0.46 mmol) and DIAD (91 μL, 0.46 mmol) were added in two portions over 48 h. The reaction mixture was evaporated to dryness and the residue was subjected to FCC using the solvent system N as eluant, to afford compound **12** containing TPPO in the molar ratio 1:3 (by ^{1}H- NMR). This mixture was portioned between EtOAc and 2N HCl, the aqueous layer was neutralized with NaOH and extracted with EtOAc. The organic layer was dried and evaporated to dryness to leave pure compound **12** which was crystallized on standing. Yield: 102 mg (60%).

4.1.7 tert-*Butyldiphenylsilyl (*E*)-3-(4-(*tert-*butyldiphenylsilyloxy)-3-methoxyphenyl)-acrylate* (**19**)

To a solution of FerA (0.19 g, 1 mmol) in dry DMF (1.5 mL) were added sequentially, under argon, imidazole (0.16 g, 2.4 mmol) and TBDPSCl (0.6 mL, 2.3 mmol) and the mixture was stirred at rt for 3 h. The reaction mixture was then diluted with water and extracted thrice with Et_2O. The organic phases were combined and washed thrice with water and once with brine, dried over Na_2SO_4 and evaporated dryness under reduced pressure. The residue was subjected to FCC, using the solvent system O as eluant, to afford pure ester **19**.

Yield: 0.54 g (81%); pale-yellow oil; R_f (O) 0.33; MS (ESI) *m/z* 693.31 $(M+Na)^+$; ^{1}H-NMR: δ 7.74-7.70 (m, 8H), 7.64 (d, *J* = 15.6 Hz, 1H), 7.46-7.35 (m, 12H), 6.97 (d, *J* = 1.8 Hz, 1H), 6.88 (dd, *J* = 7.8 and 1.8 Hz, 1H), 6.71 (d, *J* = 7.8 Hz, 1H), 6.35 (d, *J* = 15.6 Hz, 1H), 3.63 (s, 3H), 1.16 (s, 9 H), 1.14 (s, 9H) ppm; ^{13}C-NMR: δ 166.1, 150.9, 147.6, 146.0, 135.3 (2C), 135.2 (2C), 133.1 (2C), 132.1 (2C), 130.0 (4C), 129.8 (4C), 128.1, 127.7 (4C), 127.6 (4C), 122.4, 120.4, 117.3, 111.0, 55.4, 27.0 (3C), 26.6 (3C), 19.8, 19.2 ppm.

*4.1.8 (*E*)-3-(4-(*tert-*Butyldiphenylsilyloxy)-3-methoxyphenyl)acrylic acid (**20**)*

To an ice-cold solution of ester **19** (0.37 g, 0.55 mmol) in MeOH (7 mL) and THF (2.5 mL) was added an aq. solution of 10% K_2CO_3 (2.3 mL) and the mixture was stirred at rt for 1 h. The reaction mixture was diluted with cold brine (7 mL), cooled to 0 °C, adjusted to pH 4-5 with a cold aq. solution of 5% citric acid and extracted thrice with EtOAc. It should be noted that during acidification ferulic acid was also produced. The organic phases were combined and washed twice with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure at a bath temperature not exceeding 30 °C. The residue was subjected to FCC, using the solvent system P for elution, to afford pure acid **20**.

Yield: 0.14 g (61%); yellow oil; R_f (P) 0.20;MS (ESI) *m/z*:433.21 $(M+H)^+$; ^{1}H-NMR: δ 7.80-7.76 (m, 4H), 7.72 (d, *J* = 16.2 Hz, 1H), 7.50 (tt, *J* = 7.2 and 1.4 Hz, 2H), 7.44 (t, *J* = 7.2 Hz, 4H), 7.04 (d, *J* = 1.5 Hz, 1H), 6.95 (dd, *J*= 8.4 and 1.5 Hz, 1H), 6.78 (d, *J*= 8.4 Hz, 1H), 6.32 (d, *J*= 16.2 Hz, 1H), 3.69 (s, 3H), 1.20 (s, 9H) ppm. ^{13}C-NMR: δ 172.1, 150.9, 149.7, 149.2, 135.3 (4C), 133.1 (2C), 129.8 (2C), 127.7, 127.6 (4C), 122.5, 120.4, 114.7, 111.2, 55.3, 26.6 (3C), 19.8 ppm.

*4.1.9 2,5-Dioxopyrrolidin-1-yl (E)-3-(4-(*tert*-butyldiphenylsilyloxy)-3-methoxyphenyl)acrylate (**21**)*

To an ice-cold solution of acid **20** (0.12 g, 0.28 mmol) in dry THF (1 mL) were added HOSu (0.08 g, 0.72 mmol) and DCC (0.08 g, 0.4 mmol) under argon and the mixture was stirred for 30 min at 0 °C and 5 h at rt. TLC analysis indicated that the reaction was not completed, therefore, additional DCC (0.01 g, 0.05 mmol) was added in the same manner and the reaction mixture was further stirred overnight at rt. Then, water (0.15 mL) and gl. AcOH (0.05 mL) were added and after 30 min of stirring, the precipitate was filtered off and washed on the filter with cold EtOAc. The filtrate was diluted with EtOAc, washed thrice with an aq. solution of 5% $NaHCO_3$, twice with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system P as eluant to afford pure 'active' ester **21**.

Yield: 126 mg (85%); yellow oil; R_f (P); 0.34; MS (ESI) *m/z*: 1081.23 $(2M+Na)^+$, 568.31 $(M+K)^+$, 552.50 $(M+Na)^+$; ^{1}H-NMR: δ 7.77 (d, J = 16.2 Hz, 1H), 7.70-7.67 (m, 4H), 7.43-7.39 (m, 2H), 7.37-7.33 (m, 4H), 6.95 (d, J = 1.8 Hz, 1H), 6.89 (dd, J = 7.8 and 1.8 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.36 (d, J = 16.2 Hz, 1H), 3.60 (s, 3H), 2.86 (br.s, 4H), 1.12 (s, 9H) ppm; ^{13}C-NMR: δ 169.4 (2C), 162.3, 151.0, 150.2, 148.8, 135.3 (4C), 132.9 (2C), 129.9 (2C), 127.6 (4C), 127.2, 123.1, 120.5, 111.3, 108.8, 55.3, 26.6 (3C), 25.6 (2C), 19.8 ppm; Anal. Calcd for $C_{30}H_{31}NO_6Si$: C, 68.03; H, 5.90; N, 2.64. Found: C, 68.34; H, 5.49; N, 2.41.

*4.1.10 (3-Amino-2,6-dichlorophenyl)methanol (**23**)*

To a solution of nitro compound **S11** (1.0 g, 4.5 mmol) in aq. MeOH (80%) (16.2 mL) were added $FeCl_3 \cdot 6H_2O$ (36.0 mg) and activated carbon (36.0 mg) and the mixture was heated to reflux. Then, $N_2H_4 \cdot H_2O$ (0.53 mL, 10.7 mmol) was added drop-wise and the reaction mixture was stirred at the same temperature for 2 h. TLC analysis indicated that the reaction was not completed, therefore, additional $N_2H_4 \cdot H_2O$ (0.53 mL, 10.7 mmol) was added and after 2 more hours at the same temperature, the reaction was completed. The reaction mixture was left to attain rt and then diluted with EtOAc. The organic phase was washed once with an aq. solution of 5% $NaHCO_3$ and twice with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system S as eluant, to afford pure aniline derivative **23.**

Yield: 0.7 g (81%); white solid, mp 97.5-98 °C; R_f (J) 0.21; ^{1}H-NMR: δ 7.13 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 4.95 (s, 2H), 4.13 (br.s, 2H) ppm; ^{13}C-NMR: δ 142.4, 135.6, 128.3, 123.7, 120.5, 115.9, 60.7 ppm; Anal. Calcd for $C_7H_7Cl_2NO$: C, 43.78; H, 3.67; N, 7.29. Found: C, 43.54; H, 3.90; N, 7.51.

4.1.11 (*3-Azido-2,6-dichlorophenyl)methanol (**24**)*

To an ice-cold solution of aniline derivative **23** (0.69 g, 3.6 mmol) in TFA (10 mL) was added $NaNO_2$ (0.29 g, 4.3 mmol) portion-wise over 15 min. After 30 min of stirring at 0 °C, the complete conversion of compound **23** to its corresponding diazonium salt was confirmed by TLC analysis and then, NaN_3 (0.27 g, 4.2 mmol) was added portion-wise over 5 min and the mixture was stirred at rt for 30 more minutes. The completion of the reaction was confirmed by TLC analysis and the *β*-naphthol test. The reaction mixture was then diluted with H_2O (12 mL), adjusted to pH 7-8 with solid K_2CO_3 and extracted four times with Et_2O. The organic phases were combined and washed twice with H_2O and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system I, to afford pure azide **24**.

Yield: 0.62 g (79%); beige solid; mp 87-88 °C; R_f (I) 0.33; MS (ESI) *m/z*:217.34 $(M)^+$; ^{1}H-NMR: δ 7.36 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 4.96 (s, 2H), 2.12 (br. s, 1H) ppm; ^{13}C-NMR: δ 137.5, 137.0, 131.4, 128.8, 126.7, 119.7, 60.4 ppm; Anal. Calcd for $C_7H_5Cl_2N_3O$: C, 38.56; H, 2.31; N, 19.27. Found: C, 38.22; H, 2.59; N, 19.54.

*4.1.12 2-(Prop-2-ynyl)isoindoline-1,3-dione (**25**)*

To a suspension of phthalimide (0.74 g, 5 mmol) and TPP (1.57 g, 6 mmol) in THF dry (10 mL) was added propargyl alcohol (0.35 mL, 6 mmol) under argon. The mixture was then cooled to 0 °C, followed by the drop-wise addition of DIAD (1.2 mL, 6 mmol). The reaction mixture was stirred at rt for 2 h and then evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system R as eluant, to afford compound **25**.

Yield: 0.56 g (60%); white solid; mp 139-141 °C; R_f (R) 0.21; ^{1}H-NMR: δ7.91-7.87 (m, 2H), 7.76-7.72 (m, 2H), 4.46 (d, J = 2.4 Hz, 2H), 2.22 (t, J = 2.4 Hz, 1H) ppm; ^{13}C-NMR: δ167.0 (2C), 134.2 (2C), 132.0 (2C), 123.6 (2C), 77.2, 71.5, 27.0 ppm.

*4.1.13 2-((1-(2,4-Dichloro-3-(hydroxymethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-isoindoline-1,3-dione (**14**)*

To a solution of azide **24** (0.74 g, 3.4 mmol) and alkyne **25** (0.76 g, 4.1 mmol) in DCM (20 mL) were added H_2O (20 mL), $CuSO_4 \cdot 5H_2O$ (0.12 g, 0.49 mmol) and sodium ascorbate (0.16 g, 0.82 mmol) under argon and the reaction mixture was stirred at rt for 3 h. The resulting precipitate was collected by vacuum filtration and then dissolved on the filter with boiling-hot $CHCl_3$. The filtrate was immediately washed twice with water, dried over Na_2SO_4 and evaporated under reduced pressure to afford pure compound **14**.

Yield:0.96 g (70%); yellow solid; mp 194.5-195.5 °C; R_f (J) 0.07; MS (ESI) *m/z*:425.32 $(M+Na)^+$; ^{1}H-NMR (d_6-DMSO): δ 8.52 (s, 1H), 7.95-7.90 (m, 2H), 7.89-7.85 (m, 2H), 7.68 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 5.42 (t, J = 5.4 Hz, 1H), 4.96 (s, 2H), 4.76 (d, J = 5.4 Hz, 2H) ppm; ^{13}C-NMR (d_6-DMSO): δ 167.8 (2C), 143.0, 138.5, 137.2, 135.1 (2C), 134.5, 132.1 (2C), 132.0, 129.4, 129.0, 126.0, 123.8 (2C), 59.2, 33.3 ppm; Anal. Calcd for $C_{18}H_{12}Cl_2N_4O_3$: C, 53.62; H, 3.00; N, 13.89. Found: C, 53.88; H, 2.79; N, 13.62.

4.1.14 2-Amino-N-*(2,4-dichloro-3-(hydroxymethyl)phenyl)*-N-*methylacetamide (**26**)*

To an ice-cold solution of **S16** (0.35 g, 0.7 mmol) in dry THF (4.6 mL) was added TBAF·$3H_2O$ (0.32 g, 1.0 mmol) under argon and the mixture was stirred at rt for 3 h. The reaction mixture was then diluted with DCM and washed twice with brine. The aqueous phases were re-extracted with DCM and then, the organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was subjected to FCC, using the solvent system $CHCl_3$/MeOH=8:2 as eluant, to afford 0.11 g crude compound **26**as colorless oil, which contains a small quantity of TBAF (molar ratio 11:1by ^{1}H-NMR) and was used as such to the next step.

R_f (D): 0.23; MS (ESI) *m/z*: 263.04 $(M+H)^+$; ^{1}H-NMR: δ 7.40 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 4.94 (s, 2H), 3.19 (s, 3H), 3.07 (d, J = 16.8 Hz, 1H), 2.97 (d, J = 16.8 Hz, 1H), 2.55 (br.s, 3H) ppm; ^{13}C-NMR: δ 172.3, 139.0, 138.3, 136.3, 135.3, 129.7, 129.5, 60.2, 43.6, 35.9 ppm.

*4.1.15 (*E*)-3-(4-(*tert*-Butyldiphenylsilyl)-3-methoxyphenyl)*-N-*(2-((2,4-dichloro-3-(hydroxymethyl)phenyl)(methyl)amino)-2-oxoethyl)acrylamide (**27**)*

A solution of **26** (80 mg, 0.3 mmol) and dry Et_3N (90µL, 0.64 mmol) in dry DMF (1 mL) was added under argon to **21** (0.19 g, 0.36 mmol). The reaction mixture was stirred at rt for 1 h and then, it was diluted with $CHCl_3$. The organic phase was washed once with an aq. solution of 5% $NaHCO_3$, twice with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system M as eluant, to afford pure compound **27**.

Yield:174 mg (85%); colourless oil; R_f (M) 0.34; MS (ESI) *m/z*: 699.19 $(M+K)^+$; ^{1}H-NMR: δ 7.69 (d, *J* = 7.8 Hz, 4H), 7.46-7.31 (m, 9H), 6.90 (d, *J* = 1.2 Hz, 1H), 6.81 (dd, *J* = 8.1 and 1.2 Hz, 1H), 6.67 (d, *J* = 8.1 Hz, 1H), 6.26 (d, *J* = 15.6 Hz, 1H), 6.53 (unresolv. t, 1H), 4.99 (ABq, *J* = 12.0 Hz, 2H), 3.87 (dd, *J* = 18.0 and 4.5 Hz, 1H), 3.63 (dd, *J* = 18.0 and 4.5 Hz, 1H), 3.59 (s, 3H), 3.24 (s, 3H), 1.11 (s, 9H) ppm; ^{13}C-NMR: δ 168.4, 165.9, 150.7, 147.0, 141.4, 138.3, 138.2, 136.9, 135.4, 135.3 (4C), 135.2, 133.3, 129.9, 129.8, 129.7 (2C), 128.3, 127.6 (4C), 121.4, 120.3, 117.9, 111.2, 60.5, 55.3, 42.1, 36.0, 26.6 (3C), 19.8 ppm.

*4.1.16 (*E*)-*N*-(2-((3-(Bromomethyl)-2,4-dichlorophenyl)(methyl)amino)-2-oxoethyl)-3-(4-(*tert*-butyldiphenylsilyloxy)-3-methoxyphenyl)acrylamide (**28**)*

To a solution of alcohol **27** (82 mg, 0.12 mmol) in THF dry (0.2 mL) were added CBr_4 (0.12g, 0.36 mmol) and TPP (94 mg, 0.36 mmol) at 0 °C in three portions over 2 h and the mixture was stirred at rt after each addition, protected from light. An hour after the last addition, TLC analysis indicated that the reaction was not completed, therefore, the reaction mixture was further stirred at rt overnight. Then, additional CBr_4 (0.12 g, 0.36 mmol) and TPP (94 mg, 0.36 mmol) were added and the reaction was completed within an hour. The mixture was evaporated to dryness under reduced pressure and the residue was subjected to FCC, using the solvent system K as eluant, to afford pure bromide**28**.

Yield: 64 mg (72%); white foam; R_f (K) 0.4; MS (ESI) *m/z*: 761.08 $(M+Na)^+$; ^{1}H-NMR: δ 7.69 (unresolv. dd, *J* = 7.2 Hz, 4H), 7.47-7.38 (m, 4H), 7.34 (t, *J* = 7.2 Hz, 4H), 7.26 (t, *J* = 4.2 Hz, 1H), 6.90 (d, *J* = 1.2 Hz, 1H), 6.82 (dd, *J* = 7.8 and 1.2 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.51 (unresolv. t, 1H), 6.26 (d, *J* = 15.6 Hz, 1H), 4.76 (ABq, *J* = 10.2 Hz, 2H), 3.90 (unresolv. dd, *J* = 18 Hz, 1H), 3.59 (s, 3H), 3.59 (unresolv. dd, *J* = 18 Hz, 1H), 3.25 (s, 3H), 1.11 (s, 9H) ppm; ^{13}C-NMR: δ 168.3, 165.9, 150.7, 147.0, 141.4, 138.5, 136.7, 136.3, 135.3 (4C), 135.0, 133.2 (2C), 130.0,

129.9, 129.7 (2C), 128.3, 127.6 (4C), 121.5, 120.3, 117.8, 111.1, 55.3, 42.0, 36.0, 27.1, 26.6 (3C), 19.8 ppm.

*4.1.17 (*E*)-3-(4-(*tert*-Butyldiphenylsilyloxy)-3-methoxyphenyl)-N-(2-((2,4-dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)oxy)methyl)phenyl)(methyl)-amino)-2-oxoethyl)acrylamide (**29**)*

To a solution of bromide **28** (46 mg, 0.062 mmol) and quinolinol **2** (17 mg, 0.064 mmol) in dry DMF (0.4 mL) was added K_2CO_3 (26 mg, 0.19 mmol) under argon and the reaction mixture was stirred at rt for 3 h. Then, it was diluted with $CHCl_3$ and washed thrice with water. The organic phase was dried over Na_2SO_4, evaporated to dryness under reduced pressure and the residue was subjected to FCC, using the solvent system B as eluant, to afford pure compound **29**. During this reaction, 5 mg of compound **6** were also produced and isolated from the aforementioned FCC as a pale yellow solid.

Yield: 25 mg (44%); yellow oil; R_f (B) 0.26; MS (ESI) *m/z*: 925.29 $(M+H)^+$.

*4.1.18 (*E*)-*N*-(2-((2,4-dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)oxy)methyl)phenyl)(methyl)amino)-2-oxoethyl)-3-(4-hydroxy-3-methoxyphenyl)-acrylamide (**6**)*

To an ice-cold solution of compound **29** (25 mg, 0.028 mmol) in dry THF (0.2 mL) was added TBAF·$3H_2O$ (13 mg, 0.04 mmol) under argon and the mixture was stirred at rt for 3 h. The reaction mixture was then diluted with $CHCl_3$, washed once with an aq. solution of 5% $NaHCO_3$ and twice with water. The organic phase was dried over Na_2SO_4, evaporated to dryness under reduced pressure and the residue was subjected to FCC, using the solvent system B as eluant. Compound **6** was then, treated with a small volume of Et_2O and refrigerated overnight. The precipitate was collected by vacuum filtration, washed on the filter with ice-cold Et_2O and dried under vacuum.

Yield:12 mg (64%); beige solid; mp 176.8-179.1 °C; R_f (B) 0.1; MS (ESI) *m/z*: 687.17 $(M+H)^+$; ^{1}H-NMR: δ 8.65 (unresolv. dt, J = 4.2 Hz, 1H), 7.95 (unresolv. dd, J = 8.4 Hz, 1H), 7.77 (td, J = 7.8 and 1.2 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 15.6 Hz, 1H), 7.46-7.40 (m, 2H), 7.31-7.25 (m, 3H), 7.01-6.96 (m, 2H), 6.86 (d, J = 7.8 Hz, 1H), 6.72 (s, 1H), 6.67 (unresolv. t, 1H), 6.34 (d, J = 15.6 Hz, 1H), 5.61 (ABq, J = 10.8 Hz, 2H), 5.42 (s, 2H), 3.93 (dd, J = 18.0 and 3.9 Hz, 1H), 3.87 (s, 3H), 3.64 (dd, J = 18.0 and 3.9 Hz, 1H), 3.25 (s, 3H), 2.65 (s, 3H) ppm; ^{13}C-NMR: δ

168.5, 166.0, 161.0, 159.4, 156.1, 153.9, 149.4 (2C), 147.7, 146.9, 141.2, 138.3, 138.1, 137.0, 136.5, 135.0, 130.2, 129.7, 127.2, 124.8, 123.0, 122.4, 121.3, 121.2, 117.8, 115.5, 114.8, 114.2, 109.6, 102.5, 70.8, 68.0, 55.9, 42.2, 36.1, 26.0 ppm; Anal. Calcd for $C_{36}H_{32}Cl_2N_4O_6$: C, 62.89; H, 4.61; N, 8.15. Found: C, 62.65; H, 4.81; N, 8.37.

4.1.19 N-*(2,4-dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)oxy)-methyl)phenyl)-2-(1,3-dioxoisoindolin-2-yl)*-N-*methylacetamide (**30**)*

To a solution of quinolinol **2** (0.19 g, 0.72 mmol) in DMF dry (1 mL) were added sequentially alcohol **3** (0.24 g, 0.6 mmol) and TPP (0.18 g, 0.7 mmol) under argon. The resulting suspension was cooled to 0 °C and then DIAD (0.14 mL, 0.7 mmol) was added drop-wise. The reaction mixture was stirred at rt for 30 min and at 65 °C for 1h. Then, it was allowed to attain rt, followed by the addition of alcohol **3** (0.1 g, 0.26 mmol), TPP (77 mg, 0.3 mmol) and DIAD (60 µL, 0.3 mmol) in the same manner. The reaction mixture was further stirred at rt for 30 min and at 65-70 °C overnight. Then, it was evaporated to dryness under reduced pressure and the residue was subjected to FCC, using the solvent system A as eluant, to afford pure compound **30**.

Yield:0.32 g (70%); beige solid; mp 239.0-240.5 °C; R_f (A) 0.33; MS (ESI) *m/z*: 663.17 $(M+Na)^+$, 641.31 $(M+H)^+$; ^{1}H-NMR (d_6-DMSO): δ 8.62 (m, 1H), 7.93-7.84 (m, 6H), 7.83-7.78 (m, 2H), 7.63 (d, J = 7.8 Hz, 1H),7.43 (t, J = 7.8 Hz, 1H), 7.41-7.38 (m, 2H), 7.07 (s, 1H), 5.54 (ABq, J = 11.4 Hz, 2H), 5.40 (ABq, J = 13.2 Hz, 2H), 4.04 (d, J = 16.8 Hz, 1H), 3.83 (d, J = 16.8 Hz, 1H), 3.14 (s, 3H), 2.54 (s, 3H) ppm;^{13}C-NMR (150 MHz, d_6-DMSO): δ 167.6 (2C), 165.7, 160.7, 159.2, 156.3, 154.1, 149.7, 141.3, 139.0, 137.7, 137.4, 136.1, 135.2 (2C), 134.7, 132.4, 131.9 (2C), 130.6, 125.4, 123.8 (2C), 123.7, 122.0, 121.0, 115.2, 113.6, 103.4, 79.6, 71.0, 67.8, 36.2, 26.0 ppm; Anal. Calcd for $C_{34}H_{26}Cl_2N_4O_5$: C, 63.63; H, 4.09; N, 8.73. Found: C, 63.88; H, 3.79; N, 8.54.

4.1.20 *2-Amino*-N-*(2,4-dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)oxy)methyl)phenyl)*-N-*methylacetamide (**31**)*

To a suspension of compound **30** (0.32 g, 0.5 mmol) in EtOH (4 mL) was added $N_2H_4 \cdot H_2O$ (73 µL, 1.5 mmol) and the reaction mixture was heated under reflux for 1 h. Then, it was evaporated to dryness under reduced pressure and the residue was

dissolved in $CHCl_3$. The organic phase was washed once with an aq. solution of 5% $NaHCO_3$ and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system E as eluant, to afford compound **31**.

Yield:0.1 g (40%); yellow solid; R_f (E) 0.14; MS (ESI) *m/z*:511.16 $(M+H)^+$; ^{1}H-NMR: δ8.64 (m, 1H), 7.95 (dd, *J* = 8.4 and 0.6 Hz, 1H), 7.75 (td, *J* = 7.8 and 1.8 Hz,1H), 7.58 (d, *J* = 7.8 Hz,1H), 7.45 (d, *J*= 8.4 Hz,1H), 7.37 (t, *J* = 7.8 Hz,1H), 7.29-7.26 (m, 1H), 7.25-7.22 (m, 2H), 6.71 (s, 1H), 5.64 (s, 2H), 5.40 (s, 2H), 3.20 (s, 3H), 3.09 (d, *J* = 17.4 Hz, 1H), 2.97 (d, *J* = 17.4 Hz, 1H), 2.66 (s, 3H), 1.93 (br.s, 2H) ppm; ^{13}C-NMR: δ 173.1, 161.3, 159.8, 156.5, 154.3, 149.8, 142.4, 139.4, 138.1, 137.4, 137.0, 135.4, 130.7, 129.9, 125.1, 123.4, 121.7, 121.6, 116.2, 115.2, 102.8, 71.2, 68.7, 44.2, 36.3, 26.7 ppm.

*4.1.21 (2*E*,4*E*,6*E*,8*E*)*-N-*(2-((2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)-quinolin-8-yl)oxy)methyl)phenyl)(methyl)amino)-2-oxoethyl)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenamide (**7**)*

To a suspension of **31** (51 mg, 0.10 mmol) and atRA (33 mg, 0.11 mmol) in dry DMF (0.2 mL) were added sequentially HBTU (53 mg, 0.14 mmol) and dry Et_3N (43 µL, 0.31 mmol) and the resulting solution was stirred at rt for 1 h. Then, it was diluted with $CHCl_3$ and washed once with an aq. solution of 5% $NaHCO_3$ and once with brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using EtOAc as eluant, to afford pure compound **7**.

Yield:40 mg (50%); pale-green solid; R_f (G) 0.22; MS (ESI) *m/z*: 793.17 $(M+H)^+$, ^{1}H-NMR: δ 8.64 (unresolv. dt, *J* = 4.2 Hz, 1H), 7.95 (unresolv. dd, *J* = 7.8 Hz, 1H), 7.75 (td, *J* = 7.8 and 1.2 Hz, 1H), 7.59 (d, *J* = 7.8 Hz,1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.31-7.25 (m, 3H), 6.90 (dd, *J* = 15 and 11.1 Hz, 1H), 6.71 (s, 1H), 6.44 (unresolv. t, 1H), 6.24 (d, *J* = 16.2 Hz, 1H), 6.23 (d, *J* = 15 Hz, 1H), 6.12 (d, *J* = 16.2 Hz, 1H), 6.11 (d, *J* = 11.1 Hz, 1H), 5.74 (s, 1H), 5.63 (ABq, *J* = 10.2 Hz, 2H), 5.41 (s, 2H), 3.87 (dd, *J* = 18 and 4.8 Hz, 1H), 3.54 (dd, *J* = 18 and 3.6 Hz, 1H), 3.24 (s, 3H), 2.66 (s, 3H),2.31 (s,3H), 2.02 (t, *J* = 6.0 Hz, 2H), 1.98 (s, 3H), 1.71 (s, 3H), 1.64-1.58 (m, 2H), 1.49-1.44 (m, 2H), 1.02 (s, 6H) ppm; ^{13}C-NMR: δ 168.5, 166.8, 159.3, 156.1, 149.4, 149.0, 138.7, 138.3, 138.2, 137.7, 137.4, 137.0, 136.6, 135.6, 130.2, 129.9, 129.8, 129.7, 129.6, 128.2, 124.8, 123.0, 121.3, 121.2, 120.8, 115.6,

114.6, 102.4, 70.7, 68.2, 42.0, 39.6, 38.6, 35.9, 34.3, 33.1, 31.6, 29.7, 29.0 (2C), 22.7, 21.7, 19.2, 14.1, 13.6, 12.9 ppm; Anal. Calcd for $C_{46}H_{50}Cl_2N_4O_4$: C, 69.60; H, 6.35; N, 7.06. Found: C, 69.89; H, 6.12; N, 6.82.

*4.1.22 2-((1-(3-(Bromomethyl)-2,4-dichlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-isoindoline-1,3-dione (**32**)*

To a solution of alcohol **14** (0.65 g, 1.6 mmol) in dry THF (2.5 mL) were added CBr_4 (1.6 g, 4.8 mmol) and TPP (1.26 g, 4.8 mmol) at 0 °C in three portions over 2 h and the mixture was stirred at rt after each addition, protected from light. An hour after the last addition, the reaction mixture was evaporated to dryness under reduced pressure and the residue was subjected to FCC, using the solvent system J as eluant, to afford pure bromide **32**.

Yield:0.35 g (47%); white solid, m.p. 153-154.5 °C; R_f (J) 0.37; MS (ESI) *m/z*:488.91 $(M+Na)^+$, 504.85 $(M+K)^+$; ^{1}H-NMR: δ8.03 (s, 1H), 7.90-7.85 (m, 2H), 7.76-7.71 (m, 2H), 7.48 (ABq, *J* =9.0 Hz, 2H), 5.11 (s, 2H), 4.79 (s, 2H) ppm; ^{13}C-NMR:δ167.6 (2C), 142.8, 137.2, 135.7, 134.4, 134.2 (2C), 132.0 (2C), 130.9, 129.0, 128.2, 125.1, 123.6 (2C), 32.9, 27.0 ppm; Anal. Calcd for $C_{18}H_{11}BrCl_2N_4O_2$: C, 46.38; H, 2.38; N, 12.02. Found: C, 46.11; H, 2.60; N, 12.27.

*4.1.23 2-((1-(2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)-oxy)-methyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)isoindoline-1,3-dione (**33**)*

***Method A*.** To a solution of quinolinol **2** (0.15 g, 0.58 mmol) in dry DMF (1 mL) were added sequentially **14** (0.15 g, 0.36 mmol) and TPP (0.1 g, 0.4 mmol) under argon. The resulting suspension was cooled to 0 °C and then DIAD (79 μL, 0.4 mmol) was added dropwise. The reaction mixture was stirred at rt for 30 min and at 60-70 °C for 1h. Then, it was allowed to attain rt, followed by the addition of **14** (0.15 g, 0.36 mmol), TPP (0.1 g, 0.4 mmol) and DIAD (79 μL, 0.4 mmol) in the same manner. The reaction mixture was further stirred at rt for 30 min and at 60-70 °C for 2 h. TLC analysis indicated that the reaction was not completed. Therefore, additional TPP (0.2 g, 0.8 mmol) and DIAD (0.16 mL, 0.8 mmol) were added in two portions, one portion every 24 h, where the reaction mixture was stirred at 40 °C for 20 h and at 60-70 °C for 2 h. Finally, it was subjected to FCC, using the solvent system U as eluant. The isolated product was treated with EtOAc and refrigerated for 1 h. The

precipitate was collected by vacuum filtration, washed on the filter with ice-cold EtOAc and dried under vacuum to afford pure **33**.

Yield:56 mg (15%); white solid.

Method B. To a suspension of **32** (0.34 mg, 0.73 mmol) and quinolinol **2** (0.19 g, 0.73 mmol) in dry DMF (4.5 mL) was added K_2CO_3 (0.3 g, 2.19 mmol) under argon and the reaction mixture was stirred at rt for 3 h. Then, it was diluted with H_2O and the suspension was extracted twice with DCM. The organic phase was washed twice with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting solid was treated with boiling EtOAc and then, left to attain rt. The precipitate was collected by vacuum filtration, washed on the filter with EtOAc and dried under vacuum to afford pure **33**. Due to the insolubility of compound **33** in $CDCl_3$, or $(CD_3)_2SO$ or CD_3OD, NMR spectra could not be obtained. Therefore, the compound was processed to the next step as described below.

Yield:0.4 g (84%); beige solid, mp 249-249.5 °C; R_f (B) 0.39; MS (ESI) *m/z*:651.12 $(M+H)^+$; Anal. Calcd for $C_{34}H_{24}Cl_2N_6O_4$: C, 62.68; H, 3.71; N, 12.90. Found: C, 62.39; H, 3.88; N, 13.12.

*4.1.24 (1-(2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)oxy)-methyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methanamine (**34**)*

A suspension of **33** (0.4 g, 0.61 mmol) in DMF (4 mL) was heated to 70-80 °C and once the solid was dissolved, MeOH (0.5 mL) and $N_2H_4 \cdot H_2O$ (59.0 μL, 1.21 mmol) was added dropwise. The reaction mixture was stirred at the same temperature for 1 h. Then, it was diluted with an aq. solution of 5% $NaHCO_3$ and extracted thrice with DCM. The organic phase was washed once with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system D as eluant, to afford compound **34**.

Yield:0.14 g (45%); slightly grey thick oil; R_f (D) 0.15; MS (ESI) *m/z*: 521.38 $(M+H)^+$; ^{1}H-NMR (CD_3OD): δ 8.60 (unresolv. dt, J = 4.2 Hz, 1H), 8.29 (s, 1H), 7.93 (td, J = 7.2 and 1.2 Hz, 1H),7.87 (d, J = 8.4 Hz, 1H), 7.74-7.70 (m, 2H), 7.67 (d, J = 9Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.43 (dd, J = 7.2and 4.2Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 6.99 (s, 1H), 5.59 (s, 2H), 5.43 (s, 2H), 4.05 (s, 2H), 2.61 (s, 3H) ppm; ^{13}C-NMR (CD_3OD): δ 165.2, 163.5, 159.6, 157.4, 152.7, 151.2, 144.1, 142.6, 141.7,

138.4, 138.0, 136.9, 132.8, 128.9, 128.6, 128.5, 127.4, 126.1, 124.8, 118.0, 114.3, 106.6, 74.3, 69.9, 39.8, 27.4 ppm.

*4.1.25 (*E*)-4-(3-(((1-(2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)oxy)methyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-3-oxoprop-1-en-1-yl)*-N*-methylbenzamide (**8**)*

To a solution of **34** (89 mg, 0.17 mmol) and **4** (39 mg, 0.19 mmol) in dry DMF (0.3 mL) were added sequentially HBTU (91 mg, 0.24 mmol) and dry Et_3N (74 μL, 0.53 mmol) and the reaction mixture was stirred at rt for 1 h. Then, it was diluted with $CHCl_3$ and washed once with an aq. solution of 5% $NaHCO_3$ and once with brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system V as eluant. The product was crystallized from DCM after overnight refrigeration. The precipitate was then, collected by vacuum filtration, washed on the filter with ice-cold DCM and dried under vacuum to afford pure **8**.

Yield: 92 mg (76%); white solid, mp 157-160 °C; R_f (C) 0.27;MS (ESI) *m/z*: 708.19 $(M+H)^+$;^{1}H-NMR: δ8.64 (unresolv. dt, *J* = 4.2 Hz, 1H), 8.03 (unresolv. t, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.90 (s, 1H), 7.92-7.87 (m, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.76 (td, *J* = 7.8 and 1.2 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.51 (d,*J* = 15.6 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.31-7.26 (m, 4H), 6.81 (d, *J* = 15.6 Hz, 1H), 6.70 (s, 1H), 5.56 (s, 2H), 5.40 (s, 2H), 4.71 (d, *J* = 4.8 Hz, 2H), 2.77 (d, *J* = 4.2 Hz, 3H), 2.38 (s, 3H) ppm; ^{13}C-NMR: δ166.9, 166.1, 161.1, 159.5, 155.9, 153.8, 149.5 (2C), 144.8, 140.8, 139.5, 138.7, 137.5, 137.1 (2C), 134.9, 134.1, 133.7, 132.3, 128.8, 128.6, 127.6, 127.5, 124.9, 124.7, 123.1, 122.9, 121.3, 121.2, 114.9, 111.0, 102.9, 70.9, 66.4, 34.9, 26.6, 25.2 ppm; Anal. Calcd for $C_{37}H_{31}Cl_2N_7O_4$: C, 62.72; H, 4.41; N, 13.84. Found: C, 62.55; H, 4.61; N, 14.01.

*4.1.26 2-Methyl-8-(*o*-nitrophenylsulfonylamido)-4-(pyridin-2-yl)methoxyquinoline (**35**)*

To an ice-cold solution of **3** (0.27 g, 1 mmol) in dry DCM (4.5 mL) was added dry Et_3N (0.3 mL, 2.15 mmol) and then NsCl (0.25 g, 1.1 mmol) portion-wise over 50 min, under argon. The mixture was stirred at 0 °C for 30 min and at rt for 1h. TLC analysis indicated that the reaction was not completed, therefore, additional dry Et_3N (0.15 mL, 1.07 mmol) and NsCl (0.16 g, 0.7 mmol) were added in the same manner over 2 h. The reaction mixture was further stirred at rt overnight and then, diluted

with DCM. The organic phase was washed once with an aq solution of 5% $NaHCO_3$, twice with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system B as eluant to afford pure compound **35**.

Yield:0.41 g (91%); orange solid; mp 161-162.5 °C; R_f (B); 0.48; MS (ESI) *m/z*: 451.13 $(M+H)^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 10.15 (br.s, 1H),8.62 (d, *J* = 4.2 Hz, 1H), 8.09 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.94 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.91 (dd, *J* = 8.4 and 1.2 Hz, 1H), 7.81 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.73 (td, *J* = 7.8 and 1.2 Hz, 1H) 7.59 (td, J = *7*.8 and1.2 Hz, 1H), 7.56 (td, J = *7*.8 and1.2 Hz, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.27(m, 1H), 6.68 (s, 1H), 5.36 (s, 2H), 2.61 (s, 3H) ppm; ^{13}C-NMR: δ 161.1, 159.4, 155.8, 149.4, 148.1, 139.5, 137.1, 133.6, 133.2, 132.4, 132.3, 131.3, 125.4, 124.8, 123.1, 121.3, 119.8, 117.1, 116.4, 102.8, 71.0, 25.6 ppm; Anal. Calcd for $C_{22}H_{18}N_4O_5S$: C, 58.66; H, 4.03; N, 12.44. Found: C, 58.93; H, 3.89; N, 12.11.

*4.1.27*N-*(2,4-Dichloro-3-((*N-*(2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)-2-nitrophenylsulfonamido)methyl)phenyl)-2-(1,3-dioxoisoindolin-2-yl)*-N-*methyl-acetamide (**36**)*

To a suspension of **35** (0.29 g, 0.65 mmol) and **3** (0.31 g, 0.78 mmol) in dry THF (0.8 mL) was added TPP (0.2 g, 0.78 mmol) and the mixture was cooled to 0 °C. Then, was added DIAD (0.15 mL, 0.78 mmol) and the reaction mixture was heated to 50 °C for 2 h. TLC analysis indicated that the reaction was not completed. Therefore, additional TPP (79 mg, 0.3 mmol) and DIAD (59 μL, 0.3 mmol) were added and the mixture was further stirred at 50 °C for 2 h. Then, it was evaporated to dryness under reduced pressure and the residue was subjected to FCC, using EtOAc as eluant, to afford **36**as an inseparable mixture with TPPO (0.59 g), which was used as suchto the next step.

Orange foam; R_f (N) 0.15; MS (ESI) *m/z*: 847.02 $(M+Na)^+$.

*4.1.28*N-*(2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)amino)-methyl)phenyl)-2-(1,3-dioxoisoindolin-2-yl)*-N-*methylacetamide (**37**)*

To a solution of crude **36** (0.59 g) in dry DMF (3 mL) were added Na_2CO_3 (0.32 g, 3 mmol) and PhSH (0.21 mL, 2 mmol) and the reaction mixture was stirred vigorously at 40-50 °C for 2 h. Then, it was diluted with water and extracted twice with $CHCl_3$.

The organic phase was washed once with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting oil was treated with Et_2O and refrigerated overnight. The precipitate was collected by vacuum filtration, washed on the filter with ice-cold Et_2O and dried under vacuum to afford pure **37**.

Yield:0.22 g (52% over the two steps); yellow solid; mp 257-259 °C; R_f (G); 0.35; MS (ESI) *m/z*:640.15 $(M+H)^+$; ^{1}H-NMR: δ 8.61 (unresolv. dt, *J* = 4.2 Hz, 1H), 7.86-7.83 (m, 2H), 7.72 (td, *J* = 7.8 and 1.8 Hz, 1H), 7.71-7.69 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.51 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.25 (dd, *J* = 6.6 and 4.8 Hz, 1H), 7.00 (d, *J* = 7.2 Hz, 1H), 6.70 (t, *J* = 6.6 Hz, 1H), 6.64 (s, 1H), 5.36 (s, 2H), 4.87 (d, *J* = 6.6 Hz, 2H), 4.06 (ABq, *J* = 16.8 Hz, 2H), 3.23 (s, 3H), 2.59 (s, 3H) ppm; ^{13}C-NMR: δ 167.7 (2C), 165.7, 161.0, 157.1, 156.4, 149.3, 143.3, 138.9, 138.8, 137.5, 136.9 (2C), 135.8, 134.0 (2C), 132.2, 129.7, 129.4, 125.7, 123.5 (2C), 122.8, 121.1, 120.0, 119.8, 108.9, 106.6, 102.2, 70.5, 44.0, 39.5, 36.2, 25.8 ppm´Anal. Calcd for $C_{34}H_{27}Cl_2N_5O_4$: C, 63.76; H, 4.25; N, 10.93. Found: C, 63.46; H, 4.50; N, 11.24.

4.1.29 2-Amino-N-*(2,4-dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)amino)methyl)phenyl)*-N-*methylacetamide (**38**)*

To a suspension of **37** (0.19 g, 0.3 mmol) in EtOH (2.5 mL) was added $N_2H_4{\cdot}H_2O$ (29 µL, 0.6 mmol) and the reaction mixture was heated under reflux for 1 h. Then, it was diluted with an aq. solution of 5% $NaHCO_3$ and extracted twice with DCM. The organic phase was washed once with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using the solvent system Was eluant, to afford pure amine **38**.

Yield: 0.15 g (97%); yellow foam; R_f (C); 0.26; MS (ESI) *m/z*:510.16 $(M+H)^+$; ^{1}H-NMR: δ 8.62 (unresolv. dt, *J* = 4.8 Hz, 1H), 7.73 (td, *J* = 7.8 and 1.2 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.53 (dd, *J* = 7.8 and 0.6 Hz, 1H),7.43 (d, *J* = 8.4 Hz, 1H), 7.35 (t, *J*= 7.8 Hz, 1H), 7.26 (t, *J*= 7.8 Hz, 1H), 7.19 (d, *J*= 8.4, 1H), 6.97 (d, *J*= 7.2, 1H), 6.66 (s, 1H), 6.61 (unresolv. t, 1H), 5.39 (s, 2H), 4.82 (d, *J*= 1.8 Hz, 2H), 3.22 (s, 3H), 3.08 (d, *J* = 17.1 Hz, 1H), 2.99 (d, *J* = 17.1 Hz, 1H), 2.59 (s, 3H), 1.71 (br.s, 2H) ppm; ^{13}C-NMR: δ 172.8, 161.1, 157.1, 156.4, 149.3, 143.3, 139.2, 138.8, 137.3, 137.0, 136.5, 135.6, 129.6, 129.3, 125.7, 122.9, 121.1, 119.8, 108.9, 106.4, 102.3, 70.6, 44.0, 43.9, 36.0, 25.8 ppm.

*4.1.30 (E)-4-(3-((2-((2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)amino)methyl)phenyl)(methyl)amino)-2-oxoethyl)amino)-3-oxoprop-1-en-1-yl)-N-methylbenzamide (**10**)*

To a solution of **38** (87 mg, 0.17 mmol) in dry DMF (0.3 mL) were added dry Et_3N (50 μL, 0.36 mmol) and **S7** (63 mg, 0.21 mmol) under argon and the reaction mixture was stirred at rt for 1.5 h. Then, it was subjected twice to FCC, using the solvent system Was eluant. The resulting oil was treated with boiling EtOAc and then, refrigerated overnight. The precipitate was collected by vacuum filtration, washed on the filter with ice-cold EtOAc and dried under vacuum to afford pure compound **10**.

Yield:80 mg (68%); beige solid; mp 225-228.5 °C; R_f (E) 0.5; MS (ESI) *m/z*: 697.22 $(M+H)^+$; ^{1}H-NMR: δ 8.61 (unresolv. dt, J = 4.8 Hz, 1H), 7.75-7.70 (m, 3H), 7.58 (d, J = 7.8 Hz, 1H), 7.55-7.48 (m, 4H), 7.47 (d, J = 8.4 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.28-7.23 (m, 2H), 6.97 (d, J = 7.2 Hz, 1H), 6.73 (unresolv. t, 1H), 6.65 (s, 1H), 6.56 (unresolv. q, 1H), 6.50 (d, J = 15.6 Hz, 1H), 6.26 (unresolv. t, 1H), 5.38 (s, 2H), 4.81 (unresolv. d, 2H), 3.95(dd, J = 17.4 and 4.8 Hz, 1H), 3.63 (dd, J = 17.4 and 2.4 Hz,1H), 3.25 (s, 3H), 3.00 (d, J = 4.8 Hz, 3H), 2.59 (s, 3H) ppm; ^{13}C-NMR: δ 168.3, 167.5 (2C), 165.2, 161.1, 157.0, 156.4, 149.3 (2C), 143.3, 140.0, 138.3, 137.6, 137.2, 137.0, 135.4, 129.9, 129.3, 127.9 (2C), 127.4 (2C), 125.7, 122.9, 122.0, 121.1, 119.8, 108.9 (2C), 106.4, 102.3, 70.6, 44.1, 42.1, 36.1, 26.8, 25.7 ppm; Anal. Calcd for $C_{37}H_{34}Cl_2N_6O_4$: C, 63.70; H, 4.91; N, 12.05. Found: C, 63.88; H, 4.79; N, 11.82.

*4.1.31 (2E,4E,6E,8E)-N-(2-((2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)-quinolin-8-yl)amino)methyl)phenyl)(methyl)amino)-2-oxoethyl)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenamide (**11**)*

To a solution of **38** (77 mg, 0.15 mmol) in dry DMF (0.3 mL) were added dry Et_3N (45 μL, 0.32 mmol) and **22** (72.0 mg, 0.18 mmol) under argon and the reaction mixture was stirred at rt for 1.5 h. TLC analysis indicated that the reaction was not completed, thus, the mixture was further stirred at rt overnight. Then, it was diluted with $CHCl_3$ and washed twice with water and once with brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using EtOAc as eluant, to afford pure compound **11**.

Yield:50 mg (42%); yellow solid; mp 127-130 °C; R_f (G) 0.37; MS (ESI) *m/z*: 814.35 $[M+Na]^+$, 792.38 $(M+H)^+$; ^{1}H-NMR: δ 8.62 (d, J = 4.2 Hz, 1H), 7.73 (td, J = 7.8 and

0.6 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.28-7.22 (m, 2H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.91 (dd, *J* = 14.4 and 11.4 Hz, 1H), 6.65 (s, 1H), 6.39 (t, *J* = 4.2 Hz, 1H), 6.24 (d, *J* = 14.4 Hz, 2H), 6.13 (d, *J* = 15.6 Hz, 2H), 5.74 (s, 1H), 5.39 (s, 2H), 4.81 (s, 2H), 3.89 (dd, *J* = 18.3 and 4.8 Hz, 1H), 3.56 (dd, *J* = 18.3 and 3.6 Hz, 1H), 3.24 (s, 3H), 2.59 (s, 3H), 2.31 (s, 3H), 2.04-2.00 (m, 2H), 1.98 (s, 3H), 1.71 (s, 3H), 1.64-1.59 (m, 2H), 1.49-1.44 (m, 2H), 1.03 (s, 6H) ppm; ^{13}C-NMR: δ 168.5, 166.7, 161.1, 157.0, 156.4, 149.3, 149.0, 143.4, 138.9, 138.8, 138.4, 137.8, 137.5, 137.4, 137.1, 137.0, 135.5 (2C), 129.9, 129.8 (2C), 129.6, 129.3, 128.2, 125.7, 122.8, 121.1, 120.8, 119.8, 108.9, 106.4, 102.3, 70.6, 44.1, 41.9, 39.7, 36.0, 34.3, 33.1, 29.0 (2C), 25.7, 21.7, 19.2, 13.6, 12.8 ppm; Anal. Calcd for $C_{46}H_{51}Cl_2N_5O_3$: C, 69.69; H, 6.48; N, 8.83. Found: C, 70.08; H, 6.32; N, 8.51.

*4.1.32*N-*(2,6-Dichloro-3-(4-((1,3-dioxoisoindolin-2-yl)methyl)-1*H*-1,2,3-triazol-1-yl)benzyl)*-N-*(2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)-2-nitrobenzenesulfonamide (**39**)*

To a suspension of **35** (0.11 g, 0.25 mmol) and **14** (0.12 g, 0.30 mmol) in dry THF (0.3 mL) was added TPP (79 mg, 0.3 mmol) and the mixture was cooled to 0 °C. Then DIAD (59 μL, 0.3 mmol) was added and the reaction mixture was heated at 40-50 °C for 2.5 h. TLC analysis indicated that the reaction was not completed, therefore, additional TPP (40 mg, 0.15 mmol) and DIAD (30 μL, 0.15 mmol) were added and the mixture was further stirred at the same temperature for 1 h and at rt overnight. Then, it was evaporated to dryness under reduced pressure and the residue was subjected to FCC, using EtOAc as eluant, to afford 0.3 g crude **39**as an inseparable mixture with TPPO, which was used as suchto the next step.

Orange foam; R_f (K) 0.12; MS (ESI) *m/z*: 835.15 $(M+H)^+$.

*4.1.33 2-((1-(2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)-amino)methyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)isoindoline-1,3-dione (**40**)*

To a solution of crude **39** (0.3 g) in dry DMF (2.5 mL) were added Na_2CO_3 (0.11 g, 1 mmol) and PhSH (77 μL, 0.75 mmol) and the reaction mixture was stirred vigorously at rt for 2 h.TLC analysis indicated that the reaction was not completed, therefore, it was heated at 40-50 °C for 1 h and then, additional Na_2CO_3 (56 mg, 0.5 mmol) and PhSH (39 μL, 0.38 mmol) were added and the reaction mixture was further stirred at

rt for 48 h. Then, it was diluted with H_2O and extracted thrice with $CHCl_3$. The organic phase was washed once with water and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting oil was treated with Et_2O and refrigerated overnight. The precipitate was collected by vacuum filtration, washed on the filter with ice-cold Et_2O and dried under vacuum to afford **40**.

Yield:70 mg (43% for the last two steps); yellow solid; mp 258-260 °C; R_f (G); 0.34; MS (ESI) *m/z*: 650.12 $(M+H)^+$; ^{1}H-NMR: δ 8.62 (unresolv. dt, *J* = 4.2 Hz, 1H), 8.01 (s, 1H), 7.88-7.85 (m, 2H), 7.73 (td, *J* = 7.8 and 1.8 Hz, 1H), 7.73-7.70 (m, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.52 (dd, *J* = 8.4 and 1.2 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.26-7.24 (m, 1H), 6.94 (d, *J* = 6.6 Hz, 1H), 6.65 (s, 1H), 6.56 (t, *J* = 6 Hz, 1H), 5.38 (s, 2H), 5.09 (s, 2H), 4.84 (d, *J* = 6 Hz, 2H), 2.58 (s, 3H) ppm; ^{13}C-NMR: δ 167.6 (2C), 161.0, 157.1, 156.4, 149.3, 143.2, 142.6, 138.7, 137.8, 137.0 (2C), 136.9, 134.3, 134.1 (2C), 132.0, 131.5, 128.9, 127.6, 125.7, 125.2, 123.5 (2C), 122.8, 121.1, 119.7, 108.8, 106.1, 102.3, 70.5, 43.8, 32.9, 25.8 ppm´Anal. Calcd for $C_{34}H_{25}Cl_2N_7O_3$: C, 62.78; H, 3.87; N, 15.07. Found: C, 62.50; H, 3.65; N, 15.38.

*4.1.34 N-(3-(4-(Aminomethyl)-1H-1,2,3-triazol-1-yl)-2,6-dichlorobenzyl)-2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-amine (**42**)*

A suspension of **40**(70 mg, 0.11 mmol) in DMF (0.5 mL) was heated at 80 °C and then, MeOH (0.15 mL) and $N_2H_4{\cdot}H_2O$ (11 μL, 0.23 mmol) were added. The reaction mixture was stirred at 80 °C for 2 h. TLC analysis indicated that the reaction was not completed, therefore, additional $N_2H_4{\cdot}H_2O$ (22 μL, 0.46 mmol) was added in two portions every two hours and finally was stirred at 80 °C overnight. The reaction mixture was, then, diluted with 5% $NaHCO_3$ aq. solutionand extracted thrice with $CHCl_3$. The organic phase was washed once with H_2O and once with brine, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to FCC, using $CHCl_3$ as eluant. The resulting solid (40 mg) was shown by ^{1}H-NMR and ^{13}C-NMR spectroscopy (see SM) and ESI-MS [*m/z*: 548.12 $(M+H)^+$] to be the corresponding formamide **41**. Formamide **41** was then, subjected to hydrazinolysis at refluxing EtOH (1 mL) with 50 μL $N_2H_4{\cdot}H_2O$ overnight. Additional 50 μL $N_2H_4{\cdot}H_2O$ were added and the reaction was completed after 24 h. Aqueous work-up as mentioned above gave the anticipated product as an oil which was used as such to the next coupling step without further purification.

Yield: 38 mg (72% for the last two steps); yellow oil; R_f (D); 0.35; MS (ESI) *m/z*: 520.13 $(M+H)^+$.

*4.1.35 (*E*)-4-(3-(((1-(2,4-Dichloro-3-(((2-methyl-4-(pyridin-2-ylmethoxy)quinolin-8-yl)amino)methyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-3-oxoprop-1-en-1-yl)-*N*-methylbenzamide (**9**)*

To a solution of **42** (36 mg, 0.07 mmol) in dry DMF (0.1 mL) were added dry Et_3N (21 μL, 0.15 mmol) and **S7** (27 mg, 0.09 mmol) under argon and the reaction mixture was stirred at rt for 2 h. Then, it was diluted with $CHCl_3$ and washed thrice with water and once with brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The precipitate was subjected to FCC, using the solvent system $CHCl_3$/MeOH=92:8 as eluant. The product was treated with boiling EtOAc and then, refrigerated overnight. The precipitate was collected by vacuum filtration, washed on the filter with ice-cold EtOAc and dried under vacuum to afford pure compound **9**.

Yield: 25 mg (51%); brown solid; mp 247-250 °C; R_f (C) 0.33; MS (ESI) *m/z*: 707.21 $(M+H)^+$; ^{1}H-NMR (d_6-DMSO): δ 8.75 (t, *J* = 5.4 Hz, 1H), 8.61 (d, *J* = 4.2 Hz, 1H), 8.46 (q, *J* = 4.8 Hz, 1H), 8.44 (s, 1H), 7.87 (t, *J* = 7.2 Hz, 1H), 7.85 (d, *J* = 7.8 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.63 (t, *J* = 6.6 Hz, 1H), 7.51 (d, *J* = 15.9 Hz, 1H), 7.41-7.35 (m, 2H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.03 (s, 1H), 6.96 (d, *J* = 7.8 Hz, 1H), 6.77 (d, *J* = 15.9 Hz, 1H), 6.48 (t, *J* = 5.4 Hz, 1H), 5.41 (s, 2H), 4.78 (d, *J* = 5.4 Hz, 2H), 4.57 (d, *J* = 5.4 Hz, 2H), 2.78 (d, *J* = 4.2 Hz, 3H), 2.54 (s, 3H) ppm; ^{13}C-NMR (d_6-DMSO): δ 166.5, 165.2, 161.1, 157.3, 156.4, 149.7, 145.3, 143.4, 138.6, 138.4, 137.8, 137.6, 137.2, 136.5, 135.6, 135.0, 132.0, 129.8, 129.1, 128.1 (2C), 127.8 (2C), 126.2, 125.8, 123.9, 123.6, 122.0, 119.7, 108.6, 106.3, 103.3, 71.0, 43.9, 34.7, 26.7, 25.8 ppm; Anal. Calcd for $C_{37}H_{32}Cl_2N_8O_3$: C, 62.80; H, 4.56; N, 15.84. Found: C, 62.54; H, 4.81; N, 16.01.

4.2 Biology

4.2.1 Cell cultures

MCF-7 and MDA-MB-231 breast cancer cell lines were purchased from the American Type Culture Collection (ATCC). All cells were routinely cultured in a humidified 95% air/5% CO_2 incubator at 37 °C in complete medium Dulbecco's

Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) 1.0 mM sodium pyruvate, 2 mM l-glutamine, 100 IU/mL penicillin. The synthesized compounds were dissolved in DMSO and further dilutions were conducted with culture medium.

4.2.2 Determination of IC_{50} values

Breast cancer cells were seeded in 12-well plates and were allowed to grow up to 60%–70% confluence. After renewing the culture medium, the synthesized compounds were added in a range of concentrations (0-200 μM) and cells were incubated for 24 h in serum-containing culture medium and the surviving breast cancer cells were measured manually. The percentage of living cells was plotted against the log values of compounds concentrations and a non-linear fit plot was used to estimate the IC50 value for each drug.

4.2.3 Statistical analysis

Reported values in diagrams are expressed as mean ± standard deviation (SD) bars of experiments in triplicate. Statistically significant differences were evaluated using an unpaired two-tailed t-test and were considered statistically significant at the level of at least $p \leq 0.05$. Statistical analyses and graphs were made using GraphPad Prism 5 (GraphPad Software).

Author Contributions: S. Leonardi, D. Rigopoulou, E. Vachlioti, and K. Afratis carried out the synthetic work. Z. Piperigkou and C. Koutsakis performed the biological studies; S. Leonardi and Z. Piperigkou also prepared the Supplementary Material. N. Karamanos supervised the biological evaluation. D. Papaioannou supervised the synthetic work and wrote the synthetic chemistry part of the paper. G. Rassias supervised the synthetic work, wrote the discussion of the SAR results and the proposed mechanisms of action. H. Gavras, D. Papaioannou and G. Rassias conceived and directed the whole project.

Acknowledgements

We are grateful to the Research Committee of the University of Patras for funding part of this work. We also wish to thank Mr D. Vachliotis and Dr A.K.

Andreopoulou, Laboratory of Instrumental Analysis, University of Patras, for the NMR spectra and the Elemental Analyses.

Appendix A. Supplementary material

Supplementary data to this article, including experimental procedures for the synthesis and characterization of compounds **2-5**, **S1-S17** and **FR-190997**, MS and ^{1}H and ^{13}C NMR spectra for key-intermediates and final products and dose-dependent responses of MCF-7 and MDA-MB-231 breast cancer cells to the tested compounds, can be found online at ……...

Abbreviations

APL	acute promyelotic leukemia
aq.	aqueous
atRA	*all-trans*-retinoic acid
BK	bradykinin
B1R/B2R	bradykinin B1/B2 receptor
cat.	catalytic quantity
CDI	1,1'-carbonyl diimidazole
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine
DMI	1,3-dimethyl-2-imidazolidinone
FCC	flash column chromatography
FerA	ferulic acid
GBCR	G protein-coupled receptor
HBTU	*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium

	hexafluorophosphate
HCA	hydroxycinnamic acid
HOSu	*N*-hydroxysuccinimide
Ns	nosyl (*o*-nitrosulfonyl)
Phth	phthalyl
rt	room temperature
SM	supporting material
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TNBC	Triple-negative breast cancer
TPP	triphenylphosphine
TPPO	triphenylphosphine oxide

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] M.N. Oliveira, B. Breznik, M.M. Pillat, R.L. Pereira, H. Ulrich, T.T. Lah, Kinins in Glioblastoma Microenvironment, Cancer Microenviron. 12 (2019) 77–94, doi:10.1007/s12307-019-00229-x.

[2] E. Kashuba, J. Bailey, D. Allsup, L. Cawkwell, The kinin-kallikrein system: physiological roles, pathophysiology and its relationship to cancer biomarkers, Biomarkers 18 (2013) 279–296, doi:10.3109/1354750X.2013.787544.

[3] R.C. Dutra, Kinin receptors: Key regulators of autoimmunity, Autoimmun. Rev. 16 (2017) 192–207, doi:10.1016/j.autrev.2016.12.011.

[4] P.L. da Costa, P. Sirois, I.F. Tannock, R. Chammas, The role of kinin receptors in cancer and therapeutic opportunities, Cancer Lett. 34 (2014) 527–538, doi:10.1016/j.canlet.2013.12.009.

[5] E.T. Whalley, C.D. Figueroa, L. Gera, K.D. Bhoola, Discovery and therapeutic potential of kinin receptor antagonists, Expert Opin. Drug Discov. 7 (2012) 1129–1148, doi:10.1517/17460441.2012.729038.

[6] D.P. Sun, Y.W. Lee, J.T. Chen, Y.W. Lin, R.M. Chen, The Bradykinin-BDKRB1 Axis Regulates *Aquaporin 4* Gene Expression and Consequential Migration and Invasion of Malignant Glioblastoma Cells via a Ca^{2+}-MEK1-ERK1/2-NF-κB Mechanism, Cancers 12 (2020) 667, doi:10.3390/cancers12030667.

[7] M.N. Oliveira, M.M. Pillat, H. Motaln, H. Ulrich, T.T. Lah, Kinin-B1 Receptor Stimulation Promotes Invasion and is Involved in Cell-Cell Interaction of Co-Cultured Glioblastoma and Mesenchymal Stem Cells, Sci. Rep. 8 (2018) 1299, doi:10.1038/s41598-018-19359-1.

[8] N.F. Nicoletti, J. Sénécal, V.D. da Silva, M.R. Roxo, N.P. Ferreira, R.L.T. de Morais, J.B. Pesquero, M.M. Campos, R. Couture, F.B. Morrone, Primary Role for Kinin B_1 and B_2 Receptors in Glioma Proliferation, Mol. Neurobiol. 5 (2017) 47869–7882, doi:10.1007/s12035-016-0265-9.

[9] P.L.N. da Costa, D. Wynne, T. Fifis, L. Nguyen, M. Perini, C. Christophi, The kallikrein-Kinin system modulates the progression of colorectal liver metastases in a mouse model, BMC Cancer 18 (2018) 382, doi:10.1186/s12885-018-4260-6.

[10] L. Molina, C.E. Matus, A. Astroza, F. Pavicic, E. Tapia, C. Toledo, J.A. Perez, F. Nualart, C.B. Gonzalez, R.A. Burgos, C.D. Figueroa, P. Ehrenfeld, M.T. Poblete, Stimulation of the bradykinin B1 receptor induces the proliferation of estrogen-sensitive breast cancer cells and activates the ERK1/2 signalling pathway, Breast Cancer Res. Treat. 118 (2009) 499–510, doi: 10.1007/s10549-009-0314-4.

[11] W. Wang, Y. Zhou, R. Wei, G. Jiang, F. Li, X. Chen, X. Wang, D. Ma, L. Xi, Bradykinin promotes proliferation, migration, and invasion of cervical cancer cells through STAT3 signalling pathways, Oncol. Rep. 42 (2019) 2521–2527, doi:10.3892/or.2019.7380.

[12] A.G. Maria, P. Dillenburg-Pilla, R.I. Reis, E.M. Floriano, C. Tefé-Silva, S.G. Ramos, J.B. Pesquero, C. Nahmias, C.M. Costa-Neto, Host kinin B1 receptor

plays a protective role against melanoma progression, Sci. Rep. 6 (2016) 22078, doi:10.1038/srep22078.

[13] A.G. Maria, P. Dillemburg-Pilla, M. de T. Durand, E.M. Floriano, A.O. Manfiolli, S.G. Ramos, J. B. Pesquero, C. Nahmias, C.M. Costa-Neto, Activation of the Kinin B1 Receptor by Its Agonist Reduces Melanoma Metastasis by Playing a Dual Effect on Tumor Cells and Host Immune Response, Front. Pharmacol. 10 (2019) 1106, doi:10.3389/fphar.2019.01106.

[14] G. Morissette, S. Houle, L. Gera, J.M. Stewart, F. Marceau, Antagonist, partial agonist and antiproliferative actions of B-9870 (CU201) as a function of the expression and density of the bradykinin B1 and B2 receptors, Br. J. Pharmacol. 150 (2007) 369–379, doi:10.1038/sj.bjp.0706982.

[15] Y. Sawada, H. Kayakiri, Y. Abe, T. Mitsutani, N. Inamura, M. Asano, C. Hatori, I. Aramori, T. Oku, H. Tanaka, Discovery of the first non-peptide full agonists for the human bradykinin B(2) receptor incorporating 4-(2-picolyloxy)quinoline and 1-(2-picolyl)benzimidazole frameworks, J. Med Chem. 47 (2004) 2853–2863, doi:10.1021/jm030468n.

[16] E.K. Dziadulewicz, Non-peptide ligands for bradykinin receptors 1995-2004, Expert Opin. Ther. Patents 15 (2005) 829–859, and references cited therein, doi.org/10.1517/13543776.15.7.829.

[17] H. Heitsch, Non-peptide Antagonists and Agonists of the Bradykinin B_2 Receptor, Curr. Med. Chem. 9 (2002) 913–928, and references cited therein, doi: 10.2174/0929867024606722.

[18] I. Aramori, J. Zenkoh,N. Morikawa, M. Asano, C. Hatori, H. Sawai, H. Kayakiri,S. Satoh, T. Inoue, Y. Abe, Y. Sawada, T. Mizutani, N. Inamura, K. Nakahara, H. Kojo, T. Oku, Y. Notsu, Nonpeptide Mimic of Bradykinin with Long-Acting Properties at the Bradykinin B_2 Receptor, Mol. Pharmacol. 52 (1997) 16–20, doi: 10.1124/mol.52.1.16.

[19] Y. Sawada, H. Kayakiri, Y.Abe; T. Mizutani, N. Inamura, M. Asano, I. Anamori, C. Hatori, T. Oku, H. Tanaka, A New Class of Nonpeptide Bradykinin B_2 Receptor Ligand Incorporating a 4-Aminoquinoline Framework. Identification

of a Key Pharmacophore to Determine Species Difference and Agonist/Antagonist Profile, J. Med. Chem. 47 (2004) 2667−2677, doi.org/10.1021/jm030326t.

[20] Y. Sawada, H. Kayakiri, Y. Abe, K. Imai, T. Mizutani, N. Inamura, M. Asano, I. Aramori, C. Hatori, A.Katayama, T. Oku, H. Tanaka, A New Series of Highly Potent Non-Peptide Bradykinin B_2 Receptor Antagonists Incorporating the 4-Heteroarylquinoline Framework. Improvement of Aqueous Solubility and New Insights into Species Differences, J. Med. Chem. 47 (2004) 1617−1630, doi: 10.1021/jm030159x.

[21] Y. Abe, H. Kayakiri, S. Satoh, T. Inoue, Y. Sawada, N. Inamura, M. Asano, I. Aramori, C. Hatori, H. Sawai, T. Oku, H. Tanaka, A Novel Class of Orally Active Non-Peptide Bradykinin B_2 Receptor Antagonists. 3. Discovering Bioisosteres of the Imidazo[1,2-a]pyridine Moiety, J. Med. Chem. 41 (1998) 4062–4079, doi:10.1021/jm980300f.

[22] Y. Abe, H. Kayakiri, S. Satoh, T. Inoue, Y. Sawada, N. Inamura, M. Asano, I. Aramori, C. Hatori, H. Sawai, T. Oku, H. Tanaka, A Novel Class of Orally Active Non-Peptide Bradykinin B_2 Receptor Antagonists. 4. Discovery of Novel Frameworks Mimicking the Active Conformation, J. Med. Chem. 41 (1998) 4587–4598, doi:10.1021/jm980330i.

[23] N.A. Sharif, P. Katoli, D. Scott, L. Li, C. Kelly, S. Xu, S. Husain, C. Toris, C. Crosson, FR-190997, a nonpeptide bradykinin B2-receptor partial agonist, is a potent and efficacious intraocular pressure lowering agent in ocular hypertensive cynomolgus monkeys, Drug Dev. Res. 75 (2014) 211−223, doi:10.1002/ddr.21174.

[24] V.R.Solomon, H. Lee, Quinoline as a Privileged Scaffold in Cancer Drug Discovery, Curr. Med. Chem. 18 (2011) 1488−1508, doi: 10.2174/092986711795328382.

[25] R. Musiol, An overview of quinoline as a privileged scaffold in cancer drug discovery, Expert Opin. Drug Disc. 18 (**2011**) 1488−1508, doi: 10.1080/17460441.2017.1319357.

[26] P. De, M. Baltas, F. Bedos-Belval, Cinnamic Acid Derivatives as Anticancer Agents-A Review, Curr. Med. Chem. 18 (2011) 1672–1703, doi: 10.2174/092986711795471347.

[27] E. Pontiki, A. Peperidou, I. Fotopoulos, D. Hadjipavlou-Litina, Cinnamate Hybrids: A Unique Family of Compounds with Multiple Biological Activities, Curr. Pharm. Biotechnol. 19 (2018) 1019–1048, doi: 10.2174/1389201019666181112102702.

[28] S. Greco, C. Storelli, S. Marsigliante, Protein kinase C (PKC)-delta/-epsilon mediate the PKC/Akt-dependent phosphorylation of extracellular signal-regulated kinases 1 and 2 in MCF-7 cells stimulated by bradykinin, J. Endocrinol. 188 (2006) 79–89, doi:10.1677/joe.1.06433.

[29] S. Greco, M.G. Elia, A. Muscella, S. Romano, C. Storelli, S. Marsigliante, Bradykinin stimulates cell proliferation through an extracellular-regulated kinase 1 and 2-dependent mechanism in breast cancer cells in primary culture, J. Endocrinol. 186 (2005) 291-301, doi:10.1677/joe.1.06052

[30] S. Greco, A. Muscella, M.G. Elia, S. Romano, C. Storelli, S. Marsigliante, Mitogenic signalling by B2 bradykinin receptor in epithelial breast cells. J. Cell Physiol. 201 (2004) 84–96, doi:10.1002/jcp.20052.

[31] C. Dubuc, M. Savard, V. Bovenzi, A. Lessard, A. Fortier, J. Côté, W. Neugebauer, F. Rizzolio, S. Geha, A. Giordano, S. Chemtob, F. Gobeil, Targeting intracellular B2 receptors using novel cell-penetrating antagonists to arrest growth and induce apoptosis in human triple-negative breast cancer, Oncotarget, 9 (2018) 9885–9906, doi:10.18632/oncotarget.24009.

[32] B.E. Halcrow, W.O. Kermack, Nitration of 4-Chloro- and 4-Hydroxy-quinaldine, J. Chem. Soc. (1945) 415–417. For an alternative method see: E.A. Shmoilova, O.V. Dyablo, A.F. Pozharskii, Synthesis of 4,5-bis(dimethylamino)quinolines and the dual direction of their protonation, Chem. Heter. Comp. 49 (2013) 1308–1322. doi: 10.1007/s10593-013-1380-4.

[33] T. Garnelis, C.M. Athanassopoulos, D. Papaioannou, I.M. Eggleston, A.H. Fairlamb, Very Short and Efficient Syntheses of the Spermine Alkaloid

Kukoamine A and Analogs Using Isolable Succinimidyl Esters, Chem. Lett. 34 (2005) 264–265, doi: 10.1246/cl.2005.264.

[34] G. Magoulas, D. Papaioannou, E. Papadimou, D. Drainas, Preparation of spermine conjugates with acidic retinoids with potent ribonuclease P inhibitory activity, Eur. J. Med. Chem. 44 (2009) 2689–2695, doi.org/10.1016/j.ejmech.2009.01.001.

[35] S. Kalantzi, C.M. Athanassopoulos, R. Ruonala, Y. Helariutta, D. Papaioannou, General Approach for the Liquid-Phase Fragment Synthesis of Orthogonally Protected Naturally Occurring Polyamines and Applications Thereof, J. Org. Chem. 84 (2019) 15118−15130, doi.org/10.1021/acs.joc.9b02066.

[36] Y. Ikeda, I. Hayashi, E. Kamoshita, A. Yamazaki, H. Endo, K. Ishihara, S. Yamashina, Y. Tsutsumi, H. Matsubara, M. Majima, Host stromal bradykinin B2 receptor signalling facilitates tumor-associated angiogenesis and tumor growth, Cancer Res. 64 (2004) 5178–5185, doi:10.1158/0008-5472.CAN-03-3589.

[37] L. Barki-Harrington, A.L. Bookout, G. Wang, M.E. Lamb, L.M. Leeb-Lundberg, Y. Daaka, Requirement for direct cross-talk between B1 and B2 kinin receptors for the proliferation of androgen-insensitive prostate cancer PC3 cells, Biochem. J. 371 (2003) 581–587, doi:10.1042/BJ20021708.

[38] I.I. Kramarenko, T.A. Morinelli, M.A. Bunni, J.R. Raymond, Sr, M.N. Garnovskaya, The bradykinin B(2) receptor induces multiple cellular responses leading to the proliferation of human renal carcinoma cell lines, Cancer Manag. Res. 4 (2012) 195–205, doi:10.2147/CMAR.S31847.

[39] V. Sgnaolin, T.C.B. Pereira, M.R. Bogo, R. Zanin, A.M.O. Battastini, F.B. Morrone, M.M. Campos, Functional and molecular characterization of kinin B1 and B2 receptors in human bladder cancer: implication of the PI3Kγ pathway, Invest. New Drugs 31 (2013) 812-822, doi:10.1007/s10637-012-9907-6.

[40] N.F. Nicoletti, T.C. Erig, R.F. Zanin, T.C. Brandão, T.C.B. Perreira, M.R. Bogo, M.M. Campos, F.B. Morrone, Mechanisms involved in kinin-induced glioma

cells proliferation: the role of ERK1/2 and PI3K/Akt pathways, J. Neurooncol. 120 (2014) 235–244, doi:10.1007/s11060-014-1549-4.

[41] M.T. Bawolak, S. Fortin, J. Bouthillier, A. Adam, L. Gera, R. C-Gaudreault, F. Marceau, Effects of inactivation-resistant agonists on the signalling, desensitization and down-regulation of bradykinin B(2) receptors, Br. J. Pharmacol. 158 (2009) 1375–1386, doi:10.1111/j.1476-5381.2009.00409.x.

[42] M.T. Bawolak, C. Roy, L. Gera, F. Marceau, Prolonged signalling and trafficking of the bradykinin B2 receptor stimulated with the amphibian peptide maximakinin: insight into the endosomal inactivation of kinins, Pharmacol Res. 65 (2012) 247–253, doi:10.1016/j.phrs.2011.11.004.

[43] L. Gera, M.T. Bawolak, C. Roy, R. Lodge, F. Marceau, Design of fluorescent bradykinin analogs: application to imaging of B2 receptor-mediated agonist endocytosis and trafficking and angiotensin-converting enzyme, J. Pharmacol. Exp. Ther. 337 (2011) 33–41, doi: 10.1124/jpet.110.177147.

[44] F. Marceau, H. Bachelard, J. Bouthillier, J.-P. Fortin, G. Morissette, M.-T. Bawolak, X. Charest-Morin, L. Gera, Bradykinin receptors: Agonists, antagonists, expression, signalling, and adaptation to sustained stimulation, Int. Immunopharmacol. 82 (2020) 106305, doi:10.1016/j.intimp.2020.106305.

[45] F. Gobeil Jr, M. Montagne, N. Inamura, D. Regoli, Characterization of non-peptide bradykinin B2 receptor agonist (FR 190997) and antagonist (FR 173657), Immunopharmacology 43 (1999) 179–185, doi:10.1016/s0162-3109(99)00129-0.

[46] K. Chiba, K. Adachi, Discovery of fingolimod, the sphingosine 1-phosphate receptor modulator and its application for the therapy of multiple sclerosis, Future Med. Chem. 4 (2012) 771–781, doi:10.4155/fmc.12.25.

[47] D.R. Jeffery, K.W. Rammohan, K. Hawker, E. Fox, Fingolimod: a review of its mode of action in the context of its efficacy and safety profile in relapsing forms of multiple sclerosis, Expert Rev. Neurother. 16 (2016) 31–44, doi:10.1586/14737175.2016.1123094.

[48] C. Volpi, C. Orabona, A. Macchiarulo, R. Bianchi, P. Puccetti, U. Grohmann, Preclinical discovery and development of fingolimod for the treatment of multiple

sclerosis, Expert Opin. Drug Discov. 14 (2019) 1199–1212, doi:10.1080/17460441.2019.1646244.

[49] V.V. Gurevich, E.V. Gurevich, Biased GPCR signalling: Possible mechanisms and inherent limitations, Pharmacol. Ther. 211 (2020) 107540, doi:10.1016/j.pharmthera.2020.107540.

[50] P.-Y. Jean-Charles, S. Kaur, S.K. Shenoy, G Protein–Coupled Receptor Signalling Through β-Arrestin–Dependent Mechanisms, J. Cardiovasc. Pharmacol. 70 (2017) 142–158, doi:10.1097/fjc.0000000000000482.

[51] T.J. Fernandez, M. De Maria, B.T. Lobingier, A cellular perspective of bias at G protein-coupled receptors, Protein Sci. 29 (2020) 1345–1354, doi:10.1002/pro.3872.

[52] M. Takano, A. Kanoh, K. Amako, M. Otani, K. Sano, M. Kanazawa-Hamada, S. Matsuyama, Nuclear localization of bradykinin B(2) receptors reflects binding to the nuclear envelope protein lamin C, Eur. J. Pharmacol. 723 (2014) 507–514, doi:10.1016/j.ejphar.2013.09.054.

[53] M. Takano, S. Matsuyama, Intracellular and nuclear bradykinin B2 receptors, Eur. J. Pharmacol. 732 (2014) 169–172, doi:10.1016/j.ejphar.2014.03.011.

[54] M. Savard, D. Barbaz, S. Bélanger, W. Müller-Esterl, G. Bkaily, P. D'orléans-Juste, J. Côté, V. Bovenzi, F. Gobeil Jr, Expression of endogenous nuclear bradykinin B2 receptors mediating signalling in immediate early gene activation, J. Cell Physiol. 216 (2008) 234–244, doi:10.1002/jcp.21398.

[55] S. Sposini, A.C. Hanyaloglu, Evolving View of Membrane Trafficking and Signalling Systems for G Protein-Coupled Receptors, Prog. Mol. Subcell Biol. 57 (2018) 273–299, https://doi.org/10.1007/978-3-319-96704-2_10.

[56] A.C. Hanyaloglu, Advances in Membrane Trafficking and Endosomal Signalling of G Protein-Coupled Receptors, Int. Rev. Cell Mol. Biol. 339 (2018) 93–131, doi:10.1016/bs.ircmb.2018.03.001.

[57] K. Eichel, M. von Zastrow, Subcellular Organization of GPCR Signalling, Trends Pharmacol. Sci. 39 (2018) 200–208, doi:10.1016/j.tips.2017.11.009.

[58] B.T. Lobingier, M. von Zastrow, When trafficking and signalling mix: How subcellular location shapes G protein-coupled receptor activation of heterotrimeric G proteins, Traffic 20 (2019) 130–136. doi:10.1111/tra.12634.

[59] https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL130517/

[60] https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL99869/

[61] https://www.cancerrxgene.org/ (each drug searched independently)

Declaration of interests

✓ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: