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Allene as an Alternative Functional Group for Drug Design: Effect of C–C Multiple Bonds Conjugated with Quinazolines on the Inhibition of EGFR Tyrosine Kinase

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A series of allenic quinazolines were synthesized as receptor tyrosine kinase inhibitors by using a simple protocol for palladium-catalyzed allene transformation. Among the compounds synthesized, two allenic 4-anilinoquinazolines selectively suppressed epidermal growth factor receptor (EGFR) tyrosine kinase activity in vitro. According to immunoblot analysis, the allenic quinazolines inhibited the EGF-mediated phosphorylation of EGFR and its downstream kinases in A431 cells. Furthermore, one of these allenic quinazolines decreased the proliferation of A431 cells through the induction of cell-cycle arrest and apoptosis.

Introduction

In the course of drug development, modification of known drug structures or lead compounds provides an improvement in efficacy and minimizes undesirable biological activities. Although various functional groups such as halides, esters, amines, nitriles, acetylenes, as well as vinyl, hydroxy, and nitro groups have been examined in functional group modification efforts, allenic groups have not received very much attention. Allenes have extraordinary properties such as the potential for axial chirality and a higher reactivity than alkenes. Therefore, the introduction of an allenic moiety onto an existing molecular scaffold has the potential to improve a compound's biological and pharmacological properties. Owing to limitations in synthesis, there are only a few examples of allene-containing compounds that are biologically active. Derivatives of prostaglandin E are lipid mediators converted from arachidonic acid by cyclooxygenase and produce gastrointestinal anti-secretory and anti-ulcer effects (Figure 1).[1] The allenic prostaglandin analogue enprostil was found to be a highly potent inhibitor of gastric acid secretion in a variety of species through selective agonism of the prostaglandin E receptor subtype 3 (EP3).[2,3] Furthermore, enprostil has anti-inflammatory activity by suppressing the production of interleukin 8 in human colonic epithelial cell lines.[4] An allenic derivative of the neurosteroid allopregnanolone possesses anticonvulsant and anxiolytic activity.[5,6] Allopregnanolone has been reported to be a potent positive allosteric modulator of γ-aminobutyric acid (GABA) action at the $GABA_A$ receptor,[7] and introduction of an allene group onto allopregnanolone potently increased $GABA_A$ receptor modulating activity, with 70-fold greater efficacy than allopregnanolone itself.[5]

Epidermal growth factor receptor (EGFR) tyrosine kinase plays a fundamental role in signal transduction pathways. EGFR and its ligands (EGF and TGF-α) have been implicated in numerous tumors of epithelial origin[8,9] and proliferative disorders of the epidermis such as psoriasis.[10] Therefore, the design of small-molecule EGFR tyrosine kinase inhibitors is an attractive approach for the development of new therapeutic agents.[11–14] Gefitinib (ZD-1839, Iressa)[15,16] and erlotinib (OSI-774, Tarceva)[17] have both been approved for the treatment of non-small-cell lung cancer (NSCLC) as EGFR tyrosine kinase inhibitors. Although therapeutic responses to both inhibitors can persist for as long as 2–3 years, the mean duration of response in most cases of NSCLC is only 6–8 months.[18–20] Mechanisms of the drug resistance are not well understood,[21] however, their modification is still a significant requirement in this area.

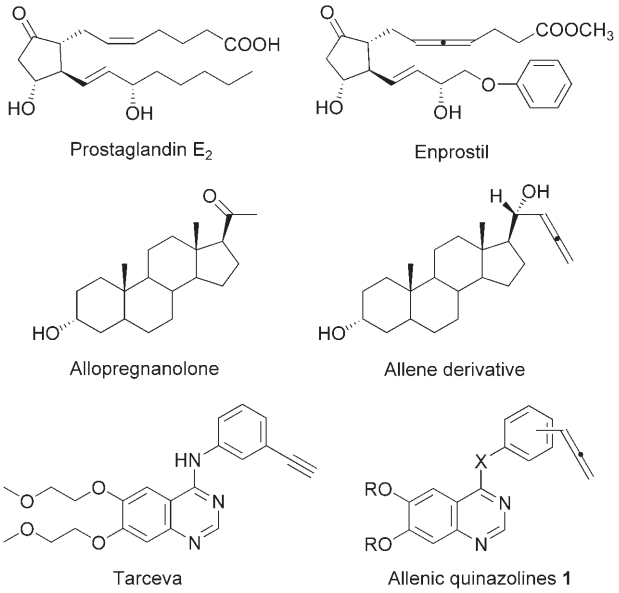

Figure 1. Biologically active allene-containing compounds.

Recently we developed a simple protocol for the introduction of an allene moiety into various electrophiles under palladium-catalyzed conditions.[22] This allene formation enabled us

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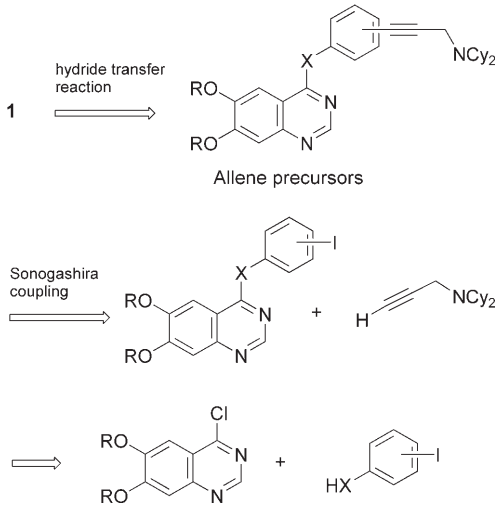
to synthesize various allene-conjugated heterocycles.[23–25] We were therefore interested in the utility of allene as an alternative functional group for pharmaceutical drug design, and we focused on an acetylene moiety in Tarceva.[26] According to the X-ray crystal structure, the acetylene group is located in the hydrophobic kinase pocket of EGFR.[27] Herein we demonstrate the effect of C–C multiple bonds in quinazolines on the inhibition of EGFR tyrosine kinase activity by replacement with allenyl, vinyl, and ethyl groups in the framework.

Results and Discussion

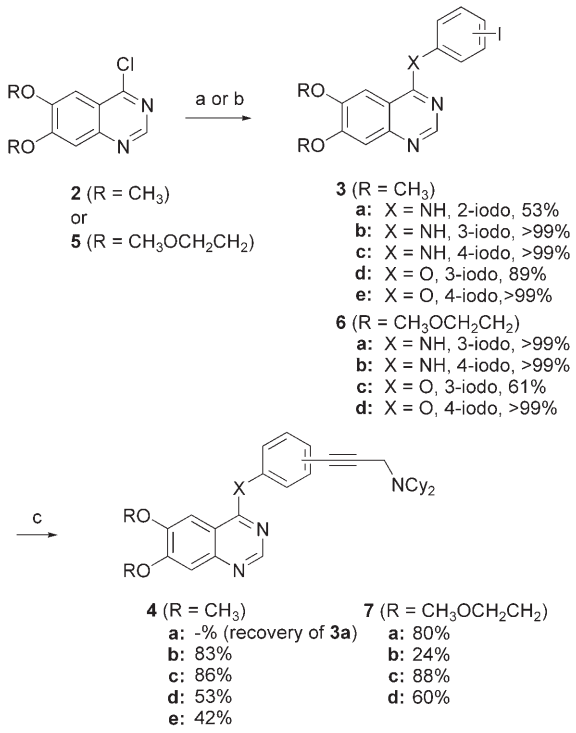
Chemistry

A retrosynthesis of the allenic quinazolines **1** is shown in Scheme 1. A key step in their synthesis is the palladium-catalyzed hydride transfer from the allene precursors, which were synthesized from the 4-iodoanilinoquinazoline compounds and *N*,*N*-dicyclohexylprop-2-ynylamine via the Sonogashira coupling reaction. The 4-iodoanilinoquinazolines were synthesized from the corresponding 4-chloroquinazolines and iodoanilines or iodophenols according to reported procedures, with modification.[28]



Scheme 1. Retrosynthesis of the allenic quinazolines.

Nucleophilic substitution of 4-chloro-6,7-dimethoxyquinazoline **2** with 2-, 3-, and 4-iodoanilines proceeded in isopropanol to give the iodoanilinoquinazolines **3a**–**c** in yields of 53–99% (Scheme 2). The reaction of **2** with 3- and 4-iodophenols required basic conditions, and the iodophenoxyquinazolines **3d** and **3e** were obtained in 89 and >99% yields, respectively. Introduction of a propargylic amine moiety as an allene precursor into iodo compounds **3a**–**e** was carried out by the Sonogashira coupling reaction with *N*,*N*-dicyclohexylprop-2-ynylamine. Unexpectedly, the 2-iodo derivative **4a** did not undergo Sonogashira coupling with *N*,*N*-dicyclohexylprop-2-ynylamine as a result of steric hindrance, and the starting material was recovered. The coupling reaction of iodo compounds **3b**–**e** with



Scheme 2. Reagents and conditions: a) 2-, 3-, or 4-iodoaniline, *i*PrOH, reflux; b) 3- or 4-iodophenol, K_2CO_3, *i*PrOH, reflux; c) *N*,*N*-dicyclohexylprop-2-ynylamine, $[Pd(PPh_3)_4]$ (5 mol%), CuI (10 mol%), triethylamine, CH_3CN, 60 °C.

N,*N*-dicyclohexylprop-2-ynylamine proceeded in the presence of $[Pd(PPh_3)_4]$ (5 mol%) and CuI (10 mol%) in acetonitrile at 60 °C to afford the corresponding allene precursors **4b**–**e** in yields of 42–86%. Similarly, 6,7-bis-(2-methoxyethoxy)quinazoline derivatives **7a**–**d** were also synthesized from the chloroquinazoline **5** via compounds **6a**–**d**.

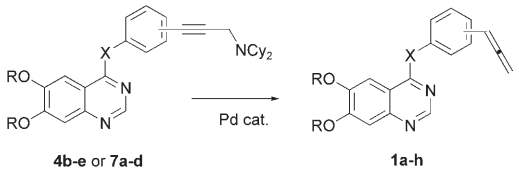
We next examined the allene transformations. The allene precursors **4b**–**e** and **7a**–**d** underwent palladium-catalyzed hydride transfer to afford allenic quinazolines **1a**–**h** as shown in Table 1. The reactions proceeded in the presence of $[Pd_2(dba)_3]\cdot CHCl_3$ (2.5 mol%) and $(C_6F_5)_2PC_2H_4P(C_6F_5)_2$ (10 mol%) in $CHCl_3$ at 100 °C, giving the corresponding allenes **1a**–**h** in yields of 39–99%. Furthermore, to compare the inhibitory potency of the allenic group with other C–C multiple bond moieties against EGFR tyrosine kinase, we synthesized vinyl- and ethyl-group-conjugated quinazolines **8** and **9** (Scheme 3).

Biological results

In vitro kinase inhibition assays

The inhibitory activity of the allenic quinazolines against EGFR, HER-2, Flt-1, and KDR tyrosine kinases was determined by measuring the levels of phosphorylation of the tyrosine-kinase-specific peptides in vitro,[29] and the results are listed in Table 2. Among the allenic quinazolines, **1a** and **1e**, which have an allene moiety at the *meta* position of their aniline rings, showed significant inhibitory activity against EGFR tyrosine kinase, and their IC_{50} values were determined to be 38.7 and

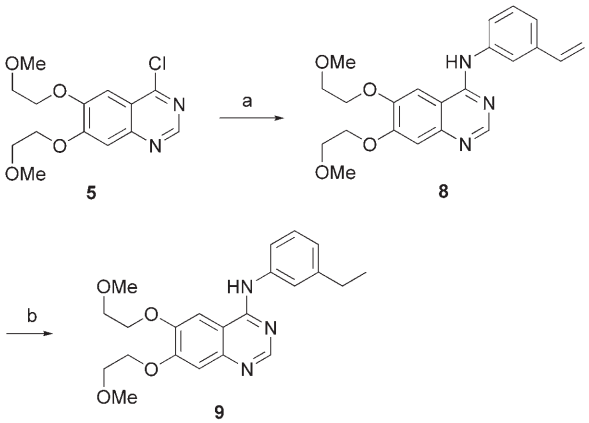
Table 1. Synthesis of allenic quinazolines **1** from **4b–e** or **7a–d** via palladium-catalyzed hydride transfer.[a]



4b-e or **7a-d** **1a-h**

Compd	R	X	Position[b]	Yield [%]
1a	CH_3	NH	3′	66
1b	CH_3	NH	4′	39
1c	CH_3	O	3′	46
1d	CH_3	O	4′	>99
1e	$CH_3OC_2H_4$	NH	3′	49
1f	$CH_3OC_2H_4$	NH	4′	39
1g	$CH_3OC_2H_4$	O	3′	55
1h	$CH_3OC_2H_4$	O	4′	61

[a] Reactions were carried out in the presence of $[Pd_2(dba)_3]\cdot CHCl_3$ (2.5 mol%) and $(C_6F_5)_2PC_2H_4P(C_6F_5)_2$ (10 mol%) in $CHCl_3$ at 100 °C in a vial tube. [b] Refers to the allene moiety position on the aniline or phenoxy groups of compounds **1**.



Scheme 3. Reagents and conditions: a) 3-vinylaniline, *i*PrOH, reflux, 79%; b) H_2, Pd-C, EtOH, 30%.

96.0 nM, respectively. However, the *para*-substituted allene derivatives **1b** and **1f** had weaker EGFR inhibitory activity. Allenic phenoxyquinazolines **1c**, **1d**, **1g**, and **1h** were not effective for the inhibition of EGFR tyrosine kinase. Furthermore, weak or no inhibitory activities against HER-2, Flt-1, and KDR, were observed in all cases (Table 2). These results indicate that the introduction of an allene group at the *meta* position of the 4-anilinoquinazoline is essential for selective suppression of EGFR tyrosine kinase activity, although the potency is not as high as that of Tarceva. Furthermore, we compared the inhibitory potency of C–C multiple bonds conjugated with anilinoquinazolines toward EGFR tyrosine kinase. Anilinoquinazolines substituted with vinyl and ethyl groups (respectively compounds **9** and **8**) showed selective inhibitory activity against EGFR tyrosine kinase as shown in Table 2, and the efficient groups for inhibitory activity are ethynyl, vinyl, allenyl, and ethyl, in order of decreasing potency.

Table 2. In vitro inhibition of receptor tyrosine kinase activity by allenic quinazolines **1a–h**, **8** and **9**.[a]

Compd	EGFR	HER2	Flt-1	KDR
1a	38.7±8.5 nM (81.6±0.6)	>1 μM (−)[b]	>1 μM (33.6±1.2)	>1 μM (34.8±4.2)
1b	865.2±3.4 nM (56.3±1.8)	>1 μM (−)	>1 μM (−)	>1 μM (31.2±2.4)
1c	>1 μM (44.3±6.0)	>1 μM (12.7±8.04)	>1 μM (−)	>1 μM (27.2±1.2)
1d	>1 μM (11.9±3.1)	>1 μM (−)	>1 μM (33.9±5.5)	>1 μM (37.7±5.8)
1e	96.0±3.4 nM (92.9±1.6)	>1 μM (−)	>1 μM (−)	>1 μM (44.2±2.8)
1f	>1 μM (49.2±2.7)	>1 μM (−)	>1 μM (43.9±0.75)	>1 μM (46.2±0.6)
1g	>1 μM (45.0±4.0)	>1 μM (−)	>1 μM (−)	>1 μM (27.8±1.2)
1h	>1 μM (25.3±6.8)	>1 μM (−)	>1 μM (−)	>1 μM (49.6±0.2)
8	11.9±4.2 nM (71.3±6.1)	>1 μM (−)	>1 μM (30.6±8.0)	>1 μM (30.1±2.6)
9	348.7±17.4 nM (62.5±5.3)	>1 μM (−)	>1 μM (−)	>1 μM (−)
Tarceva	4.5±0.3 nM (98.4±0.5)	>1 μM (26.2±2.7)	>1 μM (−)	>1 μM (37.9±3.5)

[a] The first value given in each case is the drug concentration required to inhibit phosphorylation of the poly(Glu/Tyr) substrate by 50% (IC_{50}), and was determined from semi-logarithmic dose–response plots; results represent the mean ±SD of two or three independent experiments performed in duplicate. The second value given (in parentheses) is the percent inhibition of phosphorylation at a drug concentration of 1 μM. [b] (−): No inhibitory effect was observed.

Inhibition of EGF-induced phosphorylation

To determine the inhibitory activity of the current allenic quinazolines against EGFR tyrosine kinase at the cellular level, we examined their effect on the EGF-mediated signaling pathway in EGFR-overexpressing A431 cells. As shown in Figure 2, treat-

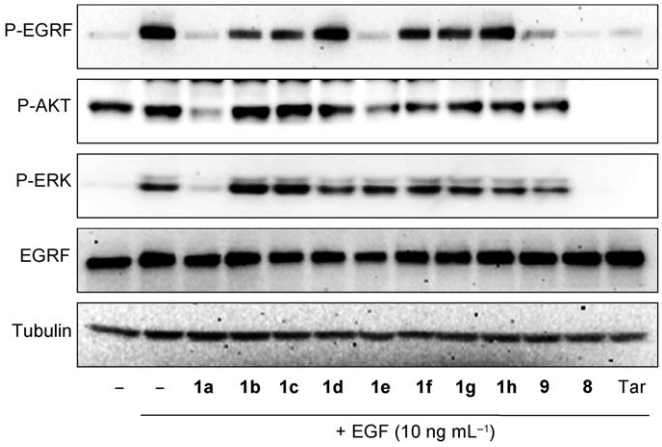


Figure 2. Inhibition of EGF-induced phosphorylation of EGFR and its downstream kinases. A431 cells were pre-incubated with the allenic quinazolines **1**, **8**, **9** (at 1 μM), or Tarceva (Tar; at 0.1 μM), and then stimulated with EGF (10 ng mL^{-1}). The levels of each protein indicated were detected by immunoblot analysis with the specific antibody.

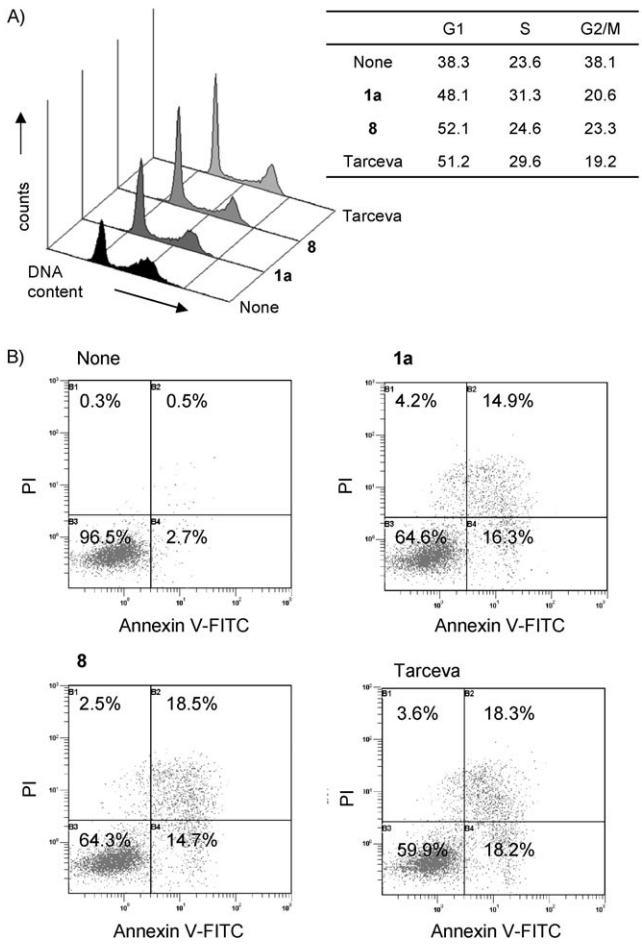
ment of A431 cells with EGF (10 ng mL^{-1}) induced phosphorylation of EGFR and its downstream kinases ERK and Akt, which play an important role in the regulation of cell proliferation and apoptosis, respectively.[30] In agreement with the results of kinase assay, allenic quinazolines **1a** and **1e**, and compounds **8** and **9** potently suppressed EGF-induced phosphorylation of EGFR and downstream kinases at a compound concentration of 1 μM. Tarceva also showed complete inhibitory activity at this concentration. These results indicate that allenic quinazolines suppress the EGFR-mediated signaling pathway through inhibition of EGFR tyrosine kinase activity.

Cell growth inhibition assays

We next examined the effects of the compounds on the proliferation of various human carcinoma cell lines by use of the MTT assay in order to implicate the inhibitory activity of the allenic quinazolines against EGFR tyrosine kinase in cell growth. As shown in Table 3, compounds **1a**, **1e**, and **8** significantly suppressed the proliferation of EGFR-overexpressing A431 cells, with GI_{50} values of 0.60, 1.14, and 0.23 μM, respectively, although significant cell growth inhibition was not observed in other cell lines. These results suggest that the decrease in A431 cell proliferation could be through EGFR inhibition by compounds **1a**, **1e**, and **8**.

Cell-cycle effect and apoptotic activity of 1a

As it has been reported that inhibition of EGFR leads to the induction of cell-cycle arrest and apoptosis,[31] we next conducted a flow cytometry analysis using A431 cells. Treatment of A431 cells with **1a**, **8**, and Tarceva at a concentration of 1 μM for 24 h led to profound changes in cell-cycle profiles as shown in Figure 3A. The population of cells in the G1 phase increased, while the G2/M cell population dramatically decreased. Moreover, to characterize the mechanism of cell death induced by **1a** and **8**, a biparametric cytofluorimetric analysis was performed using FITC-labeled annexin V and propidium iodide (PI), which respectively stain phosphatidylserine residues and DNA.[32] Annexin V is a Ca^{+}-dependent phospholipid binding protein with a high affinity for phosphatidylserine. A431 cells were treated with **1a** (1 μM) for 24 h and then stained with PI and FITC-labeled annexin V to specifically detect the exposure of phosphatidylserine residues at the cell surface. The resulting green (FITC) and red (PI) fluorescence emissions were monitored by flow cytometry. As shown in Figure 3B, the number of annexin-V-positive and PI-negative cells increased upon treatment with **1a**, and the population of early and late apoptotic cells increased, respectively, to 16.3 and 14.9% at 24 h. These results indicate that apoptosis is induced by **1a**. Tarceva also exhibited higher apoptotic activity than **1a**, and



	G1	S	G2/M
None	38.3	23.6	38.1
1a	48.1	31.3	20.6
8	52.1	24.6	23.3
Tarceva	51.2	29.6	19.2

Figure 3. Induction of cell-cycle arrest and apoptosis by compounds **1a** and **8**. A431 cells were incubated for 24 h with compounds **1a**, **8**, or Tarceva (each at 1 μM). A) Cells were harvested and stained with propidium iodide (PI). B) Cells were stained with annexin V–FITC and PI. The cell-cycle phases and apoptosis were determined by a flow cytometer.

Table 3. Effects of allenic quinazolines **1a**–**h**, **8**, and **9** on the proliferation of human cancer cells.[a]

Compd	GI_{50} [μM]					
	A431	SKBR3	KatoIII	HepG2	HCT15	SW480
1a	0.60 ± 0.01	4.48 ± 0.20	4.33 ± 0.08	6.21 ± 0.27	6.37 ± 0.02	8.41 ± 0.17
1b	9.82 ± 0.01	> 10	4.09 ± 0.37	> 10	6.90 ± 0.42	> 10
1c	6.07 ± 0.10	> 10	7.57 ± 1.00	> 10	> 10	> 10
1d	> 10	> 10	3.50 ± 0.08	> 10	> 10	> 10
1e	1.14 ± 0.11	3.16 ± 0.86	4.66 ± 0.16	> 10	7.32 ± 1.48	6.43 ± 0.49
1f	5.86 ± 0.03	1.43 ± 0.33	4.07 ± 0.42	> 10	> 10	5.76 ± 0.17
1g	> 10	> 10	> 10	> 10	> 10	> 10
1h	> 10	> 10	4.14 ± 0.45	> 10	> 10	> 10
8	0.23 ± 0.02	8.57 ± 0.53	7.38 ± 0.41	> 10	> 10	7.40 ± 0.55
9	6.57 ± 0.48	9.75 ± 0.15	3.98 ± 0.64	> 10	> 10	> 10
Tarceva	0.14 ± 0.01	1.94 ± 0.09	4.42 ± 0.02	> 10	> 10	5.92 ± 0.20

[a] Cells were incubated for 72 h with various concentrations (10 nM to 10 μM) of compounds, and then the rates of viable cells were determined by MTT assay. The drug concentration required to inhibit cell growth by 50% (GI_{50}) was determined from semi-logarithmic dose–response plots, and results represent the mean ± SD of triplicate samples.

the respective population of early and late apoptotic cells was 18.2 and 18.3% at 24 h. These results indicate that the antiproliferative effect of compound **1a** and Tarceva toward A431 cells is associated with the induction of cell-cycle arrest and apoptosis.

Docking simulations

To get a better understanding of the structure–activity relationships of the C–C multiple-bond-containing functional groups conjugated with quinazolines, we performed molecular docking simulations using the binding mode of Tarceva in the crystal structure of EGFR (PDB code: 1M17).[27] As shown in Figure 4A, compound **1e** bound the ATP binding site of the EGFR kinase pocket in a manner similar to that of Tarceva, and hydrogen bonds were observed between the backbone amide nitrogen of Met769 and the N1 nitrogen atom of quinazoline, and between a water molecule and the N3 nitrogen atom of quinazoline. LigScore2[33] values were calculated at 5.38 for **1e** and 5.89 for Tarceva. However, compound **1g**, which has a 3-allenylphenoxy group instead of a 3-allenylanilino group, exhibited a different binding mode from Tarceva: the 3-allenenylphenoxy group is located near Val821 (Figure 4B). Although similar hydrogen bond interactions between **1e** and Tarceva were observed, as depicted in Figure 4A, those hydrogen bonds were not observed in the binding mode of **1g**. Therefore, this binding mode may cause the weaker inhibitory activity of **1g** against EGFR tyrosine kinase observed in both the in vitro and cellular assays. We also carried out docking simulations for compounds **8** and **9**. Both showed docking modes similar to that of Tarceva, and their respective LigScore2 values were calculated at 5.39 and 5.89. These results also support their potency as inhibitors of EGFR tyrosine kinase activity.

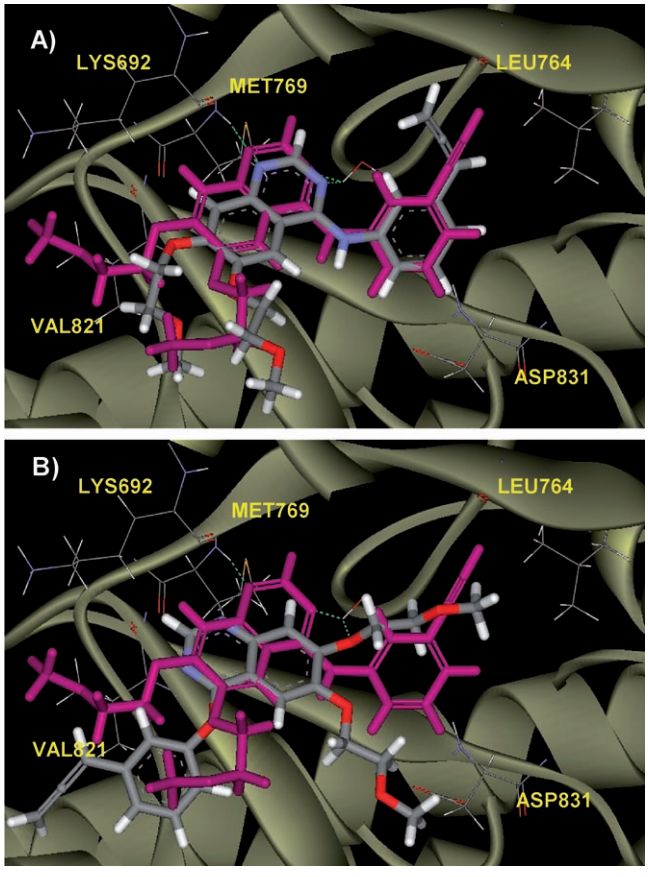


Figure 4. An overlay of allenic quinazolines A) **1e** (gray) and B) **1g** (gray) docked into the active site of the EGFR kinase domain. The docking model was calculated with the DS-Modeling program version 1.7, based on the crystallographic data (PDB code: 1M17) of EGFR tyrosine kinase in complex with Tarceva (in red). Significant hydrogen bonds between molecules and the kinase domain are indicated by green dashed lines.

Conclusions

We synthesized allene-conjugated quinazolines as EGFR tyrosine kinase inhibitors by using our allene transformation protocol. Among the compounds synthesized, allenes **1a** and **1e**, substituted at the *meta* position of the 4-anilinoquinazoline, selectively suppress EGFR tyrosine kinase activity. Moreover, these compounds inhibit EGF-mediated phosphorylation of EGFR and its downstream kinases in A431 cells, resulting in cell-cycle arrest and apoptosis. The C–C multiple-bond-containing functional groups attached at the *meta* position of 4-anilinoquinazoline affected kinase inhibition, and the most efficient groups for inhibitory activity are ethynyl, vinyl, allenyl, and ethyl, in order of decreasing potency. Although Tarceva, equipped with the C≡C triple bond of its ethynyl group, is more potent at inducing apoptosis in A431 cells through inhibition of the EGF-mediated phosphorylation signaling pathway, the findings presented herein show the potential for developing biologically and pharmacologically active compounds by introducing allenes as alternative functional groups onto the existing molecular scaffold. Experiments to gain further insight into the applications of allene moieties are currently in progress in our laboratory.

Experimental Section

Chemistry: ^{1}H NMR and ^{13}C NMR spectra were measured on Jeol JNM-AL 300 (300 MHz) and Varian Unity-Inova 400 (400 MHz) spectrometers. ^{1}H NMR and ^{13}C NMR chemical shifts are expressed in δ (ppm), and coupling constants are expressed in Hz. IR spectra were measured on a Shimadzu FTIR-8200A spectrometer. Analytical TLC was performed on glass plates (Merck Kieselgel 60 F_{254} or RP-18 F_{254}, layer thickness: 0.2 mm). Samples were visualized by UV light (254 nm), I_2, or $KMnO_4$. Column chromatography was performed on silica gel (Merck Kieselgel 70–230 mesh). All reactions were carried out under an argon atmosphere using standard Schlenk techniques. Most chemicals were of analytical grade and used without further purification.

(6,7-Dimethoxyquinazolin-4-yl)-(2-iodophenyl)amine (3a): A mixture of 4-chloro-6,7-dimethoxyquinazoline **2** (112 mg, 0.5 mmol) and 2-iodoaniline (120 mg, 0.55 mmol) was dissolved in *i*PrOH (10 mL) and held at reflux for 16 h. After the reaction mixture was cooled to room temperature, the precipitate was filtered and washed with *i*PrOH and H_2O. The white solid obtained was dried under reduced pressure to give **3a** (108 mg, 0.265 mmol, 53%); mp: 204–205 °C, ^{1}H NMR (400 MHz, CD_3OD): δ = 8.48 (s, 1H), 7.93

(d, $J=7.6$ Hz, 1H), 7.83 (s, 1H), 7.38–7.45 (m, 2H), 7.13 (s, 1H), 7.09 (t, $J=7.6$ Hz, 7.6 Hz, 1H), 3.98 (s, 3H), 3.95 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=159.3$, 153.0, 149.6, 141.0, 140.5, 137.0, 131.0, 130.6, 108.3, 107.8, 103.7, 100.5, 99.1, 57.4, 57.2 ppm; IR (KBr): $\tilde{\nu}=$ 3393, 3030, 2712, 2347, 1630, 1572, 1510, 1435, 1362, 1281, 1236, 1157, 1072, 1020, 986, 854 cm^{-1}; MS (EI) *m/z* 407 [M^+]; Anal. calcd for $C_{16}H_{14}IN_3O_2$: C 47.19, H 3.47, N 10.32, found: C 47.02, H 3.35, N 10.40.

(6,7-Dimethoxyquinazolin-4-yl)-(3-iodophenyl)amine (3b) was synthesized from 4-chloro-6,7-dimethoxyquinazoline **1** (224 mg, 1.0 mmol) and 3-iodoaniline (240 mg, 1.1 mmol) using the procedure described for **3a** to give **3b** (405 mg, 1.00 mmol, >99%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.60$ (s, 1H), 8.06 (s, 1H), 7.86 (s, 1H), 7.63 (d, $J=8.4$ Hz, 1H), 7.58 (d, $J=8.0$ Hz, 1H), 7.14 (t, $J=8.4$ Hz, 8.0 Hz, 1H), 7.10 (s, 1H), 3.97 (s, 3H), 3.96 ppm (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): $\delta=167.9$, 166.4, 163.4, 160.8, 157.2, 151.5, 143.6, 141.9, 140.9, 132.5, 120.2, 116.7, 112.0, 103.9, 71.2, 66.4, 58.9, 58.3 ppm; Anal. calcd for $C_{16}H_{14}IN_3O_2$: C 47.19, H 3.47, N 10.32, found: C 47.15, H 3.42, N 10.04.

(6,7-Dimethoxyquinazolin-4-yl)-(4-iodophenyl)amine (3c) was synthesized from 4-chloro-6,7-dimethoxyquinazoline **2** (224 mg, 1.0 mmol) and 4-iodoaniline (240 mg, 1.1 mmol) using the procedure described for **3a** to give **3c** (407 mg, 1.00 mmol, >99%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.73$ (s, 1H), 8.02 (s, 1H), 7.86 (d, $J=8.8$ Hz, 2H), 7.58 (d, $J=8.8$ Hz, 2H), 7.25 (s, 1H), 4.11 ppm (t, $J=1.6$ Hz, 2.0 Hz, 6H); ^{13}C NMR (75 MHz, CD_3OD): $\delta=$ 159.1, 153.0, 149.5, 139.2, 138.1, 136.9, 136.8, 127.5, 108.9, 103.8, 100.4, 91.7, 57.4, 57.3 ppm; Anal. calcd for $C_{16}H_{14}IN_3O_2$: C 47.19, H 3.47, N 10.32, found: C 47.09, H 3.28, N 10.17.

4-(3-Iodophenoxy)-6,7-dimethoxyquinazoline (3d): A mixture of 4-chloro-6,7-dimethoxyquinazoline **2** (224 mg, 1.0 mmol), 3-iodophenol (240 mg, 1.1 mmol), and K_2CO_3 (276 mg, 2.0 mmol) was dissolved in *i*PrOH (10 mL) and held at reflux for 4 h. After the reaction mixture was cooled to room temperature, the precipitate was filtered and washed with *i*PrOH and H_2O. The white solid obtained was dried under reduced pressure to give **3d** (362 mg, 0.89 mmol, 89%) as a white solid; mp: >300 °C, ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 8.62 (s, 1H), 7.64 (d, $J=4.4$ Hz, 1H), 7.62 (s, 1H), 7.49 (s, 1H), 7.31 (s, 1H), 7.16–7.26 (m, 2H), 4.05 (s, 3H), 4.045 ppm (s,3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=165.0$, 156.0, 152.81, 152.75, 150.3, 149.5, 134.9, 131.2, 130.9, 121.5, 110.5, 106.9, 100.8, 93.9, 56.4, 56.3 ppm; IR (KBr): $\tilde{\nu}=3421$, 3082, 2368, 1618, 1585, 1420, 1371, 1236, 1207, 993, 908, 856 cm^{-1}; MS (EI) *m/z* 408 [M^+]; Anal. calcd for $C_{16}H_{13}IN_2O_3$: C 47.08, H 3.21, N 6.86, found: C 47.31, H 3.46, N 6.65.

4-(4-Iodophenoxy)-6,7-dimethoxyquinazoline (3e) was synthesized from 4-chloro-6,7-dimethoxyquinazoline **2** (224 mg, 1.0 mmol), 4-iodophenol (242 mg, 1.1 mmol), and K_2CO_3 (276 mg, 2.0 mmol) using the procedure described for **3d** to give **3e** (408 mg, 1.00 mmol, >99%) as a white solid; mp: 172–174 °C, ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.60$ (s, 1H), 7.77 (d, $J=8.8$ Hz, 2H), 7.51 (s, 1H), 7.31 (s, 1H), 7.02 (d, $J=8.8$ Hz, 2H), 4.05 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=165.0$, 155.9, 152.7, 152.4, 150.3, 149.5, 138.8, 124.2, 110.6, 100.8, 89.9, 56.41, 56.35 ppm; IR (KBr): $\tilde{\nu}=3435$, 3058, 2932, 2837, 2366, 1890, 1686, 1568, 1502, 1421, 1379, 1213, 991, 907, 843 cm^{-1}; MS (EI) *m/z* 408 [M^+]; Anal. calcd for $C_{16}H_{13}IN_2O_3$: C 47.08, H 3.21, N 6.86, found: C 47.15, H 3.50, N 6.89.

[3-(3-Dicyclohexylaminoprop-1-ynyl)phenyl]-(6,7-dimethoxyquinazolin-4-yl)amine (4b): Propargyldicyclohexylamine (0.051 mL, 0.46 mmol) and triethylamine (0.77 mL, 0.7 mmol) were added to a mixture of **3b** (170 mg, 0.42 mmol), CuI (7.9 mg, 0.042 mmol), and [$Pd(PPh_3)_4$] (24 mg, 0.021 mmol) in CH_3CN (2 mL), and the reaction mixture was stirred at 60 °C. The reaction progress was monitored by TLC. After **3b** was consumed, the reaction was quenched by a solution of saturated aqueous NH_4Cl, washed with a solution of saturated aqueous NaCl, and then dried over $MgSO_4$. After removal of solvents under reduced pressure, the residue was purified by column chromatography on silica gel with EtOAc to give **4b** (173 mg, 0.350 mmol, 83%) as a brown solid; mp: 126–127 °C, ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.65$ (s, 1H), 7.74 (s, 1H), 7.67 (d, $J=$ 8.0 Hz, 1H), 7.16–7.32 (m, 4H), 3.99 (s, 3H), 3.98 (s, 3H), 3.70 (s, 2H), 2.85 (m, 2H), 1.91 (d, $J=10.8$ Hz, 4H), 1.79 (d, $J=12.8$ Hz, 4H), 1.62 (d, $J=12.4$ Hz, 2H), 1.07–1.42 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=156.3$, 154.7, 153.7, 149.4, 147.0, 138.5, 128.7, 127.3, 124.8, 124.4, 121.7, 109.0, 107.6, 99.9, 89.6, 83.1, 57.5, 56.34, 56.31, 35.6, 31.1, 26.13, 26.11 ppm; IR (KBr): $\tilde{\nu}=3337$, 2930, 2853, 2363, 1624, 1578, 1431, 1389, 1252, 1219, 1144, 1001, 851 cm^{-1}; MS (ESI) *m/z* 499 ([*M*+H]$^+$); Anal. calcd for $C_{31}H_{38}N_4O_2$: C 74.67, H 7.68, N 11.24, found: C 74.53, H 7.87, N 11.15.

[4-(3-Dicyclohexylaminoprop-1-ynyl)phenyl]-(6,7-dimethoxyquinazolin-4-yl)amine (4c) was synthesized from **3c** (99.7 mg, 0.245 mmol) using the procedure described for **4b** to give **4c** (105 mg, 0.212 mmol, 86%) as a white solid; mp: 200–201 °C, ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.66$ (s, 1H), 7.64 (d, $J=8.8$ Hz, 2H), 7.41 (d, $J=8.8$ Hz, 2H), 7.25 (s, 1H), 7.00 (s, 1H), 4.02 (s, 3H), 4.01 (s, 3H), 3.69 (s, 2H), 2.81 (m, 2H), 1.89 (d, $J=12.4$ Hz, 4H), 1.78 (d, $J=13.2$ Hz, 4H), 1.60 (s, 2H), 1.11–1.41 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=156.2$, 154.7, 153.5, 149.5, 147.4, 138.1, 132.1, 121.4, 119.4, 109.2, 107.6, 99.5, 89.1, 83.1, 57.5, 56.1, 35.7, 31.2, 26.20, 26.19 ppm; IR (KBr): $\tilde{\nu}=3387$, 2928, 2363, 1578, 1510, 1421, 1246, 995, 851 cm^{-1}; MS (EI) *m/z* 408 [M^+]; Anal. calcd for $C_{31}H_{38}N_4O_2$: C 74.67, H 7.68, N 11.24, found: C 74.68, H 7.56, N 11.22.

Dicyclohexyl-{3-[3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl]-prop-2-ynyl}amine (4d) was synthesized from **3d** (142 mg, 0.34 mmol) using the procedure described for **4b** to give **4d** (92.2 mg, 0.185 mmol, 53%) as a brown solid; mp: 64–65 °C, ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.64$ (s, 1H), 7.55 (s, 1H), 7.42 (dd, $J=$ 8.0, 7.6 Hz, 1H), 7.36 (s, 1H), 7.35 (d, $J=7.6$ Hz, 1H), 7.29 (s, 1H), 7.22 (d, $J=8.0$ Hz, 1H), 4.08 (s, 6H), 3.84 (s, 2H), 2.97 (m, 2H), 1.96 (d, $J=11.2$ Hz, 4H), 1.81 (d, $J=12.8$ Hz, 4H), 1.63 (d, $J=12.4$ Hz, 2H), 1.09–1.48 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=165.2$, 155.9, 152.8, 152.2, 150.2, 149.3, 129.5, 129.1, 125.1, 124.7, 121.9, 110.6, 88.5, 83.6, 57.7, 56.4, 56.3, 35.4, 30.3, 26.00, 25.97 ppm; IR (KBr): $\tilde{\nu}=3423$, 2930, 2853, 2372, 1618, 1570, 1502, 1418, 1377, 1236, 1211, 1186, 1153, 997, 910, 853 m^{-1}; MS (EI) *m/z* 499 [M^+]; Anal. calcd for $C_{31}H_{37}N_3O_3$: C 74.52, H 7.46, N 8.41, found: C 74.66, H 7.62, N 8.45.

Dicyclohexyl-{3-[4-(6,7-dimethoxyquinazolin-4-yloxy)phenyl]-prop-2-ynyl}amine (4e) was synthesized from **3d** (100 mg, 0.25 mmol) using the procedure described for **4b** to give **4d** (51.3 mg, 0.103 mmol, 42%) as a white solid; mp: 67–68 °C, ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.63$ (s, 1H), 7.54 (s, 1H), 7.51 (dd, $J=$ 2.0 Hz, 6.4 Hz, 2H), 7.32 (s, 1H), 7.20 (dd, $J=2.0$ Hz, 6.4 Hz, 2H), 4.070 (s, 3H), 4.068 (s, 3H), 3.72 (s, 2H), 2.84 (m, 2H), 1.92 (d, $J=$ 11.2 Hz, 4H), 1.81 (d, $J=12.4$ Hz, 4H), 1.63 (d, $J=12.4$ Hz, 2H), 1.09–1.43 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=165.2$, 155.8, 152.8, 151.8, 150.2, 149.3, 132.8, 121.8, 121.6, 110.6, 106.7, 100.9, 89.6, 82.6, 57.4, 56.33, 56.27, 35.6, 31.2, 26.23, 26.16 ppm; IR (KBr): $\tilde{\nu}=3449$, 2928, 2365, 1578, 1502, 1421, 1379, 1215, 997, 905, 849 m^{-1}; MS (EI) *m/z* 499 [M^+]; Anal. calcd for $C_{31}H_{37}N_3O_3$: C 74.52, H 7.46, N 8.41, found: C 74.80, H 7.52, N 8.35.

[6,7-Bis(2-methoxyethoxy)quinazolin-4-yl]-(3-iodophenyl)amine (6a) was synthesized from 4-chloro-6,7-di(methoxyethoxy)quinazoline **5** (235 mg, 0.72 mmol) and 3-iodoaniline (175 mg, 0.8 mmol) using the procedure described for **3a** to give **6a** (373 mg, 0.72 mmol, >99%) as a white solid; mp: 108–109 °C, ^{1}H NMR (400 MHz, CD_3OD): δ = 8.62 (s, 1H), 8.07 (s, 1H), 7.90 (s, 1H), 7.64 (d, J = 9.4 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.16 (s, 1H), 7.15 (t, J = 9.2 Hz, 8.4 Hz, 1H), 4.29 (m, 4H), 3.78 (m, 4H), 3.38 (s, 3H), 3.37 ppm (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ = 160.0, 158.5, 152.1, 149.7, 139.4, 137.0, 136.8, 134.4, 131.6, 124.8, 108.9, 105.2, 101.6, 94.2, 71.7, 71.64, 70.7, 70.5, 59.5 ppm; IR (KBr): $\tilde{\nu}$ = 3510, 3322, 3071, 2928, 2365, 1626, 1576, 1514, 1429, 1240, 1124, 1061, 991, 930, 868 cm^{-1}; MS (EI) *m/z* 495 [M^+]; Anal. calcd for $C_{20}H_{22}IN_3O_4$: C 48.50, H 4.48, N 8.48, found: C 48.55, H 4.61, N 8.29.

[6,7-Bis(2-methoxyethoxy)quinazolin-4-yl]-(4-iodophenyl)amine (6b) was synthesized from 4-chloro-6,7-di(methoxyethoxy)quinazoline **5** (212 mg, 0.65 mmol) and 4-iodoaniline (157 mg, 0.72 mmol) using the procedure described for **3a** to give **6b** (337 mg, 0.65 mmol, >99%) as a white solid; mp: 145 °C, ^{1}H NMR (400 MHz, CD_3OD): δ = 8.54 (s, 1H), 7.87 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.14 (s, 1H), 4.27 (m, 4H), 3.78 (m, 4H), 3.38 (s, 3H), 3.37 ppm (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ = 159.9, 158.5, 152.1, 149.7, 139.2, 138.1, 127.4, 109.3, 105.2, 102.0, 101.7, 91.9, 71.7, 71.6, 70.7, 70.5, 59.5 ppm; IR (KBr): $\tilde{\nu}$ = 3411, 2917, 2821, 2640, 2369, 1634, 1514, 1487, 1364, 1281, 1234, 1126, 870 cm^{-1}; MS (EI) *m/z* 495 [M^+]; Anal. calcd for $C_{20}H_{22}IN_3O_4$: C 48.50, H 4.48, N 8.48, found: C 48.28, H 4.41, N 8.47.

4-(3-Iodophenoxy)-6,7-bis(2-methoxyethoxy)quinazoline (6c) was synthesized from 4-chloro-6,7-di(methoxyethoxy)quinazoline **5** (230 mg, 0.71 mmol) and 3-iodophenol (175 mg, 0.8 mmol) using the procedure described for **3d** to give **6c** (354 mg, 0.710 mmol, >99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (s, 1H), 7.65 (d, J = 7.2 Hz, 1H), 7.64 (s, 1H), 7.53 (s, 1H), 7.31 (s, 1H), 7.19–7.26 (M, 2H), 4.32 (q, 4H), 3.89 (q, 4H), 3.50 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.8, 155.4, 152.7, 152.6, 149.7, 149.3, 134.8, 131.0, 130.8, 121.4, 110.4, 107.6, 102.2, 93.8, 70.5, 70.3, 68.6, 68.4, 59.2 ppm; IR (KBr): $\tilde{\nu}$ = 3398, 2882, 2367, 1923, 1616, 1570, 1502, 1375, 1209, 1120, 1028, 968, 862 cm^{-1}; MS (EI) *m/z* 496 [M^+]; Anal. calcd for $C_{20}H_{21}IN_2O_5$: C 48.40, H 4.26, N 5.64, found: C 48.25, H 4.53, N 5.81.

4-(4-Iodophenoxy)-6,7-bis-(2-methoxyethoxy)quinazoline (6d) was synthesized from 4-chloro-6,7-di(methoxyethoxy)quinazoline **5** (230 mg, 0.71 mmol) and 4-iodophenol (175 mg, 0.8 mmol) using the procedure described for **3d** to give **6d** (354 mg, 0.71 mmol, >99%) as a white solid; mp: 137 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (s, 1H), 7.78 (d, J = 9.2 Hz, 2H), 7.55 (s, 1H), 7.31 (s, 1H), 7.04 (d, J = 9.2 Hz, 2H), 4.33 (m, 4H), 3.89 (m, 4H), 3.50 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.0, 155.5, 152.7, 152.4, 149.7, 149.4, 138.7, 120.2, 110.5, 107.7, 102.4, 89.9, 70.6, 70.4, 68.7, 68.5, 59.3 ppm; IR (KBr): $\tilde{\nu}$ = 3471, 2921, 2885, 2360, 1618, 1568, 1379, 1211, 1128, 1042, 907, 851 cm^{-1}; MS (EI) *m/z* 496 [M^+]; Anal. calcd for $C_{20}H_{21}IN_2O_5$: C 48.40, H 4.26, N 5.64, found: C 48.71, H 4.43, N 5.75.

[6,7-Bis(2-methoxyethoxy)quinazolin-4-yl]-[3-(3-dicyclohexylaminoprop-1-ynyl)phenyl]amine (7a) was synthesized from **6a** (215 mg, 0.43 mmol) using the procedure described for **4b** to give **7a** (200 mg, 0.34 mmol, 80%) as a white solid; mp: 72–73 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.64 (s, 1H), 7.79 (s, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.53 (bs, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.28 (s, 1H), 7.21 (s, 1H), 7.17 (d, J = 8.0 Hz, 1H), 4.27 (m, 4H), 3.83 (m, 4H), 3.76 (s, 2H), 3.46 (s, 3H), 3.45 (s, 3H), 2.94 (m, 2H), 1.96 (d, J = 11.6 Hz, 4H), 1.81 (d, J = 13.2 Hz, 4H), 1.63 (d, J = 12.4 Hz, 2H), 1.09–1.48 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.3, 154.5, 153.6, 148.8, 147.6, 138.7, 128.9, 127.2, 124.5, 121.6, 109.2, 108.8, 102.6, 88.3, 83.9, 70.9, 70.4, 69.3, 68.3, 59.3, 59.2, 58.0, 35.8, 30.8, 26.1 ppm; IR (KBr): $\tilde{\nu}$ = 3451, 2930, 2853, 2332, 1624, 1578, 1508, 1437, 1251, 1124, 1032, 862 cm^{-1}; MS (ESI) *m/z* 587 ([M+H]$^+$); Anal. calcd for $C_{35}H_{46}N_4O_4$: C 71.64, H 7.90, N 9.55, found: C 71.51, H 7.78, N 9.57.

[6,7-Bis(2-methoxyethoxy)quinazolin-4-yl]-[4-(3-dicyclohexylaminoprop-1-ynyl)phenyl]amine (7b) was synthesized from **6b** (380 mg, 0.76 mmol) using the procedure described for **4b** to give **7b** (107 mg, 0.18 mmol, 24%) as a white solid; mp: 90–91 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.64 (s, 1H), 7.76 (bs, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.30 (s, 1H), 7.18 (s, 1H), 4.22 (qui, 4H), 3.79 (m, 4H), 3.72 (s, 2H), 3.42 (s, 6H), 2.86 (m, 2H), 1.92 (d, J = 11.2 Hz, 4H), 1.80 (d, J = 12.8 Hz, 4H), 1.63 (d, J = 12.4 Hz, 2H), 1.09–1.44 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.2, 154.4, 153.5, 148.7, 147.5, 138.3, 132.1, 121.3, 119.1, 109.3, 108.6, 102.6, 88.5, 83.4, 70.8, 70.3, 69.1, 68.2, 59.21, 59.16, 57.6, 35.7, 31.1, 26.2 ppm; IR (KBr): $\tilde{\nu}$ = 3568, 2928, 2853, 2365, 1616, 1570, 1427, 1383, 1215, 1128, 1029, 907, 862 cm^{-1}; MS (ESI) *m/z* 587 ([M+H]$^+$); Anal. calcd for $C_{35}H_{46}N_4O_4$: C 71.64, H 7.90, N 9.55, found: C 71.49, H 8.10, N 9.42.

(3-{3-[6,7-Bis(2-methoxyethoxy)quinazolin-4-yloxy]phenyl}prop-2-ynyl)dicyclohexylamine (7c) was synthesized from **6c** (285 mg, 0.58 mmol) using the procedure described for **4b** to give **7c** (297 mg, 0.51 mmol, 88%) as a white solid; mp: 84 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (s, 1H), 7.47 (s, 1H), 7.31 (t, J = 7.6 Hz, 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.22 (s, 1H), 7.20 (s, 1H), 7.10 (d, J = 7.6 Hz, 1H), 4.24 (m, 4H), 3.80 (m, 4H), 3.61 (s, 2H), 3.41 (s, 6H), 2.73 (m, 2H), 1.81 (d, J = 11.2 Hz, 4H), 1.69 (d, J = 12.0 Hz, 4H), 1.52 (d, J = 12.4 Hz, 2H), 0.98–1.31 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.0, 155.3, 152.7, 152.1, 149.5, 149.2, 129.3, 128.9, 125.4, 124.6, 121.4, 110.5, 107.6, 102.3, 90.4, 82.3, 70.5, 70.3, 68.6, 68.3, 59.2, 57.3, 35.5, 31.1, 26.1, 26.0 ppm; IR (KBr): $\tilde{\nu}$ = 3589, 2932, 2428, 1944, 1612, 1566, 1500, 1416, 1371, 1245, 1182, 1030, 912, 862 cm^{-1}; MS (ESI) *m/z* 588 ([M+H]$^+$); Anal. calcd for $C_{35}H_{45}N_3O_5$: C 71.52, H 7.72, N 7.15, found: C 71.73, H 7.89, N 7.34.

(3-{4-[6,7-Bis(2-methoxyethoxy)quinazolin-4-yloxy]phenyl}prop-2-ynyl)dicyclohexylamine (7d) was synthesized from **6d** (345 mg, 0.7 mmol) using the procedure described for **4b** to give **7d** (208 mg, 0.42 mmol, 60%) as a white solid; mp: 120 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (s, 1H), 7.56 (s, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.34 (s, 1H), 7.19 (d, J = 8.4 Hz, 2H), 4.33 (m, 4H), 3.89 (m, 4H), 3.73 (s, 2H), 3.50 (s, 6H), 2.86 (m, 2H), 1.94 (d, J = 9.2 Hz, 4H), 1.81 (d, J = 12.4 Hz, 4H), 1.64 (d, J = 12.8 Hz, 2H), 1.10–1.46 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.2, 155.6, 152.9, 152.0, 149.8, 149.4, 132.9, 122.0, 121.5, 110.7, 107.8, 102.5, 89.0, 83.1, 70.6, 70.5, 68.8, 68.6, 59.4, 57.6, 35.6, 31.0, 26.23, 26.20 ppm; IR (KBr): $\tilde{\nu}$ = 3398, 2934, 2856, 2559, 2363, 1616, 1570, 1427, 1375, 1215, 1126, 1028, 908, 862 cm^{-1}; MS (EI) *m/z* 587 [M^+]; Anal. calcd for $C_{35}H_{45}N_3O_5$: C 71.52, H 7.72, N 7.15, found: C 71.44, H 7.64, N 7.31.

(6,7-Dimethoxyquinazolin-4-yl)-(3-propa-1,2-dienylphenyl)amine (1a): A mixture of **4b** (150 mg, 0.3 mmol), $[Pd_2dba_3]\cdot CHCl_3$ (7.8 mg, 7.5 μmol), and $(C_6F_5)_2PCH_2CH_2P(C_6F_5)_2$ (25 mg, 30 μmol) was dissolved in $CHCl_3$ (2 mL) and stirred at 100 °C in a vial tube.[23] The reaction progress was monitored by TLC. After **4b** was consumed, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with hexane/EtOAc (1:2) to give **1a** (66.0 mg, 0.20 mmol, 66%) as a yellow solid; mp: 105–106 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.66 (s, 1H), 7.65 (bs, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.52 (s, 1H), 7.30 (t, J = 7.6 Hz,

8.0 Hz, 1H), 7.23 (s, 1H), 7.15 (s, 1H), 7.06 (d, J=8.0 Hz, 1H), 6.14 (t, J=6.8 Hz, 1H), 5.13 (d, J=6.8 Hz, 2H), 3.97 (s, 3H), 3.94 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=209.8, 156.5, 154.7, 153.6, 149.4, 147.4, 138.9, 134.9, 129.2, 122.8, 120.8, 120.1, 109.1, 107.7, 99.6, 93.8, 79.0, 56.2 ppm; IR (KBr): $\tilde{\nu}$=3275, 2930, 2835, 2365, 1942, 1654, 1578, 1503, 1423, 1394, 1339, 1245, 1215, 1142, 999, 851 cm^{-1}; MS (EI) m/z 319 [M^+]; Anal. calcd for $C_{19}H_{17}N_3O_2$: C 71.46, H 5.37, N 13.16, found: C 71.46, H 5.57, N 13.43.

(6,7-Dimethoxyquinazolin-4-yl)-(4-propa-1,2-dienylphenyl)amine (1b) was synthesized from **4c** (63 mg, 0.15 mmol) using the procedure described for **1a** to give **1b** (19 mg, 0.060 mmol, 39%) as a yellow solid; mp: 168–169 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.65 (s, 1H), 7.62 (d, J=8.8 Hz, 2H), 7.31 (d, J=8.8 Hz, 2H), 7.23 (s, 1H), 7.10 (s, 1H), 6.16 (t, J=6.8 Hz, 1H), 5.16 (d, J=6.8 Hz, 2H), 3.99 (s, 3H), 3.98 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=209.8, 154.7, 153.5, 149.4, 147.3, 137.2, 129.9, 127.2, 122.2, 109.0, 107.6, 99.5, 93.4, 78.9, 56.2 ppm; IR (KBr): $\tilde{\nu}$=3357, 2929, 2365, 1942, 1578, 1514, 1420, 1339, 1244, 1144, 995, 924, 854 cm^{-1}; MS (EI) m/z 319 [M^+]; Anal. calcd for $C_{19}H_{17}N_3O_2$: C 71.46, H 5.37, N 13.16, found: C 71.57, H 5.27, N 12.93.

6,7-Dimethoxy-4-(3-propa-1,2-dienylphenoxy)quinazoline (1c) was synthesized from **4d** (77 mg, 0.15 mmol) using the procedure described for **1a** to give **1c** (23 mg, 0.071 mmol, 46%) as a white solid; mp: 106–107 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.64 (s, 1H), 7.56 (s, 1H), 7.42 (t, J=7.6 Hz, 8.0 Hz, 1H), 7.33 (s, 1H), 7.23 (d, J=7.6 Hz, 1H), 7.20 (s, 1H), 7.10 (d, J=8.0 Hz, 1H), 6.20 (t, J=6.8 Hz, 1H), 5.16 (d, J=6.8 Hz, 2H), 4.07 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=210.0, 165.4, 155.8, 153.0, 152.9, 150.2, 149.4, 136.1, 129.8, 124.3, 120.4, 119.9, 110.7, 106.8, 101.0, 93.5, 79.2, 56.4, 56.3 ppm; IR (KBr): $\tilde{\nu}$=3415, 2930, 2835, 2368, 1942, 1618, 1572, 1502, 1420, 1379, 1241, 1209, 1134, 995, 851 cm^{-1}; MS (EI) m/z 320 [M^+]; Anal. calcd for $C_{19}H_{16}N_2O_3$: C 71.24, H 5.03, N 8.74, found: C 71.15, H 5.13, N 8.94.

6,7-Dimethoxy-4-(4-propa-1,2-dienylphenoxy)quinazoline (1d) was synthesized from **4d** (51 mg, 0.10 mmol) using the procedure described for **1a** to give **1c** (33 mg, 0.10 mmol, >99%) as a white solid; mp: 155–156 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.63 (s, 1H), 7.56 (s, 1H), 7.41 (d, J=8.4 Hz, 2H), 7.33 (s, 1H), 7.20 (d, J=8.4 Hz, 2H), 6.21 (t, J=6.8 Hz, 1H), 5.18 (d, J=6.8 Hz, 2H), 4.075 (s, 3H), 4.071 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=209.9, 165.5, 155.8, 153.0, 151.3, 150.2, 149.3, 131.7, 127.9, 122.1, 110.7, 106.8, 101.0, 93.3, 79.0, 56.38, 56.35 ppm; IR (KBr): $\tilde{\nu}$=2959, 2365, 1938, 1578, 1421, 1379, 1215, 1132, 995, 907, 854 cm^{-1}; MS (EI) m/z 320 [M^+]; Anal. calcd for $C_{19}H_{16}N_2O_3$: C 71.24, H 5.03, N 8.74, found: C 71.36, H 5.31, N 8.93.

[6,7-Bis-(2-methoxyethoxy)quinazolin-4-yl]-(3-propa-1,2-dienylphenyl)amine (1e) was synthesized from **7a** (74 mg, 0.13 mmol) using the procedure described for **1a** to give **1e** (25 mg, 0.062 mmol, 49%) as a yellow solid; mp: 83 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.64 (s, 1H), 7.61 (d, J=8.0 Hz, 1H), 7.57 (s, 1H), 7.33 (t, J=7.6 Hz, 8.0 H, 1H), 7.22 (s, 1H), 7.21 (s, 1H), 7.08 (d, J=7.6 Hz, 1H), 6.18 (t, J=6.8 Hz, 1H), 5.17 (d, J=6.8 Hz, 2H), 4.26 (q, J=3.6 Hz, 4H), 3.84 (q, J=4.8 Hz, 4H), 3.46 (s, 3H), 3.45 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=209.7, 156.6, 154.3, 153.6, 148.5, 147.2, 139.0, 134.7, 129.0, 122.5, 120.7, 120.1, 109.2, 108.4, 102.7, 93.8, 78.8, 70.7, 70.3, 68.9, 68.2, 59.09, 59.05 ppm; IR (KBr): $\tilde{\nu}$=3385, 2930, 2366, 1942, 1622, 1578, 1508, 1427, 1246, 1124, 1032, 862 cm^{-1}; MS (EI) m/z 407 [M^+]; Anal. calcd for $C_{23}H_{25}N_3O_4$: C 67.80, H 6.18, N 10.31, found: C 67.63, H 6.43, N 10.14.

[6,7-Bis-(2-methoxyethoxy)quinazolin-4-yl]-(4-propa-1,2-dienylphenyl)amine (1f) was synthesized from **7b** (82 mg, 0.14 mmol) using the procedure described for **1a** to give **1e** (29 mg, 0.070 mmol, 39%) as a yellow solid; mp: 62–63 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.63 (s, 1H), 7.73 (bs, 1H), 7.64 (d, J=8.4 Hz, 2H), 7.29 (d, J=8.4 Hz, 2H), 7.26 (s, 1H), 7.16 (s, 1H), 6.16 (t, J=6.8 Hz, 7.2 Hz, 1H), 5.15 (d, J=6.8 Hz, 2H), 4.19 (bs, 4H), 3.77 (dt, J=4.4 Hz, 13.6 Hz, 4H), 3.41 (s, 3H), 3.40 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=209.7, 156.4, 154.3, 153.7, 148.6, 147.4, 137.4, 129.6, 127.2, 122.1, 109.2, 108.6, 102.6, 93.5, 78.9, 70.8, 70.3, 69.0, 68.2, 59.21, 59.15 ppm; IR (KBr): $\tilde{\nu}$=3401, 2926, 2367, 1942, 1624, 1578, 1511, 1425, 1246, 1207, 1124, 1032, 860 cm^{-1}; MS (EI) m/z 407 [M^+]; Anal. calcd for $C_{23}H_{25}N_3O_4$: C 67.80, H 6.18, N 10.31, found: C 67.72, H 6.00, N 10.44.

6,7-Bis-(2-methoxyethoxy)-4-(3-propa-1,2-dienylphenoxy)quinazoline (1g) was synthesized from **7c** (110 mg, 0.19 mmol) using the procedure described for **1a** to give **1g** (43 mg, 0.10 mmol, 55%) as a white solid; mp: 82–83 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.62 (s, 1H), 7.57 (s, 1H), 7.41 (t, J=8.0 Hz, 1H), 7.32 (s, 1H), 7.23 (d, J=8.0 Hz, 1H), 7.20 (s, 1H), 7.09 (d, J=8.0 Hz, 1H), 6.19 (t, J=6.8 Hz, 1H), 5.16 (d, J=6.8 Hz, 2H), 4.33 (t, J=4.8 Hz, 3.6 Hz, 4H), 3.84 (q, J=6.0 Hz, 3.6 Hz, 4H), 3.51 (s, 3H), 3.50 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=210.0, 165.4, 155.5, 153.0, 152.9, 149.7, 149.2, 136.1, 129.8, 124.3, 120.4, 119.8, 110.7, 107.7, 102.7, 93.5, 79.2, 70.6, 70.4, 68.8, 68.5, 59.4 ppm; IR (KBr): $\tilde{\nu}$=3423, 2928, 2365, 1944, 1719, 1618, 1578, 1501, 1425, 1381, 1236, 1126, 1034, 930, 862 cm^{-1}; MS (ESI) m/z 409 ([M+H]$^+$); Anal. calcd for $C_{23}H_{24}N_2O_5$: C 67.63, H 5.92, N 6.86, found: C 67.36, H 5.84, N 6.63.

6,7-Bis(2-methoxyethoxy)-4-(4-propa-1,2-dienylphenoxy)quinazoline (1h) was synthesized from **7d** (120 mg, 0.21 mmol) using the procedure described for **1a** to give **1h** (52 mg, 0.13 mmol, 61%) as a white solid; mp: 128 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.61 (s, 1H), 7.57 (s, 1H), 7.40 (d, J=8.8 Hz, 2H), 7.31 (s, 1H), 7.20 (d, J=8.8 Hz, 2H), 6.21 (t, J=6.8 Hz, 1H), 5.17 (d, J=6.8 Hz, 2H), 4.33 (t, J=4.8 Hz, 4H), 3.91 (m, Hz, 4H), 3.50 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=209.9, 165.5, 155.5, 153.0, 151.3, 149.7, 149.3, 131.7, 127.9, 122.1, 110.7, 107.8, 102.7, 93.3, 79.0, 70.6, 70.4, 68.7, 68.5, 59.4 ppm; IR (KBr): $\tilde{\nu}$=3451, 2885, 2822, 2363, 1935, 1616, 1570, 1506, 1425, 1383, 1213, 1128, 1031, 907, 874 cm^{-1}; MS (EI) m/z 408 [M^+]; Anal. calcd for $C_{23}H_{24}N_2O_5$: C 67.63, H 5.92, N 6.86, found: C 67.54, H 5.69, N 6.60.

[6,7-Bis-(2-methoxyethoxy)quinazolin-4-yl]-(3-vinylphenyl)amine (8) was synthesized from 4-chloro-6,7-di(methoxyethoxy)quinazoline **5** (50 mg, 0.16 mmol) and 3-vinylaniline (19 mg, 0.16 mmol) using the procedure described for **3a** to give **8** (50 mg, 0.12 mmol, 79%) as a white solid; mp: 190 °C, ^{1}H NMR (400 MHz, CD_3OD): δ=8.17–8.14 (m, 2H), 7.90 (s, 1H), 7.74 (d, J=7.6 Hz, 1H), 7.28–7.19(m, 3H), 6.62 (dd, J=11.2, 10.8, 17.6 Hz, 1H), 5.73 (d, J=18.0, 1H), 5.24 (d, J=16.8, 1H), 4.57 (m, 2H), 4.06 (m, 2H), 3.82 (m, 2H), 3.71 (m, 2H),3.45 (s, 3H), 3.43 ppm (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ=156.5, 150.2, 146.7, 138.4, 137.0, 136.0, 128.9, 124.2, 123.2, 121.6, 115.0, 107.2, 105.1, 100.1, 76.6, 70.6, 70.1, 69.8, 69.1, 59.3, 59.2 ppm; IR (KBr): $\tilde{\nu}$=3364.7, 2931.6, 2823.6, 2362.5, 2343.4, 1633.5, 1569.9, 1512.1, 1409.9, 1363.6, 1278.7, 1234.4, 1218.9, 1199.5, 1128.3, 1072.3, 1029.3, 998.3, 896.8 cm^{-1}; HRMS (ESI) m/z 396.1929 [M+H$^+$], 418.1743 [M+Na$^+$].

[6,7-Bis-(2-methoxyethoxy)quinazolin-4-yl]-(3-ethylphenyl)amine (9). A solution of the [6,7-Bis-(2-methoxyethoxy)quinazolin-4-yl]-(3-vinylphenyl)amine (**8**) (40 mg, 0.1 mmol) in 5 mL EtOH was stirred with 10 mg 10% Pd-C under hydrogen overnight. The catalyst was filtered off, and the solvent was then removed by rotary evaporation to provide **9** (10 mg, 0.03 mmol, 30%) as a white solid; mp: 154 °C; ^{1}H NMR (400 MHz, CD_3OD): δ=8.64 (s, 1H), 7.54 (d, J=

7.6 Hz, 1H), 7.47 (s, 1H), 7.42–7.20(m, 3H), 6.99 (d, $J=7.6$ Hz, 1H), 4.23 (m, 4H), 3.80 (m, 4H), 3.44 (m, 4H), 2.67 (q, $J=7.6$ Hz, 2H), 1.25 ppm (t, $J=7.6$, 3H); ^{13}C NMR (75 MHz, CD_3OD): $\delta=156.5$, 154.5, 153.8, 148.7, 147.5, 145.2, 138.5, 128.9, 123.9, 121.9, 121.4, 119.4, 109.2, 108.8, 102.9, 70.9, 70.4, 69.2, 68.2, 59.2, 28.8, 15.4 ppm; IR (KBr): $\tilde{\nu}=3855.4$, 2931.6, 2827.4, 1622.0, 1579.6, 1533.3, 1508.2, 1446.5, 1394.4, 1244.0, 1207.4, 1124.4, 1031.8, 931.6, 862.1 cm^{-1}; HRMS (ESI) *m/z* 398.2080 [M+H$^+$], 420.1899 [M+Na$^+$].

Kinase assays: The kinase activity of various tyrosine kinases was determined by ELISA.[29] EIA/RIA strip-well plates (Corning) were coated with poly(Glu/Tyr, 4:1) peptide (Sigma, 50 μg mL^{-1}, 100 μL per well) by incubation overnight at 4 °C in phosphate-buffered saline (PBS). Kinase reactions were performed in the plates by the addition of 50 μL kinase buffer (50 mM HEPES, 125 mM NaCl, 10 mM $MgCl_2$, pH 7.4) containing ATP, 10 ng recombinant EGFR, HER2, Flt-1, or KDR (Invitrogen, catalytic domain), and inhibitors. After 20 min, the plates were washed three times with wash buffer (0.1% Tween 20 in PBS) and incubated for 20 min with 50 μL per well of horseradish peroxidase (HRP)-conjugated anti-phosphotyrosine antibody (0.2 μg mL^{-1}, Santa Cruz). After two washes, the plates were developed by the addition of tetramethylbenzidine (50 μL per well, Sigma) and stopped by addition of H_2SO_4 (2 N, 50 μL per well). The absorbance at 450 nm was measured by a 96-well plate reader (Tecan).

Cell growth assays: The human cancer cell lines A431 (epidermoid carcinoma), SKBR3 (breast cancer), KatoIII (gastric cancer), HepG2 (liver cancer), HCT115 (colon cancer), and SW480 (colon cancer) were used for the cell growth assays. Each cell line (5×10^3 cells per well of a 96-well plate) was incubated at 37 °C for 72 h in 100 μL RPMI-1640 medium containing various concentrations of the allenic quinazolines. After incubation, MTT (10 μL, 5 mg mL^{-1} in PBS; MTT = 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) was added to each well, and the cells were further incubated at 37 °C for 4 h. After removal of the medium, DMSO (100 μL) was added, and the absorbance at 570 nm was determined. The drug concentration required to inhibit cell growth by 50% (GI_{50}) was determined from semi-logarithmic dose–response plots.

Immunoblot analysis: After stimulation for the specified period, the cells were washed three times with PBS, dipped in 100 μL ice-cold lysis buffer (20 mM HEPES, pH 7.4, 1% Triton X-100, 10% glycerol, 1 mM sodium vanadate, 5 μg mL^{-1} leupeptin, and 1 mM EDTA) for 15 min, and disrupted with a Handy Sonic Disrupter, and the lysate was boiled for 5 min in a sample buffer (50 mM Tris, pH 7.4, 4% sodium dodecyl sulfate (SDS), 10% glycerol, 4% 2-thioethanol, and 0.05 mg mL^{-1} bromophenol blue) at a ratio of 4:1. The proteins were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred onto a PVDF membrane (Millipore). Antibodies used for immunoblotting were phospho-EGFR (Tyr1174) antibody (Santa Cruz), EGFR antibody (Santa Cruz), phospho-Akt (Ser473) antibody (Cell Signaling), phospho-p44/42 MAP kinase (Tyr204) antibody (Santa Cruz) and α-tubulin (Santa Cruz). After incubation with HRP-conjugated secondary antibody (Santa Cruz), the blot was treated with ECL kit (Promega), and the levels of each protein were visualized by a ChemiDoc XRS image analyzer (Bio-Rad).

Cell-cycle analysis: After incubation of A431 cells with the drugs, the cells were washed with PBS and fixed with 70% EtOH for 2 h at 4 °C. The cells were incubated for 30 min at 37 °C in 1 mL RNase solution (0.25 mg mL^{-1} in PBS), and further incubated for 30 min at 4 °C in a propidium iodide (PI) staining solution (0.05 mg mL^{-1} in PBS). The suspension was then passed through a nylon mesh filter (40 μm) and analyzed by a Cytomics FC500 flow cytometer (Beckman Coulter).

Detection of apoptosis: Phosphatidylserine externalization was measured using an annexin V–FITC kit (Beckman Coulter) according to the manufacturer's instructions. After incubation of A431 cells with the drugs, the cells were suspended in 100 μL 1× binding buffer, and 5 μL annexin V–FITC (5 μg mL^{-1}) and 2.5 μL PI (250 μg mL^{-1} in PBS) were added. The cell suspensions were incubated for 10 min at 4 °C and analyzed by a flow cytometer.

Molecular docking simulations: Docking simulations were performed using the X-ray crystallographic structure of the EGFR kinase domain in complex with Tarceva (PDB code: 1M17).[27] The Tarceva binding site in the EGFR kinase domain was for docking of allenic quinazolines **1e** and **1g** with the DS-Modeling program (v. 1.7, Accelrys, San Diego, CA, USA). The "rank-by-rank" strategy was adopted to perform consensus scorings because the results given by the scoring functions (LigScore2, PLP, PMF, and LUDI) have different units. Each scoring function involved in a given consensus-scoring scheme was applied to rank the conformations of the ligand. The final rank of a certain conformation was its average rank received from all the scoring functions.[33]

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