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Synthesis and structure–activity relationship of 4-(2-aryl-cyclopropylamino)-quinoline-3-carbonitriles as EGFR tyrosine kinase inhibitors

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Abstract—Synthesis and structure–activity relationship of a series of 4-(2-aryl-cyclopropylamino)-quinoline-3-carbonitrile derivatives as EGFR inhibitors is described. Compounds **29** and **30** showed potent in vitro inhibitory activity in the enzymatic assay as well as in the functional cellular assay. They are moderately selective against other types of tyrosine kinases.

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein that belongs to the erbB family of closely related cell membrane receptors that includes EGFR (erbB-1 or HER1), erbB-2 (HER2), erbB-3 (HER3), and erbB-4 (HER4). It is a 170 kDa protein composed of three major functional domains: an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain. Binding of ligands to EGFR leads to autophosphorylation of the receptor tyrosine kinase and subsequent activation of signal transduction pathways. EGFR plays an important role in initiating the signaling that directs the behavior of epithelial cells and tumors of epithelial cell origin.[1] EGFR is highly expressed in many human cancers (e.g., bladder, cervical, head and neck, and ovarian) and has been found to be associated with poor prognosis and correlated with decreased survival. EGFR is overexpressed in 40%–80% of non-small-cell lung cancers (NSCLC), depending on histology.[2a] Its pivotal role in governing cellular proliferation, survival, and metastasis makes EGFR an attractive molecular target, especially for the treatment of solid tumors.[3]

Small molecule EGFR inhibitors have been shown to be effective antitumor agents. Iressa (**1**) and Tarceva (**2**), two closely related quinazoline-based EGFR inhibitors, have efficacy against several types of cancers in human clinical trials and were approved for the treatment of NSCLC and colon cancers. Irreversible EGFR inhibitors such as EKB569 (**3**) and a number of small molecule EGFR inhibitors are also undergoing clinical trials.[2b] In addition, several antibodies (e.g., cetuximab, panitumumab, matuzumab, and nimotuzumab that bind to the extracellular domain of the EGFR and antisense oligonucleotides against EGFR receptors are either approved or undergoing clinical trials for the treatment of cancers. Most EGFR inhibitors such as **1**–**3** possess an anilinyl group at the C-4 position of the quinazoline or quinoline-3-carbonitrile core structures. Herein, we describe the synthesis and structure–activity relationship of a new series of quinoline-3-carbonitrile derivatives (**4**) as potent EGFR inhibitors. These compounds possess an arylcyclopropylamino group at the C-4 position of the quinoline-3-carbonitrile core structure. Some of the compounds showed excellent EGFR inhibitory activity both in enzymatic and cell-based assays (Fig. 1).

6,7-Dialkoxyquinoline-3-carbonitrile derivatives **7** were prepared as shown in Scheme 1. Thus, 4-chloro-7-hydroxy-6-methoxy-quinoline-3-carbonitrile (**5**)[4] underwent Mitsunobu reaction with a series of substituted

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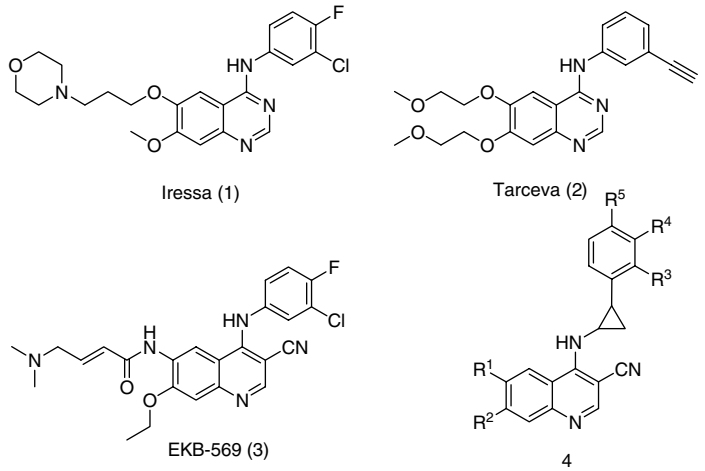
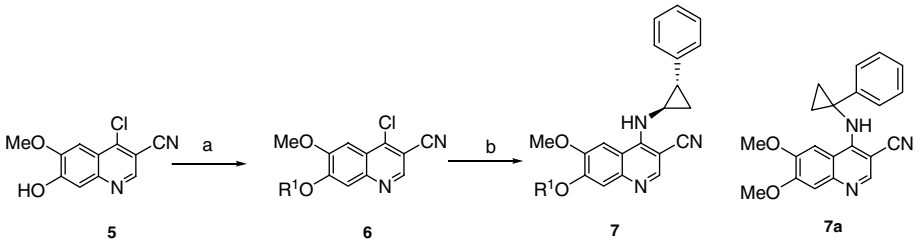
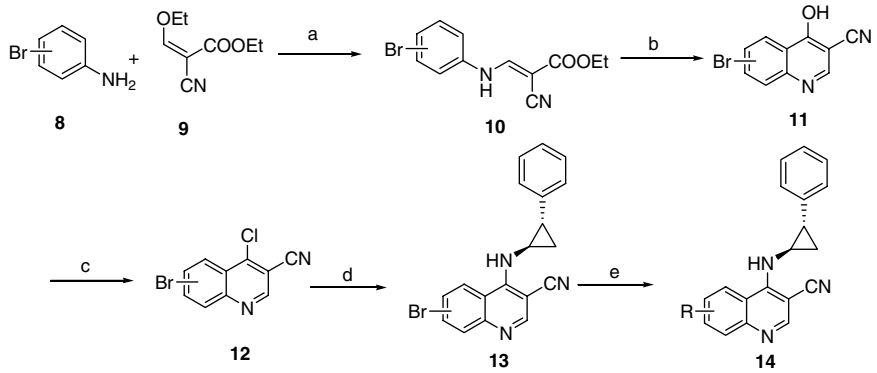


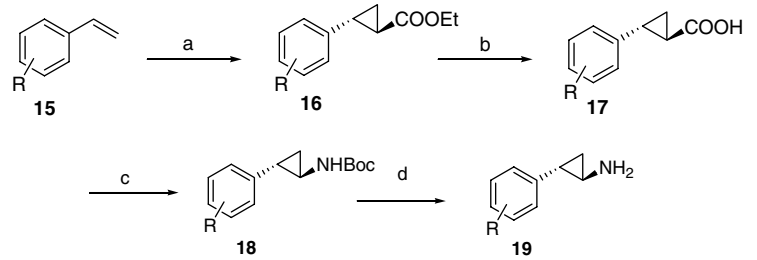
Figure 1. EGFR inhibitors.



Scheme 1. Reagents and conditions: (a) R^1OH, PPh_3P, DIAD DCM, 0 °C, rt; (b) (±)-2-*trans*-phenylcyclopropyl amine, 2-ethoxyethanol, pyridine hydrochloride, reflux, 3 h.



Scheme 2. Reagents and conditions: (a) Toluene, reflux, 2 h; (b) Dowtherm A, reflux, 10 h; (c) Oxalyl chloride, DCM, rt, 48 h; (d) (±)-2-*trans*-phenylcyclopropylamine, 2-ethoxyethanol, pyridine hydrochloride, reflux, 4 h; (d) $RB(OH)_2$, $Pd(PPh_3)_4$, aq K_2CO_3; DME, reflux 12 h.

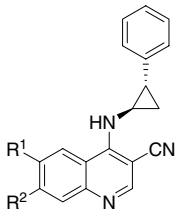


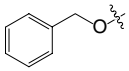
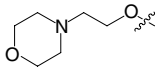
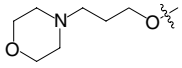
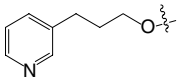
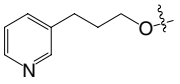
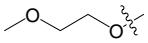
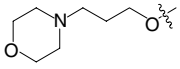
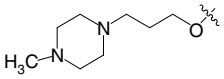
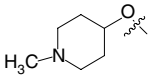
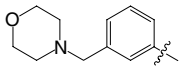
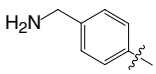
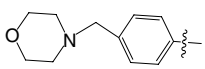
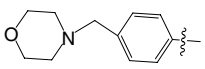
Scheme 3. Reagents and conditions: (a) Ethyl diazoacetate, Cuacac, 40 °C, 12 h; (b) 5 N NaOH, dioxane, reflux, 1 h; (c) DPPA, Et_3N, cyclohexane, *t*-BuOH, 70 °C, 18 h, $(Boc)_2O$, 3 h; (d) 1 N HCl in ether, 6 h.

alcohols to give the 6-methoxy-7-alkoxy-4-chloroquinoline-3-carbonitrile intermediate (**6**). Amination of **6** with *trans*-2-phenylcyclopropylamine furnished the desired 4-(2-phenylcyclopropylamino)-6,7-dialkoxyquinoline-3-carbonitrile derivatives (**7**). The similar sequence of synthetic steps yielded 6-alkoxy-7-methoxy analogs from the corresponding 4-chloro-6-hydroxy-7-methoxyquinoline-3-carbonitrile precursor. Reaction of 1-phenylcyclopropylamine with 4-chloro-6,7-dimethoxy-quinoline-3-carbonitrile gave compound **7a** in good yield.

Analogs with substitutions at either the C-6 or C-7 position were synthesized as shown in Scheme 2. Reaction of 3- or 4-bromoaniline (**8**) with 2-cyano-3-ethoxy-acrylic acid ethyl ester (**9**) provided the corresponding 2-cyano-3-(phenylamino)acrylate derivatives (**10**), which gave

Table 1. 4-(2-Phenylcyclopropylamino)-6- or 7-alkoxyquinoline-3-carbonitriles[a]



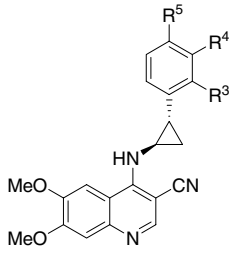
Compound	R^1	R^2	K_i (nM)	IC_{50} (cell, nM)
20	OMe	OMe	24	90
21	H	OMe	180	2580
22	OMe	H	360	8270
23		OMe	120	1610
24		OMe	64	405
25		OMe	52	na
26		OMe	68	68
27	OMe		220	173
28	OMe		60	52.8
29	OMe		8.4	5
30	OMe		3	5.7
31	OMe		360	360
32		H	340	5260
33		H	40	2100
34		H	400	2530
35	H		80	234

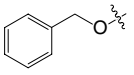
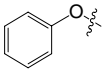
[a] K_i value was measured based on an 11-point curve, performed in duplicate. IC_{50} value was an average of two or more independent experiments.[6,7]

rise to 4-hydroxyl-quinoline-3-carbonitriles under thermal cyclization (Dowtherm) condition.[4] Chlorination with oxalyl chloride followed by amination furnished brominated intermediates (**13**). Finally, Suzuki coupling with appropriate boronic acids from commercial sources provided the desired 6- or 7-substituted products (**14**, **32**–**35**).

Scheme 3 depicts the synthesis of substituted *trans*-phenylcyclopropylamino derivatives.[5] Thus, substituted vinyl benzenes (**15**) were converted into *trans*-2-phenylcycloproanecarboxylic acid esters (**16**). Hydrolysis followed by Curtius rearrangement gave rise to the Boc protected amines (**17**), which were deprotected to give the desired substituted (±)-2-*trans*-phenylcyclopropylamines (**19**). Coupling of these amines with 4-chloro-3-cyanoquinolines provided (±)-2-*trans*-phenylcyclopropylamino derivatives.

Table 2. Substituted 4-(2-phenylcyclopropylamino)-6, 7-dimethoxyquinoline-3-carbonitriles[a]



Compound	R^3	R^4	R^5	K_i (nM)
36	H	H	Cl	44
37	H	OMe	OMe	n.a.
38	H	OMe		n.a.
39	H	H		80
40	H	H	Br	220
41	H	H	OMe	n.a
42	H	Br	H	52
43	F	H	H	36

[a] K_i value was measured based on an 11-point curve, performed in duplicate.

The compounds prepared as above were tested in enzymatic assay using fluorescent polarization.[6] Selected compounds were further profiled on their ability to inhibit the EGF-stimulated autophosphorylation at Y1068 in A431 cells.[7] The potency of 4-(2-phenylcyclopropylamino)-6,7-dialkoxyquinoline-3-carbonitriles is similar to that of 4-anilino-6,7-dialkoxyquinoline-3-carbonitriles[3] or quinazoline-based EGFR inhibitors.[4] For example, 6,7-dimethoxy-4-(2-phenylcyclopropylamino)-quinoline-3-carbonitrile (**20**) showed a K_i at 24 nM, which is comparable to 4-(3-methylanilino)-6,7-dimethoxyquinoline-3-carbonitrile (K_i: 44 nM, data not shown).[4] However, the closely related analog **7a** is inactive, indicating the orientation of aryl group at C-4 position plays a key role for the activity.

The effects of substitutions at C-6 and C-7 positions were first investigated. Removal of one of the two methoxy groups at 6 or 7 positions (**21** and **22**) led to a dramatic reduction of kinase inhibitory activity and significant loss of cellular activities indicating that both the alkoxy groups at 6 and 7 positions are required for potency. With this result, we investigated other alkoxy groups to find a replacement of the methoxy group that would improve other properties such as cellular activity while retaining the potency in the enzymatic assay. Replacement of 6-methoxy with a benzyloxy (**23**) resulted in a fivefold decrease in activity. Other basic nitrogen-containing side chains, notably 2-(1-morpholinyl) ethoxy (**24**), 3-(1-morpholinyl) propyloxy (**25**) or 3-(3-pyridinyl) propyloxy (**26**), at C-6 position retained the in vitro potency. The same side chains tended to increasethe activity when placed at C-7 position as compared to the C-6 position. For example, 3-(1-morpholinyl) propyloxy at C-7 position (**29**) dramatically increased potency with K_i of 8.4 nM and showed excellent cellular activity with IC_{50} at ~5 nM. 4-[(*trans*-2-Phenylcyclopropyl)amino]-6-methoxy-7-[3-(4-methylpiperazinyl) propoxy]quinoline-3-carbonitrile (**30**) was the most

Table 3. Selectivity profile for selected compounds[a]

Compound	EGFR K_i (μM)	ABL K_i (μM)	SYK K_i (μM)	Flt-1 K_i (μM)	KDR K_i (μM)	SRC K_i (μM)
20	0.024	0.95	0.25	1.6	2.1	0.15
21	0.18	>10	>10	>10	>10	0.17
22	0.36	>10	>10	>10	>10	0.21
23	0.12	>10	>10	>10	2.6	0.33
24	0.064	>10	>10	>10	0.15	>10
25	0.052	1.8	>10	0.91	0.56	>10
26	0.068	1.8	>10	1.71	0.65	>10
27	0.22	0.3	0.14	1.9	0.099	0.049
28	0.06	0.39	0.16	2.9	0.14	0.11
29	0.008	0.2	>10	0.35	0.42	0.045
30	0.003	0.044	0.041	0.11	0.3	0.027
31	0.36	>10	>10	1.5	>10	>10
32	0.34	>10	>10	>10	>10	0.28
33	0.04	0.77	0.45	1.4	1.5	0.086
34	0.4	>10	>10	>10	>10	0.57
35	0.08	0.3	0.3	0.92	0.17	0.14

[a] K_i value was measured based on an 11-point curve, performed in duplicate at ATP concentrations equal to respective K_m value of the enzymes.

potent EGFR inhibitor in this chemical class. It is interesting to note that compounds **32**–**35** that contain no alkoxy groups at C-6 or C-7 positions showed reasonable in vitro activity but there was a >50-times shift in cellular activity, especially for those compounds with C-6 substitutions (Table 1).

Effect of substituents on the phenyl ring of the phenylcyclopropylamine moiety on the activity was explored next and is summarized in Table 2. 6,7-Dimethoxyquinoline-3-carbonitrile was chosen as the core structure for exploration due to its ease of synthesis. Substitutions at C-5′ position (R^5), except halogens, are less tolerated especially when the C-4′ position was simultaneously substituted (cf. **37**, **38**, and **41**). Halogen atoms fluorine, chlorine, and bromine are well tolerated in all three positions (**36**, **40**, **42**, and **43**).

In conclusion, a series of 4-(2-phenylcyclopropylamino)-quinoline-3-carbonitriles were prepared and their activity as EGFR inhibitors was determined. Alkoxy substituents at both C-6 and C-7 positions enhanced the activity. Compounds with basic amine-containing groups at C-7 position showed single digit nM potency in the cellular phosphorylation assay. Most reported EGFR inhibitors with quinazoline or quinoline-3-carbonitrile core structures contain a substituted aniline moiety at the C-4 position. We have shown that a phenylcyclopropylamine moiety could be an excellent surrogate for the aniline moiety, leading to the identification of current compound series. All these compounds showed moderate to excellent selectivity over other kinases listed in Table 3.

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6. The protein tyrosine kinase activity of EGFR was monitored by measuring the initial velocity of turnover of a peptide substrate $pp60^{v\text{-}src}$ (Biosource). Fluorescence polarization (FP), in which the size and shape of a fluorescent molecule is determined by the ratio of fluorescence intensity from parallel and polarized light (anisotropy), was used to evaluate the phosphorylation of $pp60^{v\text{-}src}$ (phospho-$pp60^{v\text{-}src}$) (Turek et al., (2001) *Analytical Biochemistry* 299, 45–53). The anisotropic change was recorded continuously for 20 minutes to monitor the progression of an EGFR reaction. Since the anisotropy change does not linearly correspond to the concentration of the phosphorylated $pp60^{v\text{-}src}$ (phospho-$pp60^{v\text{-}src}$), an authentic reaction product peptide, phospho-$pp60^{v\text{-}src}$ (Biosource), was used to convert the time-courses of anisotropy changes to the time-courses of EGFR reactions. The initial velocities of EGFR reactions were obtained by linear regressional fitting of the converted reaction time-courses. All the assays were performed in 96-well microtiter plates (LJL, HE, black 96-well plates) using recombinant human EGFR (Invitrogen). In a typical 96-well plate assay, the decrease in the anisotropy (λ_{ex} = 485 nm, λ_{em} = 530 nm) (increase in the concentration of phospho-$pp60^{v\text{-}src}$) of a 12 μL assay solution in each well was monitored continuously using an Analyst HT or GT multi-well plate reader (Molecular Devices). Each 12 μL assay solution contained 10 mM Hepes·Na^+ (pH 7.5), 2.5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.5 mM DTT, 1 nM Invitrogen EGFR, 1X antibody/tracer detection solution (Panvera/Invitrogen), 10 μM ATP, 5 μM $pp60^{v\text{-}src}$ peptide, 1% DMSO, and 0.0098–10 μM testing compound. Assays were initiated with the addition of the ATP substrate. Data were fitted to the Dixon competitive inhibition equation using Grafit 5.0 (Erithacus Software).
7. Cell-based EGFR autophosphorylation (Y1068) was performed with A431 cells in a 96-well format. Briefly, A431 cells (60,000 per well) were incubated at 37 °C overnight in 200 μL of Dulbecco's Modified Eagle's Medium (DMEM, GIBCOBRL, Cat#11995-073) containing 10% FBS. After removal of the medium, EGFR inhibitor in 100 μL DMEM was added and incubated for 60 min at 37 °C. Human EGF (40 ng/well) in DMEM was added and incubated for additional 20 min. Routine wash and 60 μL PIPA-2 buffer were added to lyse the cells. After centrifugation, the supernatant of the cell lysates was detected with ELISA kit for pY1068 according to the manufacturer's instruction (Biosource).