Chapter 18
Synthesis of Heterocycle-Appended 4-Aminoquinazolines with Antiproliferative Properties and Potential to Inhibit Tyrosine Kinases

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Abstract The 4-anilinoquinazoline derivatives have established themselves as inhibitors of epidermal growth factor receptor (EGFR) tyrosine kinase phosphorylation. Molecular hybridization to replace the aniline moiety with a heterocyclic scaffold has been found to lead to heterocycle-appended quinazolines with enhanced biological properties and reduced side effects. We have also merged 7-amino-2-arylindole and 7-amino-2-arylbenzofuran moieties with the 2-aryl-4-chloroquinazolines to afford indole- and benzofuran-appended 4-aminoquinazoline analogues of gefitinib. The prepared molecular hybrids were evaluated for antigrowth effect against a panel of EGFR-positive cell lines, such as the human lung cancer (A549), epithelial colorectal adenocarcinoma (Caco-2), hepatocellular carcinoma (C3A), breast adenocarcinoma (MCF-7) and cervical cancer (HeLa) cell lines. These compounds were also evaluated for their ability to inhibit EGFR tyrosine kinase phosphorylation complemented with molecular docking into the adenosine triphosphate (ATP) binding site.

18.1 Introduction

Nitrogen-containing small heterocycles such as quinazolines and indoles continue to attract considerable attention in targeted therapies as antitumor drugs. The 4-anilinoquinazolines shown in Fig. 18.1, for example, have been found to produce anticancer activity through inhibition of the epidermal growth factor receptor tyrosine

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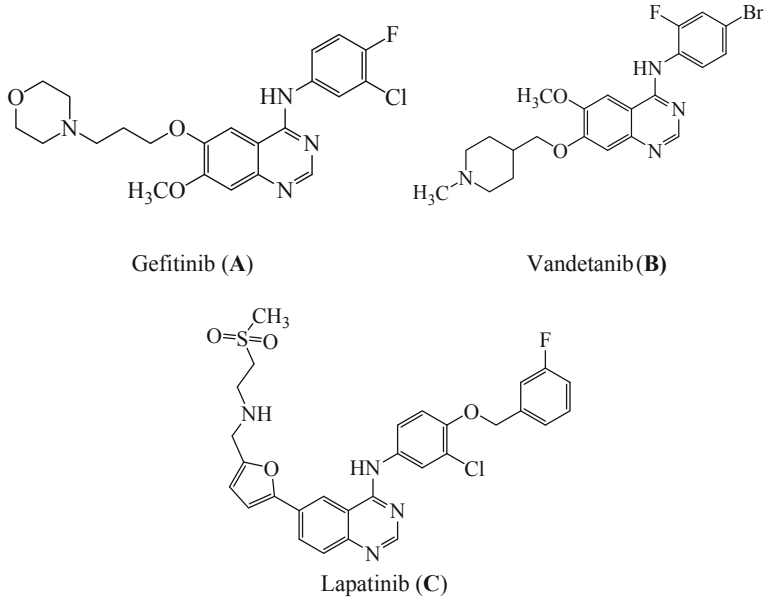


Fig. 18.1 Examples of the medicinally important 4-anilinoquinazolines with anticancer properties

kinase (EGFR-TK) phosphorylation, which results from competitive binding at the ATP site [1–3]. This receptor is overexpressed in various types of tumours including colon, non-small cell lung, prostate, breast and ovarian cancers [4, 5]. The EGFR continues to be an attractive target for the design and development of compounds that can specifically bind to it and inhibit its tyrosine kinase (TK) activity and its signal transduction pathway in cancer cells [6]. Gefitinib (**A**) shown in Fig. 18.1, is an example of a poly-substituted 4-anilinoquinazoline drug used for the treatment of non-small-cell lung cancer (NSCLC) with sensitive mutations of the EGFR [7]. Vandetanib (**B**), on the other hand, has been found to be a selective inhibitor that targets the vascular endothelial growth factor receptor (VEGFR) and the EGFR signal transduction pathways for the treatment of breast cancer [8]. Lapatinib (**C**) also shown in Fig. 18.1 is a 6-heteroaryl substituted 4-anilinoquinazoline derivative, which is an oral dual TK inhibitor that targets both EGFR and HER2 to inhibit the proliferation of breast cancer cells [9].

Most of the EGFR-TK inhibitors have a common 4-aminoquinazoline core and only differ in terms of the substituents and side chains. Structure–activity relationship studies investigating the ability of the 4-anilinoquinazolines to inhibit EGFR-TK activity revealed that both of the quinazoline nitrogen atoms are essential for anticancer activity [10]. Any nitrogen substitution in the ring system of a quinazoline resulted in decreased biological activity. Replacement of the quinazoline ring with a quinoline moiety, for example, resulted in 200-fold less affinity for EGFR [10]. The aniline moiety bearing lipophilic substituents such as chloro, bromo and tri-

fluoromethyl group was also important as it occupies the lipophilic pocket [11]. Drug resistance is the major problem in almost half of the small molecules that were developed for reversibly inhibiting EGFR tyrosine kinase [12]. This resulted when the amino acid threonine 790 was substituted by methionine (T790M) in the ATP binding pocket of EGFR causing resistance in inhibitors such as gefitinib and erlotinib [13].

In the design of new drugs, hybridization approach to merge two or more pharmacophores has been found to provide a more general method to obtain compounds with improved biological activities capable of overcoming multi-drug resistance. Cediranib/Recentin™ (**D**) shown in Fig. 18.2 is an example of an indole-ether quinazoline hybrid, which is a highly potent, orally bioavailable and selective vascular endothelial growth factor receptor (VEGFR) inhibitor for the treatment of cancer [14, 15]. 4-(1-Benzyl-1*H*-indol-3-yl)-6,7-dimethoxyquinazoline (**E**) represented in Fig. 18.2, on the other hand, is an example of an indole-quinazoline hybrid that was found to exhibit moderate ErbB-2 activity with little or no activity against EGFR [16]. The analogous 4-(indole-3-yl)quinazolines were found to be highly potent EGFR-TK inhibitors with excellent cytotoxic properties against several cancer cell lines [17]. The N^4-(1*H*-indol-5-yl)quinazoline-4,6-diamines (**F**), on the other hand, were found to exhibit significant anti-inflammatory properties and dose-dependent lipopolysaccharide-induced TNF-α and IL-6 release [18].

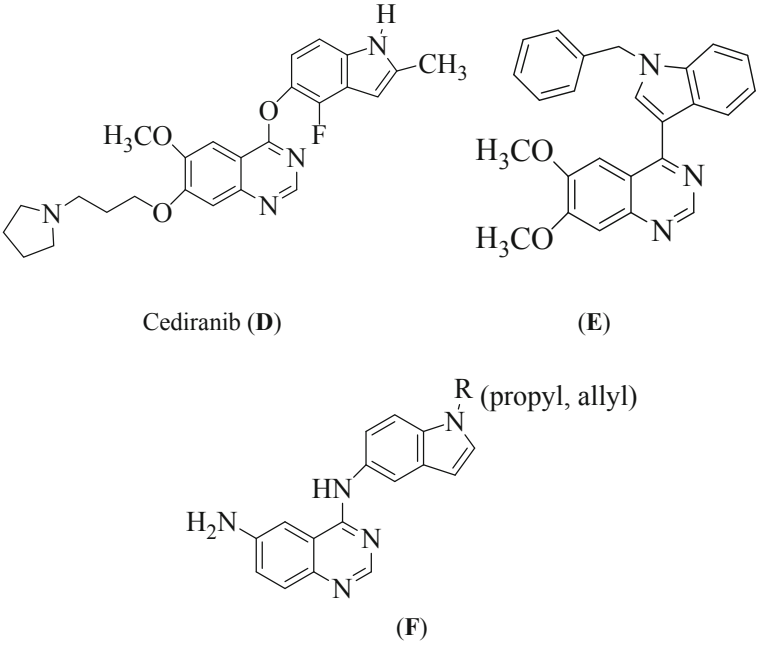


Fig. 18.2 Examples of indole-appended quinazoline hybrids of biological importance

Literature search revealed several methods for merging the quinazoline moiety with other scaffolds to form molecular hybrids with enhanced biological properties. These methods are discussed below.

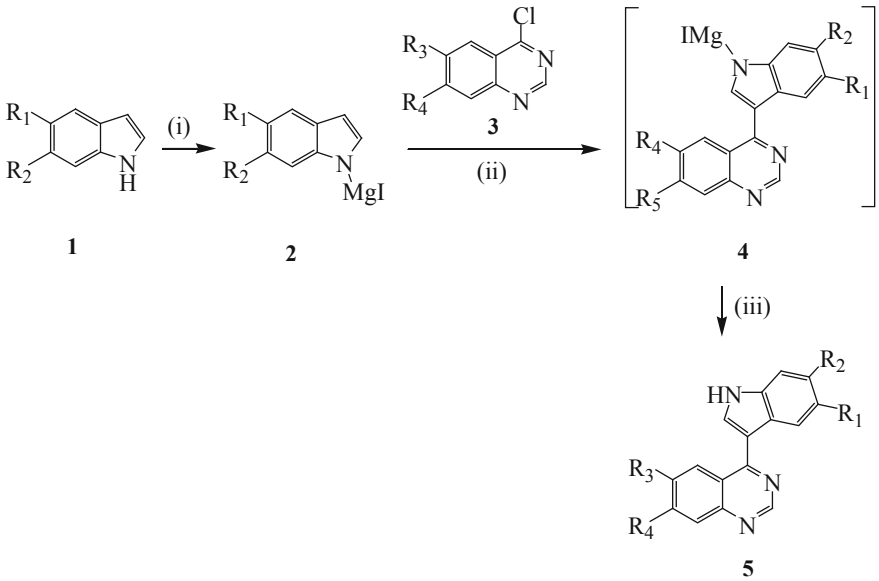
18.2 Methods for Preparation of Indole-Quinazoline Hybrids

The 4-(indole-3-yl)quinazolines **5** shown in Scheme 18.1 were previously prepared via the reaction of indolyl-magnesium derivatives **2** with 4-chloroquinazolines **3** in diethyl ether under reflux followed by hydrolysis of the intermediates **4** [17]. The indole-quinazoline hybrids **5b–f** were found to be potent inhibitors of EGFR-TK activity with IC_{50} values ranging from 131 to 533 nM [16]. In addition, compounds **5c** and **5f** were also found to inhibit human epidermal growth factor receptor 2-tyrosine kinase (HER-2-TK) at 100 nM.

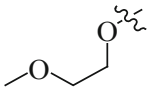
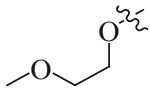
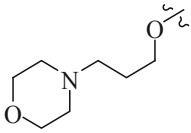
Aluminium chloride-catalyzed cross-coupling of indoles **6** with 2,4-dichloroquinazoline **7** in dry dichloroethane under nitrogen atmosphere at 75–80 °C previously afforded 2-chloro-4-(3-indolyl)quinazolines **8** (R = H, $–CH_3$) (Scheme 18.2) [19]. Compounds **8** were further reacted with cyclic amines to form 2-amino-4-(3-indolyl)quinazolines, which were found to exhibit anti-leishmanial and/or anti-proliferative activities against the prostate carcinoma (DU145), breast adenocarcinoma (MCF-7), oral epidermal carcinoma (KB) and cervical carcinoma (C33A) [19].

Indium(III) chloride ($InCl_3$)-catalyzed hybridization of indole **9** with 4-chloroquinazoline **10a** (R = H, R′ = N, R″ = Ph) or **10b** (R = Cl, R′ = CH, R″ = H) in acetonitrile under microwave irradiation at 150 W and 120 °C afforded the 4-(1*H*-indol-3-yl)-2-phenylquinazoline **11a** (R = H, R′ = N, R″ = Ph) in 78% yield or its analogous 7-chloro-4-(1*H*-indol-3-yl)quinolone **11b** (R = Cl, R′ = CH, R″ = H) in 89% yield (Scheme 18.3) [20]. The use of phenol as a catalyst in the reaction of **9** with **10b** afforded **11b** as a major product along with a 4-phenoxyquinoline. Furthermore, **11b** was obtained in 82, 83, and 84% yields when boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$), indium(III) trifluoromethanesulfonate or gallium trichloride were used as catalysts, respectively. However, the use of ytterbium(III) trifluoromethanesulfonate or cerium ammonium nitrate as catalysts led to the recovery of the starting material.

N′-(2-Cyano-4-nitro-phenyl)-*N,N*-dimethylformamidine **13** was reacted with 5-aminoindoles **14** in acetic acid under reflux to afford (6-nitro-quinazolin-4-yl)-(1*H*-indol-5-yl)-amines **15** (R = $–CH_3$, propyl, allyl) (Scheme 18.4) [17]. Compound **13** used as precursor was, in turn, prepared via the condensation of 2-amino-5-nitrobenzonitrile **12** with dimethylformamide dimethyl acetal in toluene under reflux (Scheme 18.4). Compounds **15** were further reduced to their corresponding N^4-(1*H*-indol-5-yl)-quinazoline-4,6-diamine derivatives which were also found to exhibit



1 2 4 5

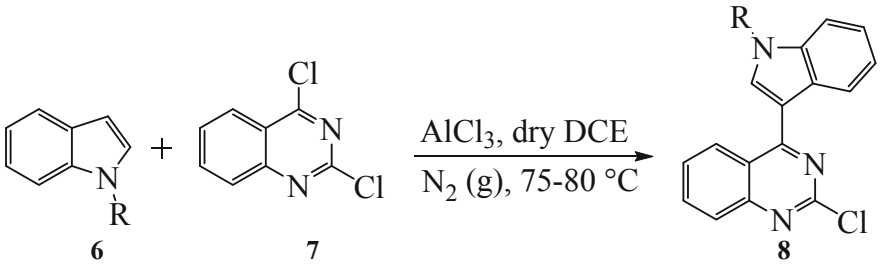
5	R_1	R_2	R_3	R_4	%Yield
a	H	H	H	H	45
b	Br	H	$-OCH_3$	$-OCH_3$	14
c	Cl	F	$-OCH_3$	$-OCH_3$	13
d	$-CH_3$	F	$-OCH_3$	$-OCH_3$	23
e	Cl	F			10
f	Cl	F		$-OCH_3$	21

Reagents and conditions: (i) Mg, I_2, CH_3I, Et_2O, 5 °C; (ii) Et_2O, reflux; (iii) H_2O

Scheme 18.1 Hybridization of **2** with **3** to afford **5**

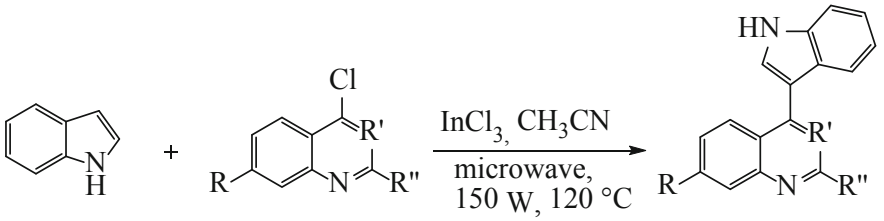
anti-inflammatory properties against lipopolysaccharide-induced TNF-α and IL-6 expression [17].

We considered our previous work on the antiproliferative properties of the 2-arylindoles [21] and the 4-anilinoquinazolines [22, 23] in combination with the literature analyses on bioactive compounds containing these moieties and decided to merge the 4-aminoquinazoline moiety with the indole and benzofuran rings, respectively.



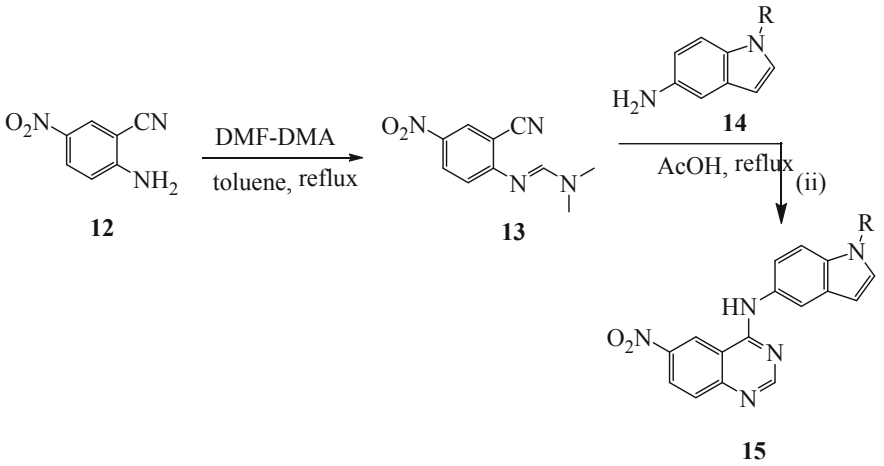
6 7 8

Scheme 18.2 Cross-coupling of **6** with **7** to afford **8**



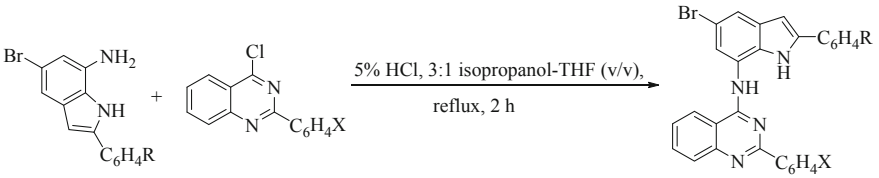
9 **10a**,**b** **11a**,**b**

Scheme 18.3 Hybridization of **9** with **10** to afford **11**



12 **13** **15**

Scheme 18.4 Preparation of **15** from reaction of **13** with **14**



16a–d **17a:** X = H **18a–d**

17b: X = 4-F **18e–h**

18	R	%Yield of **18**
a	H	81
b	4-F	79
c	3-Cl	75
d	4-OMe	83
e	H	78
f	4-F	80
g	3-Cl	78
h	4-OMe	67

Scheme 18.5 Amination of **17** with **16** to afford indole-quinazoline hybrids **18**

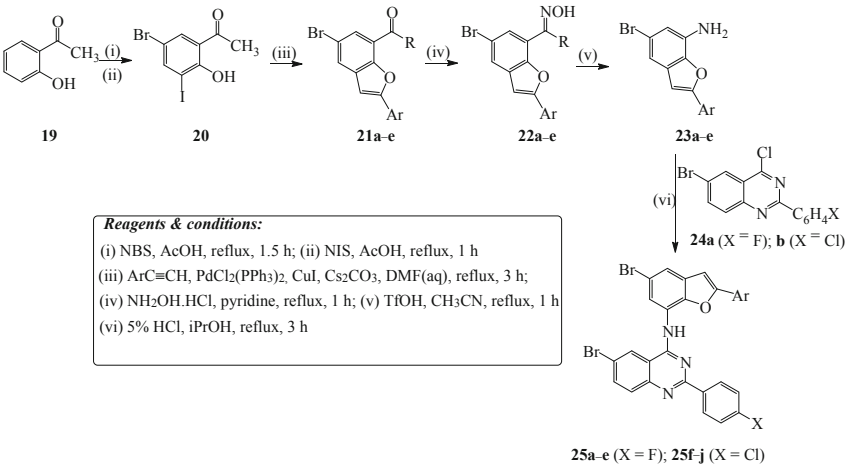
18.3 Preparation of Indole-Appended Aminoquinazoline Hybrids

The indole-appended 4-aminoquinazolines **18a–h** were prepared via HCl catalyzed amination of the electrophilic 2-aryl-4-chloroquinazoline derivatives **17a** and **17b** with 7-aminoindoles **16a–d** in tetrahydrofuran-isopropanol (THF-iPrOH) mixture under reflux (Scheme 18.5) [24]. Compounds **18a–h** were evaluated for antigrowth effect in vitro against the human lung cancer (A549), epithelial colorectal adenocarcinoma (Caco-2), hepatocellular carcinoma (C3A), breast adenocarcinoma (MCF-7) and cervical cancer (HeLa) cell lines. Hybrids **18f** and **18g** were found to have cytotoxicity against most of the cancer cell lines and to inhibit the EGFR-TK activity against gefitinib (IC_{50}38.9 nM) as a reference standard with the IC_{50} values 52.5 and 40.7 nM, respectively [24].

18.4 Preparation of Benzofuran-Appended Aminoquinazoline Hybrids

Despite what looks like a simple molecular framework, a thorough literature search revealed that no attempts have been made before towards the synthesis of benzofuran-

appended quinazoline hybrids in which the two pharmacophores are linked through a heteroatom bridge. We reacted the nucleophilic 7-aminobenzofurans **23a–e** with the electrophilic 6-bromo-4-chloro-2-(4-halogenophenyl)quinazoline **24a** (X = F) or **24b** (X = Cl) in the presence of 5% HCl in isopropanol (iPrOH) under reflux for 4 h (Scheme 18.6) [25]. We successfully isolated compounds characterized using a combination of spectroscopic (NMR, IR, MS) and single crystal X-ray diffraction technique as the corresponding benzofuran-aminoquinazoline hybrids **25a–e** or **25f–j**, respectively. Since the molecular construct of compounds **25a–j** resembles that of the EGFR-TK inhibitor, gefitinib, we evaluated them for antiproliferative effect against a panel of EGFR-positive cancer cell lines, namely, the A549, Caco-2, C3A (HepG2/C3A) and HeLa cell lines [25]. Among them compound **25d** showed significant cytotoxicity against the C3A cell line ($LC_{50} = 9.0\ \mu M$) when compared to gefitinib ($LC_{50} = 5.01\ \mu M$) and compound **25j** was also found to exhibit increased cytotoxicity against the Caco-2 cells ($LC_{50} = 18.4\ \mu M$) more so than gefitinib ($LC_{50} = 27.9\ \mu M$). Mechanistic studies demonstrated that the benzofuran-appended aminoquinazoline hybrids **25d** and **25j** induced apoptosis via activation of caspase-3 pathway. Moreover, compounds **25d** and **25j** exhibited significant and moderate inhibitory effects against EGFR ($IC_{50} = 29.3$ and 61.5 nM, respectively) when compared to gefitinib ($IC_{50} = 33.1$ nM).



Ar	%Yield **21**	%Yield **22**	%Yield **23**	%Yield **25a–e**	%Yield **25f–j**
C_6H_5-	73 (**21a**)	93 (**22a**)	62 (**23a**)	75 (**25a**)	83 (**25f**)
3-FC_6H_4-	76 (**21b**)	86 (**22b**)	78 (**23b**)	82 (**25b**)	81 (**25g**)
4-FC_6H_4-	76 (**21c**)	72 (**22c**)	71 (**23c**)	87 (**25c**)	78 (**25h**)
3-ClC_6H_4-	74 (**21d**)	81 (**22d**)	65 (**23d**)	78 (**25d**)	67 (**25i**)
4-$CF_3OC_6H_4$-	77 (**21e**)	78 (**22e**)	85 (**23e**)	82 (**25e**)	84 (**25j**)

Scheme 18.6 Amination of **24** with **23** to afford benzofuran-aminoquinazoline hybrids **25**

18.5 Conclusions and Perspective

We have demonstrated that the indole or benzofuran moieties can be linked with the quinazoline scaffold through an amino bridge to produce molecular hybrids with structural resemblance to the medicinally important 4-anilinoquinazoline derivatives. The prepared molecular hybrids have been evaluated for cytotoxicity in vitro against a panel of cancer cell lines and for inhibitory effect against the EGFR-TK phosphorylation. Since these compounds may also target proteins other than EGFR, future studies will also be extended to other types of protein kinases to explore the mechanism of action and selectivity of the title compounds. The observed results and structure activity relationship (SAR) form a basis for the design and synthesis of more potent heterocycle-appended aminoquinazoline hybrids in which the two pharmacophores are linked by a heteroatom bridge.

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