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N-substituted 2′-(aminoaryl)benzothiazoles as kinase inhibitors: Hit identification and scaffold hopping

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ABSTRACT

Starting with a hit from vHTS attained by a docking procedure of virtual compounds into ATP pockets of different kinases applying the 4SCan® technology, variations of the adenine mimic resulted in the identification of promising scaffolds, giving rise to in vitro IC_{50} values in the nanomolar range on different kinases down to 63 nM.

Protein kinases play a vital role in cell signal transduction and proliferation of cells. Focusing on cancer, deregulation of activity or expression levels of certain kinases is implicated and linked to different stages of tumor genesis, promotion, survival and metastasis (for a selection, see Table 1).[1–3] Small molecule kinase inhibitors are usually addressing the ATP binding site,[1,3–7] which is highly conserved among the 518 kinases being encoded in the human genome. Thus, selectivity for certain kinases might only be achievable by addressing side pockets of the binding site or inactive state conformations.[1,3,4,7–10] A different approach aims at multi-target inhibitors, which might even be advantageous for successfully tackling cancer cells in terms of efficacy and avoiding quick development of resistances.[1,4,6,11–16] With around 165 kinases being identified to be of potential relevance in cancer cells,[1,17] the approach described in here aimed at identifying small molecule inhibitors with an interesting inhibition profile on selected kinases, which displayed a scattered activity within different tumor stages as depicted in Table 1. The inhibition of kinases whose functionality is essential for non-tumor cells, like the insulin receptor (InsR) had to be avoided. In short: selectively unselective kinase inhibitors were envisaged. An initial screen was performed usually on a set of 16 kinases (Table 1) and InsR.[18]

Hit finding was accomplished by virtual high throughput screening (vHTS) based on docking experiments into the active site as deduced from crystal structures of four different kinases out of the proliferation, survival and angiogenesis section (Aurora A, Akt1, IGF1R, VEGFR2) using the 4SCan® technology.[19] From a database of 3.3 Mio commercially available compounds, around 250 were selected for biological testing according to their docking scores and simultaneously covering structural diversity. With an initial hit rate of 16% in an in vitro kinase screen (residual activity at 10 μM below 50% on at least one of the tested kinases), several different entities were identified as potential hit structures, including acyl-thiourea derivatives. These were next submitted to a virtual 2D similarity search within the same database for a hit validation, resulting in the identification of compound **1** displaying several excellent inhibitory activities with IC_{50} values below 1 μM, even down to 190 nM on the Src kinase (Fig. 1).

Besides the excellent inhibitory activity at Src and, similarly, at EGFR and IGF1R, several derivatives of this class of compounds displayed inhibitory activities to the InsR in a low micromolar range, compound **1** even with an IC_{50} of 900 nM. Furthermore, the scaffold of **1** incorporated a pharmacologically unfavourable thiourea linker.[20] Thus, our approach was a suitable replacement of this unit, keeping the benzothiazole moiety constant. Certain *N*-unsubstituted (aminophenyl)benzothiazoles like **2** and **3** (Fig. 1) are known from literature to be able to unfold an antitumor activity, even though not by inhibition of protein kinases but by preferentially activating metabolic pathways via the aryl hydrocarbon receptor within sensitive tumor cells.[21] For our synthetic purposes, several differently substituted 2′-(3-aminophenyl)- and 2′-(4-ami-

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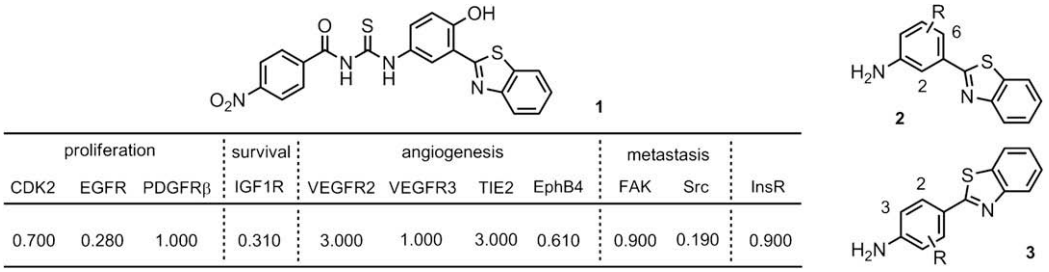
Table 1
Selection of kinases for initial screening

Proliferation	Survival	Angiogenesis	Metastasis
Aurora A	Akt1	VEGFR2 (KDR)	FAK
Aurora B	IGF1R	VEGFR3 (FLT4)	Src
CDK2		TIE2	
CDK4		EphB4	
EGFR			
ErbB2 (HER2)			
PDGFRβ			
PLK1			

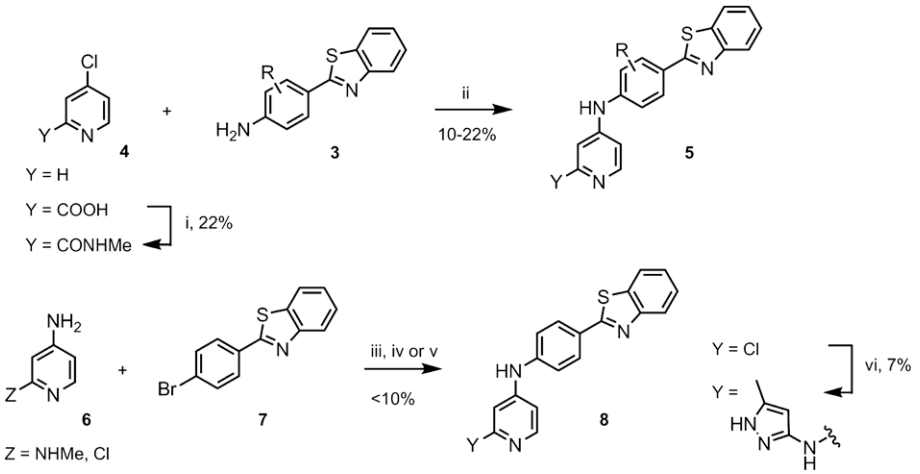
nophenyl)benzothiazoles **2** and **3** were prepared (Fig. 1; R = Me, Cl, OH and OMe, varying positions) as building blocks and initially tested in vitro for their inhibitory activity on 16 kinases, also including DF 203 (**3**, R = 3-Me) – one of the above mentioned, most potent compounds with antiproliferative activity on MCF-7 cells[21] – only to verify that these compounds were not remarkably potent on these targets, with IC_{50} values ranging around 10 μM at best. The syntheses of 2′-(3-aminophenyl)- or 2′-(4-aminophenyl)benzothiazole derivatives as well as 2′-(4-bromophenyl)benzothiazole (**7**, Scheme 1) followed a literature procedure, in which condensation of 2-aminothiophenols with differently substituted amino- or bromobenzoic acids, respectively, was achieved in polyphosphoric acid at 185 °C.[22]

According to literature data it was assumed that the attachment of an "adenine mimic" at the aniline nitrogen of building blocks **2** and **3** should result in improved inhibitory activities, due to its capability to effectively bind into the hinge region of the protein[23] – which is most likely not adequately addressed by a thiourea unit as present in the initial hit molecule. Thus the effect of several such groups was evaluated for both benzothiazole scaffolds with the amino group being located in the 3- or 4-position of the aryl ring. Synthetic realization of such derivatives is only depicted for the linear 4-aminoaryl series, though, but was similar for the second, bent 3-aminorayl series (Schemes 1 and 2). Guided by detailed scaffold analyses in the literature,[8,11,23–26] focus was laid on the incorporation of pyrimidines, purines, quinolines and quinazolines. Further variations of these core fragments evolved from a superposition of structures with the suggested 5-point pharmacophore for promiscuous kinase inhibitors,[9] which allowed for a rough estimation of possible different binding modes within the hinge region and thus for a generation of further substitution ideas (Fig. 2). In most cases, attachment of the heterocycles envisaged was achieved by treating the (aminophenyl)benzothiazoles **2** and **3** with the respective chlorinated heterocycle (Schemes 1 and 2), which was realized in DMF at 160 °C for 4-chloropyridines **4** and in ethylene glycol at 110–160 °C for mono-chlorinated pyrimidine-based heterocycles **11**, **13**, and 4,7-dichloroquinoline (not shown, yields: 5–45%) with optional acceleration of reaction rates by addition of HCl in dioxane or NaI. For substrates 2,4-dichloropyrimidine (**9**) or –quinazoline **15**, a regioselective twofold amination was based on different reactivities in the 2- and 4-position of these substrates[27]: amination of the more reactive 4-position was achieved with the corresponding amine in alcoholic solution (ethanol or *n*-butanol) and added base at ambient temperatures (for pyrimidines) or moderate heating (for quinazolines), the second amination at the 2-position succeeded under similar conditions but now without any base present and at elevated temperatures, either with or without purification of the intermediates formed

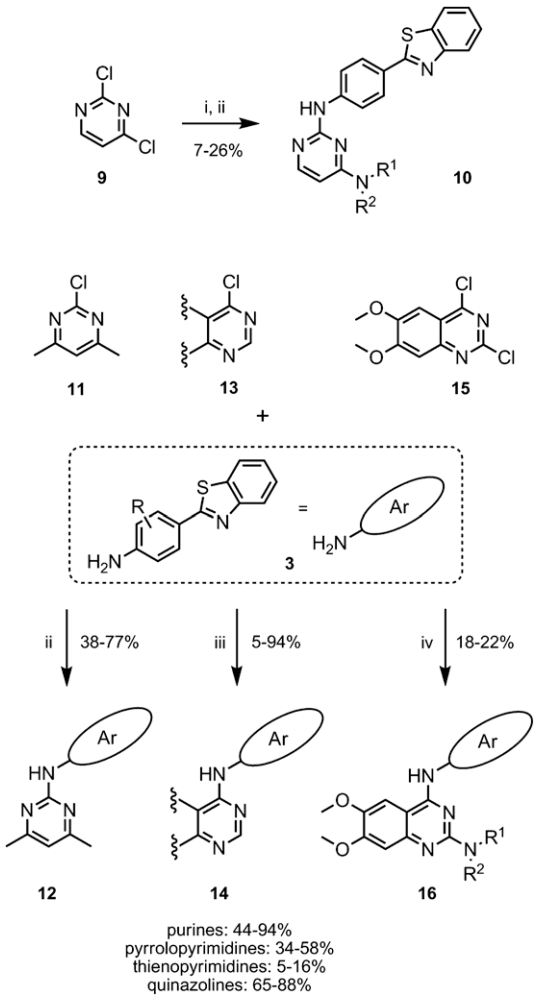


proliferation			survival	angiogenesis				metastasis		
CDK2	EGFR	PDGFRβ	IGF1R	VEGFR2	VEGFR3	TIE2	EphB4	FAK	Src	InsR
0.700	0.280	1.000	0.310	3.000	1.000	3.000	0.610	0.900	0.190	0.900

Figure 1. IC_{50} values [μM] on selected kinases for the initial hit molecule, and structures of building blocks **2** and **3**.



Scheme 1. Reagents and conditions: (i) $H_2NMe{\cdot}HCl$, EDC, DIEA, DMF, r.t., 48 h; (ii) DMF, 160 °C, 8 h; optional for acceleration of otherwise sluggish reactions: HCl in dioxane (4.0 M, 1.0 equiv); (iii) Pd_2dba_3 (0.04 equiv), Xantphos (0.12 equiv), K_3PO_4 (1.4 equiv), dioxane, 100 °C, 20 h; (iv) $Pd(OAc)_2$ (0.1 equiv), JohnPhos (0.2 equiv), K_3PO_4 (1.4 equiv), DME, 100 °C, 20 h; (v) Pd_2dba_3 (0.05 equiv), JohnPhos (0.2 equiv), NaO*t*Pent (1.4 equiv), toluene, 110 °C, 20 h; (vi) 5-methyl-1*H*-pyrazol-3-amine (1.5 equiv), HCl in dioxane (4.0 M, 1.0 equiv), ethylene glycol, 160 °C, 24 h; yields are not optimized.



Scheme 2. Reagents and conditions: (i) aliphatic amine or 5-methyl-1*H*-pyrazol-3-amine, DIEA, EtOH, r.t., 20 h; for 5-methyl-1*H*-pyrazol-3-amine, additional 4 h at 50 °C; (ii) 2′-(4-aminopheny)benzothiazole, ethylene glycol, 160 °C, 3–6 h; (iii) ethylene glycol, 110 °C, 3 h; optional for acceleration of otherwise sluggish reactions: HCl in dioxane (4.0 M, 1.0 equiv), additional 3 h at 120 °C; (iv) DIEA (1.0 equiv), *n*BuOH, 120 °C, 20 h, then aliphatic amine or 5-methyl-1*H*-pyrazol-3-amine (2.0 equiv), 140 °C, 3 h; yields are not optimized.

upon first amination. For a few substrates, palladium-catalyzed amination reactions had to be conducted: substituted 4-aminopyridines **6** were arylated with 2′-(4-bromophenyl)benzothiazole (**7**) using Xantphos[28] or biphenyl-2-yl-di-*tert*-butyl-phosphine (JohnPhos)[29] as ligand, in poor yields though following either variant (<10%; Scheme 1).[30] Interestingly, 2-chloro-4-(4′-methyl-piperazin-1′-yl)-pyrimidine – obtained from 2,4-dichloropyrimidine (**9**) and *N*-methylpiperazine – could not be converted into the corresponding diaminated substrates **10** applying a second nucleophilic substitution step as for all other similar substrates, the second amination had to be replaced by a Pd-catalyzed amination reaction as well.[30]

With an attachment of 6-membered heterocycles to the linear or bent (aminophenyl)benzothiazole fragments **2** and **3**, both of the series examined displayed some inhibitory activities with a focus on Aurora A (only the linear series is depicted in Table 2, for a bent derivative, see Table 5). For attaining inhibitory activities with IC_{50} values around 1 μM, the presence of a hydrogen bond donor for binding to the hinge region was required within the adenine mimic but not a sufficient precondition, though (cf. entry 4). The only example with highlighted inhibitory activities within the EGFR/ErbB2 group (IC_{50} = 1100 nM on ErbB2) was the pyridinecarboxamide (entry 2) – a moiety also found in Sorafenib[12,31] (Fig. 2). Obviously, best interaction within the hinge region was attained among the 6-membered heterocycles by the addition of an aminopyrazole unit – as present in the Aurora inhibitor VX-680[32] (Fig. 2) – which resulted in best inhibitory activities for both, linear and bent 2′-

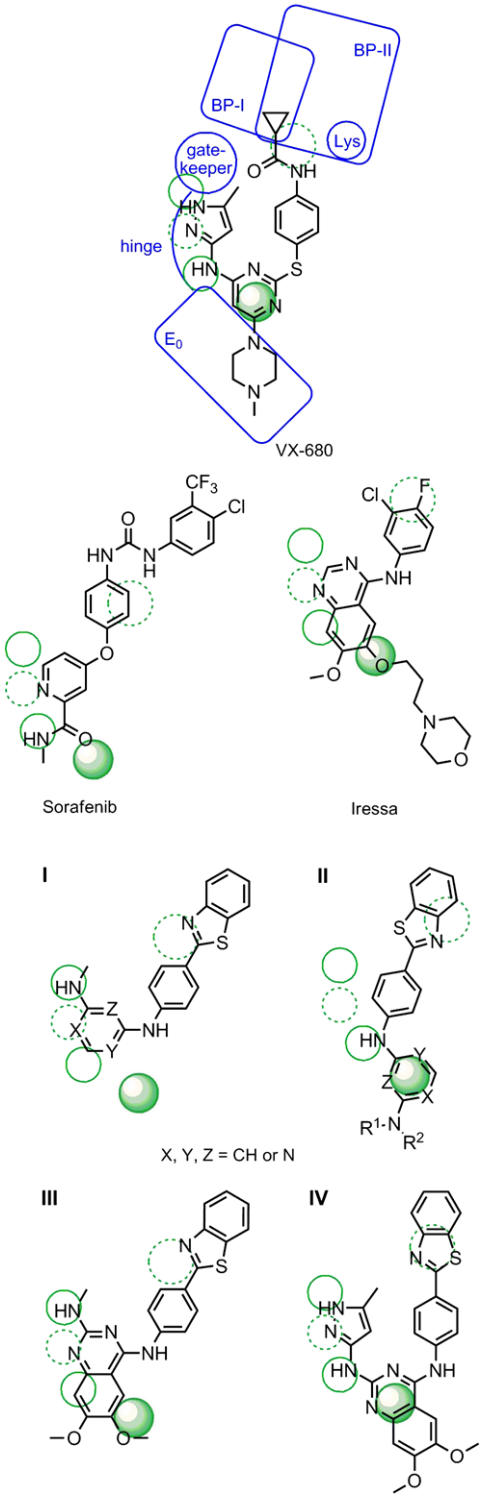
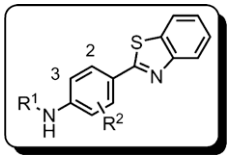
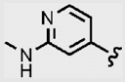
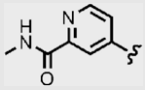
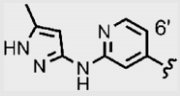
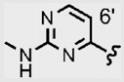
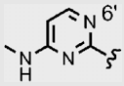
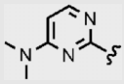
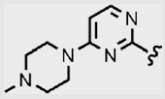
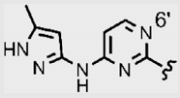


Figure 2. Reference compounds in superposition with the pharmacophore for promiscuous kinase inhibitors (green)[9,33] and discussion of binding modes for representatives of the arylbenzothiazole series. Lined green circles = hydrogen bond donors within the ligand, dotted green circles = hydrogen bond acceptor, full green circle = aromatic region;[9] blue areas = simplified pocket nomenclature according to Liao.[33]

Table 2
In vitro IC_{50} values [μM] on selected kinases out of a panel of 16; n.d. = not determined



Entry	R^1	R^2	Proliferation				Angiogenesis		
			Aurora A	Aurora B	EGFR	ErbB2	VEGFR2	VEGFR3	InsR
1		H	>10	>10	>10	>10	>10	6.000	>10
2		H	>10	>10	(2.000)	(1.100)	3.600	>10	>10
3		H	(1.300)	(1.800)	>10	>10	8.800	6.500	>10
4		H	>10	>10	>10	>10	8.900	6.900	9.700
5		H	(1.500)	>10	>10	>10	>10	>10	>10
6		H	>10	>10	>10	n.d.	>10	n.d.	>10
7		H	(0.840)	>10	>10	>10	>10	5.200	>10
8		H	(0.370)	7.000	>10	n.d.	>10	n.d.	>10
9		2-OMe	(0.320)	3.100	>10	>10	>10	>10	>10

(≤0.5 μM)
(0.501–2.0 μM)

(aminophenyl)benzothiazoles, with IC_{50}s down to 320 nM and a clear Aurora selectivity for the former (Table 2, entries 8 and 9) and even 160 nM for the latter (Table 5, entry 31), which proved to be less selective (also 190 nM at Src). According to literature,[26,33] incorporation of such a hinge region binding motif would position the arylbenzothiazole from the front cleft towards the back cleft, occupying the intersection of binding pockets I and II (BP-I and BP-II, respectively; Fig. 2) according to the nomenclature established by Liao,[33] an area wide enough to host either linear or bent arylbenzothiazole moieties. Attachment of an otherwise unsubstituted 4-pyridyl resulted in compounds offering only one possibility for hydrogen bonding to the hinge region, either by the pyridine itself (mode I, X = N; Fig. 2) or by the anilinic NH (mode II, X = N) – which is apparently not sufficient within this structural class of compounds (no inhibitory activities detected).

For linear (aminophenyl)benzothiazoles, an additional 3′-methylamino group on the pyridyl resulted in some inhibitory activity on VEGFR3 (entry 1), which was found to be in a similar range for the methylamino group being positioned between the two endocyclic nitrogens of a pyrimidinyl (around 6 μM, entry 4), but clearly better (by a factor of 4) for the N-shifted pyrimidinyl of entry 5 with an IC_{50} of 1.5 μM on Aurora A. The gain in inhibitory activity as compared to simply pyridyl might be explained by binding mode I (Fig. 2), in which the additional methylamino group would match a hydrogen bond donor unit of the 5-point pharmacophore model. Comparing compounds of entries 3 and 8, both containing the aminopyrazole unit enabling strong interactions within the hinge region, a 4-pyridyl unit was again less favourable by a factor of 4 as compared to the pyrimidinyl: IC_{50} value of 1300 nM at Aurora A for the former (entry 3) vs. around 350 nM for the latter derivatives (entries 8, 9). Consequently, a heterocyclic nitrogen in 6′-position seemed to be essential for attaining optimized inhibitory activities (cf. entries 1, 3 and 4 with 5, 8 and 9). Incorporation of an endocyclic nitrogen next to the anilinic attachment site was identified to be a privileged substructure for kinase inhibitors, no statement, however, was made on which of the two

possible positions (2′ or 6′) to be preferred.[26] Even though structures of compounds of entries 8 and 9 possess an array probably coinciding with all areas of the postulated 5-point pharmacophore of promiscuous kinase inhibitors[9] (cf. superposition with VX-680 in Fig. 2, wherein the benzothiazole replaces the cyclopropylamide unit, with the nitrogen of the thiazole unit matching the hydrogen bond acceptor region of the pharmacophore), these derivatives displayed high selectivity for Aurora A, which, however, was less emphasized for the bent variant of entry 31 (Table 5).

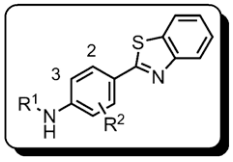
When the scaffold offered no hydrogen bond donor properties within the adenine mimic (entries 6, 7 and also 4-pyridyl), a complete loss of inhibitory activity was found, with 4-piperazinylpyrimidin-2-yl being an exception (IC_{50} = 840 nM at Aurora A; entry 7). Incorporation of a piperazine unit was likewise deduced from VX-680, in our case, however, generating a substrate which might accomplish hinge region binding only by its anilinic NH. This fact may be compensated by a decent orientation of the arylbenzothiazole within the adenine pocket towards BP-II in the back cleft and positioning of the piperazine within the secondary hydrophobic patch E_0 (mode II, Fig. 2).[33]

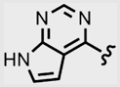
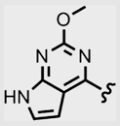
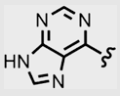
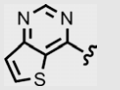
Next, bicyclic heteroaromatics were investigated for their ability to bind within the hinge region: attachment of pyrrolopyrimidines,[34] purines[35] and thienopyrimidines[11] to the (aminoaryl) benzothiazole fragments (Table 3). With the (all N)-heterocycle scaffolds, a methoxy substitution of the arylbenzothiazole proved to be advantageous, resulting in generally better inhibitory activities (entries 10, 12, 14 vs. 11, 13, 15). Variation of the heterocycle resulted in different kinases to be the preferred target with IC_{50} values around 1 μM (R^2 = 2-OMe): EGFR for an unsubstituted pyrrolopyrimidine (entry 11), VEGFR2 for methoxypyrrolopyrimidine (entry 13) and TIE2 for purine (entry 15). With the pyrrolopyrimidine as core fragment, the methoxy group R^2 (entry 11) seemed to be the decisive selectivity factor, as its unsubstituted (aminophenyl)benzothiazole analog (entry 10) was rather equi-active on EGFR, IGF1R, VEGFR2, TIE2 and Src. Incorporation of thieno[3,2-d]pyrimidin-4-yl resulted in rather selective Aurora B inhibitors depending on an appropriate choice of the substituent within the arylbenzothiazole unit (entries 16–19): the fluoro-substituted derivative (entry 19) displayed a good inhibitory activity with an IC_{50} value of 290 nM with almost complete selectivity.

Investigating the influence on inhibitory activity for quinoline/quinazoline derivatives, the starting scaffold was a 7′-chloroquinoline,[36] to which were added additional substituents and heteroatoms (Table 4). Using the former adenine mimic (entries 20–23), substituents at the aryl unit of the 2′-(4-aminophenyl)benzothiazole showed a drastic effect, in that either no inhibitory activity was detectable (entry 21, R^2 = 2-OMe) or a broadly scattered inhibition profile was identified with several sub-micromolar IC_{50} values. Generally, these compounds were rather inactive at Aurora A, a slight preference for Src, VEGFR2 (cf. Ref. 37) and Aurora B was deducible, with best IC_{50} values around 500 nM (entries 22 and 23).

Keeping the chloro-substituent in 7′-position but adding a methoxy group and an additional heterocyclic nitrogen (quinazoline framework)[38] emphasized the preference for Src (now 300 nM) and resulted in a certain selectivity over other kinases (entry 24). Replacement of 7′-Cl by an additional methoxy yielded rather selective kinase inhibitors with good IC_{50} values below 400 nM on different kinases, depending on the substituent attached to the arylbenzothiazole (entries 25–28): Aurora B

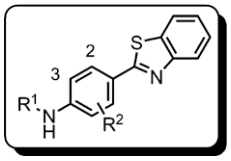
Table 3
In vitro IC_{50} values [μM] on selected kinases out of a panel of 16; n.d. = not determined; for InsR, all IC_{50} > 10 μM, except for entry 10: IC_{50} = 6.95 μM

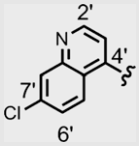
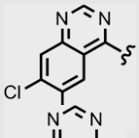
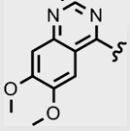
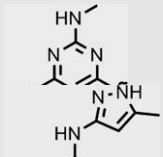
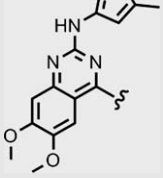


Entry	R^1	R^2	Proliferation				Survival IGF1R	Angiogenesis			Metastasis Src
			Aurora A	Aurora B	EGFR	ErbB2		VEGFR2	VEGFR3	TIE2	
10		H	8.000	>10	(1.300)	3.100	(1.500)	(1.200)	2.100	(1.900)	(1.500)
11		2-OMe	>10	>10	(0.720)	(1.600)	3.600	>10	>10	8.600	>10
12		H	>10	>10	7.700	>10	4.400	10.000	>10	8.700	6.400
13		2-OMe	>10	>10	5.100	>10	2.900	(0.960)	7.700	2.500	2.100
14		H	>10	>10	7.800	n.d.	>10	>10	n.d.	n.d.	>10
15		2-OMe	5.800	>10	2.600	>10	>10	9.200	3.400	(1.300)	>10
16		H	2.800	(0.720)	>10	>10	>10	>10	>10	>10	>10
17		2-OMe	>10	>10	>10	>10	>10	>10	>10	>10	>10
18		2-Cl	>10	>10	>10	>10	>10	>10	>10	4.400	>10
19		3-F	4.000	(0.290)	>10	>10	>10	>10	>10	4.100	>10

(≤0.5 μM)
(0.501–2.0 μM)

Table 4
In vitro IC_{50} values [μM] on selected kinases out of a panel of 16; n.d. = not determined



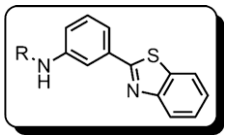
Entry	R^1	R^2	Proliferation			Survival IGF1R	Angiogenesis			Metastasis Src	InsR
			Aurora A	Aurora B	EGFR		VEGFR2	TIE2	EphB4		
20		H	5.700	(0.750)	5.300	(0.860)	(0.640)	7.000	(1.400)	(0.630)	2.700
21		2-OMe	>10	>10	>10	>10	>10	>10	>10	>10	>10
22		2-Cl	>10	>10	(0.800)	(0.800)	(0.600)	>10	(0.800)	(0.500)	>10
23		3-F	>10	(0.510)	>10	(1.100)	(0.800)	(1.000)	(1.000)	(0.700)	>10
24		H	2.700	>10	>10	3.300	(1.000)	>10	>10	(0.300)	>10
25		H	(0.350)	(0.135)	(1.500)	>10	6.800	n.d.	>10	>10	>10
26		3-Me	4.400	3.700	(0.240)	>10	3.400	(0.840)	>10	>10	>10
27		2-OH	>10	4.200	2.200	>10	6.900	n.d.	>10	>10	>10
28		2-OMe	>10	>10	(0.063)	>10	>10	n.d.	>10	>10	>10
29		H	>10	>10	>10	>10	>10	>10	>10	>10	(0.630)
30		H	6.000	>10	>10	(1.650)	>10	>10	3.300	3.000	2.150

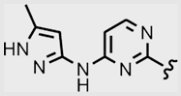
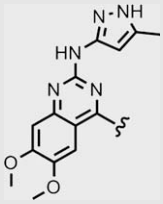
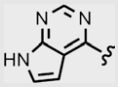
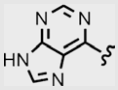
(≤0.5 μM)
(0.501–2.0 μM)

(IC_{50} = 135 nM; entry 25) or EGFR (IC_{50} = 240 nM and excellent 63 nM; entries 26 and 28). With a 2-methoxy substituent (entry 28), complete selectivity for EGFR was achieved within the panel of kinases tested. The 4′-(arylamino)-6′,7′-dialkoxyquinoline pattern represents a privileged scaffold for EGFR inhibition[8,11,25] with one of the most prominent representatives being Iressa (Fig. 2), which is already FDA approved for the treatment of metastatic non-small cell lung cancer as a selective EGFR/ErbB2 inhibitor.[8,11,39] Binding mode of the compounds of Table 4 bearing the 6′,7′-dimethoxyquinazoline (entries 25–28) should be comparable to that of Iressa, with an additional stretching into the intersection area of BP-I and -II (Fig. 2). Based on such a binding mode, knowledge of the 5-point pharmacophore for promiscuous kinase binding[9] would suggest an attachment of an additional hydrogen bond donor within the 2′-position of the quinazoline to probably allow for enhanced binding within the hinge region (mode III and IV, Fig. 2). However, such substitutions proved to be detrimental to inhibitory activities: with a 2′-methylamino substitution in place, only an IC_{50} of 950 nM was attained for PDGFRβ, paralleled by an IC_{50} of 630 nM for InsR (entry 29). With aminopyrazole being attached as a self-sustaining unit for hinge region binding, a mediocre IC_{50} value of 1650 nM was achieved on IGF1R (entry 30), and again, inhibition of the InsR was found to be within the same range (IC_{50} = 2150 nM). Such a general inhibition level is comparable to the aminopyrazole-pyridine derivative of entry 3 (Table 2), which was likewise found to display IC_{50} values only around 1.5 μM (on Aurora A though), but clearly unfavourable to those from the pyrimidine series (Table 2, entries 8 and 9).

All adenine mimics depicted in Tables 2–4 were likewise attached to the bent 2′-(3-aminophenyl)benzothiazole derivatives **2**. Utilizing pyridine and pyrimidine moieties, results were rather comparable to those of the linear series (cf. Table 2), giving rise to moderate IC_{50} values around 1 μM especially on Aurora A, but now sporadically also on Aurora B and VEGFR3, with the most promising variation implementing an aminopyrazole unit (Table 5, entry 31). Incorporation of the latter unit proved to be mandatory for attaining any moderate inhibitory activity at all for compounds based on the different quinazoline frameworks (entry 32), resulting in a broad inhibition panel with several IC_{50} values around 1 μM – but clearly being disadvantageous as compared to the results of the corresponding pyrimidine (IC_{50} = 160 nM on Aurora A, entry 31). Similarly, bent derivatives of the purine-derived adenine mimics (cf. Table 3) displayed rather poor inhibitory activities, only for a pyrrolopyrimidine and a purine variation IC_{50} values around 1 μM were attained: on TIE2 in the former (entry 33) and rather non-selectively on IGF1R, VEGFR2, EphB4 and Src in the latter case (entry 34). Within the bent series, however, selectivity over the InsR regularly became an issue, the insulin receptor

Table 5
In vitro IC_{50} values [μM] on selected kinases out of a panel of 16; n.d. = not determined; IC_{50} values [μM] for InsR: 1.6 (entry 31), 0.94 (entry 32), >10 (entries 33, 34)



Entry	R	Proliferation				Survival IGF1R	Angiogenesis				Metastasis Src
		Aurora A	Aurora B	EGFR	PDGFRβ		VEGFR2	VEGFR3	TIE2	EphB4	
31		(0.160)	2.000	1.900	>10	(0.1000)	7.500	2.500	>10	>10	(0.190)
32		5.500	>10	3.300	(1.100)	(0.810)	1.900	1.600	4.400	(0.780)	(0.960)
33		>10	6.400	>10	>10	>10	>10	>10	(1.200)	>10	>10
34		>10	>10	1.700	n.d.	(1.200)	(0.870)	n.d.	n.d.	(0.840)	(1.100)

(≤0.5 μM)
(0.501–1.5 μM)

was likewise inhibited with IC_{50} values around 1–3 μM in several cases (selected data shown in Table 5).

According to the results described herein, the 4′-(arylamino)-6′,7′-dimethoxyquinazoline scaffold was identified as a most promising starting point for further optimizations, with inhibitory activities found to be already around 100 nM, not displaying selectivity issues over InsR, and focussing on kinases important for tumor proliferation. The importance of the arylbenzothiazole unit for tuning target specificity and selectivity emanates already from the examples discussed within this contribution and became even more obvious through our optimization efforts, which will be described in due course.[40] Additionally, an Iressa analog based on the 6′,7′-dimethoxyquinazoline moiety (in analogy to entries 25–28) displayed excellent activities on EGFR and ErbB2 (IC_{50} = 1 and 24 nM, respectively), whereas a replacement of the benzothiazole in compound entry 25 by an isoindoline resulted in a general significant loss of activity with the best IC_{50} of 920 nM being determined on Aurora B.[41] Consequently, incorporation of larger and rather linear anilines than those implemented in Tarceva and Iressa results in a shift of activity from the ErbB family kinases towards Aurora kinases, with the nature of said group simultaneously altering the selectivity profiles. Furthermore, the arylbenzothiazole moiety extends into an area of the binding pocket not addressed by Tarceva or Iressa, offering new opportunities for a fine tuning of selectively unselective kinase inhibitors.

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41. IC_{50} values [μM] below 10 μM for a) the Iressa analog *N*-(3-chloro-4-fluorophenyl)-6′,7′-dimethoxyquinazolin-4′-amine: 6.5 (Aurora A), 7.4 (Aurora B), 0.001 (EGFR), 0.024 (ErbB2), 3.0 (PDGFRβ), 0.51 (VEGFR2), 2.4 (VEGFR3), 8.3 (TIE2), 0.48 (EphB4), 2.7 (Src); and b) *N*-[4-(isoindolin-2-yl)phenyl]-6′,7′-dimethoxyquinazolin-4′-amine: 5.3 (Aurora A), 0.92 (Aurora B), 6.6 (CDK4), 1.6 (EGFR), 7.5 (IGF1R), 7.9 (Src).