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Discovery of dually acting small-molecule inhibitors of cancer-resistance relevant receptor tyrosine kinases EGFR and IGF-1R†‡

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Novel benzo-anellated furo- and pyrrolo[2,3-*b*]pyridines with a 4-benzylamine substitution have been evaluated as inhibitors of the epidermal growth factor receptor (EGFR). Substituent effects on the determined protein kinase affinity have been discussed based on varied benzylamine residues at the differently substituted molecular scaffolds. Docking studies were carried out in order to explore the potential binding modes of the novel inhibitors. The observed activity data encouraged the measurement of the inhibition of the insulin-like growth factor receptor (IGF-1R), which is known to play an important role in the cancer-resistance development against EGFR inhibitors *via* receptor heterodimerizations with IGF-1R. We identified novel dual inhibitors of both kinases and report their first cancer cell growth inhibition data.

Introduction

The understanding of cell regulating processes during the past decades helped to identify deregulated cellular pathways in cancer cells which result from an abnormal activity of regulating protein kinases.[1,2] Such protein kinases can be found either overexpressed or overactivated in cancer cells so that the cells show enhanced proliferation rates, escape from apoptosis and finally show an aggressive invasive growth into neighboured tissues.[1,3,4] Growth factors play a central role in such a tumor progression.[4] They can be differentiated in exogenous and cellular factors which contribute not only to the tumor growth but also to tumor-induced angiogenesis.[4] Among the various receptor tyrosine kinases which are involved in cell signalling processes after an exogenous stimulus, the epidermal growth factor receptor (EGFR) is a prominent kinase which contributes to pathogenesis and progression of various types of cancer.[4,5] Recent studies document high expression rates for EGFR and the specific EGFR-binding ligand TGF-α in breast cancer and non-small lung cancer.[4,6,7] So, EGFR represents an important target structure for the development of inhibitors in anticancer therapy.[8] Small molecule inhibitors have been developed which address the intracellular ATP-binding site of EGFR and also monoclonal antibodies were found which block the extracellular ligand binding and receptor activation.[9,10] Certain problems with those early small molecule inhibitors have been resistance development resulting from gene mutations.[11,12] While single mutations which cause amino acid exchanges in the ATP binding regions have been tolerated, mutations in the encoding exon 20 led to the inactivity of known inhibitors.[13–15] Anticancer therapies using antibodies are critical because of immense costs and also in those cases resistance developments occur.[16,17] Therefore, there is a strong demand for novel, structurally diverse small molecule inhibitors which show an alternative inhibitor binding and are more effective concerning the resulting costs for therapy.[16]

The quinazoline scaffold is a part of prominent EGFR inhibitors like erlotinib with a 4-amino residue.[18] Thienopyrimidines have been described as alternative EGFR inhibitors with partly disappointing cellular activities as far as reported.[19] In those thienopyrimidines, the phenyl part of the quinazoline scaffold has been replaced with a thiophene ring, which was substituted with various phenylic residues attached to the 2-position of the thiophene. We synthesized novel benzo-anellated furo[2,3-*b*]pyridines as potential novel EGFR inhibitors. We evaluated their EGFR inhibiting properties and further varied the molecular scaffold by replacing the furan residue with a pyrrole residue in the respective structures. In addition to the inhibition of EGFR, we evaluated the affinities of all compounds towards the insulin-like growth factor receptor (IGF-1R), which is known to play a

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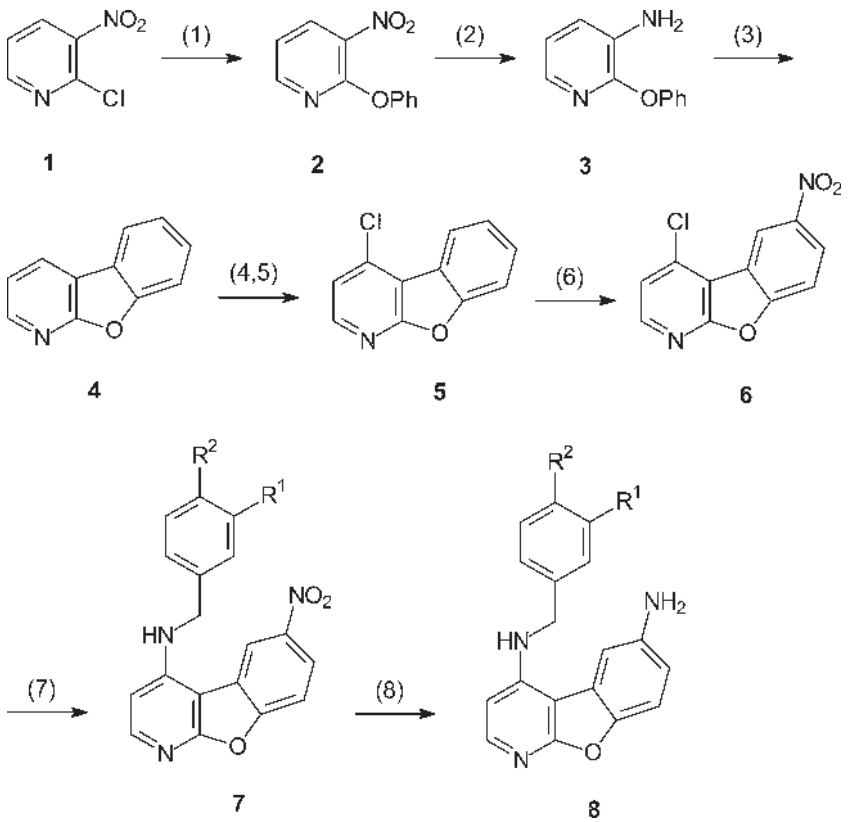
central role in EGFR-mediated cancer-resistances due to the heterodimerization of EGFR with IGF-1R as will be discussed below. In this way, we identified the first promising dual EGFR and IGF-1R inhibitors for which their first cellular anti-cancer screening data will be reported.

Results and discussion

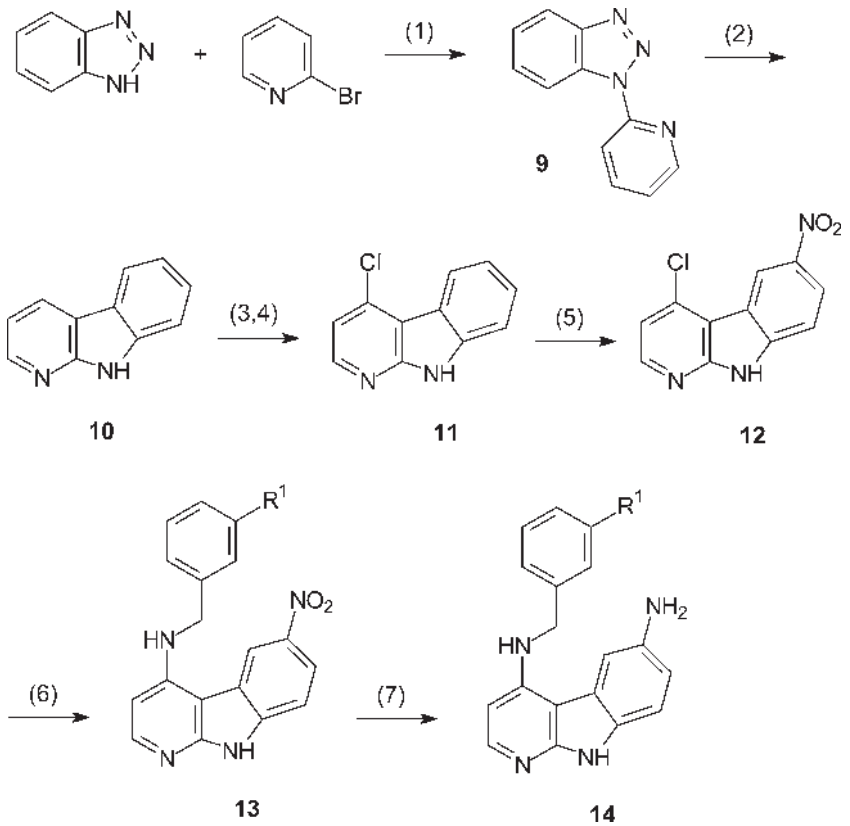
The benzo-anellated furo[2,3-*b*]pyridine scaffold **4** was formed from 3-amino-2-phenoxy-pyridine (**3**) in a copper-catalyzed reaction after treatment with sodium nitrite in sulfuric acid. The precursor of compound **3** with a nitro function at the 3-position of the pyridine had been treated with hydrogen in a palladium-catalyzed reaction to reduce the nitro function. The 2-phenoxy group of precursor **2** had been introduced by treatment of the commercially available 2-chloro-3-nitropyridine (**1**) with phenol using sodium hydride. The 4-chloro substitution in compound **5** had been introduced using phosphorus oxychloride after activation of the nitrogen as *N*-oxide with *meta*-chloroperbenzoic acid (*m*CPBA), so that the chlorine atom was preferably introduced into the 4-position of the activated scaffold. The 6-nitro function had been selectively introduced into compound **5** with fuming nitric acid at low temperature and then the varying benzylamines reacted with the given 6-nitro compound **6** to form the target structure series **7** under reflux conditions. The given benzylamine compound series was finally treated with tin(II) chloride in hydrochloric acid to yield the 6-amino compound series **8** (Scheme 1).

The benzo-anellated pyrrolo[2,3-*b*]pyridine **10** was formed from (2-pyridyl) benzotriazole (**9**) in a polyphosphoric acid (PPA)-catalyzed reaction. The benzotriazole **9** resulted from the reaction of 2-bromopyridine and benzotriazole in toluene under reflux conditions. The 4-chloro function had been introduced in derivative **11** after activation of the nitrogen of compound **10** using hydrogen peroxide in acetic acid to give the *N*-oxide that reacted with phosphorus oxychloride to give the 4-chloro derivative **11**. The 6-nitro function was introduced similarly to compound **7** with fuming nitric acid. The 4-benzylamino compound series **13** resulted from the direct heating of the 4-chloro-6-nitro compound **12** with the benzylamines at varying temperatures. Finally, the 6-amino compound series **14** formed after treatment of the 6-nitro function of the benzylamine substituted compounds **13** with tin(II) chloride under hydrochloric acid conditions (Scheme 2).

The EGFR-wild type (wt) affinities have been determined in an ATP competition assay as described in the ESI.† In short, the reduced amount of phosphorylated EGFR substrate has been measured using a scintillation technique under



Scheme 1 Formation of the benzo-anellated furo[2,3-*b*]pyridine **4** with reaction conditions and yields: (1) NaH, phenol, toluene, reflux, 89%; (2) $Pd/BaSO_4$, H_2, 98%; (3) H_2SO_4, $NaNO_2$, 0 °C, Cu, 55 °C, acetone, 22%; (4) *m*CPBA, $CHCl_3$, reflux, 82%; (5) $POCl_3$, $CHCl_3$, reflux, 69%; (6) HNO_3, 0 °C, 97%. Derivatization reaction to target structure series **7** and the final reaction to compound series **8** with reaction conditions and yields: (7) benzylamine cpd., reflux, 1.4–57%; (8) $SnCl_2$, HCl, reflux, 18–96%.



Scheme 2 Formation of the benzo-anellated pyrrolo[2,3-*b*]pyridine **10** with reaction conditions and yields: (1) toluene, reflux, 97%; (2) PPA, 170 °C, 36%; (3) H_2O_2, HAc, reflux, 73%; (4) $POCl_3$, DMF, 0 °C, 58%; (5) HNO_3, 0 °C, 75%. Derivatization reaction to target structure series **13** and the final reaction to compound series **14** with reaction conditions and yields: (6) benzylamine cpd., 145–160 °C; 43–90%; (7) $SnCl_2$, HCl, reflux; 41–71%.

varied inhibitor concentrations. From the resulting IC_{50} values, the affinity constants have been calculated. For the 3-methoxybenzylamine derivative **7a**, we found a low micromolar affinity towards EGFR (Table 1).

When the methoxy function was moved to the 4-position of the benzylamine, as shown in derivative **7b**, the affinity slightly increased. The 3-chloro benzylamine compound **7c** showed an improved affinity compared to the 3-methoxy compound. If placed into the 4-position of the benzylamine, as shown in compound **7d**, the affinity was lowered. Next we investigated the influence of a bromo substituent, as shown in compounds **7e** and **7f**. The 3-bromo substitution was comparably more favourable than the investigated chloro and methoxy substitutions leading to a submicromolar affinity in compound **7e** towards EGFR. When moving the bromo substituent to the 4-position, we found a similar decrease in the affinity for compound **7f** to what has been stated for the chloro substituent movement. Then we tested derivative **7g**, which has a 3-amino substituent. The compound showed the poorest affinity among all derivatives so far. If replaced with a nitro function, as in derivative **7h**, we found a submicromolar affinity towards EGFR. Summarizing the given results we can state that a 3-substitution in the benzylamine residue is more favourable than a 4-substitution. Best affinities have been found for the 3-bromo and the

Table 1 Inhibitory activities towards EGFR-wt and IGF-1R determined as K_i values of our benzylamine substituted target compounds **7a–h**, **8a–g**, **13a–c** and **14a–c**

			K_i value[a]	
Cpd.	R^1	R^2	EGFR-wt (μM)	IGF-1R (μM)
7a	OMe	H	2.74 ± 0.07	4.56 ± 0.04
7b	H	OMe	2.05 ± 0.02	3.40 ± 0.02
7c	Cl	H	1.36 ± 0.03	2.85 ± 0.28
7d	H	Cl	2.52 ± 0.12	3.54 ± 0.08
7e	Br	H	0.68 ± 0.02	2.61 ± 0.06
7f	H	Br	0.81 ± 0.01	2.87 ± 0.24
7g	NH_2	H	3.59 ± 0.06	6.18 ± 0.11
7h	NO_2	H	0.93 ± 0.04	2.39 ± 0.04
8a	OMe	H	0.17 ± 0.01	0.54 ± 0.05
8b	H	OMe	n.a.[b]	n.a.[b]
8c	Cl	H	0.40 ± 0.04	4.60 ± 0.05
8d	H	Cl	n.a.[b]	n.a.[b]
8e	Br	H	0.23 ± 0.02	n.a.[b]
8f	H	Br	n.a.[b]	n.a.[b]
8g	NH_2	H	0.94 ± 0.03	8.93 ± 0.98
13a	OMe	—	0.33 ± 0.04	0.89 ± 0.02
13b	Cl	—	0.08 ± 0.02	0.28 ± 0.04
13c	Br	—	0.11 ± 0.02	0.32 ± 0.07
14a	OMe	—	0.32 ± 0.01	3.56 ± 0.01
14b	Cl	—	0.14 ± 0.01	1.92 ± 0.02
14c	Br	—	0.11 ± 0.02	2.10 ± 0.03

[a] Mean of two determinations. [b] Not active, IC_{50} value >1000 μM.

3-nitro substitution reactions, whereas the 3-amino substitution is unfavourable.

Then we investigated our 6-amino substituted series **8**. The 3-methoxy substitution in derivative **8a** led to a more than fifteenfold higher affinity towards EGFR than the same substitution in compound **7a**. However, if moved to the 4-position, the resulting compound **8b** was found inactive as an EGFR inhibitor. A similar strong difference in activity was found in the case of the chloro-substituted compounds **8c** and **8d**. While the 3-chloro substitution resulted in a favourable submicromolar affinity, the 4-chloro substitution caused a loss of affinity. So we found the same tendency that a 3-substitution with either methoxy or chloro was favourable reaching low submicromolar ranges, whereas the 4-substitution led to inactive compounds. The tendency was also confirmed for the bromo-substituted compounds. The 3-bromo substitution in compound **8e** led to a lower micromolar affinity whereas the 4-bromo substituted compound **8f** was not active. Finally, the 3-amino substitution in derivative **8g** was much more favourable than that of the 6-nitro compound **7g**.

The given results prove that the 3-benzylamine substitutions are more favourable in the 6-amino compound series **8** than in the 6-nitro series **7**, whereas benzylamine substitutions in the 4-position are not tolerated in the 6-amino compound series **8**. However, also in the 6-nitro series **7**, decreased affinities have been found for the 4-substituted benzylamino compounds when compared to those 3-substituted benzylamine derivatives.

Next we investigated the varied benzylamine substitutions in our pyrrolopyridine series **13**. The 3-methoxy substitution of derivative **13a** was more favourable than that of the furopyridine compound **7a**. The 3-chloro function in compound **13b** led to a more than fifteenfold higher affinity towards EGFR than that in the corresponding furopyridine derivative **7c**. This compound showed a nanomolar affinity with a K_i value of 82 nM. Also the 3-bromo compound **13c** showed a higher affinity towards EGFR than the respective compound **7e**. So we found the investigated 3-benzylamine substituted pyrrolopyridines with the 6-nitro functions to be more active as EGFR inhibitors than the furopyridines reaching nanomolar affinity ranges.

With the 6-amino function, we found almost the same affinities for the 3-methoxy benzylamino substitution in compound **14a** as in derivative **13a**. However, the 3-chloro substitution in the 6-amino compound **14b** was less favourable than that in the 6-nitro derivative **13b**. The 3-bromo substitution in compound **14c** with the 6-amino function was as favourable as in the 6-nitro compound **13c**. If we compare the pyrrolopyridines with the 6-amino function with the corresponding furopyridines, we found the 3-chloro and 3-bromo benzylamine residues to be more favourable in the pyrrolopyridines. However, some higher affinity towards EGFR resulted for the 3-methoxy benzylamine substitution in the furopyridine **8a** with the 6-amino function if compared to the pyrrolopyridine **14a**.

So we identified most favourable 3-benzylamine substituted derivatives with highest affinities towards EGFR in the 6-amino furopyridines series on one hand and in the 6-nitro pyrrolopyridine series on the other hand reaching nanomolar ranges.

Resistance developments of the established EGFR inhibitors have recently been described to result from the heterodimerization of one EGFR receptor half with other receptors.[20,21] After binding of the extracellular ligand, one EGFR receptor half normally undergoes homodimerization to give a functional receptor dimer that is autophosphorylated at the cytoplasmatic tyrosine residues.[22,23] In the following cell signalling proteins dock to the phosphorylated receptor site and are activated by phosphorylation to proceed in the cell signalling process.[21,24] Alternatively, to the primary homodimerization one EGFR receptor half may dimerize with another receptor.[20,21] In that case an inhibiting compound may not prevent the EGFR activity and resistance results. That kind of anticancer drug resistance mechanism has been described for erlotinib as a consequence of heterodimerization with IGF-1R in an aggressive type of non-small lung cancer.[20]

So there has been strong interest to develop dually acting inhibitors of both EGFR and IGF-1R. Such novel inhibitors ideally have a novel molecular scaffold different from the established EGFR inhibitors so that potential resistance development as result of EGFR mutations is lowered. Our novel furo- and pyrrolopyridines own such a novel molecular scaffold and so we investigated them to act not only as EGFR inhibitors as demonstrated, but also as IGF-1R inhibitors to compete with the second EGFR resistance mechanism of receptor heterodimerization.

First we investigated the furopyridines **7** and **8** as IGF-1R inhibitors in the ATP competition assay as described for the inhibition of EGFR. Compound **7a** showed a lower micromolar affinity towards IGF-1R, which slightly improved in compound **7b** with the 4-methoxy substitution. In the case of the varying chloro substitutions in derivatives **7c** and **7d**, we found a slightly better affinity for the 3-chloro derivative **7c** whereas the 4-chloro compound **7d** was as active as the 4-methoxy compound **7b**. The compounds with bromo substituents showed best results both in the 3- and the 4-positions if compared to the varied methoxy and chloro positions. The 3-amino function in compound **7g** showed decreased affinities compared to the corresponding bromo function. The 3-nitro derivative **7h** was the best IGF-1R inhibitor of this class of 6-nitro furopyridines. Favourably, that compound was also the best EGFR inhibitor within this group of 6-nitro substituted compounds, so that we identified a first dual inhibitor.

Then we investigated the next group of furopyridines with the 6-amino function as IGF-1R inhibitors. The 3-methoxy function in derivative **8a** was very favourable reaching submicromolar affinity ranges. When the methoxy function was moved to the 4-position, the activity of the resulting compound **8b** was lost. A similar result was found for the varying chloro substitutions in compounds **8c** and **8d**. While the

3-chloro derivative **8c** showed a micromolar activity, the 4-chloro compound **8d** was found inactive. However, the substitution of the bigger bromine atom led to inactive compounds both in the 3- and the 4-positions of the benzylamine residues in compounds **8e** and **8f**. For the 3-amino substitution in derivative **8g**, we found a lowered IGF-1R affinity compared to the 3-methoxy and 3-chloro substituted compounds **8a** and **8c**. In our second group of furopyridines, we found a tendency similar to our first group with the 6-nitro function concerning the IGF-1R affinity. Obviously, the 4-substituted benzylamino function is less or unfavourable for substituents as demonstrated. With the 3-methoxy benzylamine compound **8a**, we identified our second dual inhibitor of both EGFR and IGF-1R with mainly improved affinities if compared to the best dual inhibitor of the 6-nitro furopyridine series **7h**.

Finally, we determined the IGF-1R inhibitory activities of our pyrrolopyridines starting with the 6-nitro substituted compounds **13a–c**. All compounds showed submicromolar affinities with the 3-chloro substitution of derivative **13b** being the best one for the inhibitory activities. The 3-methoxy substitution of compound **13a** was less favourable and the 3-bromo substitution in derivative **13c** was just slightly poorer than the 3-chloro function substitution. With a substituent ranking from chloro to bromo and, finally, methoxy of decreased affinities towards IGF-1R, we found a similar tendency in activities for the furo- and pyrrolopyridine series of the 6-nitro substitution. The pyrrolopyridines, however, showed an up to tenfold higher affinity towards IGF-1R than the furopyridines. In the compound series with the 6-amino pyrrolopyridines **14a–c**, the ranking of the 3-benzylamino acids was the same as that of the 6-nitro series with the 3-chloro substituent of compound **14b** being the best one, followed by the bromo substituent in derivative **14c** and, finally, the methoxy residue in compound **14a**. However the affinities of compounds **14a–c** were about sevenfold lower than those of the 6-nitro series **13a–c**. All 6-nitro derivatives **13a–c** were dual inhibitors of EGFR and IGF-1R with compound **13b** being the best one with a nanomolar EGFR inhibitory activity and a lower submicromolar affinity towards IGF-1R.

In order to provide a potential rationalisation for the detected *in vitro* activity, docking studies were done for the two kinases EGFR and IGF-1R in their active form. The inhibitors were docked into the ATP binding pocket using the GOLD docking program (see the ESI†) and default docking settings. The benzo-anellated furo[2,3-*b*]pyridine and benzo-anellated pyrrolo[2,3-*b*]pyridine scaffolds are mimicking the adenine ring of ATP and represent hinge-binding motifs. The docking results show that the active inhibitors are able to form a hydrogen bond between the pyridine nitrogen and the backbone NH of the hinge residue Met793 (EGFR), and Met1082 (IGF-1R), respectively (Fig. 1a–d). The different orientation results from the varying size of the gatekeeper residue (Thr790 in EGFR and Met1079 in IGF-1R). The different gatekeeper residues and the size of the hydrophobic back

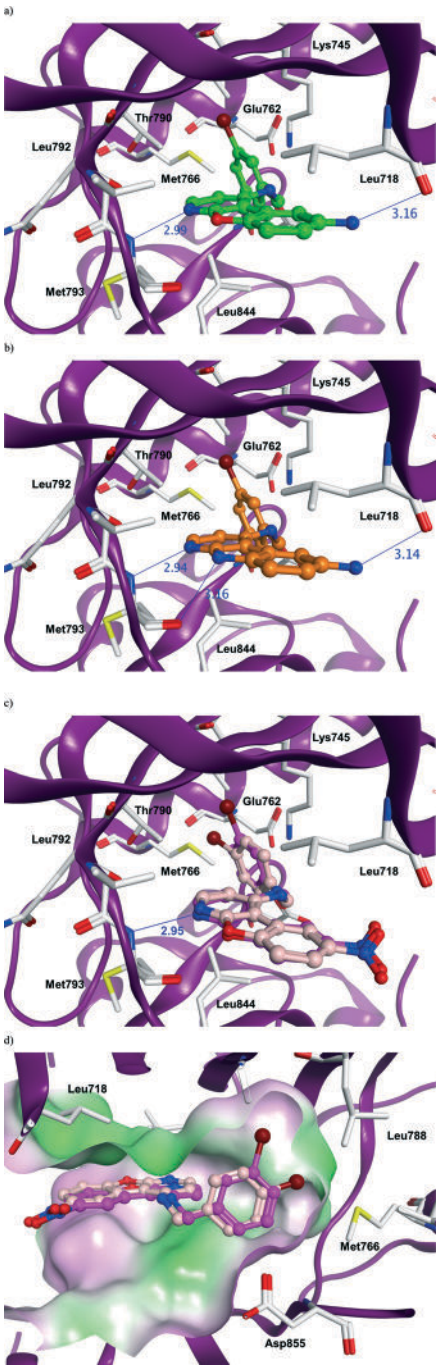


Fig. 1 Docking results for EGFR. a) The pyridine ring is accepting a hydrogen bond from the hinge backbone (NH of Met793). The 3-*meta*-bromobenzyl ring of **8e** (green) is placed in the hydrophobic pocket nearby the gatekeeper (formed by Met766, Leu788 and Thr790), whereas the 6-amino group is donating a hydrogen bond to the backbone of the P-loop residue Leu718. b) A similar binding mode is observed for the pyrrolopyridine derivatives (*e.g.* the one shown in orange is **14c**). A second hydrogen bond is observed between the pyrrolo NH and the carbonyl group of Met793. Hydrogen bond distances are given in Å. c) In the case of the 6-nitro derivatives (*e.g.* the one shown in magenta is **7e** and in pink is **7f**), both 3-*meta* and 4-*para*-substituted benzyl groups can be accommodated in the hydrophobic pocket nearby the gatekeeper. d) Molecular surface of the EGFR binding pocket – hydrophobic parts are colored green while hydrophilic regions are colored magenta. The docked inhibitors **7e** and **7f** are shown.

pocket are mainly responsible for the observed selectivity of some of the inhibitors.

The docking results for EGFR show that the pyridine ring of the inhibitors from the four series is accepting a hydrogen bond from the hinge backbone (NH of Met793). In the case of the 6-amino substituted derivatives (series **8** and **14**), a hydrogen bond to the backbone of the P-loop residue Leu718 is further fixing the position of the inhibitors. In this position, a substitution at the benzyl ring is only sterically possible in the 3-*meta* position resulting in favourable van der Waals interactions with Met766, Leu788 and Thr790 (Fig. 1a and b). In the case of the 4-*para* substitution (*i.e.* **8b**, **8d**, **8f**), a steric clash with Met766 and Leu788 would occur. In the case of the 6-nitro derivatives, this hydrogen bond is not possible resulting in a position that is slightly moved out of the pocket (Fig. 1c and d). As a consequence, both 3-*meta*- and 4-*para*-substituted benzyl groups can be accommodated in the hydrophobic pocket nearby the gatekeeper.

The docking results for IGF-1R show that similar hydrogen bonds exist between the furopyridine and pyrrolopyridine rings with the hinge region residues. The hydrophobic pocket nearby the gatekeeper Met1079 shows a different size from the pocket in EGFR. However, also here the docking of 3-*meta*-substituted benzyl derivatives gave more favourable results as shown for compound **13c** in Fig. 2a. In the case of the 4-*para*-substituted benzyl derivatives, the hydrophobic ring is adopting a different conformation that is less buried (Fig. 2b). This might explain the inactivity of some of the 4-*para*-substituted compounds (*i.e.* **8b**, **8d**, **8f**).

Next we initially screened the anticancer activity of our most promising compounds in various cancer cells lines. As discussed above, EGFR and its exogenous ligands have been reported to be predominantly overexpressed in breast cancer and non-small lung cancer. EGFR overexpression has been reported to occur in 30% of the breast cancer types.[25] We selected cell lines BT-549 and HS 578T as two breast cancer cell lines with proven EGFR-expression rates.[25,26] Furthermore, two non-small lung cancer cell lines have been selected for our initial screening. For both cell lines, an EGFR-overexpression has been documented.[27] Cellular growth inhibitory activity has been determined for one given concentration to estimate the benefit of an effective EGFR inhibition for our compounds. We selected the dual EGFR/IGF-1R inhibitors **8a** and **13b** and the only EGFR-inhibiting compound **8e**. The results for percent growth inhibition at the given screening concentration using the sulforhodamine fluorescence assay are shown in Table 2.

In the breast cancer cell line BT-549, we found growth inhibition for our compounds ranging from 29% to 43%. In the other breast cancer cell line HS 578T, we found a difference in the compound activities. Our submicromolar EGFR inhibitors **8a** and **8e** showed a poorer activity with a growth inhibition of just 16%, whereas our nanomolar EGFR inhibitor **13b** with the best IGF-1R inhibiting properties resulted in a growth inhibition of 82%.[28] In the non-small lung cancer cell lines HOP-62 and NCI-H460, we found similar differences

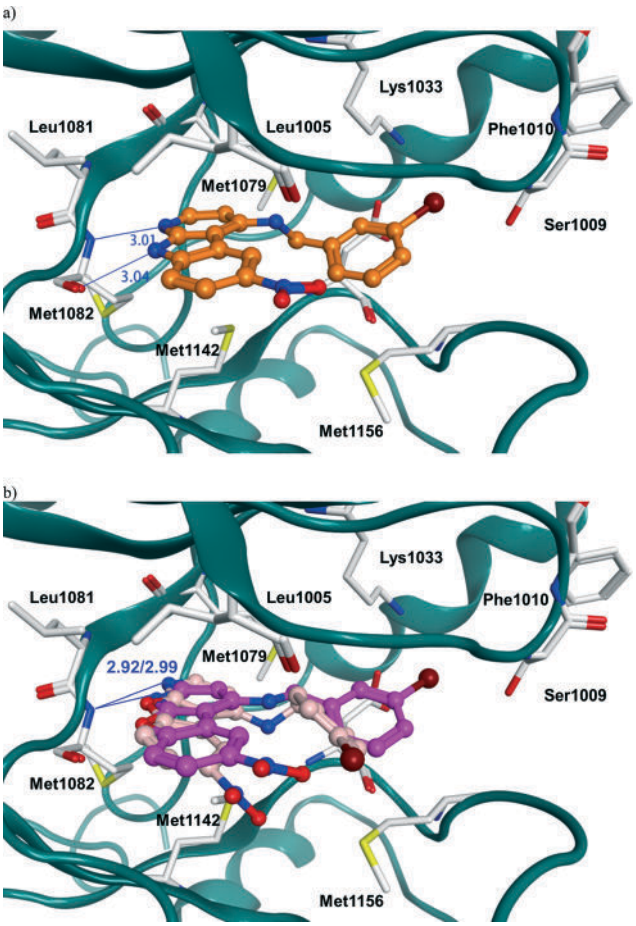


Fig. 2 Docking results for IGF-1R. a) The pyridine ring is accepting a hydrogen bond from the hinge backbone (NH of Met1082). A second hydrogen bond is observed between the pyrrolo NH and the carbonyl group of Met1082. The 3-*meta*-bromobenzyl ring of **13c** (orange) is placed nearby the gatekeeper residue. b) Docking results for the furopyridine derivatives **7e** (magenta) and **7f** (pink). In the case of the 4-*para*-substituted benzyl derivative **7f**, the hydrophobic benzyl ring is adopting a different position that is less buried.

in the activity of our submicromolar inhibitors **8a** and **8e** to the nanomolar inhibitor **13b**. We found a growth inhibition of 18% and 23% for our non IGF-1R inhibiting EGFR inhibitor **8e**. The slightly improved EGFR inhibitor with the submicromolar IGF-1R affinity of compound **8a** resulted in a

Table 2 Tumor cell growth inhibition at a concentration of 10 μM of the respective inhibitors **8a**, **8e** and **13b** in screened breast cancer and non-small lung cancer cell lines

Cancer cell line	Compound growth inhibition (%)		
	8a	**8e**	**13b**
Breast cancer			
BT-549	43	29	30
HS 578T	16	16	82
Non-small lung cancer			
HOP-62	28	18	89
NCI-H460	29	23	73

growth inhibition of 28% and 29%. Our nanomolar EGFR inhibitor **13b** with the comparably best IGF-1R affinity caused a growth inhibition of 89% and 73%.

Our first screening results prove that our inhibitor **13b** with the best dual affinities towards EGFR and IGF-1R may become a promising drug candidate to combat cell growth in non-small lung cancer.

Conclusion

It can be concluded that our novel furo- and pyrrolopyridines show EGFR and IGF-1R inhibiting activities which depend on both the benzylamino and the molecular scaffold substitutions. For the newly synthesized compound **15** of the furopyridine type with a 4-methoxybenzylamine substituent and without a substituent in the 6-position of the molecular scaffold,[29] we characterized the EGFR and IGF-1R inhibiting properties, yielding 1.43 μM for EGFR-wt and 1.03 μM for IGF-1R. That compound could have been screened for a first selective kinase inhibition in a novel project. The compound has been investigated to inhibit two EGFR mutants EGFR-T790M and -L858R with both determined inhibition values >100 μM, so that the compound was more sensitive to the wild type EGFR. Further screening efforts included EGFR-related kinases HER2 and HER4 as well as the related receptor tyrosine kinases JAK2 and 3 and TIE-2 with similar values >100 μM. If screened against a few other kinases from partly different kinase families VEGFR2 and 3, PDGFR-β and GSK-3β, we found no activity for the compound with inhibition values >100 μM as determined. So we can document the first selectivity to EGFR-wt and IGF-1R inhibition for this novel compound class.

In our discussed structure–activity compound studies, we identified the first dual inhibitors of both kinases with submicromolar and nanomolar affinities, which showed growth inhibition in EGFR-related breast and non-small lung cancer cell lines. With reference to the reported EGFR- and IGF-1R heterodimerization in such a non-small-lung cancer tumor, our dual inhibitor **13b** characterized to inhibit non-small lung cancer cell growth may become an attractive compound for further drug development.

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References

1 E. G. Giancotti, *FEBS Lett.*, 2014, **588**, 2559.
2 A. H. Bild, G. Yao, J. T. Chang, Q. Wang, A. Potti, D. Chasse, M.-B. Joshi, D. Harpole, J. M. Lancaster, A. Berchuck, J. A. Olson Jr., J. R. Marks, H. K. Dressman, M. West and J. R. Nevins, *Nature*, 2006, **439**, 353.
3 T. Wada and J. M. Penninger, *Oncogene*, 2004, **23**, 2838.
4 N. Normanno, A. De Luca, C. Bianco, L. Strizzi, M. Mancino, M. R. Macello, A. Carotenuto, G. De Feo, F. Caponigro and D. S. Salomon, *Gene*, 2006, **366**, 2.
5 M. K. Needergard, C. J. Hedegaard and H. S. Poulsen, *BioDrugs*, 2012, **26**, 83.
6 H. Masuda, D. Zhang, C. Bartholomeusz, H. Doihara, G. N. Hortobagyi and N. T. Ueno, *Breast Cancer Res. Treat.*, 2012, **136**, 331.
7 G. Metro, G. Finocchiaro, L. Toschi, S. Bartolini, E. Magrini, A. Cancellieri, R. Trisolini, L. Castaldini, G. Tallini, L. Crino and F. Cappuzzo, *Rev. Recent Clin. Trials*, 2006, **1**, 1.
8 V. Grünwald and M. Hidalgo, *Curr. Probl. Cancer*, 2002, **26**, 109.
9 J. Zhang, P. L. Yang and N. S. Gray, *Nat. Rev. Cancer*, 2009, **9**, 28.
10 E. Martinelli, R. De Palma, M. Orditura, F. De Vita and F. Ciardiello, *Clin. Exp. Immunol.*, 2009, **158**, 1.
11 H. Yasuda, E. Park, Ch. H. Yun, A. R. Lucena-Araujo, W. L. Yeo, M. S. Hubeman, D. W. Cohen, S. Nakavama, K. Ishioka, N. Yamaguchi, M. Hanna, G. R. Qxnard, C. S. Lathan, T. Moran, L. V. Sequist, J. E. Chaft, G. J. Riely, M. E. Arcila, R. A. Soo, M. Meyerson, M. J. Eck, S. S. Kobayashi and D. B. Costa, *Sci. Transl. Med.*, 2014, **6**, 225.
12 M. D. Siegelin and A. C. Borczuk, *Lab. Invest.*, 2014, **94**, 129.
13 C. M. Lovly, L. Horn and W. Pao, *My Cancer Genome*, 2015, https://www.mycancergenome.org/content/disease/lung-cancer/egfr/.
14 R. Sordella, D. W. Bell and J. Settleman, *Science*, 2004, **305**, 1163.
15 M. Ladanyi and W. Pao, *Mod. Pathol.*, 2008, **21**(Suppl. 2), S16.
16 D. G. Pfister, *J. Clin. Oncol.*, 2013, **31**, 3487.
17 A. Lange, A. Prenzler, M. Frank, M. Kirstein, A. Vogel and J. M. von Schulenburg, *Eur. J. Cancer*, 2014, **50**, 40.
18 Y. J. Yu-Jing, C. M. Zhang and Z. P. Liu, *Anti-Cancer Agents Med. Chem.*, 2012, **12**, 391.
19 S. Bugge, S. J. Kaspersen, S. Larsen, U. Nonstad, G. Bjoroy, E. Sundby and B. H. Hoff, *Eur. J. Med. Chem.*, 2014, **75**, 354.
20 F. Morgillo, J. J. Woo, E. S. Kim, W. K. Hong and H.-Y. Lee, *Cancer Res.*, 2006, **66**, 10100.
21 D. D. Wang, L. Ma, M. P. Wong, V. H. F. Lee and H. Yan, *PLoS One*, 2015, **10**, e0128360.
22 S. R. Hubbard and W. T. Miller, *Curr. Opin. Cell Biol.*, 2007, **19**, 117.
23 J. Schlessinger, *Cell*, 2000, **103**, 211.
24 T. Pawson, G. D. Gish and P. Nash, *Trends Cell Biol.*, 2001, **11**, 504.
25 M. E. Irwin, K. L. Mueller, N. Bohin, Y. Ge and J. L. Boerner, *J. Cell. Physiol.*, 2011, **226**, 2316.
26 J. L. Martin, H. C. de Silva, M. Z. Lin, C. D. Scott and R. C. Baxter, *Mol. Cancer Ther.*, 2014, **13**, 316.
27 Y.-W. Zhang, B. Staal, Y. Su, P. Swiatek, P. Zhao, B. Cao, J. Resau, R. Sigler, R. Bronson and G. F. Vande Woude, *Oncogene*, 2007, **26**, 269.
28 The increased inhibitory activities of compound **13b** may be plausible with the additional IGF-1R inhibitory properties referred to in the literature.[26] A stimulation of EGFR

expression had been documented under an insulin-like growth factor-binding protein which is known to act also *via* IGF-1R,[26] so that an IGF-1R inhibition may increase an EGFR-inhibiting effect. The only EGFR-inhibiting compound gefitinib had been reported to result in an only 20% cellular growth inhibition of HS 578T at a concentration of 4 μM.[26] That comparison may support the favour of a dual EGFR/IGF-1R inhibition as shown for our compound **13b**.

29 *N*-(4-Methoxybenzyl)benzofuro[2,3-*b*]pyridine **15**. Yield 0.08 g (61%); beige solid; mp 148–154 °C; ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 4.56 (d, J = 6.0 Hz, 2H, CH_2), 6.49 (d, J = 5.9 Hz, 1H, 3-H), 6.88 (d, J = 8.4 Hz, 2H, 2′-, 6′-H), 7.33 (d, J = 8.4 Hz, 2H, 3′-, 5′-H), 7.37–7.48 (m, 3H, 6-, 7-, *N*H), 7.63 (d, J = 7.9 Hz, 1H, 8-H), 7.91 (d, J = 5.9 Hz, 1H, 2-H), 8.43 (d, J = 7.5 Hz, 1H, 5-H); MS (EI), m/z = 304 [M^+]; IR (KBr): 3319, 1604, 1584, 1514, 1467, 746 cm^{-1}. Anal. ($C_{19}H_{16}N_2O_2$) Calc. C 75.0, H 5.3, N 9.2; found C 75.4, H 5.4, N 8.8.

