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Novel Chimeric Histone Deacetylase Inhibitors: A Series of Lapatinib Hybrides as Potent Inhibitors of Epidermal Growth Factor Receptor (EGFR), Human Epidermal Growth Factor Receptor 2 (HER2), and Histone Deacetylase Activity

Siavosh Mahboobi,*[,†] Andreas Sellmer,[†] Matthias Winkler,[†] Emerich Eichhorn,[†] Herwig Pongratz,[†] Thomas Ciossek,[‡] Thomas Baer,[‡] Thomas Maier,[‡] and Thomas Beckers*[,§]

[†]*Department of Pharmaceutical Chemistry I, University of Regensburg, D-93040 Regensburg, Germany,* [‡]*Nycomed GmbH, Therapeutic Area Oncology, Byk-Gulden Strasse 2, D-78467 Konstanz, Germany, and* [§]*Oncotest GmbH, Institute for Experimental Oncology, Am Flughafen 12-14, D-79108 Freiburg, Germany*

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Reversible lysine-specific acetylation has been described as an important posttranslational modification, regulating chromatin structure and transcriptional activity in the case of core histone proteins. Histone deacetylases (HDAC) are considered as a promising target for anticancer drug development, with **2a** as pan-HDAC inhibitor approved for cutanous T-cell lymphoma therapy and several other HDAC inhibitors currently in preclinical and clinical development. Protein kinases are a well-established target for cancer therapy with the EGFR/HER2 inhibitor **5** approved for treatment of advanced, HER2 positive breast cancer as a prominent example. In the present report, we present a novel strategy for cancer drug development by combination of EGFR/HER2 kinase and HDAC inhibitory activity in one molecule. By combining the structural features of **5** with an (*E*)-3-(aryl)-*N*-hydroxyacrylamide motif known from HDAC inhibitors like **1** or **3**, we obtained selective inhibitors for both targets with potent cellular activity (target inhibition and cytotoxicity) of selected compounds **6a** and **6c**. By combining two distinct pharmacologically properties in one molecule, we postulate a broader activity spectrum and less likelihood of drug resistance in cancer patients.

Introduction

Carcinogenesis and tumor suppression are controlled by genetic as well as epigenetic events. Unlike genetic aberrations, epigenetic changes in cancer cells such as DNA hypermethylation and histone deacetylation are largely reversible alterations. Histone deacetylase (HDAC[a]) class 1 and 2 enzymes with 11 isoforms are considered as promising targets for anticancer drug development.[1] Various inhibitors of HDAC class 1 and/or class 2 enzymes (HDACi) are currently evaluated in clinical phase 1 and 2 trials.[2−6]

Substrates of HDAC enzymes are core histone proteins H2A/B, H3, and H4, highly conserved proteins complexed with DNA and building up chromatin. Histone proteins are post-translationally modified by various means, including methylation and acetylation of N-terminal lysine residues. The reversible lysine-specific acetylation of core histone proteins has been well described as a mechanism to regulate chromatin structure, transcription factor accessibility, and finally transcriptional activity. Negatively charged DNA binds to positively charged lysine residues in the amino-terminal tails of histones, which is abrogated by lysine N-terminal acetylation.[7] HDAC isoenzymes are essential for deacetylating histone proteins, allowing tight binding of DNA and formation of a closed, transcriptionally inactive chromatin conformation. Inhibitors of HDAC reactivate gene expression but also affect acetylation of nonhistone targets which have been described.[8] On a cellular level, HDACi's reactivate tumor suppressor genes exhibit cell cycle arresting and pro-apoptotic properties.[9] Several HDACi's exhibiting the hydroxamic acid or benzamide motif as a Zn^{2+} complexing headgroup are currently in preclinical and clinical development (Figure 1, compounds 1−4) as represented by the hydroxamic acid analogues "**1** (PXD101)",[10] **2a** (phase II), "**2b** (PCI(CRA)-24781)"[11] (phase II), "3 (LBH589),"[12]or the benzamide analogue "**4** (SNDX/MS 275)" (phase II),[9] to name just a few.

Inhibitors of protein tyrosine or serine/threonine kinases are another well described drug class. The human epidermal growth factor receptor (EGFR, HER1) and the family member HER2 (c-erbB2) have been linked to various human malignancies like breast, head and neck, pancreatic, colon, gastric, and nonsmall cell lung cancer. Small synthetic inhibitors (SMI) as well as monoclonal antibodies have been developed for cancer therapy. The SMI **5** is one example

*To whom correspondence should be addressed. For medicinal chemistry (S.M.): phone, (+49) (0) 941 943 4824; fax, (+49) (0) 941-9431737; E-mail, siavosh.mahboobi@chemie.uni-regensburg.de. For pharmacology (T.B.): phone, (+49) (0) 761 5155916; fax, (+49) (0) 761 5155955; E-mail, Thomas.Beckers@oncotest.de.

[a] Abbreviations: AMC, 7-amino-4-methylcoumarin; ATCC, American Type Culture Collection; BOP, 1-benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate; CDK, cell division kinase; CML, chronic myeloid leukemia; HDACi, histone deacetylase inhibitor; HDAC, histone deacetylase; nd, not determined; InsR, insulin receptor; NSCLC, nonsmall cell lung cancer; PDGFR, platelet derived growth factor receptor; Plk1, polo-like kinase 1; PKA, protein kinase A; rHDAC, recombinant HDAC; SAHA, suberoylanilide hydroxamic acid; SDS, sodium dodecylsulfate; SDS-PAGE, SDS polyacrylamide gel electrophoresis; SMI, small molecule inhibitor; TSA, trichostatin A.

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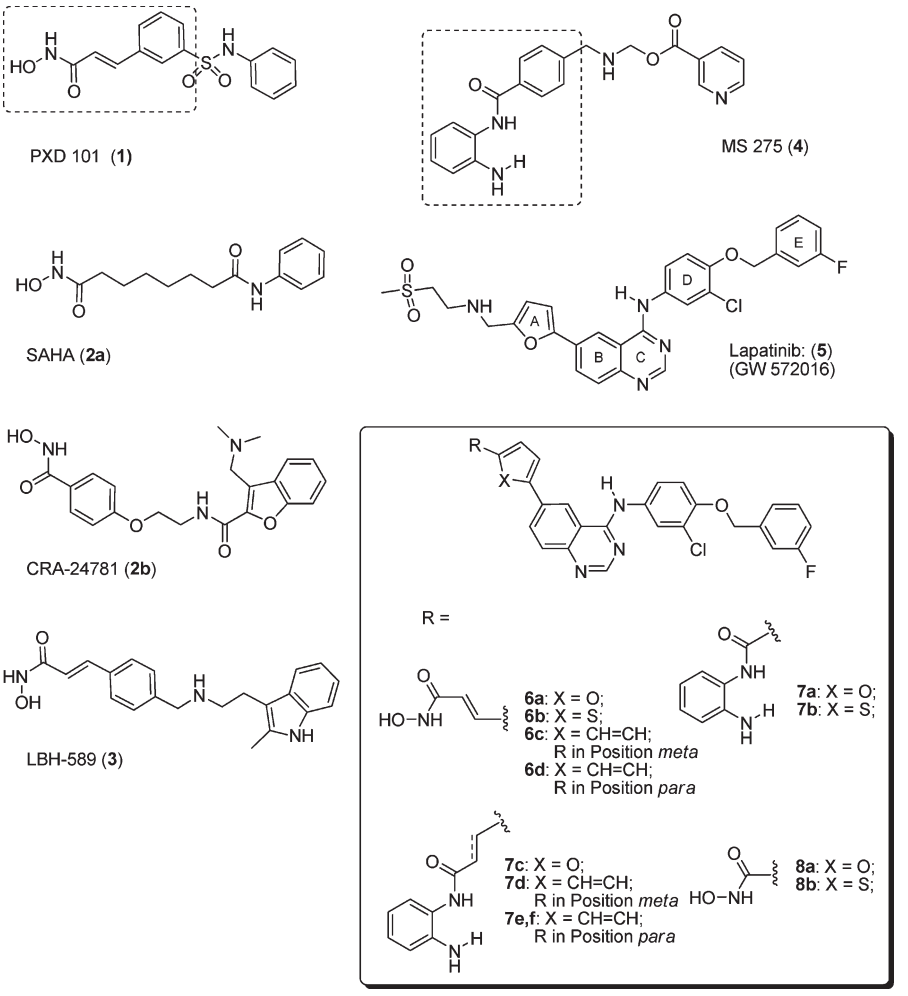


Figure 1. Chemical structures of selected HDACi's in preclinical and clinical developments (**1**−**4**), **5**, and novel chimerically designed compounds (**6**−**8**).

approved for treatment of HER2 positive advanced breast cancer in combination with Capecitabine.[13]

In this report, we present a novel strategy for cancer drug development, combining the pharmacological activity of EGFR/HER2 kinase inhibition with inhibition of HDAC class 1/2 enzymes. As a scaffold, **5** was selected to include a HDAC inhibitory headgroup ("warhead") in order to combine, in general, the antiproliferative activity of EGFR/HER2 inhibition with the pro-apoptotic, transcriptional reprogramming activity of a HDACi. Of the various HDAC inhibitory headgroups, we choose the hydroxamic acid and benzamide motifs for combination with the [3-chloro-4-(3-fluorobenzyloxy)phenyl]quinazolin-4-yl-amine core structure of **5** to obtain chimerically designed compounds.

Chemistry

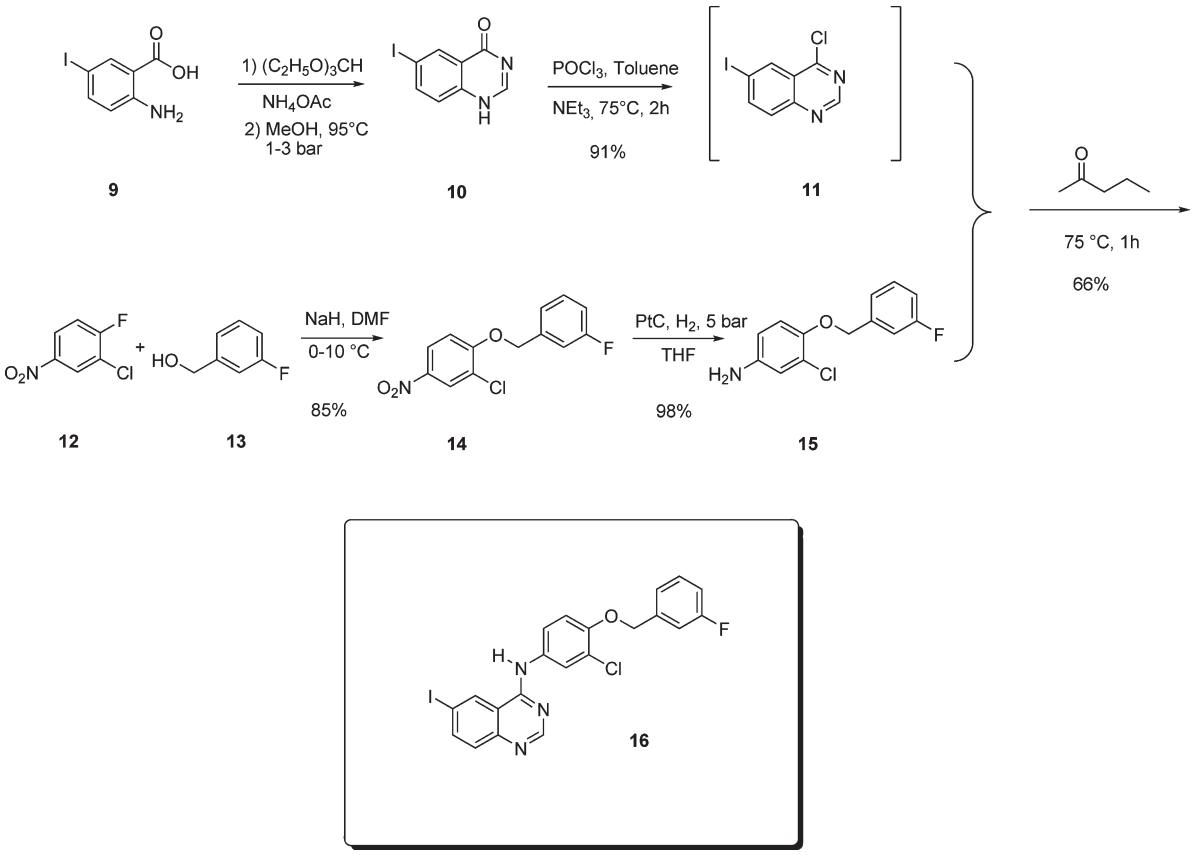
The synthetic route to obtain the desired target compounds is given in the following (Scheme 1): ring closure of 2-amino-5-iodo benzoic acid (**9**) according to Ninishino[14] formed the 1*H*-quinazolin-4-one system **10**. Chlorination with phosphorus oxychloride and one-pot S_N-reaction[15] by 3-chloro-4-(3-fluoro-benzyloxy)phenylamine (**15**), yielded the desired [3-chloro-4-(3-fluorobenzyloxy)phenyl]-(6-iodo quinazolin-4-yl)amine (**16**) as a central intermediate. The aniline derivative **15** was easily accessible from its nitro-precursor **14**[16] by catalytic reduction with hydrogen over sulfided platinum.[16] The nitro-precursor **14** itself was prepared according to Wallace et al.[16] by S_{NAr}-reaction of 2-chloro-1-fluoro-4-nitrobenzene and (3-fluorophenyl)methanol in DMF solution.

The desired furanyl- and thiophenyl- and phenyl-*N*-hydroxy-acrylamides **6a**−**6d** (Scheme 2) were obtained from **16** by Suzuki coupling with the respective formylboronic acids **17a**−**17d** analogous to Hosoya,[17] Wittig olefination,[18] and deprotection of the resulting acrylic acid *tert*-butyl esters (**19a**−**19d**) with trifluoroacetic acid. Amidation of the acrylic acids **20a**−**20d** with commercially available NH_2OTHP (*O*-(tetrahydropyran-2-yl)hydroxylamine) by use of BOP (1-benzotriazolyloxy-tris-(dimethylamino)phosphoniumhexafluorophosphat) as coupling reagent[19] and cleavage of the tetrahydro-pyran-2-yl protected acrylamides **21a**−**21d** led to the *N*-hydroxy-acrylamides **6a**−**6d**.

Analogously, the carboxylic acid (2-aminophenyl)amides **7a** and **7b** as well as the carboxylic acid hydroxyamides **8a** and **8b** were prepared as shown in Scheme 3 by amidation of the carboxylic acids with NH_2OTHP or the mono protected phenylendiamine **25**, followed by deprotection with trifluoroacetic acid (for details, see Experimental Section). The mono protected phenylendiamine **25** itself was easily accessible by reaction of *o*-phenylendiamine (**24**) with BOC_2O in THF solution.[20]

The (*E*)-*N*-(2-aminophenyl)-3-(furan-2-yl)acrylamide **7c** as well as the *N*-(2-aminophenyl)cinnamamides **7d** and **7e** were

Scheme 1. Preparation of [3-Chloro-4-(3-fluorobenzyloxy)phenyl]-(6-iodoquinazolin-4-yl)amine (**16**) As a Central Intermediate



obtained in a similar manner by amidation[19] of the acrylic acids **20a**−**20d** with the mono protected phenylendiamine **25**[20] and deprotection of the *tert*-butyl carbamates (**28a**, **28c**, and **28d**) with trifluoroacetic acid (Scheme 4). Selective catalytic hydrogenation in presence of a benzyloxy- and a chloroarylgroup by use of PtO_2 yielded the *N*-(2-aminophenyl)-3-phenylpropanamide **7f** without additional cleavage.

Results and Discussion

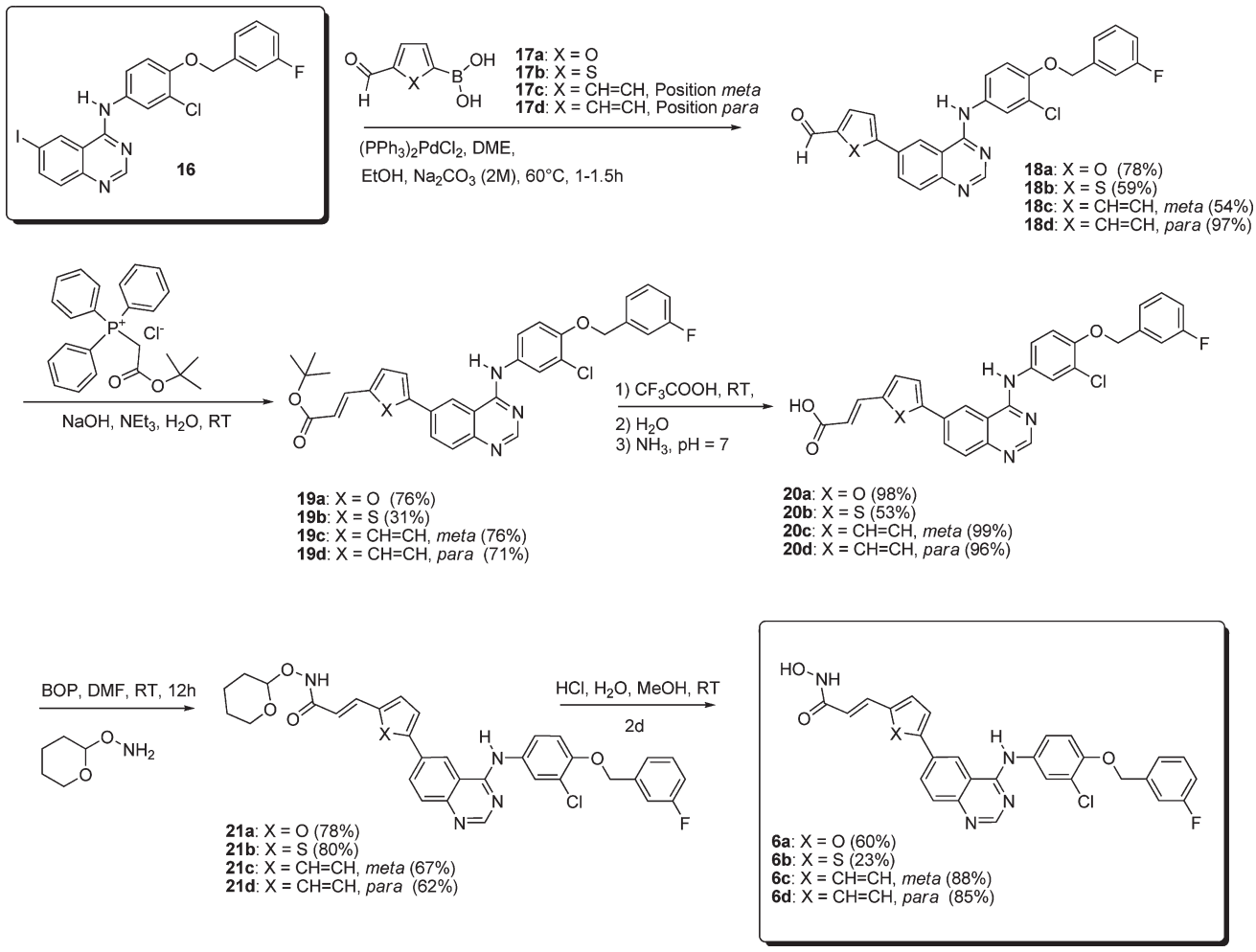
The synthesized chimeric compounds were characterized in biochemical assays for HDAC as well as protein-kinase inhibitory activity. In addition, cellular target specific activity was assessed in respective cancer cell lines. Finally, the cytotoxic activity was determined in EGFR or HER2 overexpressing cancer cell lines for selected analogues.

Pharmacological Profile in Biochemical and Cellular, Target-Specific Assays. The in vitro data for inhibition of HeLa nuclear extract HDAC activity, a mixture of HDAC isoenzymes 1, 2, 3, 5, and 8, recombinant rHDAC 1, 3, and 6 as well as cellular data for induction of histone H3 hyperacetylation are compiled in Table 1. As references, **5** as well as **2a** and **4** are included as representatives for hydroxamate and benzamide analogues. Combining the structural features a [3-chloro-4-(3-fluorobenzyloxy)phenyl]quinazolin-4-ylamine (substructure of **5** in Figure.1) with an (*E*)-3-(aryl)-*N*-hydroxyacrylamide motif taken from **1** (Figure 1; compounds **6a**−**6c**) leads to inhibition of nuclear extract HDAC, rHDAC1 and rHDAC6 in the submicromolar (**6a**) or even nanomolar range (**6c**) as well as cellular histone H3 hyperacetylation. Compounds **6a** to **6c** also display very potent and specific inhibition of EGFR and HER2 kinase activity in biochemical assays, with **6c** being only 2- to 5-fold less potent as Lapatinib (Table 2). The protein kinases PDGFRβ, InsR, Abl, CDK2, PKA, and Plk1 were chosen to evaluate kinase target selectivity.

A change of the substitution patterns from meta (**6c**) to para substitution drastically reduced HDAC inhibitory potency, as seen for **6d** and the bioisosteric built up thiophene derivative **6b**, while inhibition of EGFR and HER2 kinases was not affected. In modifications bearing the *N*-(2-aminophenyl)-benzamide substituent as a HDACi motif taken from **4**, the potent inhibition of EGFR and HER2 kinase activity and selectivity are conserved. However, no significant HDAC inhibitory activity is evident for benzamide analogues **7a** and **7b**, structurally related to **8a** and **8b**.

To investigate the effect of the system vinylogous to benzamides, included in the HDAC-inhibitory structures **6a** and **6c**, we extended the study to the vinylogous benzamides **7c**−**7e** as well as to benzamide **7f** and the arylhydroxamates **8a** and **8b**. In the resulting benzamide and arylhydroxamate series, EGFR and HER2 kinases were strongly inhibited (Table 2). Regarding inhibition of HDAC enzymes, no activity is exhibited by benzamides **7a**−**7f**, independent from the substitution patterns (ortho or para), the nature of the A-ring system (Figure.1) in the substructure of **5**, or from the linker between the benzamide structure and the A-ring system. This result is in contrast to our findings, published earlier, where combination of a benzamide motif with the imatinib pharmacophore led to chimeric HDAC class I inhibitors.[21] Weak inhibition of HeLa nuclear extract HDAC, rHDAC 1, 3, and 8 class I enzymes activity with stronger inhibition of rHDAC6 as a class 2 representative was observed for the hydroxamate **8b**. These compounds were potent inhibitors of EGFR and HER2 with presumably less selectivity (Table 2).

Scheme 2. Synthesis of *N*-Hydroxyacrylamides **6a**−**6d**



Cytotoxic Properties toward EGFR and HER2 Expressing Cancer Cell Lines. All compounds were tested for cytoxicity toward cancer cell lines either with EGFR (A431, Cal27) or HER2 (SKBR3, SKOV3) overexpression or of relevance for HDAC inhibitor testing (HeLa, A549). The data are summarized in Table 3, showing highly potent activity of **5** in the Cal27, SKBR3, and SKOV3 cell lines with a bottom plateau of the concentration−effect curve between 30% and 70%. In the EGFR overexpressing cal27 head and neck cancer cell line, **5** also strongly inhibited cellular EGF stimulated EGFR phosphorylation (IC_{50} < 32 nM) with no effect on histone H3 acetylation (Figure 2B). *N*-hydroxyacrylamide analogues **6a** and **6c** were most interesting from the initial in vitro profiling experiments. Whereas **6a** potently inhibited EGF stimulated EGFR phosphorylation in Cal27 cells (IC_{50} < 32nM), **6c** was less potent ($IC_{50} \approx 0.5$ μM) (Figure 2A). Nevertheless, **6c** was nicely inducing histone H3 hyperacetylation at 1 μM, behaving as a cellularly active, pharmacologically bifunctional compound. In the cytotoxicity profile, **6c** was broadly active with IC_{50} values ≤1 μM (Table 3) and a complete cell kill at higher concentrations.

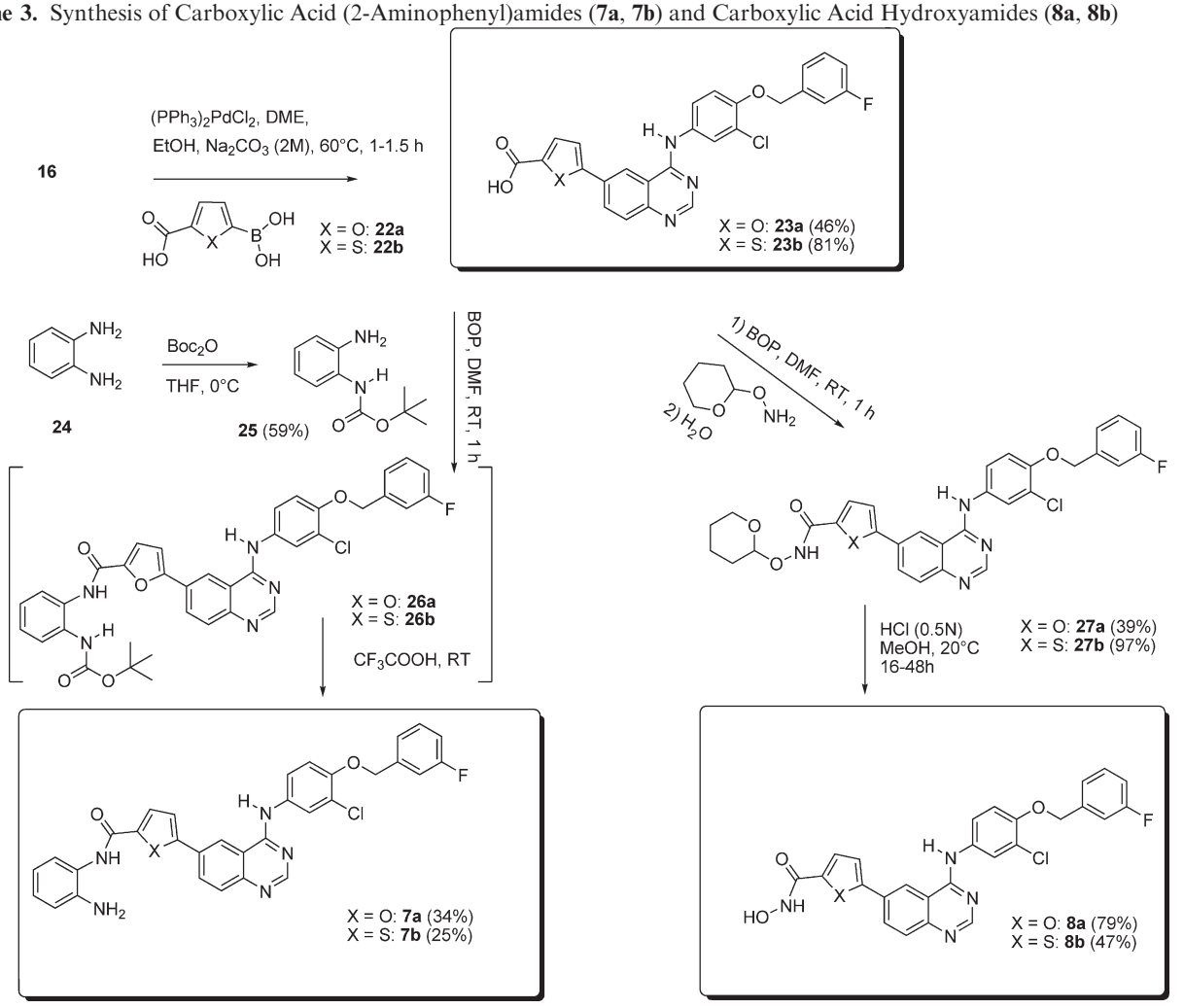
Conclusions

Transferring the (*E*)-3-(aryl)-*N*-hydroxyacrylamide motif of **5**, which is an EGFR/HER protein kinase inhibitor and a drug approved for treatment of advanced, HER2 positive breast cancer, leads to chimeric compounds with **6c** acting as truly pharmacologically chimeric compound. In biochemical assays, this prototypic analogue shows selective and potent inhibiton of EGFR/HER2 as well as HDAC enzymatic activity. In cellular assays exemplified by the head and neck cell line Cal27, EGFR phosphorylation and histone H3 acetylation are concentration dependently inhibited and induced, respectively. Between 0.1 and 1 μM concentration, maximal target inhibitory effects are visible, correlating with an IC_{50} of 0.59 μM in the cytotoxicity assay. However, transferring the HDAC inhibitory benzamide motif so far resulted in compounds with conserved kinase but no HDAC inhibitory activity. By combination of two pharmacologically distinct properties in one molecule, we postulate a broader activity spectrum with less likelihood of drug resistance. Thus, chimeric HDAC-kinase inhibitors might constitute a new class of experimental cancer drugs worth to be studied in more detail.

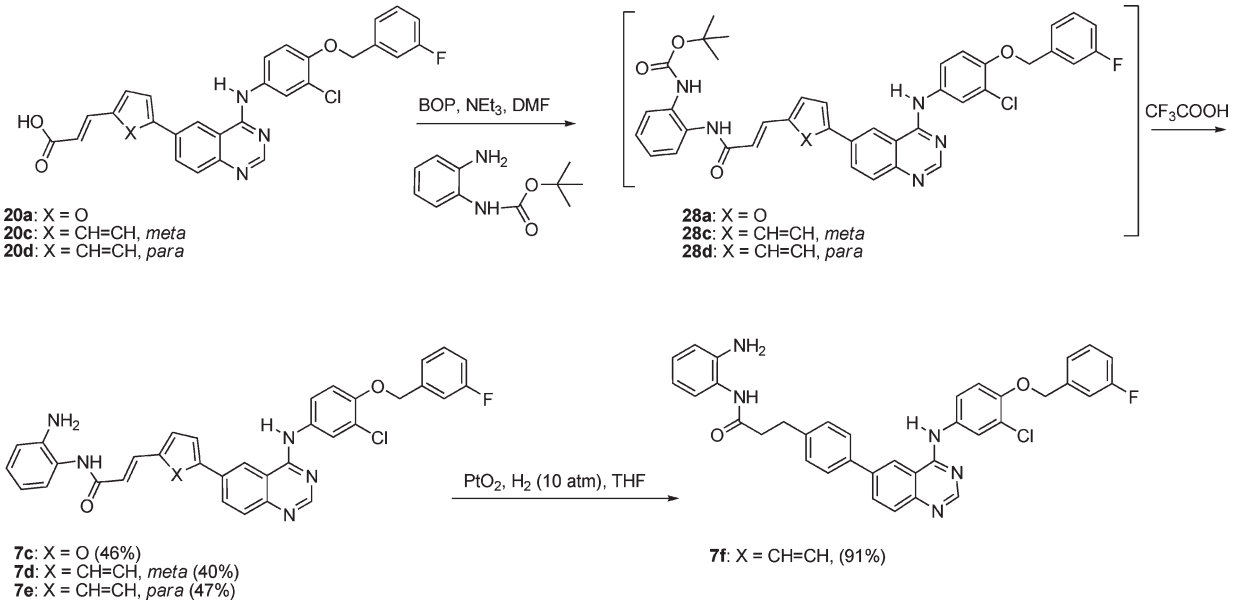
Experimental Section

Biological Methods. Biochemical HDAC Assays. For rHDAC1 and rHDAC6 expression, a clonal HEK293 (ATCC CRL1573) human kidney cell line expressing the human rHDAC1 isoenzyme bearing a C-terminal Flag epitope was provided by E. Verdin, The Gladstone Institute/San Francisco, CA, USA. Human HDAC3-Flag was coexpressed with the SMRT DAD domain in Sf21 insect cells, whereas human HDAC8-Flag was expressed

Scheme 3. Synthesis of Carboxylic Acid (2-Aminophenyl)amides (**7a**, **7b**) and Carboxylic Acid Hydroxyamides (**8a**, **8b**)

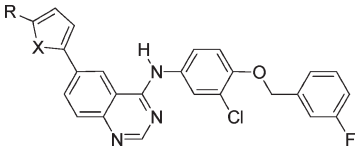


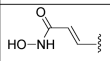
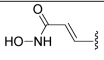
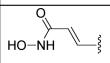
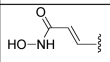
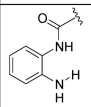
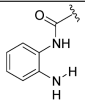
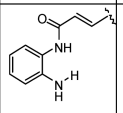
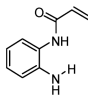
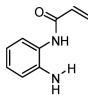
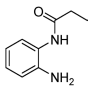
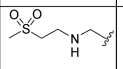
Scheme 4. Synthesis of Phenylaminoacrylamides (**7c**−**7e**) and of *N*-(2-Aminophenyl)-3-(4-(4-(3-chloro-4-(3-fluorobenzyloxy)-phenylamino)quinazolin-6-yl)phenyl)propanamide (**7f**)



without cofactor in Sf21 cells. The rHDAC proteins were purified by M2-affinity gel chromatography according to the manufacture protocol (Sigma no. A2220). Purified protein samples were routinely analyzed by SDS-PAGE (12.5% or 10% Laemmli gels) followed by Coomassie stain and Western blotting using a FLAG-specific antibody (anti M2-POX antibody,

Table 1. IC_{50}/EC_{50} Values for Chimeric Lapatinib-HDAC Inhibitors As Tested for Inhibition of HeLa Nuclear Extract HDAC Activity, Recombinant HDAC Isoenzymes, and Induction of Cellular Histone H3 K^{9+14} Hyperacetylation [a]



No.	R	X	HDAC inhibition IC_{50} [μM]	rHDAC 1 inhibition IC_{50} [μM]	rHDAC 3 inhibition IC_{50} [μM]	rHDAC 6 inhibition IC_{50} [μM]	rHDAC 8 inhibition IC_{50} [μM]	$H3K^{9+14}$ EC_{50} [μM]
6a		O	0.63	0.61	6.30	0.23	11	3.65
6b		S	50	24	> 32	1.2 (max 70%)	19	>100
6c		CH=CH *meta*	0.047	0.035	0.066	0.086	0.63	4.70
6d		CH=CH *para*	> 32	0.65	> 32	0.23	31	> 10
7a		O	> 100	> 50	> 32	> 10	> 32	>100
7b		S	>10 (max. 20%)	> 50	> 32	> 10	> 32	>100
7c		O	> 32	> 100	> 32	> 32	> 32	n.d.
7d		CH=CH *meta*	> 32	> 100	> 32	>32	> 32	≥20
7e		CH=CH *para*	>32	> 100	> 32	>32	> 32	> 100
7f		CH=CH *para*	> 32	> 32	> 32	>32	> 32	n.d.
8a		O	8.3 (max 30%)	> 50	> 32	9.8	7.7	>100
8b		S	0.43 (max. 65%)	0.94	0.56 (max. 75 %)	0.05	0.98 (max. 70 %)	>100
5		O	> 100	> 10	> 10	> 10	> 10	>30
2a			0.07	0.021	0.037	0.025	1.2	6.67
4			11.0	0.082	0.62	>30	≥ 25	2.35

[a] In general, experiments were done in replicate and mean values are shown (data for **4** and **2a** from ref 22).

Sigma no. A8592) followed by ECL-detection (GE Healthcare). In addition, protein batches were analyzed by Western blotting for various HDAC isoenzymes including HDAC1, 3, 6, and 8.

The biochemical HDAC activity assay was essentially done as described by Wegener et al.[23]

Biochemical Protein Kinase Assays. Active kinase proteins were either obtained from commercial suppliers (ProQinase,

Table 2. Inhibition of Selected Recombinant Protein Tyrosine and Serine/Threonine Kinases in Biochemical Assays, Namely Epidermal Growth Factor Receptor (EGFR), Human EGFR-Like Receptor 2 (HER2), Platelet-Derived Growth Factor Receptor β (PDGF-Rβ), Insulin Receptor (InsR), Abl1, Cell Division Kinase 2 (CDK2), Protein Kinase A (PKA), and Polo-Like Kinase 1 (PlK1)[a]

no.	EGFR IC_{50} [μM]	HER2 IC_{50} [μM]	PDGF-Rβ IC_{50} [μM]	InsR IC_{50} [μM]	Abl1 IC_{50} [μM]	CDK2 IC_{50} [μM]	PKA IC_{50} [μM]	PlK1 IC_{50} [μM]
6a	0.0025	0.0041	3.3	7.34	7.7	0.44	120	12.0
6b	0.0048	0.0035	1.1	1.9	2.7	0.44	35	2.7
6c	0.018	0.011	2.5	3.6	5.3	0.92	> 100	6.80
6d	0.018	0.012	2.3	3.7	4.9	0.85	> 100	9.1
7a	0.0029	0.0057	4.0	1.3	4.5	15.0	> 100	3.9
7b	0.0028	0.0049	2.3	2.5	6.0	2.1	> 100	5.6
7c	0.026	0.0087	3.9	5.5	4.9	1.9	> 100	8.5
7d	0.049	0.014	2.5	2.8	2.9	0.85	> 100	5.5
7e	0.029	0.015	2.7	3.0	4.9	1.2	> 100	6.2
7f	0.015	0.0047	4.7	7.1	6.9	1.4	> 100	27
8a	0.0039	0.0020	1.0	1.4	1.7	0.46	85	2.0
8b	0.0022	0.0035	1.8	1.7	1.7	0.2	> 100	2.9
5	0.0029	0.0045	8.50	17.0	23.0	11.0	> 100	> 100

[a] In general, experiments were done in replicate and mean IC_{50} values are shown.

Freiburg/Germany; InvitroGen/Panvera, Carlsbad, CA, USA) or prepared in-house. Experimental details of the flash-plate based radioactive enzyme assay have been described previously.[21]

Cellular Histone H3 Hyperacetylation Assay. To assess the cellular efficacy of a histone deacetylase inhibition, an assay was set up for use on the Cellomics "ArrayScan II" platform for a quantitative calculation of histone acetylation as described.[24]

Cellular Proliferation Assay. The antiproliferative activity of selected compounds was evaluated using the tumor cell lines HeLa (cervical carcinoma, ATCC CCL-2), A549 (NSCLC, ATCC CCL-185), SKBR-3 (breast carcinoma, ATCC HTB-30), SKOV-3 (ovarian carcinoma, ATCC HTB-77), Cal27 (tongue carcinoma, ATCC CRL-2095), and A-431 (vulva carcinoma, ATCC CRL-2592). For quantification of cellular proliferation/cytotoxicity, the Alamar Blue (Resazurin) cell viability assay was applied.[25]

Western Blot Analysis. For Western blot analysis, about 4×10^5 Cal27 cells/well in 6-well cell culture plates were treated with the test compounds for 16 h. Next, cells were stimulated with 100 ng/mL recombinant EGF for 5 min at room temperature before cell lysis. Cells were lysed in lysis buffer (50 mM Tris HCl pH8, 150 mM NaCl, 1v/v NP-40, 0.5% w/v sodium desoxycholate, 0.2% w/v disodium dodecylsulfate (SDS), 0.02% w/v NaN_3, 1 mM sodium vanadate, 20 mM NaF, 100 μg/mL PMSF, 10 mM sodium pyrophosphate, protease inhibitor mix/Roche and 50U/mL Benzonase) at 4 °C. Respective equal amounts of protein were separated by SDS-PAGE before transfer to polyvinylidendifluoride (PVDF) membrane (Biorad art. no. 162−0177) by semidry blotting. The following antibodies were used: monoclonal mouse antibody specific for β-actin (clone AC-12, Sigma art. no. A-5441), phosphorylated EGFR (Y1068, Cell Signaling), EGFR (Upstate), acetylated histone H3 (Cell Signaling), and histone H3 (Cell Signaling). As secondary antibodies, goat antirabbit IgG-HRP conjugated (Biorad: 170−6515), goat-antisheep (Santa Cruz) and goat-antimouse IgG-HRP conjugated (Biorad: 170-6516) were used.

Chemical Procedures. General. NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. IR spectra (KBr or pure solid) were measured with a Bruker Tensor 27 spectrometer. Melting points were determined with a Büchi B-545. MS spectra were measured with a Finnigan MAT 95 (EI, 70 eV), or with a Finnigan Thermo Quest TSQ 7000 (ESI) (DCM/MeOH + 10 mmol/L NH_4Ac), respectively. All reactions were carried out under nitrogen atmosphere. Elemental analyses were performed by the Analytical Laboratory of the University of Regensburg and are in a range of ±0.4% of the calculated values if not given otherwise, ensuring a purity ≥95%. Chemical names were created using ChemBioDraw Ulta 11.0 software.

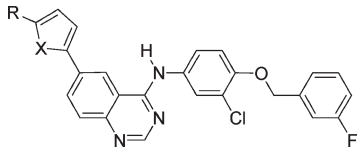
Procedures and Analytical Data. 3-Chloro-4-(3-fluorobenzyloxy)phenylamine (15). Preparation analogous lit.[16] as follows: 2-chloro-1-(3-fluorobenzyloxy)-4-nitrobenzene (**14**) (20.0 g; 70.9 mmol) was dissolved in THF (300 mL), dry sulfided platinum on carbon (Aldrich) (4.00 g) were added and the mixture was stirred under a hydrogen atmosphere (10 atm) overnight. The platinum was filtered off over celite, washed with THF, the solvent removed under reduced pressure and the residue treated with light petrol to afford the title compound as an analytically pure solid. Yield (17.49 g; 98%). ^{1}H NMR (DMSO-d_6) δ (ppm) 4.98 (s, 2H, exchangeable), 5.04 (s, 2H), 6.54 (dd, 1H, J = 8.8 Hz, ^4J = 2.6 Hz), 6.74 (d, 1H, ^4J = 2.6 Hz), 6.92 (d, 1H, J = 8.8 Hz), 7.09−7.15 (m, 1H), 7.25−7.30 (m, 2H), 7.37−7.45 (m, 1H).

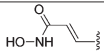
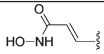
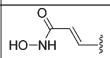
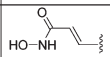
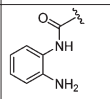
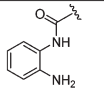
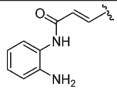
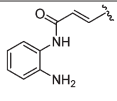
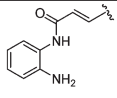
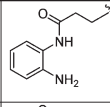
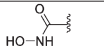
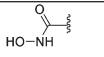
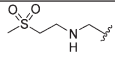
[3-Chloro-4-(3-fluorobenzyloxy)phenyl]-(6-iodoquinazolin-4-yl)amine (16).[26] Preparation analogous to lit.[15] as follows: To a mixture of 6-iodo-1*H*-quinazolin-4-one (**10**) (6.80 g; 25.0 mmol), toluene (5.0 mL), and $POCl_3$ (27.5 mmol; 2.60 mL), triethylamine (27.5 mmol; 3.81 mL) was added carefully. The mixture was heated to 80 °C for 2 h, cooled to room temperature, a solution of 3-chloro-4-(3-fluorobenzyloxy)phenylamine (**15**) (27.50 mmol; 6.92 g) in 2-butanone (20.0 mL) added, and the mixture stirred at 80 °C for another hour. The mixture was cooled to 0 °C, and the yellow precipitate was filtered off and added to a NaOH solution (1N; 150 mL) by stirring. After 30 min, the yellow solid was filtered off, washed with water and a small amount of acetone, and dried in vacuo. Yield (8.38 g; 66%) analytical pure sample. ^{1}H NMR (DMSO-d_6) δ (ppm) 5.26 (s, 2H), 7.15−7.22 (m, 1H), 7.27 (d, 1H, J = 9.1 Hz), 7.29−7.35 (m, 2H), 7.43−7.51 (m, 1H), 7.56 (d, 1H, J = 8.8 Hz), 7.74 (dd, 1H, J = 9.1 Hz, ^4J = 2.5 Hz), 8.02 (d, 1H, ^4J = 2.5 Hz), 8.12 (dd; 1H, J = 8.8 Hz, ^4J = 1.7 Hz), 8.62 (s, 1H), 8.96 (d, ^4J = 1.7 Hz), 9.90 (s, 1H, exchangeable).

General Procedure for Suzuki Coupling. Preparation analogous to Hosoya.[17] A mixture of **16** (5.00 g; 9.88 mmol), the respective arylboronic acid (**17a**, **17b**, **22b** Aldrich; **17c**, **17d** Alfa Aesar; **22a** Rare Chemicals) (13.1 mmol), $(PPh_3)_2PdCl_2$ (0.35 g; 0.5 mmol), DME (30 mL), ethanol (20 mL), and 2 M aqueous Na_2CO_3 (30 mL) was heated at 60 °C for 3 h. After cooling to room temperature, the crude product precipitated as a yellow solid. It was removed by filtration, washed with water, and dried in vacuo overnight. Crystallization from acetone afforded the title compound in sufficient purity for further synthesis. An analytical sample was obtained by column chromatography (SiO_2; CH_2Cl_2/ethyl acetate = 4:1).

Furane- and Benzene-carbaldehydes (18a, 18c). 5-{4-[3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl}furan-2-carbaldehyde (18a).[26] Yield (3.65 g, 78%); mp 229.8−234.1 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 5.26 (s, 2H), 7.17 (dt, 1H, J = 2.5 Hz, J = 8.5), 7.26−7.34 (m, 3H), 7.40 (d, 1H, J = 3.6 Hz), 7.43−7.50

Table 3. Cytotoxicity of the Chimeric HDAC Inhibitors Towards Selected Cancer Cell Lines, Namely A549 NSCL and HeLa Cervical Cancer (Source for HDAC Nuclear Activity), A431 Epidermoid Carcinoma, and Cal27 Head and Neck Cancer (EGFR Overexpression) and SKOV3 Ovarian and SKBR3 Breast Cancer (HER2 Overexpression)[a]



No.	R	X	A549 IC_{50} [μM]	HeLa IC_{50} [μM]	A431 IC_{50} [μM]	Cal27 IC_{50} [μM]	SKOV3 IC_{50} [μM]	SKBR3 IC_{50} [μM]
6a		O	3.9	3.2	1.9	0.82	2.1	0.51
6b		S	2.6	1.2	0.73	0.66	4.0	1.1
6c		CH=CH *meta*	1.2	0.97	0.72	0.59	1.0	0.35
6d		CH=CH *para*	1.1	0.92	0.51	0.28	1.2	0.33
7a		O	≥16	8.0	>5.0	0.33	>50	1.3
7b		S	9.1	3.7	2.4	0.71	3.9	0.62
7c		O	18.0	6.5	5.3	3.6	22	0.26
7d		CH=CH *meta*	27.0	8.4	12	>16	36	>50
7e		CH=CH *para*	15.0	3.1	3.6	4.0	16	17
7f		CH=CH *para*	22.0	8.3	8.6	0.2	13	4.4
8a		O	>16	9.8	>5	0.2	15	0.27
8b		S	3.4	2.9	1.9	0.65	3.1	0.42
5		O	8.5	5.9	0.97	0.007 (max 75%)	0.003 (max 70%)	0.002 (max. 30%)
2a			1.8	1.5	4.2	3.2	2.2	2.6

[a] In general, experiments were done in replicate and mean IC_{50} values are shown (data for **2a** from Beckers et al., 2007[22]).

(m, 1H), 7.68−7.74 (m, 2H), 7.84 (d, 1H, J = 8.5 Hz), 7.98 (d, 1H, J = 2.5 Hz), 8.28 (dd, 1H, J = 1.1 Hz, J = 8.8 Hz), 8.58 (s, 1H), 8.95 (d, 1H, J = 0.8 Hz), 9.66 (s, 1H), 10.10 (s, 1H, exchangeable). +p ESI m/z (%): 476 $[M + H^+]^{+\cdot}$ (37); 474 $[M + H]^+$ (100). −p ESI m/z (%): 474 $[M - H^+]^-$ (37), 472 $[M - H]^-$ (100). IR (KBr): 3399, 1673 cm^{-1}.

3-{4-[3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl}benzaldehyde (18c). Yield (2.73 g, 54%) yellow crystals; mp 243.2−247.5 °C. ^1H NMR (DMSO-d_6) δ (ppm) 5.25 (s, 2H), 7.18 (dt, 1H, J = 2.2 Hz, J = 8.3 Hz), 7.28 (d, 1H, J = 9.1 Hz), 7.33 (m, 2H), 7.47 (dt, 1H, J = 6.1 Hz, J = 8.0 Hz), 7.74 (dd, 1H, J = 2.2 Hz, J = 6.7 Hz), 7.78 (t, 1H, J = 5.4 Hz), 7.86 (d, 1H, J = 8.7 Hz), 7.97 (td, 1H, J = 1.2 Hz, J = 7.6 Hz), 8.01 (d, 1H, J = 2.6 Hz), 8.21 (m, 2H), 8.38 (t, 1H, J = 1.6 Hz), 8.60 (s, 1H), 8.85 (d, 1H, J = 1.6 Hz), 9.94 (s, 1H, exchangeable), 10.14 (s, 1H). EI-MS (70 eV) m/z (%): 483 (18) $[M^{+\cdot}]$, 374 (100) $[M\text{-}C_7H_6F^{\cdot}]^+$. IR (KBr): 3380, 2729, 1696 cm^{-1}. Anal. ($C_{28}H_{19}ClFN_3O_2 \cdot {}^1/_5H_2O$): C, H, N.

Acrylic Acid *tert*-Butyl Esters (19a−d). A mixture of the respective aryl-2-carbaldehydes **18a−18d** (1.0 mmol), (*tert*-butoxycarbonylmethyl)triphenylphosphonium chloride (0.56 g; 1.02 mmol), NaOH (0.08 g; 2.04 mmol), and NEt_3 (0.3 g; 3.06 mmol) in CH_2Cl_2 (100 mL) and water (2.04 mL) was stirred at room temperature overnight. The organic layer was separated,

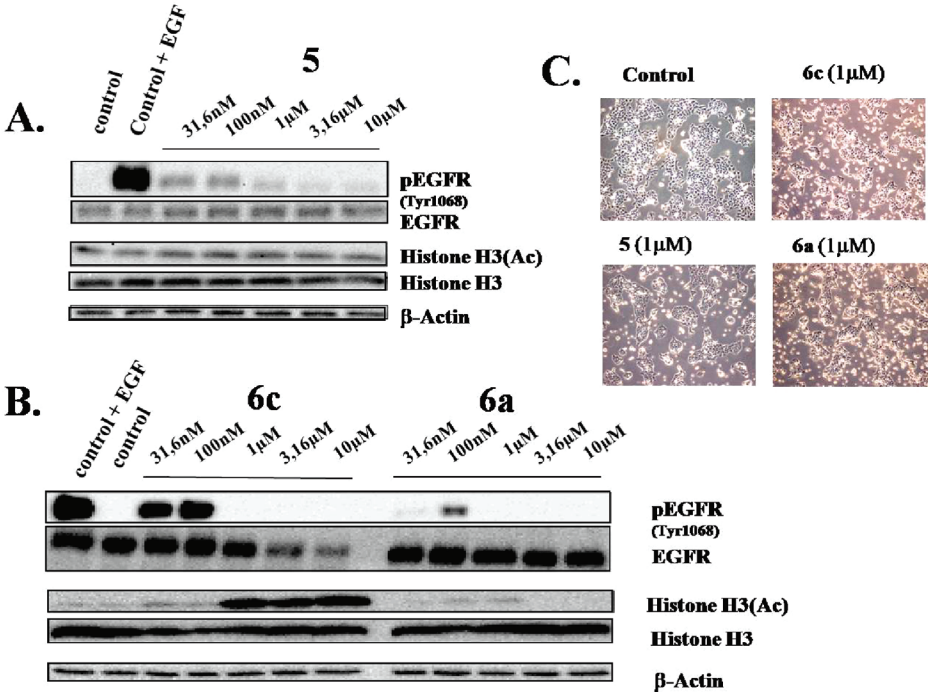


Figure 2. Western blot analysis of Cal27 head and neck cancer cells treated for 16 h with compounds **5** (A) and **6c** or **6a** (B). As a control, cells treated with 1 μM **5**, **6a**, or **6c** were documented by phase contrast microscopy in (C).

subsequently washed with satd NH_4Cl solution (3 × 50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (DCM/EE = 1:1) and precipitated by addition of light petrol.

3-{5-[4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl}furan-2-yl)acrylic Acid *tert*-Butyl Ester (19a). Yield (0.43 g, 76%) yellow crystals; mp 201.3−201.4 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 1.50 (s, 9H), 5.28 (s, 2H), 6.50 (d, 1H, J_{trans} = 15.6 Hz), 7.13 (d, 1H, J = 3.6 Hz), 7.19 (dt, 1H, J = 2.4 Hz, J = 8.6 Hz), 7.28−7.36 (m, 4H), 7.44 (d, 1H, J_{trans} = 15.6 Hz), 7.45−7.52 (m, 1H), 7.72 (dd, 1H, J = 2.5 Hz, J = 8.8 Hz), 7.82 (d, 1H, J = 8.8 Hz), 7.99 (d, 1H, J = 2.5 Hz), 8.29 (dd, 1H, J = 1.5 Hz, J = 8.6 Hz), 8.57 (s, 1H), 8.87 (d, 1H, J = 1.1 Hz), 9.97 (s, 1H, exchangeable). +p ESI m/z (%): 574 [M + H$^+$] (39), 572 [M + H$^+$] (100%). −p ESI m/z (%): 572 [M − H$^+$]$^-$ (40), 570 [M − H$^+$]$^-$. IR (KBr): 2977, 1697 cm^{-1}. Anal. ($C_{32}H_{27}ClFN_3O_4$): C, H, N.

3-{-[4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl)phenyl)acrylic Acid *tert*-Butyl Ester (19c). Yield (2.30 g, 76%) colorless crystals; mp 202.9−203.0 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 1.51 (s, 9H), 5.27 (s, 2H), 6.73 (d, 1H, J = 16.0 Hz), 7.19 (dt, 1H, J = 2.2 Hz, J = 8.2 Hz), 7.32 (m, 3H), 7.48 (m, 1H), 7.60 (t, 1H, J = 7.7 Hz), 7.69 (d, 1H, J = 16.0 Hz), 7.76 (m, 2H), 7.87 (d, 1H, J = 8.7 Hz), 7.94 (d, 1H, J = 7.9 Hz), 8.03 (d, 1H, J = 2.6 Hz), 8.20 (m, 1H), 8.29 (dd, 1H, J = 1.7 Hz, J = 8.7 Hz), 8.60 (s, 1H), 8.83 (d, 1H, J = 1.5 Hz), 9.90 (s, 1H, exchangeable). EI-MS (70 eV) m/z (%): 581 (18) [M$^{+\cdot}$], 236 (100) [M-$C_{11}H_{14}F^{\cdot}$]$^+$. IR (KBr): 3379, 2982, 1676 cm^{-1}. Anal. ($C_{34}H_{29}ClFN_3O_3$) C, H, N.

Synthesis of Acrylic Acids (20a−20d) by Cleavage of the Acrylic Acid *tert*-Butyl Esters. The respective acrylic acid *tert*-butyl ester (3.50 mmol) was dissolved in 10 mL TFA. The mixture was stirred at 20 °C for 30 min, added to water (50 mL) by stirring, and the yellow precipitate filtered off, washed with water, and dried in vacuo.

3-{5-[4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl}furan-2-yl)acrylic Acid (20a). Yield (0.18 g, 99%) yellow crystals; mp 268.8−268.9 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 5.27 (s, 2H), 6.53 (d, 1H, J_{trans} = 15.9 Hz), 7.11 (d, 1H, J = 0 3.6 Hz). 7.18 (dt, 1H, J = 2.0 Hz, J = 8.6 Hz), 7.26−7.35 (m, 4H, aromat), 7.41−7.50 (m, 1H), 7.46 (d, 1H, J_{trans} = 15.9 Hz), 7.73 (dd, 1H, J = 2.5 Hz, J = 8.9 Hz), 7.82 (d, 1H, J = 8.8 Hz), 8.00 (d, 1H, J = 2.5 Hz), 8.27 (dd, 1H, J = 1.6 Hz, J = 8.8 Hz), 8.57 (s, 1H), 8.86 (d, 1H, J = 1.4 Hz), 9.96 (s, 1H, exchangeable), 12.45 (s, 1H, exchangeable). +p ESI m/z (%): 518 [M + H$^+$] (37), 516 [M + H$^+$] (100). −p ESI m/z (%): 516 [M − H$^+$]$^-$ (40), 514 [M + H$^+$]$^+$ (100). IR (KBr): 3406, 1673 cm^{-1}. Anal. ($C_{28}H_{19}ClFN_3O_4$) C, H, N.

3-{3-[4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl}-phenyl)acrylic Acid (20c). Yield (0.78 g, 99%) orange crystals; mp 275.1−275.2 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 5.29 (s, 2H), 6.73 (d, 1H, J = 16.0 Hz), 7.19 (dt, 1H, J = 2.2 Hz, J = 8.3 Hz), 7.33 (m, 3H), 7.48 (m, 1H), 7.62 (t, 1H, J = 7.7 Hz), 7.71 (m, 2H), 7.80 (d, 1H, J = 7.8 Hz), 7.92 (m, 2H), 7.96 (d, 1H, J = 2.5 Hz), 8.19 (s, 1H), 8.42 (dd, 1H, J = 1.6 Hz, J = 8.8 Hz), 8.83 (s, 1H), 8.94 (d, 1H, J = 1.2 Hz), 10.88 (s, 1H, exchangeable), 12.58 (s, 1H, exchangeable). ES-MS (DCM/MeOH + 10 mmol/L NH_4Ac) m/z (%): 526 (100) [M + H$^+$]$^+$. IR (KBr): 3442, 2934, 1671 cm^{-1}. Anal. ($C_{30}H_{21}ClFN_3O_3 \cdot {}^3/_4$TFA) C, H, N.

General Procedure for Amidation by Use of BOP as Coupling Reagent: Preparation of compounds 21a−21d, 26a−26b and 27a−27b. A mixture of the respective carboxylic acid (1.15 mmol), BOP (0.53 g; 1.20 mmol), the respective amine (2-aminophenyl)-carbamic acid *tert*-butyl ester[20] (**25**) or *O*-tetrahydropyran-2-yl)hydroxylamine (Aldrich)) (1.15 mmol), and NEt_3 (0.24 g; 2.40 mmol) in DMF (10 mL) was stirred at room temperature for 12 h. The solution was added to water (50 mL) by stirring, the precipitating product filtered off, washed with water, and dried in vacuo. Crystallization from the respective solvent given afforded the pure compound as yellow crystals.

(*E*)-3-(5-(4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino)quinazolin-6-yl)furan-2-yl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)acrylamide (21a). Crystallization from acetone. Yield: (0.55 g, 78%); mp 232.8−232.9 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 1.54 (s, br., 3H), 1.70 (s, br 3H), 3.54 (d, br., 1H, J = 11.0 Hz), 3.92−4.02 (m, br, 1H), 4.93 (s, 1H), 5.28 (s, 2H), 6.55 (d, 1H, J_{trans} = 15.6 Hz), 7.04 (d, 1H, J = 3.3 Hz), 7.16−7.52 (m, 7H), 7.74 (dd, 1H, J = 8.5 Hz, J = 1.4 Hz), 7.84 (d, 1H, J = 8.8 Hz), 8.01 (d, 1H, J = 2.5 Hz), 8.22 (dd, 1H, J = 8.8 Hz, J = 1.4 Hz), 8.58 (s, 1H), 8.86 (s, 1H), 9.98 (s, 1H,exchangeable), 11.30 (s, 1H, exchangeable). +p ESI m/z (%): 617 [M + H$^+$] (39), 615 [M + H$^+$] (100). −p ESI m/z (%): 615

$[M - H^+]^-$ (37), 613 $[M - H^+]^-$ (100). IR (KBr): 3295, 2950, 1651 cm^{-1}. Anal. ($C_{33}H_{28}ClFN_4O_5 \cdot {}^1/_2H_2O$) C, H, N.

(*E*)-3-(3-(4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino)quinazolin-6-yl)phenyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)acrylamide (21c). Yield (0.24 g, 67%) colorless crystals. Purification by cc (SiO_2, ethyl acetate, CH_2Cl_2 1:1) and crystallization from ethyl acetate. ^{1}H NMR (DMSO-d_6) δ (ppm) 1.55 (s, 3H), 1.71 (s, 3H), 3.54 (m, 1H), 3.96 (m, 1H), 4.94 (s, 1H), 5.27 (s, 2H), 6.68 (d, 1H, J = 15.8 Hz), 7.19 (dt, 1H, J = 2.2 Hz, J = 8.2 Hz), 7.32 (m, 3H), 7.48 (m, 1H), 7.64 (m, 3H), 7.76 (dd, 1H, J = 2.5 Hz, J = 8.9 Hz), 7.88 (d, 1H, J = 8.7 Hz), 7.92 (m, 1H), 8.03 (d, 1H, J = 2.5 Hz), 8.07 (s, 1H), 8.25 (dd, 1H, J = 1.7 Hz, J = 8.7 Hz), 8.61 (s, 1H), 8.85 (d, 1H, J = 1.1 Hz), 9.95 (s, 1H, exchangeable), 11.29 (s, 1H, exchangeable). ES-MS (DCM/MeOH + 10 mmol/L NH_4Ac) m/z (%): 625 (100) $[M + H^+]$. IR (KBr): 3444, 2872, 1668 cm^{-1}. Anal. ($C_{35}H_{30}ClFN_4O_4$) C, H, N.

Synthesis of *N*-Hydroxyacrylamides (6a−6d, 8a, and 8b from the Respective (Tetrahydropyran-2-yloxy)amide: Precursors. To a stirred solution of the corresponding (tetrahydropyran-2-yloxy)amide (0.5 mmol) in MeOH (50 mL) was added 1N HCl (50 mL). The mixture was stirred at room temperature overnight. Half of the solvent was removed under reduced pressure, and the precipitating product was filtered off, crystallized from MeOH, and dried in vacuo.

3-{5-[4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino]quinazolin-6-yl}furan-2-yl)-*N*-hydroxy-acrylamide Hydrochloride Monohydrate (6a). Yield: (0.20 g, 60%) yellow crystals; mp 190.9−192.0 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 5.32 (s, 2H), 6.62 (d, 1H, J_{trans} = 15.6 Hz), 7.01 (d, 1H, J = 3.6 Hz), 7.19 (dt, 1H, J = 2.2 Hz, J = 8.9 Hz), 7.20−7.32 (m, 4H), 7.45−7.53 (m, 2H), 7.72 (dd, 1H, J = 2.5 Hz, J = 8.8 Hz), 7.95 (d, 1H, J = 2.5 Hz), 7.98 (d, 1H, J = 8.8 Hz), 8.40 (d, 1H, J = 0.8 Hz, J = 9.1 Hz), 8.91 (s, 1H), 9.40 (d, 1H, 0.8 Hz), 10.84 (s, 1H, exchangeable), 11,98 (s, 1H, exchangeable). +p ESI m/z (%): 533 $[M + H^+]$ (38), $[M + H^+]$ (531). −p ESI m/z (%): 589 $[M + Ac^-]^-$ (91), 565 $[M + Cl^-]^-$ (51), 592 $[M - H^+]^-$ (100). IR (KBr): 3418, 2855, 1660 cm^{-1}. Anal. ($C_{28}H_{20}ClFN_4O_4 \cdot H_2O \cdot HCl$) C, H, N, Cl.

(*E*)- 3-{3-[4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino)quinazolin-6-yl)phenyl)-*N*-hydroxyacrylamide (6c). Yield (0.13 g, 88%) yellow crystals. NMR (DMSO-d_6) δ (ppm) 5.32 (s, 2H), 6.70 (d, 1H, J = 15.8 Hz), 7.20 (dt, 1H, J = 2.1 Hz, J = 8.3 Hz), 7.35 (m, 3H), 7.49 (dt, 1H, J = 6.1 Hz, J = 8.0 Hz), 7.61 (dd, 2H, J = 7.8 Hz, J = 15.5 Hz), 7.70 (m, 2H), 7.97 (m, 2H), 8.04 (d, 1H, J = 8.7 Hz), 8.15 (s, 1H), 8.51 (dd, 1H, J = 1.1 Hz, J = 8.8 Hz), 8.97 (s, 1H), 9.28 (d, 1H, J = 0.9 Hz, exchangeable), 10.85 (s, 1H, exchangeable), 11.89 (s, 1H, exchangeable). ES-MS (DCM/MeOH + 10 mmol/L NH_4Ac) m/z (%): 541 (100) $[M + H^+]$. IR (KBr): 3385, 2860, 1662 cm^{-1}. Anal. ($C_{30}H_{22}ClFN_4O_3 \cdot HCl \cdot 2H_2O$) C, H, N.

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Supporting Information Available: Full experimental details for synthetically preparations, analytical data, and biological test systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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