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Novel inhibitors of epidermal growth factor receptor: (4-(Arylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanones and (1*H*-indol-2-yl)(4-(phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanones

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ABSTRACT

Several members of the quinazoline class of known tyrosine kinase inhibitors are approved anticancer agents, often showing selectivity for receptors of the HER/ErbB-family. Combining structural elements of this class with the bisindolylmethanone-structure led to a series of novel compounds. These compounds inhibited EGFR in the nanomolar range. Moreover, inhibition of EGFR autophosphorylation in intact A431 cells was shown, with IC_{50} values ranging form 0.3–1 μM for compound **42**, and 0.1–0.3 μM for **45**. In a panel of 42 human tumor cell lines the sensitivity profile of the novel compounds was shown to be similar to that of the quinazoline class of tyrosine kinase inhibitors lapatinib and erlotinib (Tarceva®).

1. Introduction

Due to the diversity in structure, synthetic and naturally occurring indoles exhibit antibacterial, fungicide, cytotoxic and antiproliferative activity. We recently reported on 2-indolylmethanones (e.g., **1a** and **1b**) as potent inhibitors of FMS-like tyrosine kinase 3 (FLT3) and platelet-derived growth factor receptor (PDGF-R) tyrosine kinase (Fig. 1).[1] Many anticancer agents which act as tyrosine kinase inhibitors selectively inhibit receptors of the HER/ErbB-family. They comprise the pyrimidine group as a core moiety, either as part of a quinazoline increment [for example see gefitinib **2** (Iressa®, Astra Zeneca, EGFR inhibitor), erlotinib **3** (Tarceva®, Roche, EGFR inhibitor), ZD6474 **4** (Astra Zeneca, VEGFR and EGFR inhibitor), lapatinib **5** (Tykerb®, Glaxo Smith Kline, EGFR and ErbB2) and tandutinib **6** (Millenium Pharmaceuticals, FLT3 inhibitor)] (Fig. 1), or, incorporated into an indole related system as shown by PKI-166 **7** (Novartis, EGFR inhibitor).

The human epidermal growth factor receptor (EGFR, HER1) and another member of this family, HER2 (ErbB2), have been linked to various human malignancies, for example breast,[2] head and neck,[3] gastric,[4] and non-small cell lung cancer.[5–8] Chronic medication often induces selection of inhibitor-resistant mutants of tyrosine kinases, thus resulting in relapse of the tumor.[9] Consequently, we are in need of compounds with novel therapeutic profiles based on alternative binding modes, well-defined kinase selectivity, and activity against clinically relevant kinase mutants.

Therefore, we prepared a series of compounds, incorporating structural elements of the quinazoline class of known tyrosine kinase inhibitors into the bisindolylmethanone-structure and investigated their biological properties. It turned out that the arylamino-increment, taken from the quinazoline based compounds described above, was essential for biological activity. The novel compounds (Fig. 1), are potent EGFR inhibitors in vitro and in intact cells.

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§ Tel.: +49 (0) 3641 9395631.

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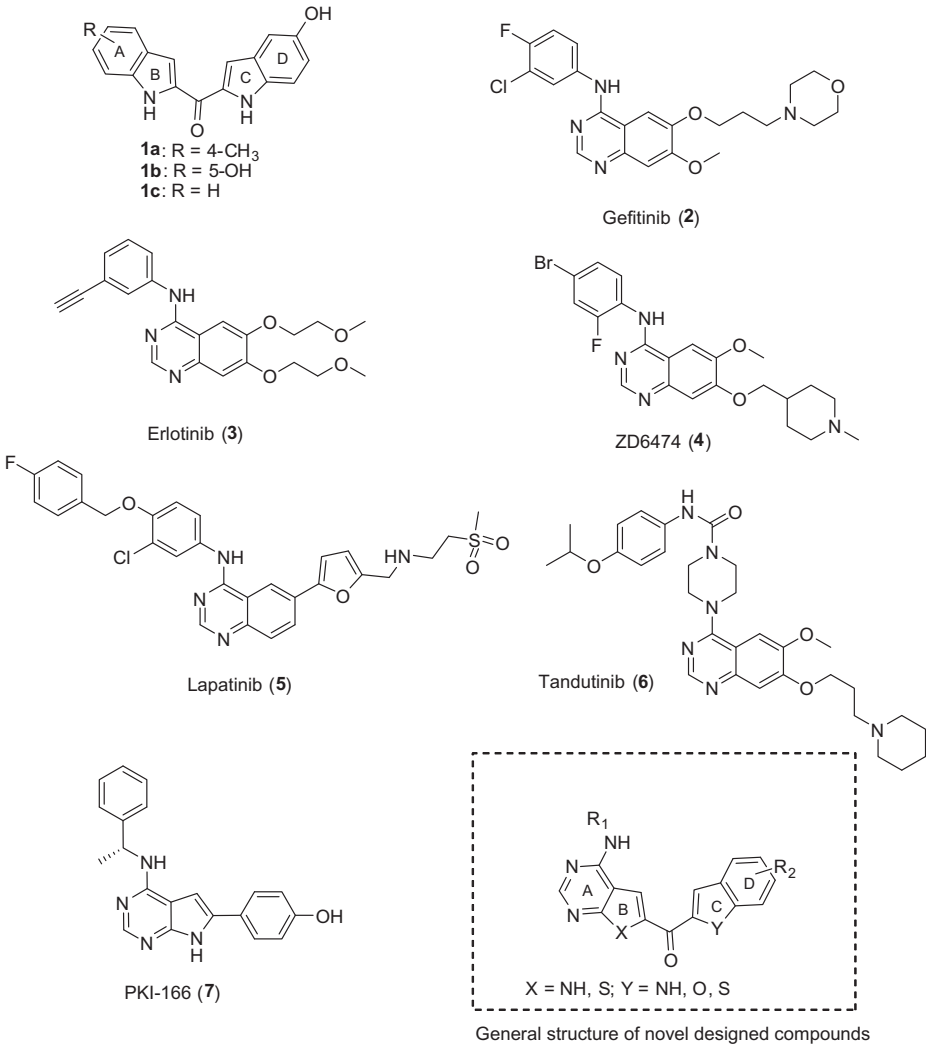


Figure 1. Structures of 2-indolylmethanones (e.g., **1a** and **1b**), well known tyrosine kinase inhibitors containing the pyrimidine core structure as part of a quinazoline system, and general structure of newly designed compounds.

2. Results and discussion

2.1. Chemistry

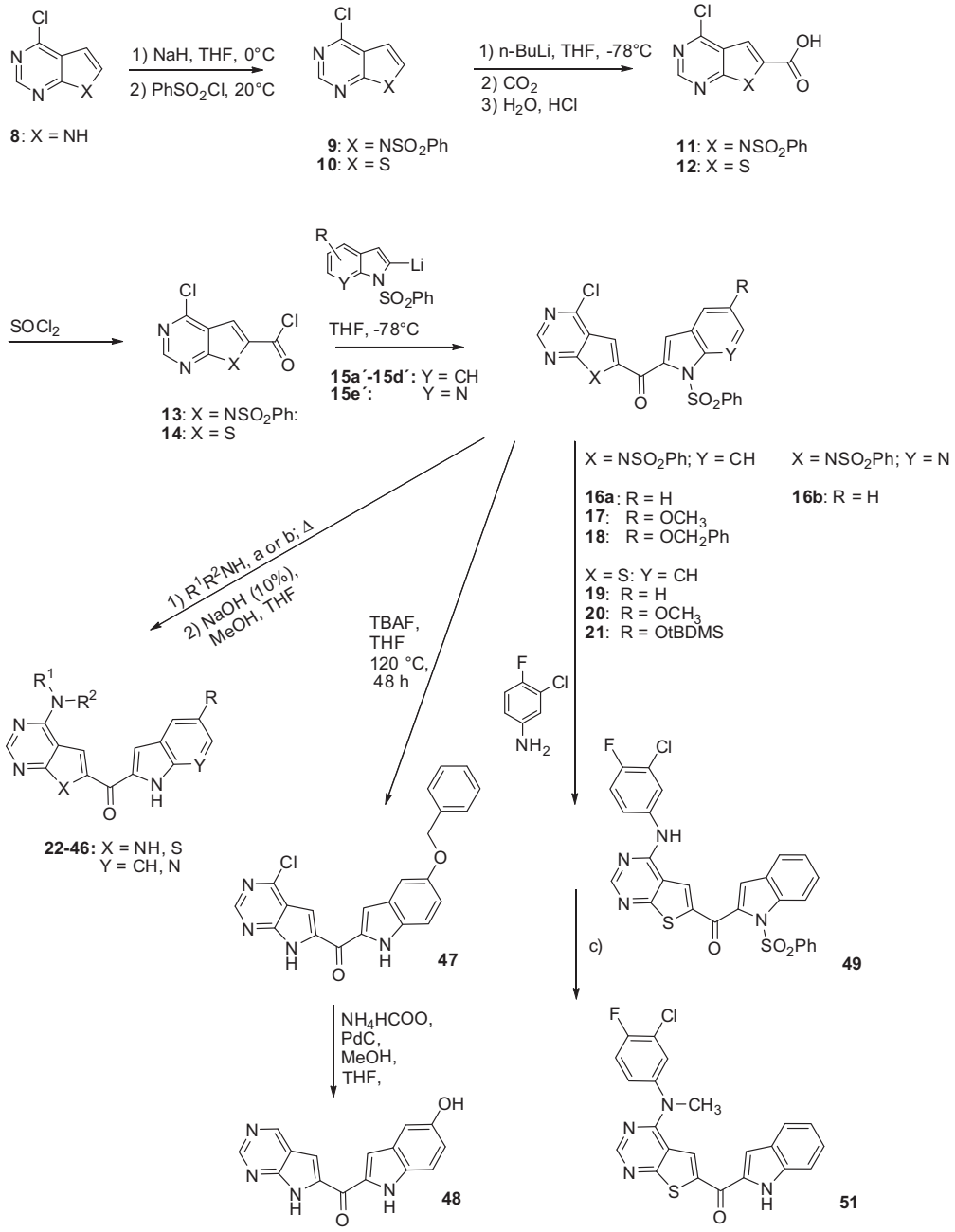
Novel compounds were synthesized as outlined in Scheme 1: Commercially available 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8**) was phenylsulfonated in THF solution using NaH as a base.[10] Lithiation of the resulting 4-chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**), or the sulfur analog 4-chlorothieno[2,3-*d*]pyrimidine (**10**) and carboxylation according to procedures described,[1] yielded the carboxylic acids **11** and **12**, respectively. Heating in an excess of thionyl chloride led to the corresponding acid chlorides and coupling with the lithiated species **15a′**–**15e′**[1] generated the central intermediates **16**–**21**. After modification by an aromatic S_N-reaction the desired compounds **22**–**46** and **51** were obtained by cleavage of the phenylsulfonyl-protecting groups. Cleavage of the phenylsulfonyl-protecting group in **18** by tetrabutylammonium fluoride in THF, followed by hydrogenolytic cleavage of the benzyloxy-group and simultaneous removing of chlorine led to **48**.

The reaction of **14** with benzofuran-2-yllithium and benzo[*b*]thiophen-2-yllithium according to Scheme 1 failed. This step was performed as shown in Scheme 2. Pd catalyzed reaction of 4-chlorothieno[2,3-*d*]pyrimidine-6-carbonyl chloride (**14**) with the 2-arylboronic acids **52** and **53**[11] led to **54** and **55**, respectively. Chlorine substitution by 3-chloro-4-fluoroaniline (**56**) led to the final compounds **57** and **58**.

2.2. Biology

In a first screening, the new compounds were checked for their activities against a panel of kinases, namely EGFR, ErbB2, VEGFR2, ABL1 wt, MET wt, and FLT3 at a concentration of 1 μM. In a second set the IC_{50} of the most potent compounds, exhibiting at least 90% inhibition of EGFR protein kinase at 1 μM was determined. The IC_{50} values reach from 5.54 nM of **44** to 43.7 nM of **43**.

The data compiled in Tables 1 and 2 show potent inhibiton of EGFR, ErbB2, and some activity at VEGFR2. No inhibitons of ABL 1 wt and MET wt were observed (not shown). Interestingly, by switching from the bisindolylemethanone system of compounds **1a**–**1c** to the (1*H*-indol-2-yl)(7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanone **48**, by introducing two nitrogen atoms into the benzene ring of one indole system, the inhibitory activity on FLT3 was completely deleted. Moreover, as shown by comparison of the data of **48** with those bearing an arylamino- or benzylamino-system connected to C-4 of ring A this substitution pattern proved to be essential for activity. By the way this modification did not restore any activity at FLT3.

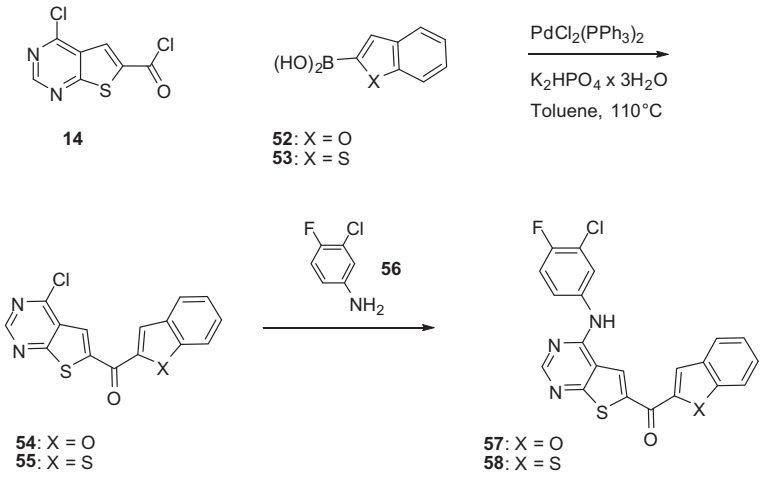


Scheme 1. Synthetic path for preparation of (4-(arylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanones and (1*H*-indol-2-yl)(4-(phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanones. For definitions of X, R, R^1 and R^2 see Tables 1 and 2. Conditions: (a) 2-butanone, 2 d; (b) *n*-butanol, 12 h; (c) (1) NaH, THF, CH_3I, (2) NaOH (10%), MeOH, THF. Formulas for **15a′**–**15e′** show the lithiated species corresponding to indole-derivatives **15a**–**15e**.

Modifications of the arylamine system itself are of minor influence. Replacement by a lipophilic substituent in position 3 of the arylamino-system, however, seems to enhance activity, as is demonstrated by compounds **23**, **24**, **25**, and **26** in comparison with **22**, **28**, **29** or **30** for X = N. Substituents at C-5 of ring D have no effect on the potency towards EGFR in this series, but reduce the activity at ErbB2 as shown by comparison of data of **25** with those of **26**. Modifications of the ring D slightly reduce the inhibition at EGFR (**27** vs **26**). In the (substituted) benzylamino compounds **31** and **32**, an additional α-methyl-group seems to be of benefit.

By introduction of sulfur instead of nitrogen in the ring B, in nearly all cases the activities at EGFR were increased (e.g. compound **34** vs **24**, **36** vs **28**, **37** vs **29**, or **38** vs **31**). Taking into account the additional influence of substitution patterns of the arylamino substituent, which in general follows the trend discussed for the series with X = N, the most active compounds **42** and **44** completely inhibit EGFR at 1.0 μM. The additional 4-fluorine substituent of the arylamino-system of **44** in comparison with **42**, enhanced the activity, as shown by the IC_{50} values (16.6 nM of **42** and 5.54 nM of **44**). To draw a comparison: the IC_{50} values of the reference compounds erlotinib and lapatinib are 3.10 and 8.90 nM, respectively.

Thus, further modifications of **44** were performed, regarding the heterocycle C, the substituent R^2 in the benzene ring D, and the alkylation of the arylamino-substituent R^1. Modification of the substituents of the ring D (H replaced by OH or OCH_3) was well tolerated for OCH_3 (compound **46**; $IC_{50\ EGFR}$ = 5.93 nM) or caused enhanced activity at VEGFR in case of **45**. However, as can be seen for **46**, N-alkylation in the arylamino substituent reduced the activity significantly (**51**). Exchange of N for S in the ring



Scheme 2. Synthesis of **57** and **58**, respectively.

C was well tolerated in **56**, introduction of oxygen in the case of **55**, however, was not.

Of compounds **42** and **45**, showing IC_{50} values of 16.6 ± 0.4 and 24.0 ± 0.2 nM, respectively, as well as most potent inhibition of ErbB2 protein kinase, EGFR autophosphorylation in intact A431 cells was determined. This phosphorylation was inhibited with IC_{50} values in a range of 0.3–1 μM (**42**) and of 0.1–0.3 μM (**45**), respectively (see Fig. 2).

To study whether the kinase inhibition by the novel compounds provokes growth inhibition in cancer cell lines, we compared a selection of the compounds exhibiting the most potent dual mode of action, namely **42, 44, 45** and **46** with lapatinib and erlotinib in 42 sensitive and resistant lines. These new compounds exhibitited at least 95% inhibition at EGFR at a concentration of 1 μM and in addition an inhibition of at least 60% at ErbB2. The cell lines represent 15 different tumor entities: bladder, colorectal, head and neck, liver, non-small cell lung, mammary, melanoma, ovarian, pancreatic, pleuramesothelioma, renal, sarcoma and urethral carcinoma.

The data of Table 3 show the sensitivity profile of **42**, **44**, **45** and **46** to be similar to those of lapatinib and erlotinib being representatives of the quinazoline class of tyrosine kinase inhibitors. The colon cancer cell line DiFi and the head and neck cancer cell line Cal27 were the most sensitive to lapatinib and to **42**, **44**, **45**, and **46**. The cell lines most sensitive for erlotinib were DiFi, Cal27, the gastric cancer cell line GXF 251L and the non-small cell lung adenocarcinoma line LXFA 629L. Sensitivity towards EGFR inhibition has been published for all four tumor models.[12,13] This may be due to amplified EGFR (copy numbers measured by SNP6.0 array or FISH).[14]

By comparing the activity profiles of the new compounds in all 42 cell lines tested with those of erlotinb and lapatinib by a Spearman rank correlation, a higher score of similarity to lapatinib compared erlotinib was found. For lapatinib a Spearman rank coefficient of rho = 0.67 (**42**), rho = 0.67 (**45**), and rho = 0.60 (**44**) was found. For erlotinib, rho = 0.51 was found for **45**, and rho $\leqslant$0.4 for **42**, **44**, and **46**. This suggests the biological activities of the novel compounds to be closer to those of the dual ErbB2/EGFR inhibitor lapatinib than to erlotinib, a very potent EGFR specific inhibitor.

Comparing their biological activities, **42**, **44**, and **45** revealed higher similarity between each other (rho 0.76–0.85) than with **46** (rho 0.46–0.63).

3. Conclusions

Combining structural elements of the quinazoline class of known tyrosine kinase inhibitors with the bisindolylmethanone structure led to a series of novel compounds, which inhibit EGFR in biochemical assays in the nanomolar range. These compounds also potently inhibit EGFR autophosphorylation in intact A431 cells with IC_{50} values in the submicromolar range. As shown by the sensitivity profile in a panel of 42 human tumor cell lines, the novel compounds are similar to lapatinib as expressed by the Spearman rank coefficient (rho 0.6–0.67). This suggests the biological activities of the novel compounds to be close to the dual ErbB2/EGFR inhibitor lapatinib.

4. Experimental section

4.1. General

NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. IR spectra (KBr) were measured with a Bruker Tensor 27 spectrometer; melting points were determined with a Büchi B545 device. MS spectra were measured with a Finnigan MAT 95 (EI, 70 eV) or with a Finnigan Thermo Quest TSQ 7000 (ESI). All reactions were carried out under nitrogen. 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 stated otherwise. Chemical names were created using ChemDraw Ultra 10.0 software.

4.2. Procedures

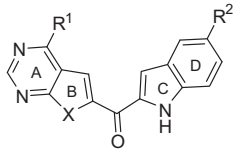
4.2.1. 4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (9)[10]

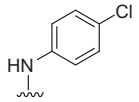
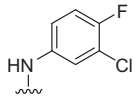
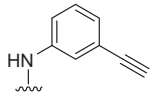
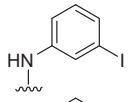
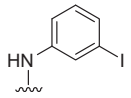
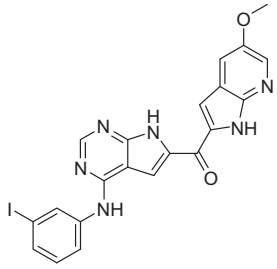
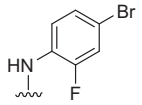
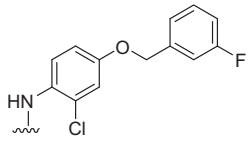
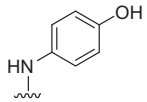
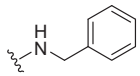
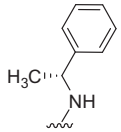
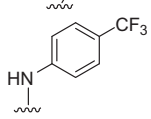
89% Yield; colourless crystals, mp 169–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.73 (d, J = 4.1 Hz, 1H), 7.55 (t, J = 7.5 Hz, 2H), 7.65 (t, J = 7.6 Hz, 1H), 7.80 (d, J = 4.1 Hz, 1H), 8.22 (d, J = 7.4 Hz, 2H), 8.78 (s, 1H), ppm. MS (EI$^+$): 293.1 ([M]$^+$, 13; ^{35}Cl), 229.2 ([M−SO_2]$^+$, 24; ^{35}Cl), 141.1.([M−$C_6H_5SO_2$]$^+$, 27; ^{35}Cl), 77.1 ([C_6H_5]$^+$, 100). Anal. ($C_{12}H_8ClN_3O_2S$; 293.73): C, H, N.

4.2.2. 4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine -6-carboxylic acid (11)

Preparation analogous to Lit.[15,16] as follows: 4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**) (10 g, 34.0 mmol) was dissolved in dry THF (300 mL) under nitrogen, cooled to −78 °C, *n*-butyllithium (1.1 equiv, 23.4 mL 1.6 M in hexane) was added and the mixture stirred at −78 °C for 4 h. Dry CO_2 gas was introduced into the solution for 2 h, then the mixture was allowed to

Table 1
Influence of modifications in the ring B on inhibition of EGFR, ErbB2, and VEGFR2 by test compounds at a concentration of 1.0 μM. Data were determined at least as duplicates and are given as mean values ± standard deviation of the mean value. For compounds exhibiting inhibition >90%, IC_{50} values were determined in addition



No.	R^1	R^2	X	EGFR inhibition in % at c = 1.0 μM	ErbB2 inhibition in % at c = 1.0 μM	VEGFR2 inhibition in % at c = 1.0 μM
48	H	OH	N	0	0	0
22		H	N	68 ± 5	24 ± 3	47 ± 2
33		H	S	80 ± 0	46 ± 2	19 ± 6
23		H	N	88 ± 3	22 ± 5	47 ± 2
44		H	S	100 ± 0 IC_{50} = 5.54 nM	80 ± 0	15 ± 7
24		H	N	83 ± 1	70 ± 3	5 ± 3
34		H	S	93 ± 1 IC_{50} = 18.7 nM	60 ± 2	9 ± 5
25		H	N	88 ± 1	52 ± 1	10 ± 7
35		H	S	73 ± 2	75 ± 1	0 ± 10
26		OCH_3	N	88 ± 0	25 ± 6	17 ± 1
27		—	—	77 ± 1	20 ± 3	20 ± 3
28		H	N	50 ± 1	14 ± 6	22 ± 6
36		H	S	82 ± 1	51 ± 3	46±8
29		H	N	77 ± 2	13 ± 2	53 ± 3
37		H	S	91 ± 1 IC_{50} = 18.5 nM	57 ± 2	29 ± 5
30		H	N	57 ± 4	7 ± 5	31 ± 5
31		H	N	55 ± 2	3 ± 1	13 ± 5
38		H	S	77 ± 2	38 ± 20	17 ± 2
32		H	N	82 ± 2	37 ± 1	28 ± 3
39		H	S	88 ± 3	61 ± 3	5 ± 3
40		H	S	43 ± 3	34 ± 6	24 ± 6

(*continued on next page*)

Table 1 (*continued*)

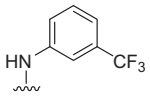
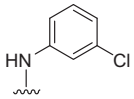
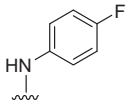
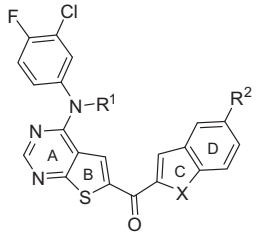
No.	R^1	R^2	X	EGFR inhibition in % at c = 1.0 μM	ErbB2 inhibition in % at c = 1.0 μM	VEGFR2 inhibition in % at c = 1.0 μM
41		H	S	79 ± 1	42 ± 9	29 ± 1
42		H	S	99 ± 1 IC_{50} = 16.6 nM	73 ± 4.	20 ± 4
43		H	S	96 ± 2 IC_{50} = 43.7 nM	50 ± 1	45 ± 1

Table 2
Inhibition of EGFR, ErbB2 and VEGFR2 by compounds **44**–**56** at a concentration of 1.0 μM. Data were determined at least as duplicates and are given as mean values ± standard deviation of the mean value.



No.	R^1	R^2	X	EGFR inhibition in % at c = 1.0 μM	ErbB2 inhibition in % at c = 1.0 μM	VEGFR2 inhibition in % at c = 1.0 μM
44	H	H	NH	100 ± 0 IC_{50} = 5.54 nM	80 ± 0	15 ± 7
45	H	OH	NH	98 ± 0 IC_{50} = 24 nM	87 ± 0	10 ± 1
46	H	OCH_3	NH	96 ± 1 IC_{50} = 5.93 nM)	60 ± 2	55 ± 8
51	CH_3	H	NH	22 ± 8	83 ± 1	23 ± 5
55	H	H	O	85 ± 14	88 ± 3	51 ± 5
56	H	H	S	99 ± 1 IC_{50} = 10.8 nM	76 ± 2	22 ± 3
Erlotinib 3				IC_{50} = 3.10 nM	IC_{50} = 300 nM	IC_{50} = 820 nM
Lapatinib 5				IC_{50} = 8.90 nM	IC_{50} = 60.0 nM	IC_{50} >10 μM

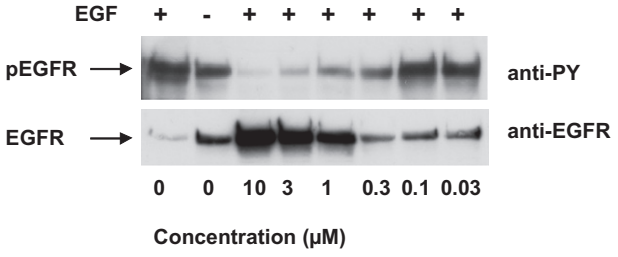


Figure 2. Inhibition of EGFR autophosphorylation in intact A431 cells by compound **45**. Cells were serum-starved over night (0.5% FCS), treated with the inhibitor at the indicated concentrations for 1 h, and then stimulated by 100 ng/ml EGF for 10 min (or left unstimulated, as indicated). Total cell lysates were analyzed by immunoblotting to detect tyrosine phosphorylation (upper panel, anti-PY), then stripped and reprobed for EGFR (lower panel). Note dose-dependent stabilization of EGFR levels by inhibitor treatment, as revealed in the anti-EGFR blot.

warm up to room temperature overnight. The solvents were removed in vacuo, and the remaining solid was suspended in water (300 mL). The suspension was filtered over a pad of Celite, the clear solution acidified with HCl by stirring; the precipitating colorless crystals were removed by filtration and dried in vacuo. If necessary, crystallization from EtOH/H_2O was performed in addition. 68% yield; colorless crystals, mp 176 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.73 (s, 1H), 7.73 (t, J = 7.5 Hz, 2H), 7.82 (t, J = 6.7 Hz, 1H), 8.31 (d, J = 7.1 Hz, 2H), 8.31 (s, 1H) ppm. MS (ESI$^+$): 379.0 ([MH$^+$+CH_3CN]$^+$,53; ^{35}Cl), 337.9 ([M+H; ^{35}Cl]$^+$, 100). Anal. ($C_{12}H_8ClN_3O_3S$; 337.74): C, H, N.

4.2.3. 4-Chlorothieno[2,3-*d*]pyrimidine-6-carboxylic acid (12)

4-Chlorothieno[2,3-*d*]pyrimidine (**10**) (5.80 g, 34.0 mmol) was dissolved in dry THF (300 mL) under nitrogen, cooled to −78 °C, *n*-butyllithium (1.1 equiv, 23.4 ml, 1.6 M in hexane) was added and the mixture stirred at −78 °C for 4 h. An excess of solid carbon dioxide was added and the mixture allowed to warm up to room temperature overnight. The solvents were removed under reduced pressure, and the remaining solid was suspended in water (300 mL). The suspension was filtered over a pad of celite and the clear solution was acidified with conc. HCl under stirring. The precipitating colourless crystals were collected by filtration and dried. 65% yield; colourless crystals; mp 195 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.07 (s, 1H) ppm. MS (ESI$^+$): 256 ([MH$^+$+CH_3CN]$^+$, 100; ^{35}Cl), 215 ([M+H]$^+$, 12; ^{35}Cl). IR (KBr): ν 3442, 3091, 1724 cm^{-1}. Anal. ($C_7H_3ClN_2O_2S$; 214.63): C, H, N.

4.2.4. 4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carbonyl chloride (13)

4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid (**11**) (6.75 g; 20 mmol) was suspended in $SOCl_2$ (50 mL) and refluxed for 3 h. Excess of $SOCl_2$ was removed under reduced pressure and the product dried in vacuo. Quantitative yield; colorless crystals, mp 124 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ

Table 3
Sensitivity profile of selected {(1*H*-indol-2-yl)(4-(phenylamino)thieno[2,3-*d*]pyrimidin-6-yl}methanones (**44**, **42**, **46** and **45**) towards a panel of different tumor cell lines by comparison with lapatinib and erlotinib (Tarceva®).

IC_{50} values [μM] in a panel of 42 human tumor cell lines (mean of 3 independent experiments)

Cell line		Lapatinib 5	Erlotinib 3	44	42	46	45
BXF	1218L	11.77	96.0	9.13	21.5	15.4	6.34
BXF	1352L	11.53	87.8	11.30	15.9	24.8	5.97
BXF	T24	9.65	13.9	11.43	23.7	17.8	9.56
CXF	269L	8.36	16.0	10.77	28.9	11.7	7.58
CXF	DIFI	0.235	0.140	1.39	1.8	1.0	0.94
CXF	HCT116	13.14	65.8	8.39	18.9	12.1	7.69
CXF	HT29	4.62	100	7.83	17.7	9.8	13.73
CXF	RKO	5.35	100	7.29	18.1	15.8	6.01
GXF	251L	1.48	0.369	2.76	4.3	3.9	4.09
GXA	MKN45	11.48	76.7	12.54	20.2	38.2	7.26
HNXF	CAL27	0.530	0.239	2.61	3.0	1.6	1.28
LIXF	575L	7.18	47.2	8.15	18.1	9.3	7.04
LXF	H460	13.46	74.3	5.82	17.7	15.0	6.00
LXFA	289L	5.79	1.78	6.68	4.7	7.1	3.25
LXFA	526L	4.21	100	10.72	13.6	28.5	4.36
LXFA	629L	2.87	0.660	4.88	6.0	5.0	2.65
LXFL	1121L	7.73	34.6	9.77	16.0	9.0	6.02
LXFL	529L	3.51	17.4	8.61	7.6	14.8	4.87
MAXF	401NL	5.80	25.0	3.89	7.3	4.2	4.09
MAXF	MCF7	4.83	100	6.96	12.5	11.3	4.22
MAXF	MDA231	7.70	34.5	8.04	23.4	17.1	7.65
MEXF	1341L	4.85	30.1	10.85	20.1	14.4	5.89
MEXF	276L	11.37	18.6	10.21	27.1	31.0	6.28
MEXF	462NL	10.13	100	8.11	24.0	16.7	6.02
OVXF	899L	3.35	8.77	5.56	13.9	19.0	4.07
OVXF	OVCAR3	13.44	100	8.45	15.3	24.6	9.08
PAXF	1657L	11.11	64.3	17.62	47.6	24.6	9.08
PAXF	546L	6.12	10.0	11.63	25.1	14.1	8.85
PAXF	PANC1	8.12	75.8	7.01	14.8	28.4	7.95
PRXF	22RV1	6.06	100	5.48	14.0	11.1	5.61
PRXF	DU145	2.99	0.966	4.36	9.2	19.4	2.96
PRXF	LNCAP	3.68	6.63	6.11	11.3	32.5	3.01
PRXF	PC3M	4.55	17.2	5.77	14.1	46.3	5.45
PXF	1118L	6.86	76.7	17.95	49.7	62.9	9.59
PXF	1752L	9.46	31.6	13.45	30.7	36.0	8.19
PXF	698L	9.49	36.4	14.28	31.0	26.7	9.23
RXF	1781L	7.67	76.7	9.05	20.0	30.4	4.87
RXF	393NL	7.77	45.6	11.76	26.4	21.1	5.87
RXF	486L	9.10	10.0	5.50	13.1	10.2	3.37
SXF	SAOS2	9.38	21.0	11.43	19.3	30.1	8.20
SXF	TE671	10.95	100	14.05	24.8	55.0	10.05
UXF	1138L	4.40	24.5	4.42	8.6	4.9	3.45
geomean IC_{50}		5.87	21.6	7.70	15.0	14.9	5.48

IC_{50} values (μM) in a panel of 42 human tumor cell lines (mean of three independent experiments).

7.26 (s, 1H), 7.62 (t, *J* = 7.4 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.74 (t, *J* = 6.9 Hz, 2H), 9.00 (s, 1H) ppm. EI-MS (70 eV) m/z (%) = 355 $[M]^{+\cdot}$ (0.3), 320 $[M\text{-}Cl^{\cdot}]^{+}$ (2.7), 291 $[M\text{-}SO_2]^{+\cdot}$ (1.9), 180 (100). The product was used without further purification.

4.2.5. 4-Chlorothieno[2,3-*d*]pyrimidine-6-carbonyl chloride (14)

4-Chlorothieno[2,3-*d*]pyrimidine-6-carboxylic acid (**12**) (2.15 g, 20.0 mmol) was suspended in $SOCl_2$ (50.0 mL) and heated to reflux for 3 h. Excess of $SOCl_2$ was removed under reduced pressure, the product dried *in vacuo* and used without further purification. Quantitative yield; beige powder, mp 88.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.01 (s, 1H), 8.34 (s, 1H) ppm. EI-MS (70 eV) m/z (%) = 235.9 $[M]^{+\cdot}$ (1.58), 233.9 $[M]^{+\cdot}$ (9.9), 232 $[M]^{+\cdot}$ (13.7), 197 $[M-Cl^{\cdot}]^{+}$ (100).

4.2.6. 1-(Phenylsulfonyl)-1*H*-indoles

65.1 mmol starting material were dissolved in dry THF (300 mL) under nitrogen, cooled to 0 °C and 1.1 equiv NaH (71.6 mmol) 2.86 g, 60% oil dispersion), were added in portions. Then the mixture was stirred for an additional h. Benzene sulfonylchloride (71.6 mmol; 9.14 mL) was added drop wise, the mixture allowed to warm to room temperature and stirred for 4 h. The suspension was poured into water (1.2 L) by stirring. After 1 h, the precipitated product was removed by filtration, dried and dissolved in the necessary amount of CH_2Cl_2. An aliquot of heptane was added, half of the solvent removed under reduced pressure and the product, precipitating as colorless crystals, removed by filtration.

4.2.6.1. 1-(Phenylsulfonyl)-1*H*-indole (15a). Preparation from 1*H*-indole as described above. 76% yield.[16]

4.2.6.2. 5-Methoxy-1-(phenylsulfonyl)-1*H*-indole (15b)[15]. Compound **15b** was prepared as described above.

4.2.6.3. 5-(Benzyloxy)-1-(phenylsulfonyl)-1*H*-indole (15c)[15]. Compound **15c** was prepared as described above.

4.2.6.4. 5-(tert-Butyldimethylsilyloxy)-1-(phenylsulfonyl)-1*H*-indole (15d)[1]. Compound **15d** was prepared as described above.

4.2.6.5. 1-(Phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine (15e). Compound **15e** was prepared from 1*H*-pyrrolo[2,3-*b*]pyridine as described above. 90% yield; colorless crystals, mp 129 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.59 (d, *J* = 3.8 Hz, 1H), 7.16 (dd, J_1 = 4.8 Hz, J_2 = 7.8 Hz, 1H) 7.43–7.48 (m, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.72 (d, *J* = 4.1 Hz, 1H), 7.83 (dd, J_1 = 1.6 Hz, J_2 = 7.7 Hz, 1H), 8.17–8.21 (m, 2H), 8.42 (dd, J_1 = 1.5 Hz, J_2 = 4.8 Hz, 1H) ppm. MS (EI$^+$): 258.0 ($[M]^{+\cdot}$, 41), 194.1 ($[M\text{-}SO_2]^{+\cdot}$, 94.3), 117.1.($[M–C_6H_5SO_2]^+$, 24.9), 77.1 ($[C_6H_5]^+$, 100) Remark: The observed loss of SO_2 in the mass spectra is in accordance with Wiegrebe, W.; Schlunegger, U. P.; Herrmann, E. G., Umlagerungen von Prolinderivaten. *Pharm. Acta. Helv.* **1974**, 49, (7/8), 253–258. Anal. ($C_{13}H_{10}N_2O_2S$; 258.30): C, H, N.

4.2.7. Preparation of (4-chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl) methanones 16–18

The respective *N*-phenylsulfonated indole derivative **15** (10 mmol) was dissolved in dry THF (50 mL) under nitrogen, cooled to −78 °C, n-butyllithium (1.1 equiv, 6.88 mL 1.6 M in hexane) was added and the mixture stirred at −78 °C for 1 h. A solution of 4-chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid chloride (**13**) (10 mmol, 3.56 g) in THF (50 mL) was cooled to −78 °C, was added rapidly in one portion and the mixture allowed to stir for half an hour at −78 °C. The solution was poured into satd. NH_4Cl solution under stirring, the organic layer separated, dried (Na_2SO_4), the solvent removed under reduced pressure and the remaining solid dissolved in a small amount of CH_2Cl_2. The solution was filtered and added to diethyl ether by stirring. The precipitated product was removed by filtration and crystallized twice form CH_2Cl_2/diethyl ether. Alternatively purification by CC (SiO_2; CH_2Cl_2/ethyl acetate 10:1) is possible.

4.2.7.1. (4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (16a). 17% yield; colorless crystals; mp 261–263 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.07 (s, 1H), 7.36–7.41 (m, 2H), 7.53–7.75 (m, 8H), 8.13 (d, *J* = 7.8 Hz, 2H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.48 (d, *J* = 7.8 Hz, 2H), 8.96 (s, 1H) ppm. MS (ESI$^+$): 576.9 ($[M+H]^+$, 100; ^{35}Cl). Anal. ($C_{27}H_{17}ClN_4O_5S_2$; 577.03): C, H, N.

4.2.7.2. (4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-yl)-methanone (16b). 24% yield; beige crystals; mp 227 °C decomp.; ^{1}H NMR1H NMR (300 MHz $CDCl_3$): δ 7.24 (s, 1H), 7.29 (s, 1H), 7.32–7.36 (m, 1H), 7.59–7.70 (m, 5H), 7.72–7.78 (m, 1H), 8.01 (dd, 1H, J_1 = 7.9 Hz, J_2 = 1.6 Hz), 8.49 -8.56 (m, 4H), 8.70 (dd, 1H, J_1 = 4.6 Hz, J_2 = 1.6 Hz), 8.98 (s, 1H) ppm. MS (ESI$^+$): 578.0 ([M+H]$^+$, 100; ^{35}Cl). Anal. ($C_{26}H_{16}ClN_5O_5S_2$: 578.02): C, H, N.

4.2.7.3. (4-Chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(5-methoxy-1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (17). 51% yield; colorless crystals; mp 245–249 °C; ^{1}H NMR (300 MHz $CDCl_3$): δ 3.85 (s, 3H), 7.01 (s, 1H), 7.02 (d, J = 2.5 Hz, 1H), 7.20 (dd, J_1 = 2.6 Hz, J_2 = 9.2 Hz, 1H), 7.34 (s, 1H), 7.52 (t, J = 7.5 Hz, 2H), 7.95–7.65 (m, 3H), 7.72 (t, J = 7.4 Hz, 1H), 8.06 (d, J = 7.1 Hz, 2H), 8.17 (d, J = 9.3 Hz, 1H), 8.49 (d, J = 7.5 Hz, 2H), 8.94 (s, 1H) ppm. MS (ESI$^+$): 607.0 ([M+H]$^+$, 100; ^{35}Cl). Anal. ($C_{28}H_{19}ClN_4O_6S_2$; 607.06): C, H, N.

4.2.7.4. (5-(Benzyloxy)-1-(phenylsulfonyl)-1*H*-indol-2-yl)(4-chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl) methanone (18). 26% yield; colorless crystals; mp 217.3–222.4 °C; ^{1}H NMR (300 MHz DMSO-d_6): δ 5.17 (s,2H), 7.34–7.49 (m, 7H), 7.52 (s, 1H), 7.66–7.90 (m, 7H), 8.05 (d, J = 8.1 Hz, 2H), 8.14 (d, J = 9.9 Hz, 1H), 8.29 (d, J = 7.4 Hz,2H), 9.04 (s, 1H) ppm. MS (ESI$^+$): 683 ([M+H]$^+$, 100; ^{35}Cl). Anal.($C_{34}H_{23}ClN_4O_6S_2$; 683.15): C, H, N.

4.2.8. Preparation of (4-chlorobenzo[*b*]thiophen-2-yl) (1-(phenylsulfonyl)-1*H*-indol-2-yl)methanones 19–21

The *N*-phenylsulfonated indole derivate **15a**–**15d** (10.0 mmol) was dissolved in dry THF (50 mL) under nitrogen and cooled to −78 °C. n-Butyllithium (1.1 equiv., 6.88 mL, 1.6 M in hexane) was added and the mixture stirred at −78 °C for 1 h. A solution of 4-chlorothieno[2,3-*d*]pyrimidine-6-carbonyl chloride (**14**) (2.33 g, 10.0 mmol) in THF (50.0 mL), cooled to −78 °C, was added in one portion and the mixture was stirred for half an hour at −78 °C. The solution was poured into satd. NH_4Cl solution under stirring. The organic layer was separated, dried (Na_2SO_4) and the solvent was removed under reduced pressure. The remaining solid was dissolved in a small amount of CH_2Cl_2. The solution was filtered and added to diethyl ether by stirring. The precipitated product was collected by filtration and precipitated twice from CH_2Cl_2/diethyl ether as described above.

4.2.8.1. (4-Chlorothieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (19). 53% yield; beige crystals; mp 224–225 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.15 (s, 1H), 8.26 (s, 1H), 8.11 (d, J = 8.2 Hz, 1H), 8.07–8.01 (m, 2H), 7.83–7.71 (m, 3H), 7.69–7.56 (m, 3H), 7.42 (t, 7.6 Hz, 1H) ppm. MS (ESI$^+$): 495 ([M+CH_3CN]$^+$, 100; ^{35}Cl), 454 ([M+H]$^+$, 32; ^{35}Cl). IR (KBr): ν 3090, 1643 cm^{-1}. Anal. ($C_{21}H_{12}ClN_3O_3S_2$; 453.92): C, H, N.

4.2.8.2. 4-Chlorothieno[2,3-*d*]pyrimidin-6-yl)(5-methoxy-1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (20). 43% yield; light yellow powder; mp 125–130 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14 (s, 1H), 8.17 (s, 1H), 8.03–7.91 (m, 3H), 7.77–7.70 (m, 1H), 7.66–7,58 (m, 3H), 7.26 (d, J_1 = 2.5 Hz, 1H), 7.18 (dd, J_1 = 2.5 Hz, J_2 = 9.2 Hz, 1H), 3.79 (s, 3H) ppm. MS (ESI$^+$): 525 ([MH+CH_3CN]$^+$, 100; ^{35}Cl), 484 ([MH]$^+$, 20). IR (KBr): ν 2923, 1647, 1525 cm^{-1}. Anal. Calcd for ($C_{22}H_{14}ClN_3O_4S_2 \times CH_3OH$; 483.95): C, H, N.

4.2.8.3. (5-(tert-Butyldimethylsilyloxy)-1-(phenylsulfonyl)-1*H*-indol-2-yl)(4-chlorothieno[2,3-*d*]pyrimidin-6-yl)methanone (21). 54% yield; light yellow powder; mp 204–205 °C; ^{1}H NMR1H NMR (300 MHz, DMSO-d_6): δ 9.16 (s, 1H), 8.23 (s, 1H), 8.01–7.94 (m, 3H), 7.78–7.71 (m, 1H), 7.67–7.58 (m, 3H), 7.20 (d, J = 2.5 Hz, 1H), 7.11 (dd, J = 2.5, 8.9 Hz, 1H), 0.96 (s, 9H), 0.20 (s, 6H) ppm. MS (ESI$^+$): 625 ([MH+CH_3CN]$^+$, 100; ^{35}Cl), 584 ([MH]$^+$, 23). IR (KBr): ν 3069, 2940, 1648 cm^{-1}. Anal. Calcd for ($C_{27}H_{26}ClN_3O_4S_2Si$; 584.18): C, H, N.

4.2.9. Preparation of (1*H*-indol-2-yl)(4-(phenylamino)-1*H*-indol-2-yl)methanone-derivatives 22–32, general procedure

A mixture of (4-chloro-7-phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (**16**) (0.7 mmol) and the suitably substituted aniline (1.0 mmol) in 16 g n-butanol was heated to 120 °C under nitrogen atmosphere for 10–12 h, cooled to rt and poured into light petroleum (100 mL). The precipitated product was filtered and the solid added to a mixture of THF: MeOH (50 mL: 50 mL) and 50 mL 10% NaOH (in water). The mixture was refluxed for 6 h. The organic solvent was removed under reduced pressure, the precipitating product was removed by filtration, dried and stirred in an organic solvent given for the respective product.

4.2.9.1. (4-(4-Chlorophenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (22). 92% yield from ethyl acetate; yellow crystals; mp 340 °C decomp.; ^{1}H NMR (300 MHz,DMSO-d_6): δ 7.14 (t, J = 7.4 Hz, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.45 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 7.4 Hz, 2H), 7.78 (d, J_2 = 8.0 Hz, 1H), 7.99 (d, J = 9 Hz, 2H), 8.04 (s, 1H), 8.44 (s, 1H), 9.91 (s, 1H) 12.03 (s, 1H), 12.48 (s, 1H), ppm. MS (ESI$^+$): 428.9 ([MH$^+$+CH_3CN]$^+$, 56; ^{35}Cl), 387.9 ([M+H]$^+$, 100; ^{35}Cl). Anal. ($C_{21}H_{14}ClN_5O \times 3/2\ H_2O$; 387.82): C, H, N.

4.2.9.2. (4-(3-Chloro-4-fluorophenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1H-indol-2-yl)methanone (23). 99% yield from ethyl acetate; yellow crystals; mp 377 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.84 (t, J = 6.85 Hz, 1H), 7.01 (t, J = 6.87 Hz, 1H), 7.31 (t, J = 9.18 Hz, 1H), 7.39 (s, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.56–7.61 (m, 2H), 7.85 (s, 1H), 8.19 (s, 1H), 8.43 (d, J = 5.49 Hz, 1H) ppm. MS (ESI$^+$): 446.9 ([M+CH_3CN]$^+$, 64), 405.9 ([M+H]$^+$, 100). Anal. ($C_{21}H_{13}ClFN_5Ox2/3\ H_2O$; 405.81): C, H, N.

4.2.9.3. (4-(3-Ethynylphenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone: (24). 61% from ethylacetate; yellow crystals; mp 390 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.23 (s, 1H), 7.12–7.20 (m, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.54 (d, J = 7.1 Hz, 2H), 7.79 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 8.05 (s, 1H), 8.19 (s, 1H), 8.47 (s, 1H), 9.87 (s, 1H), 12,04 (s, 1H), 12,71 (s, 1H), ppm. MS (ESI$^+$): 418.9 ([M+CH_3CN]$^+$, 26), 377.9 ([M+H]$^+$, 100). Anal. ($C_{23}H_{15}\ N_5O$; 377.40): C, H, N.

4.2.9.4. (1*H*-Indol-2-yl)(4-(3-iodophenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanone (25). 83% yield from ethylacetate; yellow crystals; mp 365 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.12–7.22 (m, 2H), 7.33 (t, J = 7.5 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 7.9 Hz, 2H), 7.79 (d, J = 7.9 Hz, 1H), 8.00–8.04 (m, 2H), 8.42 (s, 1H), 8.47 (s, 1H), 9.84 (s, 1H) 12.03 (s, 1H), 12.71 (s, 1H), ppm. MS (ESI$^+$): 521.0 ([MH$^+$+CH_3CN]$^+$, 63), 479.9 ([M+H]$^+$, 100). Anal. ($C_{21}H_{14}\ IN_5O$; 479.27): C, H, N.

4.2.9.5. (4-(3-Iodophenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(5-methoxy-1*H*-indol-2-yl)methanone (26). 65% yield from CH_2Cl_2/MeOH; yellow crystals; mp 355.2–356.1 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H), 6.99–7.01 (m, 1H), 7.13–7.21 (m, 2H), 7.40–7.50 (m, 3H), 8.06–8.10 (m, 1H), 8.19 (s, 1H), 8.45–8.50 (m, 2H), 10.08 (s, 1H, exchangeable), 11.9 (s, 1H, exchangeable), 12.65 (s, 1H, exchangeable) ppm. MS (ESI$^+$):

509.9 ([M+H]$^+$, 100). Anal. ($C_{22}H_{16}$ IN_5O_2 x·4/5 CH_2Cl_2; 509.30): C, H, N.

4.2.9.6. (4-(3-Iodophenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)methanone (27). 60% yield from ethyl acetate; yellow crystals; mp 365 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.16–7.25 (m, 2H), 7.42 (d, J = 7.7 Hz, 1H), 7.48 (s, 1H), 7.99–8.01 (m, 2H), 8.24 (d, J = 8.01 Hz, 1H), 8.42 (s, 1H), 8.48 (s, 2H), 9.85 (s, 1H), 12.59 (s, 1H), 12.76 (s, 1H) ppm. MS (ESI$^+$): 522.0 ([MH$^+$+CH_3CN]$^+$, 64), 480.9 ([M+H]$^+$, 80), 282.0 ([M+2H +2CH_3CN]$^{2+}$, 100). Anal. ($C_{20}H_{13}$ IN_6Ox·3/2 H_2O; 480.26): C, H, N.

4.2.9.7. (4-(4-Bromo-2-fluorophenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (28). 86% yield from ethyl acetate; yellow crystals; mp 323 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.14 (t, J = 7.5 Hz, 1H), 7.33 (t, J = 7.3 Hz, 1H), 7.45–7.54 (m, 3H), 7.69 (dd, J_1 = 2.2 Hz, J_2 = 10.1 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 8.6 Hz, 1H), 8.00 (s, 1H), 8.33 (s, 1H), 9.79 (s, 1H), 12.02 (s, 1H), 12.68 (s, 1H), ppm. MS (ESI$^+$): 492.9 ([MH$^+$+CH_3CN]$^+$, 79), 451.8 ([M+H]$^+$, 100). Anal ($C_{21}H_{13}$ $BrFN_5O$x ½ H_2O; 450.26): C, H, N.

4.2.9.8. (4-(2-Chloro-4-(3-fluorobenzyloxy)phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (29). 2-Chloro-4-(3-fluorobenzyloxy)aniline used was prepared according to Lit..[17] 67% Yield from chloroform; yellow crystals; mp 315 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6DMSO-d_6): δ 5.24 (s, 2H), 7.11–7.21 (m, 2H), 7.26–7.34 (m, 4H), 7.43–7.54 (m, 3H), 7.73–7.79 (m, 2H), 7.98 (s, 1H), 8.17 (d, J = 2.5 Hz, 1H), 8.42 (s, 1H), 9.80 (s, 1H), 12.03 (s, 1H), 12.67 (s, 1H) ppm. MS (ESI$^+$): 553 ([MH$^+$+CH_3CN]$^+$, 37), 512.1 ([M+H]$^+$, 100). Anal.($C_{28}H_{19}ClFN_5O_2$ 11/10 $CHCl_3$; 511.93): C, H, N.

4.2.9.9. (4-(4-Hydroxyphenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1H-indol-2-yl)methanone: (30). 50% yield from ethyl acetate; yellow crystals; mp 312 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.92 (d, J = 8.2 Hz, 2H), 7.14 (t, J = 7.4 Hz, 1H), 7,33 (t, J = 7.3 Hz, 1H), 7.45–7.53 (m, 4H), 7.76 (d, J = 7.9 Hz, 1H), 8.34 (s, 2H), 9.81 (s, 1H), 11.19 (s, 1H), 12.05 (s, 1H), 13.22 (s, 1H) ppm. MS (ESI$^+$): 410.9 (MH$^+$ + CH_3CN]$^+$, 34), 369.9 ([M+H]$^+$, 100). Anal.($C_{21}H_{15}N_5O_2$;369.38): C, H, N.

4.2.9.10. (4-(Benzylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (31). 70% yield from ethyl acetate; yellow crystals; mp 320 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.90 (d, J = 5,7 Hz, 2H), 7.12 (t, J = 7.5 Hz, 1H), 7.30–7.36 (m, 2H), 7.38–7.43 (m, 2H), 7.48–7.53 (m, 4H), 7.73 (d, J = 8.2 Hz, 1H), 8.22 (s, 1H), 8.41 (s, 1H), 10.19 (s, 1H), 12.06 (s, 1H), 13.34 (s, 1H) ppm. MS (ESI$^+$): 409.0 ([MH$^+$+CH_3CN]$^+$, 20), 368.0 ([M+H]$^+$, 100). Anal.($C_{22}H_{17}$ N_5O; 367.40): C, H, N.

4.2.9.11. (*R*)-(1*H*-Indol-2-yl)(4-(1-phenylethylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanone (32). 30% yield from ethyl acetate; yellow crystals; mp 288 °C decomp.; ^{1}H NMR(300 MHz, DMSO-d_6): δ 1.16 (d, J = 7.1 Hz, 3H), 5.52–5.57 (m, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.23 (t, J = 7.1 Hz, 1H), 7.28–7.36 (m, 3H), 7.43–7.53 (m, 4H), 7.78 (d, J_2 = 8.0 Hz, 1H), 7.99 (s, 1H), 8.19 (s, 1H), 8.36 (d, J = 8.0 Hz, 1H), 11.96 (s, 1H), 12.39 (s, 1H) ppm. MS (ESI$^+$): 423.0 ([MH$^+$+CH_3CN]$^+$, 13), 382.0 ([M+H]$^+$, 100). Anal.($C_{23}H_{19}$ N_5O; 381.43): C, H, N.

4.2.10. Preparation of (1*H*-indol-2-yl)(4-(phenylamino)benzo[*b*]thiophen-2-yl)methanones 33–46

Example:

4.2.10.1. (4-(4-Chlorophenylamino)thieno[2,3-*d*]pyrimidine-6-yl)(1*H*-indol-2yl)methanone (33). (4-Chlorothieno[2,3-*d*]-pyrimidine-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2yl)methanone (**19**) (0.70 g, 1.54 mmol) and 4-Chloroaniline (0.35 g, 2.74 mmol) were suspended in 2-butanone (25 mL). The mixture was heated to reflux for 2 days. After cooling to room temperature the solvent was removed under reduced pressure. The residue was dissolved in a mixture of methanole (10 mL), THF (10 mL) and aqueous NaOH (10%, 10 mL) and heated to reflux for 1.5 h. THF and methanole were removed under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was separated, dried (Na_2SO_4) and the solvent removed under reduced pressure. The precipitated product was collected by filtration and dried in vacuo.

62% Yield from CH_2Cl_2/MeOH; yellow crystals; mp 342–345 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.15 (s, 1H), 10.20 (s, 1H), 9.03 (s, 1H), 8.65 (s, 1H), 7.96–7.89 (m, 2H), 7.83 (d, J = 8.2 Hz, 1H), 7.70 (s, 1H), 7.58–7.47 (m, 3H), 7.38 (t, J = 7.4 Hz, 1H), 7.15 (t, J = 7.4 Hz, 1H) ppm. MS (ESI$^+$): 446 ([MH$^+$+ CH_3CN]$^+$, 72; ^{35}Cl), 405 ([M+H]$^+$, 100; ^{35}Cl). IR (KBr): ν 3428, 3324, 1617 cm^{-1}. Anal. ($C_{21}H_{13}ClN_4OS \times 0.33$ CH_2Cl_2, 404.87): C, H, N.

4.2.10.2. (4-(3-Ethynylphenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (34). 60% yield from methanole; yellow crystals; mp 215–219 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.15 (s, 1H), 10.17 (s, 1H), 9.02 (s, 1H), 8.68 (s, 1H), 8.07 (br s, 1H), 7.94–7.88 (m, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.72 (s, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.45, (t, J = 8.0 Hz, 1H), 7.40–7.33 (m, 1H), 7.29–7.24 (m, 1H), 7.16 (t, J = 7.5 Hz, 1H), 4.25 (s, 1H) ppm. MS (ESI$^+$): 436 ([M+CH_3CN]$^+$, 26), 395 ([M+H]$^+$, 100). IR (KBr): ν 3473, 3294 1593 cm^{-1}. Anal. Calcd for ($C_{23}H_{14}N_4OS \times 1.33$ CH_3OH; 394,45): C, H, N.

4.2.10.3. (1*H*-Indol-2-yl)(4-(3-iodophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanone (35). 47% yield; beige powder; mp 249–254 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.14 (s, 1H), 10.13 (s, 1H), 9.01 (s, 1H), 8.67 (s, 1H), 8.31 (s, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.70 (br s, 1H), 7.53 (t, J = 7.9 Hz, 2H), 7.37 (t, J = 7.7 Hz, 1H), 7.28–7.13 (m, 2H) ppm. MS (ESI$^+$): 538 ([MH$^+$+CH_3CN]$^+$, 42), 497 ([M+H]$^+$, 100). IR (KBr): ν 3447, 1622, 1591 cm^{-1}. Anal. Calcd for ($C_{21}H_{13}IN_4OS \times 1.5$ H_2O; 496.32): C, H, N.

4.2.10.4. (4-(4-Bromo-2-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (36). 68% yield; yellow crystals from ethyl acetate; mp 277–278 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.13 (s, 1H), 10.23 (s, 1H), 8.98 (s, 1H), 8.53 (s, 1H), 7.83–7.60 (m, 4H), 7.56–7.48 (m, 2H), 7.36 (t, J = 7.2 Hz, 1H), 7.15 (t, J = 7.2 Hz, 1H) ppm. MS (ESI$^+$): 508/510 ([MH$^+$+CH_3CN]$^+$, 53), 469/467 ([M+H]$^+$, 100). IR (KBr): ν 3440, 3323, 1617 cm^{-1}. Anal. Calcd for ($C_{21}H_{12}BrFN_4OS \times 0.5$ ethyl acetate; 467.31): C, H, N.

4.2.10.5. (4-(3-Chloro-4-(3-fluorobenzyloxy)phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (37). 2-Chloro-4-(3-fluorobenzyloxy)aniline used was prepared according to Lit.[17] **37** was obtained in 69% yield; yellow crystals from methanole; mp 267–269 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.13 (s, 1H), 10.11 (s, 1H), 8.97 (s, 1H), 8.61 (s, 1H), 8.03 (d, J = 2.5 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.74–7.66 (m, 2H), 7.56–7.43 (m, 2H), 7.40–7.28 (m, 4H), 7.23–7.12 (m, 2H), 5.26 (s, 2H) ppm. MS (ESI$^+$): 570 ([MH$^+$+CH_3CN]$^+$, 33; ^{35}Cl), 529 ([M+H]$^+$, 100; ^{35}Cl). IR (KBr): ν 3443, 3319, 1612 cm^{-1}. Anal. Calcd for ($C_{28}H_{18}ClFN_4O_2S \times 1.25$ CH_3OH; 528,98): C, H, N.

4.2.10.6. (4-(Benzylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (38). 58% yield; yellow crystals; mp

235–236 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.07 (s, 1H), 9.07 (t, J = 5.7 Hz, 1H), 8.88 (s, 1H), 8.47 (s, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 1.4 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.44–7.24 (m, 6H), 7.14 (t, J = 7.5 Hz, 1H), 4.82 (d, J = 5.7 Hz, 2H) ppm. MS (ESI$^+$): 426 ([MH$^+$+CH_3CN]$^+$, 24), 385 ([M+H]$^+$, 100). IR (KBr): ν 3426, 3383, 1590 cm^{-1}. Anal. Calcd for ($C_{22}H_{16}N_4OS \times 0.2\ CH_2Cl_2$; 384,45): C, H, N.

4.2.10.7. (*R*)-(1*H*-Indol-2-yl)(4-(1-phenylethylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanone (39). Preparation from *R*-(+) alpha-methylbenzylamin (ee ⩾99%) (Aldrich). 65% yield; yellow powder from ethyl acetate; mp 204–208 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.09 (s, 1H), 8.95 (s, 1H), 8.81 (d, J = 8.0 Hz, 1H), 8.42 (s, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.65 (br s, 1H), 7.56–7.42 (m, 3H), 7.39–7.31 (m, 3H), 7.24 (t, J = 7.2 Hz, 1H), 7.15 (t, J = 7.4 Hz, 1H), 5.58 (quint, J = 7.2 Hz, 1H), 1.61 (d, J = 7.2 Hz, 3H) ppm. MS (ESI$^+$): 440 ([M+CH_3CN]$^+$, 20), 399 ([M+H]$^+$, 100). IR (KBr): ν 3418, 1580 cm^{-1}. Anal. Calcd for ($C_{23}H_{18}N_4OS \times 0.25$ ethyl acetate; 398,48): C, H, N.

4.2.10.8. (1*H*-Indol-2-yl)(4-(4-(trifluoromethyl)phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanone (40). 26% yield; brown crystals from THF; mp 138 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.17 (t, J = 7.4 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.72 (s, 1H), 7.79–7.84 (m, 3H), 8.16 (d, J = 8.5 Hz, 2H), 8.72 (s, 1H), 9.07 (s, 1H), 10.36 (s, 1H), 12.15 (s, 1H) ppm. MS (ESI$^+$): 480.0 ([MH$^+$+CH_3CN]$^+$, 100), 438.9 ([M+H]$^+$,86). Anal Calcd. for ($C_{22}H_{13}F_3N_4OS$x1/3THF; 438,43): C, H, N.

4.2.10.9. (1*H*-Indol-2-yl)(4-(3-(trifluoromethyl)phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanone (41). 23% yield from ethyl acetate; brown crystals; mp 180 °C decomp..; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.16 (t, J = 7.5 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.53 (t, J = 9.6 Hz, 2H), 7.65–7.71 (m, 2H), 7.82 (d, J = 7.9 Hz, 1H), 8.25 (d, J = 8.2 Hz, 1H),8.29 (s, 1H), 8.71 (s, 1H), 9.04 (s, 1H), 10.33 (s, 1H), 12.15 (s, 1H) ppm. MS (ESI$^+$): 480.0 ([MH$^+$+CH_3CN]$^+$, 100), 438.9 ([M+H]$^+$, 81). Anal. ($C_{22}H_{13}F_3N_4O \times$ ½ ethyl acetate; 438,43): C, H, N.

4.2.10.10. (4-(3-Chlorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (42). 46% yield; brown crystals; mp 302.7 °C decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.05 (d, J = 8.0 Hz, 1H), 7.13 (t, J = 7.3 Hz, 1H), 7.30–7.37 (m, 2H) 7,52 (d, J = 8.2 Hz, 1H), 7.56–7.58 (m, 2H), 7.69 (d, J = 7.9 Hz, 1H), 7.87 (s, 1H), 8.42 (s, 1H), 8.75 (s, 1H) ppm. MS (ESI$^+$): 445.9 ([MH$^+$+CH_3CN]$^+$, 78; ^{35}Cl), 404.9 ([M+H]$^+$, 100; ^{35}Cl). Anal. ($C_{21}H_{13}Cl\ N_4OS$; 494.87): C, H, N.

4.2.10.11. (4-(4-Fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (43). 26% yield from ethyl acetate; brown crystals; mp 332.4 °C decomp..; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.16 (t, J = 7.4 Hz, 1H), 7.25–7.31 (m, 2H), 7.36 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.69 (s, 1H), 7.80–7.87 (m, 3H), 8.59 (s, 1H), 8.99 (s, 1H), 10.18 (s, 1H), 12.19 (s, 1H) ppm. MS (ESI$^+$): 429.9 ([MH$^+$+CH_3CN]$^+$,78), 388.9 ([M+H]$^+$,100). Anal. ($C_{21}H_{13}F\ N_4O \times$ ¼ ethyl acetate; 388,42): C, H, N.

4.2.10.12. (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (44). 66% yield; yellow crystals; mp 313–316 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.14 (s, 1H), 12.21 (s, 1H), 9.98 (s, 1H), 8.66 (s, 1H), 8.20 (dd J = 2.5, 6.9 Hz, 1H), 7.86–7.77 (m, 2H), 7.68 (br s, 1H), 7.57–7.45 (m, 2H), 7.37 (t, J = 7.4 Hz, 1H), 7.16 (t, J = 7.4 Hz, 1H) ppm. MS (ESI$^+$): 464 ([MH$^+$+CH_3CN]$^+$, 82; ^{35}Cl), 423 ([M+H]$^+$, 100; ^{35}Cl). IR (KBr): ν 3456, 3216, 1576 cm^{-1}. Anal. Calcd. for ($C_{21}H_{12}ClFN_4OS \times 1.5\ CH_3OH$; 422.86): C, H, N.

4.2.10.13. (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(5-hydroxy-1*H*-indol-2-yl)methanone (45). 57% yield; yellow crystals; mp 293–294 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.87 (s, 1H), 10.20 (s, 1H), 9.10 (s, 1H), 8.94 (s, 1H), 8.65 (s, 1H), 8.20 (dd, J_1 = 2.5; J_2 = 6.9 Hz, 1H), 7.83–7.77 (m, 1H), 7.55–7.46 (m, 2H), 7.35 (d, J = 8.8 Hz, 1H), 7.05 (br s, 1H), 6.92 (dd, J = 2.2, 8.8 Hz, 1H) ppm. MS (ESI$^+$): 480 ([M+H + CH_3CN]$^+$, 100; ^{35}Cl), 439 ([M+H]$^+$, 86; ^{35}Cl). IR (KBr): ν 3423, 3314, 1574 cm^{-1}. Anal. Calcd for ($C_{21}H_{12}ClFN_4O_2S$; 438.86): C, H, N.

4.2.10.14. (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(5-methoxy-1*H*-indol-2-yl)methanone (46). 63% yield; yellow crystals; mp 265–266 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, 1H), 10.21 (s, 1H), 8.95 (s, 1H), 8.66 (s, 1H), 8.21 (dd, J = 2.5, 6.9 Hz, 1H), 7.85–7.78 (m, 1H), 7.59–7.41 (m, 3H), 7.20 (d, J = 2.3 Hz, 1H), 7.03 (dd, J = 2.3, 9.1 Hz, 1H), 3.80 (s, 3H) ppm. MS (ESI$^+$): 494 ([MH$^+$+CH_3CN]$^+$, 100; ^{35}Cl), 453 ([M+H]$^+$, 88; ^{35}Cl). IR (KBr): ν 3445, 3312, 1581 cm^{-1}. Anal. Calcd. for ($C_{22}H_{14}ClFN_4O_2S \times 0.75\ H_2O$; 452.89): C, H, N.

4.2.10.15. (5-(Benzyloxy)-1*H*-indol-2-yl)(4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanone (47). (5-(Benzyloxy)-1-(phenylsulfonyl)-1*H*-indol-2-yl)(4-chloro-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanone (**18**) (1.37 g; 2.0 mmol) was dissolved in THF (75 mL), tetrabutylammoniumfluoride trihydrate (8.0 mmol; 2.52 g) was added and the mixture heated to 120 °C for 2 d in a steel vessel. The mixture was cooled to room temperature, poured into water, the precipitated product removed by filtration, dried and purified by cc (SiO_2; CH_2Cl_2, ethyl acetate 1:1). 86% yield; yellow crystals; mp 282.6–283.3 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.42 (s, 1H, exchangeable), 12.0 (s, 1H, exchangeable), 8.77 (s, 1H), 7.60–7.30 (m, 9H), 7.10 (dd, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 1H), 5.14 (s, 2H) ppm. MS (ESI$^+$): 444.0 ([MH$^+$+CH_3CN]$^+$, 100; ^{35}Cl), 402.9 ([M+H$^+$]$^+$, 44; ^{35}Cl). Anal. Calcd for ($C_{22}H_{15}ClN_4O_2$; 402,83): C, H, N

4.2.10.16. (5-Hydroxy-1*H*-indol-2-yl)(7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)methanone 48: 47. (0.50 g; 1.24 mmol) was dissolved in MeOH/THF (1:1; 300 mL), NH_4HCOO (12.4 mmol; 0.78 g) and Pd on charcoal (0.5 g; 10%) was added. The mixture was heated to reflux for 1 h, cooled to room temperature and the catalyst was removed by filtration over a pad of sodium sulfate. To the clear solution thus obtainedwater (100 mL) was added, the organic solvent removed under reduced pressure and the precipitated product removed by filtration. Purification by cc on silica gel (THF, ethyl acetate 1:4) gave the product: 0.20 g (58%) yellow crystals. mp 240 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.95 (s, 1H, exchangeable), 9.24 (s, 1H, exchangeable), 9.05 (s, 1H, exchangeable), 8.93 (s, 1H), 7.64 (s, 1H), 7.44 (d, J = 1.4 Hz, 1H), 7.34 (d, J = 9.0 Hz, 1H), 7.02 (d, J = 2.2 Hz, 1H), 6.89 (dd, J_1 = 9.0 Hz, J_2 = 2.2 Hz, 1H) ppm. MS (ESI$^+$): 320.1 ([MH$^+$+CH_3CN]$^+$, 58), 279.0 ([M+H$^+$]$^+$, 100). Anal. Calcd for ($C_{15}H_{10}N_4O_2 \times 1/3\ H_2O$; 278,27): C, H, N.

4.2.10.17. (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (49). (4-Chlorothieno[2,3-*d*]pyrimidine-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2yl)methanone (**19**) (1.80 g, 3.97 mmol) and 3-chloro-4-fluoroaniline (1.16 g, 8.00 mmol) were suspended in 2-butanone (75 mL). The mixture was heated to reflux for 2 days. After cooling to room temperature the solvent was removed under reduced pressure. Purification by cc on silica gel (CH_2Cl_2) gave the product: 1.45 g, 65% yield; light yellow powder; mp 273–274 °C;

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17 (s, 1H), 8.69 D, J = 5.8 Hz, 2H), 8.15–8.05 (m, 4H), 7.83–7.38 (m, 9H) ppm. MS (ESI$^+$): 604 ([MH + CH_3CN]$^+$, 100; ^{35}Cl), 563 ([MH]$^+$, 65; ^{35}Cl). IR (KBr): ν 3356, 1641, 1618 cm^{-1}. Anal. Calcd for ($C_{27}H_{16}ClFN_4O_3S_2$; 563.02): C, H, N.

4.2.10.18. (4-((3-Chloro-4-fluorophenyl)(methyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (50). (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone HP-1149 (**49**) (0.2 g, 0.36 mmol) were dissolved in THF (15 ml) and NaH 60% (0.02 g, 0.5 mmol) werde added. After 5 minutes CH_3I (0.5 ml, 0.8 mmol) werde added and the reaction monitored by tlc on silica gel (ethyl acetate, light petroleum 1:2). After the disapearance of the starting material 0.5 ml MeOH were added. The solvent was removed under reduced pressure and the residue was purified by cc on silica gel (ethyl acetate:light petroleum 1:2): 0.15 g, 70% yield; yellow powder; mp 224–226 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.05 (d, J = 8.2 Hz, 1H), 7.88–7.81 (m, 3H), 7.75–7.68 (m, 2H), 7.63–7.48 (m, 4H), 7.41 (m, 1H), 7.30 (m, 1H), 7.05 (s, 1H), 6.23 (s, 1H), 3.54 (s, 3H) ppm. MS (ESI$^+$): 618 ([MH+CH_3CN; ^{35} Cl]$^+$, 25), 577 ([MH]$^+$, 100; ^{35}Cl). IR (KBr): ν 2963, 1635, 1543 cm^{-1}. Anal. Calcd for ($C_{28}H_{18}ClFN_4O_3S_2$; 577.05): C, H, N.

4.2.10.19. (4-((3-Chloro-4-fluorophenyl)(methyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanone (51). (4-((3-Chloro-4-fluorophenyl)(methyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (**50**) (0.5 g, 0.87 mmol) were dissolved in a mixture of methanole (10 mL), THF (10 mL) and aqueous NaOH (10%, 10 mL) and heated to reflux for 1.5 h. THF and methanole were removed under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was separated, dried (Na_2SO_4) and the solvent removed under reduced pressure. The precipitated product was collected by filtration and dried in vacuo. 0.28 g, 74% yield; yellow powder; mp 293–294 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.02 (s, 1H), 8.69 (s, 1H), 8.06 (dd, J_1 = 2.5, J_2 = 6.5 Hz, 1H), 7.82–7.62 (m, 3H), 7.47 (d, J = 8.2 Hz, 1H), 7.34 (t, J = 7.10 Hz, 1H), 7.17 (t, J = 7.4 Hz, 1H), 6.60 (d, J = 8.8 Hz, 2H), 3.59 (s, 3H) ppm. MS (EI): 436 ([M]$^+$, 100; ^{35}Cl), 279 (22). IR (KBr): ν 3446, 3331, 1541 cm^{-1}. Anal. Calcd for ($C_{22}H_{14}ClFN_4OS$; 436.89): C, H, N.

4.2.10.20. Benzo[*b*]furan-2-yl(4-chlorothieno[2,3-*d*]pyrimidine-6-yl)methanone (54) and Benzo[*b*]thiophen-2-yl(4-chlorothieno[2,3-*d*]pyrimidine-6-yl)methanone (55). To a mixture of benzo[*b*]thiophen-2-ylboronic acid (0.64 g, 3.6 mmol), $PdCl_2(PPh_3)_2$ (42 mg, 0.06 mmol) and $K_2HPO_4 \times 3H_2O$ (1.0 g, 4.5 mmol) in dry toluene (15 mL), 4-chlorothieno[2,3-*d*]pyrimidine-6-carbonyl chloride (0.7 g, 3.0 mmol) was added. The mixture was heated to 110 °C for 3 h, cooled to room temperature and diluted with ethyl acetate (30 mL). The organic layer was washed with satd. $NaHCO_3$ solution and dried (Na_2SO_4). After removing of the solvent the product was purified by cc (SiO_2, light petroleum/ethyl acetate 4:1.

4.2.10.21. Benzofuran-2-yl(4-chlorothieno[2,3-*d*]pyrimidin-6-yl)methanone (54). 50% yield; yellow foam; mp 166 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13 (s, 1H), 8.62 (s, 1H), 8.22 (s, 1H), 7.96–7.84 (m, 2H), 7.64 (m, 1H), 7.45 (m, 1H) ppm. MS (EI): 314 ([M]$^+$, 100; ^{35}Cl), 286 (25), 197 (28), 145 (77). IR (KBr): ν 3127, 1625. Anal. Calcd for ($C_{15}H_7ClN_2O_2S$; 314.75): C, H, N.

4.2.10.22. Benzo[*b*]thiophen-2-yl(4-chlorothieno[2,3-*d*]pyrimidin-6-yl)methanone (55). 53% yield; yellow powder; mp 155–158 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13 (s, 1H), 8.71 (s, 1H), 8.50 (s, 1H), 8.19–8.12 (m, 2H), 7.75–7.51 (m, 2H) ppm. MS (ESI$^+$): 372 ([MH$^+$+CH_3CN; ^{35}Cl]$^+$, 100; ^{35}Cl). IR (KBr): ν 3092, 1615, 1551 cm^{-1}. Anal. Calcd for ($C_{15}H_7ClN_2OS_2$; 330.81): C, H, N.

4.2.10.23. Benzofuran-2-yl(4-(3-chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanone (56). According to (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (**49**) from Benz ofuran-2-yl(4-chlorothieno[2,3-*d*]pyrimidin-6-yl)methanone (**54**), (0.65 g, 2.07 mmol) and 3-Chloro-4-fluoroaniline (0.61 g, 4.20 mmol): 0.56 g, 64% yield; yellow crystals; mp 217–218 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.28 (s, 1H), 9.12 (s, 1H), 8.66 (s, 1H), 8.20 (dd, J_1 = 2.6, J_2 = 6.7 Hz, 1H), 8.12 (s, 1H), 7.95 (d, J = 8.10 Hz, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.83–7.77 (m, 1H), 7.69–7.61 (m, 1H), 7.55–7.42 (m, 2H) ppm. MS (EI): 423 ([M]$^+$, 100, ^{35}Cl), 388 (16), 194 (25). IR (KBr): ν 3430, 1578 cm^{-1}. Anal. Calcd for ($C_{21}H_{11}ClFN_3O_2S \times 0.33\ CH_3OH$; 423.85): C, H, N.

4.2.10.24. Benzo[*b*]thiophen-2-yl(4-(3-chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanone (57).

According to (4-(3-Chloro-4-fluorophenylamino)thieno[2,3-*d*]pyrimidin-6-yl)(1-(phenylsulfonyl)-1*H*-indol-2-yl)methanone (**49**) from Benzo[*b*]thiophen-2-yl(4-chlorothieno[2,3-*d*]pyrimidin-6-yl)methanone (**55**), (0.55 g, 1.66 mmol) and 3-Chloro-4-fluoroaniline (0.49 g, 3.40 mmol): 0.28 g, 38% yield; yellow crystals; mp 234–235 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19 (s, 1H), 8.95 (s, 1H), 8.68 (s, 1H), 8.55 (s, 1H), 8.21–8.13 (m, 3H), 7.80–7.74 (m, 1H), 7.65–7.44 (m, 3H) ppm. MS (EI): 439 ([M]$^+$, 100, ^{35}Cl), 404 (17). IR (KBr): ν 3444, 3091, 1621 cm^{-1}. Anal. Calcd for ($C_{21}H_{11}ClFN_3OS_2$; 439.91): C, H, N.

4.3. Biochemical and biological assays

4.3.1. Kinase activity assays

All biochemical protein kinase activity assays were performed in 96-well FlashPlates™ (Perkin Elmer, Boston, USA) in a 50 μL reaction volume. The reaction cocktail contained 20 μL of assay buffer, 5 μL of ATP solution (in H_2O), 5 μL of test compound (in 10% DMSO), 10 μL of substrate and 10 μL of purified recombinant protein kinase. Final concentration of ATP was 1 μM. The assay for all enzymes contained 70 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM Na-orthovanadate,1.2 mM DTT, 50 μg/mL PEG_{20000}, 1 μM [γ-33P]-ATP (approx. 5×10^5 cpm per well). Amount of protein kinases used per 50 μL assay were: ABL1 wt: 10 ng; EGF-R wt: 10 ng; Erb2: 100 ng; MET wt: 12 ng; VEGF-R2: 15 ng. The following substrates were used at the specified concentrations: poly(Glu, Tyr)$_{4:1}$, 0.125 μg/50 μL: EGF-R wt, ErbB2, VEGF-R2; poly(Ala, Glu, Lys, Tyr)$_{6:2:5:1}$, 0.125 μg/50 μL: ABL1 wt, MET wt.

The reaction cocktails were incubated at 30 °C for 60 min. The reaction was stopped with 50 μl of 2%(v/v) H_3PO_4, plates were aspirated and washed two times with 200 μl 0.9% (w/v) NaCl. Incorporation of $^{33}P_i$ was determined with a Microbeta™ microplate scintillation counter (Perkin Elmer, Boston, MA, USA).

The inhibition for each concentration and the compound IC_{50} values were calculated with Quattro Workflow V3.0.3 (Quattro Research GmbH, Munich, Germany). The model used for IC_{50} determination was 'Sigmoidal response (variable slope)' with parameters 'top' fixed at 100% and 'bottom' at 0%.

4.3.2. Biological assays

A431 cells were cultivated in DMEM (4.5 g glucose/l), supplemented with glutamine, and 10% fetal bovine serum (FBS). For measuring autophosphorylation of EGFR, cells were seeded at about 50% confluence into 24-well plates. After adherence, the medium was replaced with DMEM with 0.5% FBS and starvation was allowed over night. Inhibitors were then added to the plates

(final DMSO concentration 0.1%) for 1 h. EGF was added at a final concentration of 100 ng/ml for 10 min, then cells were washed with phosphate-buffered saline, and lyzed with 100 µl lysis buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10 mN sodium pyrophosphate, 2 mM EGTA, 20 µM Zn-Acetat, 50 mM NaF, freshly added: 1 mM PMSF, 5 µg/ml leupeptin, 2 mM sodium orthovanadate) per well. The extracts were subjected to SDS-polyacrylamide gel electrophoresis, and blotting to PVDF membranes. Blots were developed with anti-phosphotyrosine antibodies (4G10, Upstate, Lake Placid, USA), then stripped and probed with anti-EGFR antibodies (Transduction Laboratories, E12020; BD Biosciences, Heidelberg, Germany) using the enhanced chemiluminescence method. Films were scanned and quantified using Fuji Multi Gauge software. Ranges of IC_{50} values indicate the two tested concentrations, in between which half-maximal inhibition was observed in all experiments.

4.3.3. Sensitivity profile of selected ((1*H*-indol-2-yl)(4-(phenyl amino)thieno[2,3-*d*]pyrimidin-6-yl)methanones

Cell lines of the panel comprised 15 different tumor histotypes, each represented by one to six cell lines. They were established from cancer of the bladder (3), colon (5), head and neck (1), liver (1), lung (6), breast (3), pancreas (3), prostate (4), ovary (2), kidney (3), stomach (2) and the uterine body (1), as well as from malignant melanoma (3), sarcoma (2) and pleuramesothelioma (3). The 24 cell lines BXF 1218L, BXF 1352L, CXF 269L, GXF 251L, LIXF 575L, LXFL 1121L, LXFA 289L, LXFA 526L, LXFL 529L, LXFA 629L, MAXF 401NL, MEXF 1341L, MEXF 276L, MEXF 462NL, OVXF 899L, PAXF 1657L, PAXF 546L, PXF 1118L, PXF 1752L, PXF 698L, RXF 1781L, RXF 393NL, RXF 486L and UXF 1138L were established at Oncotest from patient-derived tumor xenografts.[18] The origin of the donor xenografts was described by Fiebig et al. 1992 and 1999.[19,20] The other 18 cell lines were either kindly provided by the NCI (Bethesda; MD), or were purchased from ATCC (Rockville, MD), DSMZ (Braunschweig, Germany) or JCRB (Osaka, Japan). Authenticity of all cell lines was proven at the by STR (short tandem repeat) analysis, a PCR based DNA-fingerprinting methodology.[21,22] All cells were grown at 37 °C in a humidified atmosphere with 5% CO_2 in RPMI 1640 medium, supplemented with 10% (v/v) fetal calf serum and 0.1 mg/mL gentamicin (medium and all components from PAA, Cölbe, Germany).

A propidium iodide based cytoxicitiy assay was used to assess the anti-cancer activity of the compounds. Cells were harvested from exponential phase cultures, counted and plated in 96 well flat-bottom microtiter plates at a cell density of 4000–40,000 cells/well. After a 24 h recovery period to allow the cells to resume exponential growth, test compounds were added in duplicates at ten concentrations and treatment continued for four days. Cells were washed with 200 µl PBS to remove dead cells, then 200 µl of a solution containing 7 µg/ml propidium iodide (PI) and 0.1% (v/v) Triton X-100 was added. After an incubation period of 1–2 hours at room temperature, fluorescence (FU) was measured using the Cytofluor 4000 microplate reader (excitation λ = 530 nm, emission λ = 620 nm) to quantify the amount of attached viable cells. Each compound was tested in three independent experiments using all 42 cancer cell lines. Relative IC_{50} values were determined by non-linear regression (log [conc. of inhibitor] versus response (% T/C)) using the GraphPad Prism® analysis software (Prism 5 for Windows, version 5.01, GraphPad Software Inc., CA). For calculation of mean IC_{50} values over the 42 cell lines tested, the geometric mean ('geomean') was used.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.11.023.

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