European Journal of Medicinal Chemistry 46 (2011) 2759–2769

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Dual IGF-1R/SRC inhibitors based on a *N*′-aroyl-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide structure

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ARTICLE INFO

Article history:
Received 18 January 2011
Received in revised form
29 March 2011
Accepted 30 March 2011
Available online 6 April 2011

Keywords:
Anticancer
Hydrazides
Indoles
Protein kinase inhibitors
Suzuki reaction

ABSTRACT

The *N*′-aroyl-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide motif was identified as a novel scaffold for the development of kinase inhibitors. Derivatives with a biphenyl element attached to the hydrazide structure proved to be submicromolar dual inhibitors of the cancer-related kinases IGF-1R and SRC. One of the most potent kinase inhibitors of the series produced a selective growth inhibition in a panel of cultivated cancer cell lines.

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1. Introduction

Because many human tumours are characterized by hyperactivity of protein kinases, the inhibition of these enzymes has been established as a cancer treatment in recent years. Given the great success of this therapeutic strategy, the search for additional protein kinase inhibitors is one of the major research topics in the oncology drug development area [1,2]. While in the beginning of the protein kinase inhibitor era selectivity towards a single target enzyme appeared desirable, it is now generally accepted that inhibiting more than one tumour-related protein kinase at a time offers the advantage of hitting the tumour in multiple aspects [3,4]. Among other advantages, the aspecificity of drugs targeting several kinases may lead to a lower risk of drug-resistance development [3,4]. Multikinase inhibitors launched recently as anticancer drugs comprise sunitinib [5], sorafenib [6] and dasatinib [7,8]. Albeit namely the latter was reported as an inhibitor of human protein kinases with high promiscuity [7], the undesired side effects of the drug are tolerable. In the framework of our ongoing studies to identify novel multikinase inhibitor chemotypes [9–12] we here report *N*′-aroyl-2-(1*H*-indol-3-yl)-2-oxoacetohydrazides as dual IGF-1R/SRC inhibitors.

The insulin-like growth factor receptor-1 tyrosine kinase (IGF-1R) constitutes an emerging novel target for anticancer therapy [13]. There are clear hints pointing to the involvement of IGF-1R in cancer development. Particularly, activation of the IGF-1R receptor kinase leads to triggering of intracellular signalling pathways promoting cell proliferation and survival, angiogenesis, metastasis and invasion [14]. Mice overexpressing the physiological activator IGF-II of the IGF-1R develop a variety of tumours, among them hepatocellular carcinomas and lymphomas [15]. Since overexpression of the receptor and its physiological ligands have been observed in several solid human tumours, small molecules inhibiting IGF-1R have been developed as potential anticancer drugs and have already advanced to clinical trials [13,16]. c-SRC is a non-receptor tyrosine kinase mediating mitogenic signals between IGF-1R and downstream signalling cascades. A variety of investigations suggest that SRC plays a prominent role in cancer cell proliferation, invasion, and motility. SRC hyperactivity has been implicated in the development and progression of human cancers. Drug development research in recent years led to several small molecule SRC inhibitors currently being evaluated in clinical phases, among them

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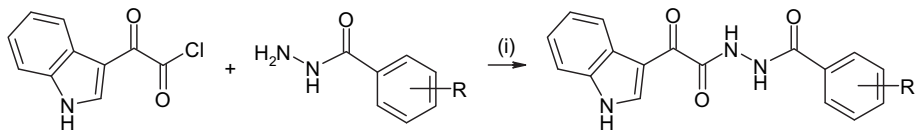
doi:10.1016/j.ejmech.2011.03.065



5 6a-q 7a-q

Scheme 1. Synthesis of *N*′-aroyl-2-(1*H*-indol-3-yl)-2-oxoacetohydrazides **7a-q**. Reagents and conditions: (i) Et_3N, MTBE, 0 °C → rt.

compounds XL-999, AZD-0530, KX010107, and Bosutinib, a dual BCR-ABL and SRC inhibitor [17]. It was reported that in cells transformed by the *src* ongogene, IGF-1R was found to be constitutively phosphorylated. This mechanism was suggested to be one of the events responsible for the transforming property of the *src* oncogene [18]. Thus, inhibiting both IGF-1R and SRC by one small molecule inhibitor could offer the advantage of interfering with two successive events of the same signalling axis, constituting a concept potentially lowering the risk for resistance development.

The identification of the dual IGF-1R/SRC inhibitor chemotype reported here resulted from an observation during a project directed to discover inhibitors of the protein kinase MET. Hyperactivity of the MET kinase is related to growth, aggressiveness and metastasis risk in several solid cancer diseases [19]. When we evaluated selected commercially available compounds in vitro in a radiometric protein kinase assay (^{33}PanQinase® Activity Assay), we identified the 2-(1*H*-indol-3-yl)-2-oxoacetohydrazide **1** as weak MET inhibitor (IC_{50} c-MET = 69 μM) [20].

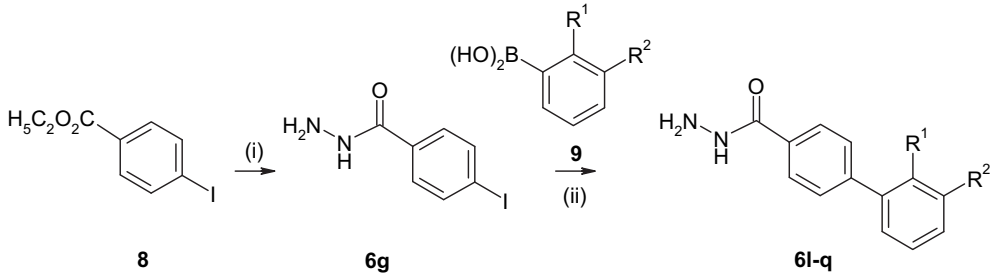
2. Chemistry

In an attempt to narrow down the pharmacophore structure element in **1**, the truncated structures **2**–**4** were synthesized following published procedures [21–25]. For the synthesis of the *N,N*′-diacylated hydrazides **7**, suitable benzoic acid hydrazides **6** were reacted with 2-(1*H*-indol-3-yl)-2-oxoacetyl chloride **5** in methyl *tert*-butyl ether (MTBE) in the presence of triethylamine (Scheme 1). The carbohydrazides **6l-q** needed as building blocks for the synthesis of **7l-q** were prepared by means of a microwave-assisted Suzuki coupling reaction catalyzed by tetrakistriphenylphospane-palladium in the presence of diazabicycloundecene (Scheme 2).

3. Biological evaluation and discussion

While none of the derivatives **2**–**4** showed improved MET inhibition, two congeners (**3b** and **4a**) exhibited a modest inhibition of IGF-1R (Table 1). Because of the overall disappointing results with **2**–**4**, we postulated that the complete *N,N*′-diacylated hydrazide structure as displayed by **1** is necessary for kinase inhibition. We continued the series with compounds **7a-k** which differ from the parent structure **1** exclusively by the substitution pattern at the phenyl ring. While none of **7a-k** exhibited MET inhibition with IC_{50} values below 10 μM, 4 congeners (**7d**, **7e**, **7g**, **7i**) showed IGF-1R inhibition in single-digit micromolar concentrations. Of note, these compounds have a more or less bulky substituent in the 4-position of the phenyl ring and inhibited SRC in the same order of magnitude as IGF-1R. We therefore extended the series to derivatives **7l-q** which are characterized by a phenyl substituent in the indicated position, constituting *N*′-acylated biphenyl-4-carbohydrazides. Actually, all novel congeners with the biphenyl element inhibited both IGF-1R and SRC in low micromolar or submicromolar concentrations (Table 1). To get preliminary information regarding bioactivity of the novel compounds in a cellular environment, compound **7o** was screened for antiproliferative activity in the in vitro cell line screening project (IVCLSP) of the National Cancer Institute (NCI) [26]. Indeed, the cell lines A549ATCC, NCI-H522 (both non-small cell lung cancer cell lines) and OVCAR-8 (ovarian cancer) were inhibited impressively (Table 2). Other cell lines were inhibited in a lower extent, indicating a selective cytotoxicity, as is expected with a protein kinase inhibitor. Inhibition of A549ATCC by **7o** coincides with the fact that this cancer cell line shows the strongest SRC expression level of all NCI panel cell lines [27].

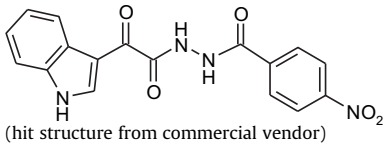
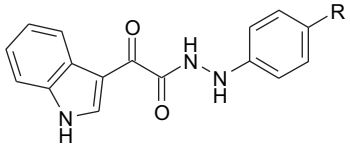
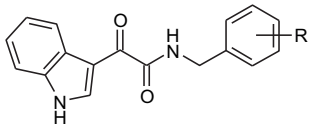
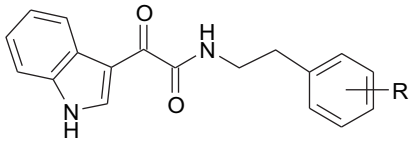
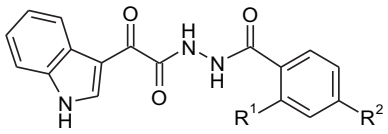
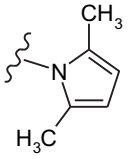
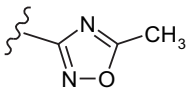
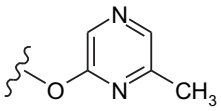
Docking of the prototype structure **7o** into published structures of IGF-1R (pdb entry 2zm3) [28] and SRC (pdb entry 2bdf) [29] produced poses showing a certain degree of similarity and explaining the gain of activity by introduction of the biphenyl structural element (Fig. 1). In both poses, elongate conformations of the inhibitors are aligned below the glycine rich loops (Leu1005-Phe1010 in IGF-1R and Leu273-Phe278 in SRC) in the cleft between the N-terminal and the C-teminal lobes of the kinases. The indole ring systems are located near the hinge area facing the entrances of the pockets. The carbonyl groups next to the indole elements are both acting as hydrogen bond acceptors towards the amino acid residue three positions downstream the gatekeeper (gk+3, Met1082 in case of IGF-1R and Met 341 in case of SRC). The benzene rings of the biphenyl element are involved in hydrophobic contacts to hydrophobic amino acid residues in the binding cleft. The *tert*-butyl phenyl ring of the inhibitor displays hydrophobic interactions with the phenylalanine residues located at the C-terminal end of the glycine rich loops (Phe1010 in IGF-1R and Phe 278 in SRC, respectively). In both poses, the ether oxygen is a hydrogen bond acceptor towards amino acids of the glycine rich loop, contacting



8 6g 6l-q

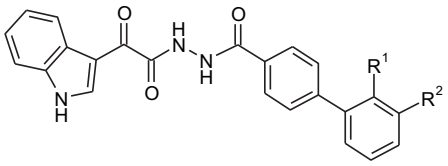
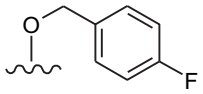
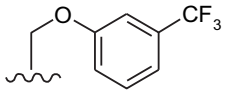
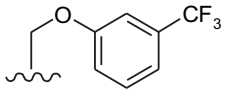
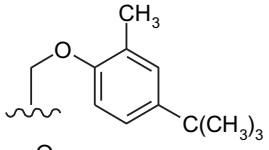
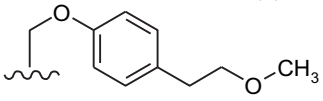
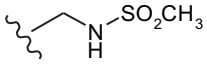
Scheme 2. Synthesis of biphenyl-4-carbohydazides **6l-q**. Reagents and conditions: (i) hydrazine hydrate, EtOH, reflux; (ii) $Pd(PPh_3)_4$, DBU, H_2O/EtOH, 150 °C, μW.

Table 1
Protein kinase inhibition by 2-(1*H*-indol-3-yl)-2-oxoacetohydrazides **1, 2, 7** and 2-(1*H*-indol-3-yl)-2-oxoacetamides **3, 4**. [a,b,c]

Entry	Structure	IC_{50} c-MET [μM]	IC_{50} IGF-1R [μM]	IC_{50} SRC [μM]
1	 (hit structure from commercial vendor)	69	>100	N.T.
2				
2a	R = −H	> 100	>100	N.T.
2b	R = −Cl	> 100	> 100	N.T.
2c	R = −Br	> 100	>100	N.T.
2d	R = −I	> 100	>100	N.T.
3				
3a	R = −H	>100	>100	N.T.
3b	R = 4-CH_3	>100	63	N.T.
3c	R = 4-Cl	>100	>100	N.T.
3d	R = 3-Cl	>100	>100	N.T.
4				
4a	R = 4-CH_3	>100	52	N.T.
4b	R = 3,4-di-OCH_3	>100	>100	N.T.
4c	R = 4-Cl	>100	>100	N.T.
4d	R = 3-Cl	>100	>100	N.T.
7a-k				
7a	R^1, R^2 = −H	>100	> 100	N.T.
7b	R^1 = H, R^2 = $-CH_3$	95	82	N.T.
7c	R^1 = H, R^2 = $-OCH_3$	96	54	N.T.
7d	R^1 = H, R^2 = $-C(CH_3)_3$	36	4.4 (±0.5)	5.9 (±2.5)
7e	R^1 = H, R^2 = $-CF_3$	49	4.1 (±2.7)	1.3
7f	R^1 = H, R^2 = −Cl	48	16	N.T.
7g	R^1 = H, R^2 = −I	14	4.0 (±0.2)	4.5 (±1.0)
7h	R^1 = F, R^2 = −H	>100	91	96
7i	R^1 = H, R^2 = 	16	4.9 (±0.6)	3.6 (±0.6)
7j	R^1 = H, R^2 = 	95	12	22
7k	R^1 = H, R^2 = 	>100	31	40

(*continued on next page*)

Table 1 (*continued*)

Entry	Structure	IC_{50} c-MET [μM]	IC_{50} IGF-1R [μM]	IC_{50} SRC [μM]
7l-q				
7l	R^2 = –H, R^1 = 	13	4.9 (±1.1)	1.9 (±0.4)
7m	R^2 = –H, R^1 = 	5.2	0.81 (±0.06)	0.62 (±0.24)
7n	R^1 = –H, R^2 = 	7.6	0.99 (±0.31)	0.72 (±0.21)
7o	R^1 = –H, R^2 = 	4.1	0.67 (±0.02)	0.52 (±0.12)
7p	R^1 = –H, R^2 = 	8.8	0.68 (±0.07)	0.38 (±0.06)
7q	R^1 = –H, R^2 = 	27	3.3 (±0.2)	2.8 (±0.2)

N.T.: not tested.
[a] Compounds **2, 3** and **4** have been described before. See experimental part for references.
[b] Values with range given in brackets represent the mean of two independent determinations.
[c] Values without range indicate a single measurement.

the side chain of Ser1009 in IGF-1R and the backbone amide of Phe278 in SRC.

According to these docking results, in both IGF-1R and SRC the inhibitor **7o** occupies not only the ATP binding pocket but also binds with its *tert*-butyl phenyl moiety to a large pocket in the neighbourhood. This binding mode may account for the selectivity for IGF-1R and SRC and needs consideration during future studies in which detailed kinetic investigations will be necessary to determine the inhibition modes (ATP-competitive or allosteric or mixed). As long as such results are not available and no crystal structure is solved for a complex between the ligands **7** and the target kinases, the binding modes presented here should be considered as working hypotheses guiding the further structure modification and optimisation process.

Table 2
Inhibition of cancer cell proliferation caused by compound **7o**[a]

Cancer cell line	Cell growth (%) in presence of **7o** (10 μM) related to untreated controls[b]
A549/ATCC	7.38
NCI-H522	−7.70
OVCAR-8	−9.59
Average of all cell lines	81.05

Negative values indicate net cell kill instead of proliferation.
[a] Data from the IVCLSP of the NCI. Cells were incubated for 48 h with the test compound. Read out by a sulforhodamine assay.
[b] Controls = 100%.

4. Conclusion

The *N′*-aroyl-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide structure was discovered as a promising template for the development of novel protein kinase inhibitors, namely of IGF-1R and/or SRC. Although a distinct compound belonging to this structure class has already demonstrated antiproliferative activity, a broad synthesis program will be necessary to increase protein kinase inhibitory potency and selectivity and to tailor ADME properties. The simple synthetic procedures and the plausible docking mode of **7o** in IGF-1R and SRC are good arguments to consider such a development campaign.

5. Experimental protocols

5.1. Synthetic chemistry

5.1.1. General

Monomode microwave device: CEM Discover focused microwave synthesis system with ChemDriver software. Melting points (mp) were determined on an electric variable heater (Barnstead

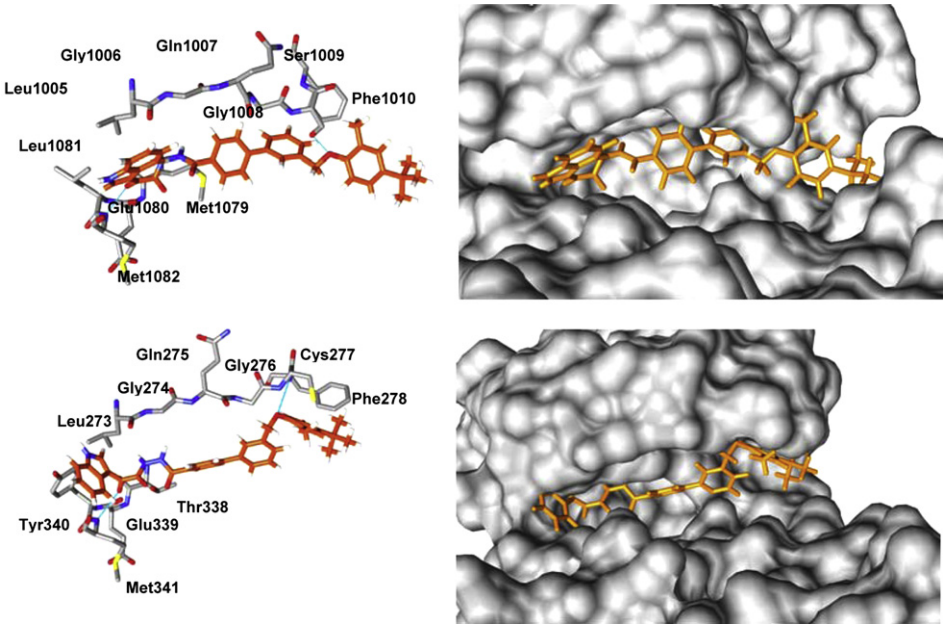


Fig. 1. Docking poses generated for inhibitor **7o** in the protein structures IGF-1R [28] (upper row) and SRC [29] (lower row). On the left side the inhibitor (H-atoms depicted, carbon atoms orange-red) is shown with the amino acids (H-atoms omitted) of the glycine-rich loop (Leu1005-Phe1010 for IGF-1R, Leu273-Phe278 for SRC) and the hinge sequence (Met1079(gatekeeper)-Met1082 for IGF-1R, Thr338(gatekeeper)-Met341 for SRC). On the pictures on the right side the surface of the protein structure is given showing the general accommodation of the inhibitor in the cleft between the N-terminal lobe (on top of the inhibitor) and the C-terminal lobe (below the inhibitor). Illustration by UCSF Chimera [36]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Electrothermal IA 9100) and were not corrected. IR spectra were recorded as KBr discs on a Thermo Nicolet FT-IR 200. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on the following instruments: Bruker Avance DRX-400 and Bruker Avance II-600, solvent DMSO-d_6 if not stated otherwise, internal standard trimethylsilane, signals in ppm (δ scale). Elemental analyses were determined on a CE Instruments FlashEA 1112 elemental analyzer (Thermo Quest).

Mass spectra were recorded on a double-focused sector field mass spectrometer Finnigan-MAT 95. Accurate measurements were conducted according to the peakmatch method using perfluorokerosene (PFK) as an internal mass reference; (EI)-MS: ionization energy 70 eV. TLC: Polygram Sil G/UV$_{254}$, Macherey-Nagel, 40 mm × 80 mm, visualization by UV illumination (254 nm). Column chromatography: silica gel 60 (Merck), column width 2.5–3 cm, column height 15–30 cm. Purity was determined by HPLC using the following devices and settings [30]: LaChrom Elite (Merck/Hitachi), pump L-2130, autosampler L-2200, diode array detector L-2450, organizer box L-2000; column Merck LiChroCART 125-4, LiChrosphere 100 RP 18, 5 μm, flow rate 1.000 mL/min, isocratic, volume of injection 10 μL; detection (DAD) at 254 and 280 nm; AUC % method; time of detection 15 min, net retention time (t_N), dead time (t_M) related to DMSO; eluent water/acetonitrile if not stated otherwise. All compounds employed in biological tests were used in ≥95% purity. The following compounds were prepared according to literature methods: **2a**-**b** [22], **3a** [23], **3b** [25], **3c**-**d** [23], **4a** [24], **4b**-**c** [21] and **6g** [31]. Compounds **5**, **6a**-**f**, **6h**, **6i**, **8** and **9** were purchased from commercial suppliers and were used without further purification.

5.1.2. *N′-(4-Bromophenyl)-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**2c**)*

A solution of triethylamine (765 μL, 5.50 mmol) in anhydrous *tert*-butyl methyl ether (10 mL) was added dropwise at 0 °C to a stirred suspension of 4-bromophenylhydrazine hydrochloride (559 mg, 2.50 mmol) in the same solvent (20 mL). After stirring for 30 min at 0 °C a suspension of 2-(1*H*-indol-3-yl)-2-oxoacetyl chloride (**5**; 521 mg, 2.51 mmol) in anhydrous *tert*-butyl methyl ether (30 mL) was slowly added. The reaction mixture was stirred at 0 °C for 15 min and then for 1.5 h at room temperature, monitoring the reaction by TLC analysis. After completion of the reaction the mixture was washed with a saturated $NaHCO_3$ aqueous solution and water (3 × 20 mL in each case). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified and isolated by silica gel chromatography (ethyl acetate/petroleum ether). Subsequent recrystallization from toluene and ethanol 70% yielded yellow crystals (5%).

Mp.: 220–223 °C (dec.); IR (KBr): 3352 cm^{-1} and 3283 cm^{-1} (NH), 1677 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 6.72–6.74 (m, 2H, ar-H), 7.27–7.31 (m, 2H, ar-H), 7.32–7.34 (m, 2H, ar-H), 7.55–7.57 (m, 1H, ar-H), 8.17 (d, 1H, J = 2.7 Hz, NH), 8.23–8.25 (m, 1H, ar-H), 8.57 (d, 1H, J = 2.5 Hz, ar-H), 10.65 (d, 1H, J = 2.7 Hz, NH), 12.29 (s, 1H, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 112.2, 113.8 (2C), 120.7, 122.3, 123.1, 130.9 (2C), 137.7 (tert. C); 109.1, 111.8, 125.4, 135.9, 147.7, 163.4, 181.7 (quat. C); $C_{16}H_{12}BrN_3O_2$ (358.19); calcd. C 53.65, H 3.38, N 11.73; found C 53.69, H 3.29, N 11.35; HPLC-purity: 98.9% at 254 nm and 97.6% at 280 nm, t_N = 2.90 min, t_M = 1.03 min (ACN/H_2O; 50:50), λ_{max}: 245 nm and 328 nm.

5.1.3. *2-(1H-Indol-3-yl)-N′-(4-iodophenyl)-2-oxoacetohydrazide (**2d**)*

To a stirred solution of 4-iodophenylhydrazine (234 mg, 1.00 mmol) in anhydrous *tert*-butyl methyl ether (8 mL) triethylamine (153 μL, 1.10 mmol) was added at 0 °C. Then a suspension of 2-(1*H*-indol-3-yl)-2-oxoacetyl chloride (**5**; 208 mg, 1.00 mmol) in anhydrous *tert*-butyl methyl ether (12 mL) was slowly added. The reaction mixture was stirred at 0 °C for 15 min and then for 1.5 h at room temperature, monitoring the reaction by TLC analysis. After completion of the reaction the mixture was washed with a saturated $NaHCO_3$ aqueous solution and water (3 × 20 mL in each case). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified and isolated by silica gel

chromatography (eluent: ethyl acetate/petroleum ether (1:1)). Subsequent recrystallization from toluene and ethanol 70% yielded yellow crystals (2%).

Mp.: 222–228 °C (dec.); IR (KBr): 3388 cm^{-1} and 3279 cm^{-1} (NH), 1685 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 6.60–6.63 (m, 2H, ar-H), 7.27–7.31 (m, 2H, ar-H), 7.46–7.48 (m, 2H, ar-H), 7.54–7.57 (m, 1H, ar-H), 8.16 (d, 1H, J = 2.8 Hz, NH), 8.22–8.25 (m, 1H, ar-H), 8.57 (d, 1H, J = 3.2 Hz, ar-H), 10.63 (d, 1H, J = 2.7 Hz, NH), 12.29 (d, 1H, J = 2.5 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 112.6, 114.7 (2C), 121.1, 122.6, 123.5, 137.0 (2C), 138.1 (tert. C); 80.0, 112.1, 125.7, 136.3, 148.5, 163.7, 182.1 (quat. C); $C_{16}H_{12}IN_3O_2$ (405.19); calcd. C 47.43, H 2.99, N 10.37; found C 47.37, H 3.00, N 9.75; MS (EI): m/z (%) = 405 $[M]^{+\cdot}$ (27); HRMS (EI): m/z $[M]^{+\cdot}$ calcd. 404.99747; found 404.99763; HPLC-purity: 98.6% at 254 nm and 97.0% at 280 nm, t_N = 3.54 min, t_M = 1.03 min (ACN/H_2O; 50:50), λ_{max}: 248 nm and 328 nm.

5.1.4. N-[2-(3-Chlorophenyl)ethyl]-2-(1H-indol-3-yl)-2-oxoacetamide (**4d**)

Triethylamine (153 μL, 1.10 mmol) was added dropwise at 0 °C to a stirred suspension of 2-(1*H*-indol-3-yl)-2-oxoacetyl chloride (**5**; 208 mg, 1.00 mmol) and 2-(3-chlorophenyl)ethylamine (139 μL, 1.00 mmol) in anhydrous *tert*-butyl methyl ether (20 mL). The reaction mixture was stirred at 0 °C for 15 min and then for 1 h at room temperature, monitoring the reaction by TLC analysis. After completion of the reaction the mixture was washed with a saturated $NaHCO_3$ aqueous solution and water (3 × 20 mL in each case). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Recrystallization of the crude product from ethanol 70% yielded colourless crystals (33%).

Mp.: 184–186 °C; IR (KBr): 3347 cm^{-1} and 3190 cm^{-1} (NH), 2938 cm^{-1} (CH aliph.), 1657 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 2.88 (t, 2H, J = 7.0 Hz, $NHCH_2CH_2$), 3.50 (dt, 2H, J = 6.4/6.8 Hz, $NHCH_2CH_2$), 7.21–7.34 (m, 6H, ar-H), 7.52–7.57 (m, 1H, ar-H), 8.21–8.27 (m, 1H, ar-H), 8.67 (s, 1H, ar-H), 8.81 (t, 1H, J = 5.8 Hz, NH), 12.24 (s, 1H, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 34.2 (CH_2), 39.2 (CH_2); 112.5, 121.2, 122.5, 123.4, 126.1, 127.5, 128.5, 130.1, 138.4 (tert. C); 112.1, 132.9, 136.3, 141.9, 163.6, 182.1 (quat. C, one signal is missing due to peak overlap); $C_{18}H_{15}ClN_2O_2$ (326.78); calcd. C 66.16, H 4.63, N 8.57; found C 65.84, H 4.60, N 8.31; HPLC-purity: 99.3% at 254 nm and 98.9% at 280 nm, t_N = 3.38 min, t_M = 1.03 min (ACN/H_2O; 55:45), λ_{max}: 256 nm and 327 nm.

5.1.5. 4-(5-Methyl-1,2,4-oxadiazol-3-yl)benzohydrazide (**6j**)

Hydrazine hydrate (485 μL, 9.98 mmol) was added dropwise to a solution of ethyl 4-(5-methyl-1,2,4-oxadiazol-3-yl)benzoate (232 mg, 1.00 mmol) in anhydrous ethanol (4 mL). The reaction mixture was refluxed for 30 h. After cooling to room temperature the resulting precipitate was collected and washed with ethanol. The crude product was used in the next step without further purification according to general procedure B yielding compound **7j**.

Yield: 84%, white powder; mp.: 237–241 °C; ^1H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 2.68 (s, 3H, CH_3), 4.57 (s, 2H, NH_2), 7.97–8.00 (m, 2H, ar-H), 8.05–8.08 (m, 2H, ar-H), 9.94 (s, 1H, NH).

5.1.6. 4-[(6-methylpyrazin-2-yl)oxy]benzohydrazide (**6k**)

Hydrazine hydrate (121 μL, 2.49 mmol) was added dropwise to a solution of ethyl 4-[(6-methylpyrazin-2-yl)oxy]benzoate (258 mg, 1.00 mmol) in ethanol (5 mL). The reaction mixture was refluxed for 32 h. After cooling to room temperature the resulting precipitate was collected and washed with ethanol. The crude product was used in the next step without further purification according to general procedure B yielding compound **7k**.

Yield: 16%, white powder; mp.: 166–167 °C; ^1H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 2.35 (s, 3H, CH_3), 4.51 (s, 2H, NH_2), 7.23–7.27 (m, 2H, ar-H), 7.86–7.90 (m, 2H, ar-H), 8.32 (d, 1H, J = 0.5 Hz, pyrazine-H), 8.34 (d, 1H, J = 0.5 Hz, pyrazine-H), 9.77 (s, 1H, NH).

5.1.7. General procedure A for the preparation of the biphenyl-4-carbohydrazides **6l-6q**

4-Iodobenzohydrazide (**6g**; 262 mg, 1.00 mmol) was reacted with the appropriate boronic acid **9** (1.00 mmol), 1,8-diazabicyclo [5.4.0]undec-7-ene (149 μL, 1.00 mmol) and 5 mol% tetrakis(-triphenylphosphine)palladium(0) (58 mg, 0.050 mmol) in water (1 mL) and ethanol (1 mL). The reaction was conducted in a microwave device using a sealed microwave reaction vessel. The reaction mixture was stirred for 10 min at 150 °C, 60 W, ramp time 5 min, maximum pressure 250 psi. After cooling to room temperature the mixture was poured into a separating funnel. Water was added and the aqueous phase was extracted with ethyl acetate (3 × 20 mL). The collected organic layers were dried over anhydrous Na_2SO_4 and evaporated. Except for **6p**, which was purified and isolated by silica gel chromatography (eluent: ethyl acetate), the crude products were used in the next step without further purification according to general procedure B yielding compounds **7l-q**.

5.1.7.1. 2′-[(4-Fluorobenzyl)oxy][1,1′-biphenyl]-4-carbohydrazide (**6l**).

Preparation according to General Procedure A yielded a yellow, viscous product (57%).

^1H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 4.54 (s, 2H, NH_2), 5.13 (s, 2H, OCH_2), 7.07 (ddd, 1H, J = 1.0/7.4/7.4 Hz, ar-H), 7.16–7.20 (m, 2H, ar-H), 7.21–7.23 (m, 1H, ar-H), 7.34–7.37 (m, 2H, ar-H), 7.40–7.44 (m, 2H, ar-H), 7.58–7.61 (m, 2H, ar-H), 7.82–7.85 (m, 2H, ar-H), 9.78 (s, 1H, NH).

5.1.7.2. 2′-{[3-(Trifluoromethyl)phenoxy]methyl}[1,1′-biphenyl]-4-carbohydrazide (**6m**).

Preparation according to General Procedure A yielded a yellow, viscous product (65%).

IR (KBr): 3310 cm^{-1} (NH), 3065 cm^{-1} (CH arom.), 2929 cm^{-1} (CH aliph.); ^1H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 4.54 (s, 2H, NH_2), 5.05 (s, 2H, OCH_2), 7.19–7.20 (m, 2H, ar-H), 7.27–7.29 (m, 1H, ar-H), 7.37–7.38 (m, 1H, ar-H), 7.47–7.49 (m, 3H, ar-H), 7.50–7.51 (m, 2H, ar-H), 7.64–7.65 (m, 1H, ar-H), 7.86–7.88 (m, 2H, ar-H), 9.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 68.0 (OCH_2); 111.1 (q, $^3J_{C,F}$ = 3.5 Hz, C–C–CF_3), 117.3 (q, $^3J_{C,F}$ = 3.9 Hz, C–C–CF_3), 118.9, 126.8 (2C), 128.0, 128.6, 128.8 (2C), 129.9, 130.0, 130.6 (tert. C); 123.9 (q, $^1J_{C,F}$ = 272 Hz, CF_3), 130.2 (q, $^2J_{C,F}$ = 32 Hz, C–CF_3), 132.1, 133.2, 140.9, 142.5, 158.2, 165.3 (quat. C).

5.1.7.3. 3′-{[3-(Trifluoromethyl)phenoxy]methyl}[1,1′-biphenyl]-4-carbohydrazide (**6n**).

Preparation according to General Procedure A yielded a yellow, viscous product (56%).

IR (KBr): 3288 cm^{-1} (NH), 3057 cm^{-1} (CH arom.), 2922 cm^{-1} und 2863 cm^{-1} (CH aliph.); ^1H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 4.56 (s, 2H, NH_2), 5.28 (s, 2H, OCH_2), 7.31 (ddd, 1H, J = 0.7/7.6/7.6 Hz, ar-H), 7.36–7.39 (m, 2H, ar-H), 7.51–7.56 (m, 3H, ar-H), 7.72 (ddd, 1H, J = 2.0/6.9/6.9 Hz, ar-H), 7.77–7.79 (m, 2H, ar-H), 7.85 (m, 1H, ar-H), 7.93–7.95 (m, 2H, ar-H), 9.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 69.4 (OCH_2); 111.3 (q, $^3J_{C,F}$ = 3.7 Hz, C–C–CF_3), 117.2 (q, $^3J_{C,F}$ = 3.9 Hz, C–C–CF_3), 119.0, 126.3, 126.4, 126.5 (2C), 127.4, 127.6 (2C), 129.2, 130.7 (tert. C); 123.9 (q, $^1J_{C,F}$ = 272 Hz, CF_3), 130.2 (q, $^2J_{C,F}$ = 32 Hz, C–CF_3), 132.2, 137.2, 139.3, 142.2, 158.5, 165.4 (quat. C).

5.1.7.4. 3′-{[4-tert-butyl-2-methylphenoxy]methyl}[1,1′-biphenyl]-4-carbohydrazide (**6o**).

Preparation according to General Procedure A yielded a yellow, viscous product (81%).

IR (KBr): 3280 cm^{-1} (NH), 3053 cm^{-1} (CH arom.), 2953 cm^{-1} und 2863 cm^{-1} (CH aliph.), 1643 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 1.24 (s, 9H, CH_3), 2.21 (s, 3H, CH_3), 4.55 (s, 2H, NH_2), 5.17 (s, 2H, OCH_2), 6.94 (d, 1H, J = 8.6 Hz, ar-H), 7.13 (dd, 1H, J = 2.2/8.2 Hz, ar-H), 7.19 (dd, 1H, J = 0.6/2.5 Hz, ar-H), 7.49 (ddd, 1H, J = 1.6/7.7/7.7 Hz, ar-H), 7.51 (dd, 1H, J = 7.6/7.6 Hz, ar-H), 7.68 (ddd, 1H, J = 1.7/7.1/7.1 Hz, ar-H), 7.75–7.77 (m, 2H, ar-H), 7.80 (m, 1H, ar-H), 7.92–7.95 (m, 2H, ar-H), 9.85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 16.4 (CH_3), 31.3 (3xCH_3), 68.9 (OCH_2); 111.2, 123.1, 125.7, 126.1, 126.5 (2C), 126.8, 127.5, 127.6 (2C), 129.1 (tert. C); 33.6, 125.1, 132.1, 138.4, 139.2, 142.4, 154.0, 165.4, 170.3 (quat. C).

*5.1.7.5. 3′-{[4-(2-Methoxyethyl)phenoxy]methyl}[1,1′-biphenyl]-4-carbohydrazide (**6p**).* Preparation according to General Procedure A yielded a yellow, viscous product (81%).

IR (KBr): 3308 cm^{-1} (NH), 3031 cm^{-1} (CH arom.), 2979 cm^{-1}, 2926 cm^{-1}, 2863 cm^{-1} und 2824 cm^{-1} (CH aliph.), 1625 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.73 (t, 2H, J = 6.9 Hz, CH_2), 3.22 (s, 3H, OCH_3), 3.47 (t, 2H, J = 7.0 Hz, CH_2), 4.58 (s, 2H, NH_2), 5.15 (s, 2H, OCH_2), 6.94–6.96 (m, 2H, ar-H), 7.13–7.16 (m, 2H, ar-H), 7.48 (m, 1H, ar-H), 7.50 (dd, 1H, J = 7.6/7.6 Hz, ar-H), 7.69 (ddd, 1H, J = 1.5/7.5/7.5 Hz, ar-H), 7.75–7.78 (m, 2H, ar-H), 7.79 (m, 1H, ar-H), 7.92–7.94 (m, 2H, ar-H), 9.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 34.4 ($OCH_2\underline{C}H_2Ar$), 57.7 (OCH_3), 68.9 (OCH_2), 72.9 (OCH_2); 114.5 (2C), 126.0, 126.2, 126.5 (2C), 127.2, 127.5 (2C), 129.1, 129.7 (2C) (tert. C); 131.1, 132.1, 138.0, 139.2, 142.2, 156.6, 165.4 (quat. C).

*5.1.7.6. N-{[4′-(Hydrazinocarbonyl)[1,1′-biphenyl]-3-yl]methyl} methanesulfonamide (**6q**).* Preparation according to General Procedure A yielded light yellow powder (50%).

^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.89 (s, 3H, CH_3), 4.25 (d, 2H, J = 6.3 Hz, CH_2), 4.54 (s, 2H, NH_2), 7.38–7.39 (m, 1H, ar-H), 7.47 (dd, 1H, J = 7.7/7.7 Hz, ar-H), 7.62–7.65 (m, 2H, ar-H, superimposed with sulfonamide-NH), 7.71 (m, 1H, ar-H), 7.75–7.77 (m, 2H, ar-H), 7.9–7.95 (m, 2H, ar-H), 9.85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 39.8 (CH_3), 45.9 (CH_2); 125.6, 126.1, 126.4 (2C), 127.2, 127.5 (2C), 129.0 (tert. C); 132.1, 139.0, 139.1, 142.3, 165.4 (quat. C).

*5.1.8. General procedure B for the preparation of the N′-aroyl-2-(1H-indol-3-yl)-2-oxoacetohydrazides **7***

Triethylamine (153 μL, 1.10 mmol) was added dropwise at 0 °C to a stirred suspension of 2-(1*H*-indol-3-yl)-2-oxoacetyl chloride (**5**; 208 mg, 1.00 mmol) and the appropriate benzohydrazide **6** (1.00 mmol) in anhydrous *tert*-butyl methyl ether (20 mL). The reaction mixture was stirred at 0 °C for 15 min and then for 1–26 h at room temperature, monitoring the reaction by TLC analysis. After completion of the reaction the solvent was removed in vacuo and the residue was washed with a saturated $NaHCO_3$ aqueous solution, water (3 × 20 mL in each case) and petroleum ether. The crude product was purified by recrystallization from the given solvent.

*5.1.8.1. N′-Benzoyl-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7a**).* Preparation according to General Procedure B. Reaction time: 2.5 h. Recrystallization from ethanol 96% yielded light yellow crystals (30%).

Mp.: 305–309 °C (dec.); IR (KBr): 3300 cm^{-1} (NH), 1710 cm^{-1} and 1639 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 7.27–7.33 (m, 2H, ar-H), 7.52–7.57 (m, 2H, ar-H), 7.57–7.59 (m, 1H, ar-H), 7.62 (tt, 1H, J = 1.3/6.2 Hz, ar-H), 7.94–7.97 (m, 2H, ar-H), 8.23–8.28 (m, 1H, ar-H), 8.72 (d, 1H, J = 3.1 Hz, ar-H), 10.57 (s, 1H, NH), 10.72 (s, 1H, NH), 12.33 (d, 1H, J = 2.8 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 112.6, 121.2, 122.7, 123.7, 127.5 (2C), 128.5 (2C), 132.0, 138.5 (tert. C); 112.7, 125.7, 132.3, 136.5, 163.7, 165.5, 182.4 (quat. C); $C_{17}H_{13}N_3O_3$ (307.30); calcd. C 66.44, H 4.26, N 13.67; found C 66.25, H 4.19, N 13.45; HPLC-purity: 99.8% at 254 nm and 99.8% at 280 nm, t_N = 4.19 min, t_M = 1.09 min (ACN/H_2O; 30:70), λ_{max}: 330 nm.

*5.1.8.2. 2-(1H-Indol-3-yl)-N′-(4-methylbenzoyl)-2-oxoacetohydrazide (**7b**).* Preparation according to General Procedure B. Reaction time: 4 h. Recrystallization from ethanol 96% yielded colourless crystals (24%).

Mp.: 301–305 °C (dec.); IR (KBr): 3301 cm^{-1} and 3147 cm^{-1} (NH), 3057 cm^{-1} (CH arom.), 2916 cm^{-1} (CH aliph.), 1709 cm^{-1} and 1638 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.39 (s, 3H, CH_3), 7.28–7.32 (m, 2H, ar-H), 7.34–7.35 (m, 2H, ar-H), 7.56–7.59 (m, 1H, ar-H), 7.84–7.85 (m, 2H, ar-H), 8.23–8.25 (m, 1H, ar-H), 8.72 (d, 1H, J = 2.9 Hz, ar-H), 10.51 (s, 1H, NH), 10.70 (s, 1H, NH), 12.35 (d, 1H, J = 2.5 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 20.1 (CH_3); 111.7, 120.2, 121.8, 122.7, 126.5 (2C), 128.1 (2C), 137.6 (tert. C); 111.6, 124.7, 128.5, 135.5, 141.1, 162.7, 164.4, 181.5 (quat. C); $C_{18}H_{15}N_3O_3$ (321.33); calcd. C 67.28, H 4.71, N 13.08; found C 67.64, H 4.78, N 12.98; HPLC-purity: 99.6% at 254 nm and 99.4% at 280 nm, t_N = 2.43 min, t_M = 1.02 min (ACN/H_2O; 40:60), λ_{max}: 246 nm and 329 nm.

*5.1.8.3. 2-(1H-Indol-3-yl)-N′-(4-methoxybenzoyl)-2-oxoacetohydrazide (**7c**).* Preparation according to General Procedure B. Reaction time: 23 h. Recrystallization from ethanol 96% yielded yellow powder (24%).

Mp.: 300–305 °C (dec.); IR (KBr): 3284 cm^{-1} (NH), 1679 cm^{-1} and 1644 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 3.84 (s, 3H, OCH_3), 7.06–7.08 (m, 2H, ar-H), 7.27–7.32 (m, 2H, ar-H), 7.56–7.58 (m, 1H, ar-H), 7.92–7.94 (m, 2H, ar-H), 8.23–8.26 (m, 1H, ar-H), 8.72 (d, 1H, J = 3.1 Hz, ar-H), 10.41 (s, 1H, NH), 10.63 (s, 1H, NH), 12.32 (d, 1H, J = 2.5 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 55.4 (OCH_3); 112.7, 113.8 (2C), 121.2, 122.7, 123.6, 129.4 (2C), 138.5 (tert. C); 112.6, 124.4, 125.7, 136.5, 162.1, 163.8, 165.0, 182.5 (quat. C); $C_{18}H_{15}N_3O_4$ (337.33); calcd. C 64.09, H 4.48, N 12.46; found C 63.98, H 4.54, N 12.47; HPLC-purity: 99.8% at 254 nm and 99.9% at 280 nm, t_N = 5.12 min, t_M = 1.02 min (ACN/H_2O; 30:70), λ_{max}: 258 nm and 329 nm.

*5.1.8.4. N′-(4-tert-Butylbenzoyl)-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7d**).* Preparation according to General Procedure B. Reaction time: 22 h. Recrystallization from ethanol 96% yielded light yellow crystals (56%).

Mp.: 270–271 °C (dec.); IR (KBr): 3340 cm^{-1}, 3277 cm^{-1} and 3158 cm^{-1} (NH), 2952 cm^{-1}, 2903 cm^{-1} and 2867 cm^{-1} (CH aliph.), 1694 cm^{-1} and 1653 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 1.32 (s, 9H, CH_3), 7.27–7.33 (m, 2H, ar-H), 7.55–7.56 (m, 2H, ar-H), 7.56–7.59 (m, 1H, ar-H), 7.87–7.89 (m, 2H, ar-H), 8.23–8.26 (m, 1H, ar-H), 8.72 (s, 1H, ar-H), 10.51 (s, 1H, NH), 10.71 (s, 1H, NH), 12.35 (s, 1H, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 30.8 (3xCH_3); 112.6, 121.1, 122.6, 123.6, 125.2 (2C), 127.3 (2C), 138.5 (tert. C); 34.6, 112.5, 125.6, 129.4, 136.4, 154.8, 163.6, 165.3, 182.4 (quat. C); $C_{21}H_{21}N_3O_3$ (363.41); calcd. C 69.41, H 5.82, N 11.56; found C 69.17, H 5.70, N 11.39; HPLC-purity: 99.9% at 254 nm and 99.7% at 280 nm, t_N = 2.97 min, t_M = 1.01 min (ACN/H_2O; 50:50), λ_{max}: 246 nm and 329 nm.

*5.1.8.5. 2-(1H-Indol-3-yl)-2-oxo-N′-[4-(trifluoromethyl)benzoyl]acetohydrazide (**7e**).* Preparation according to General Procedure B. Reaction time: 6.5 h. Recrystallization from ethanol 96%/toluene (1:1) yielded light yellow crystals (39%).

Mp.: 325–330 °C (dec.); IR (KBr): 3359 cm^{-1} and 3293 cm^{-1} (NH), 1709 cm^{-1} and 1645 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 7.28–7.33 (m, 2H, ar-H), 7.56–7.59 (m, 1H,

ar-H), 7.94–7.96 (m, 2H, ar-H), 8.12–8.14 (m, 2H, ar-H), 8.23–8.26 (m, 1H, ar-H), 8.71 (d, 1H, $J = 3.2$ Hz, ar-H), 10.85 (s, 2H, 2xNH), 12.37 (d, 1H, $J = 2.1$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 111.5, 119.9, 121.5, 122.4, 124.4 (q, 2C, $^{3}J_{C,F} = 3.6$ Hz, C–C–CF_3), 127.2 (2C), 137.3 (tert. C); 111.2, 122.6 (q, $^{1}J_{C,F} = 273$ Hz, CF_3), 124.5, 130.5 (q, $^{2}J_{C,F} = 32$ Hz, C–CF_3), 134.8, 135.2, 162.2, 163.1, 180.8 (quat. C); $C_{18}H_{12}F_3N_3O_3$ (375.30); calcd. C 57.61, H 3.22, N 11.20; found C 57.59, H 3.14, N 10.86; HPLC-purity: 99.9% at 254 nm and 99.8% at 280 nm, $t_N = 2.55$ min, $t_M = 1.02$ min (ACN/H_2O; 45:55), λ_{max}: 254 nm and 331 nm.

*5.1.8.6. N′-(4-Chlorobenzoyl)-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7f**).* Preparation according to General Procedure B. Reaction time: 2 h. Recrystallization from ethanol 70%/toluene (3:1) yielded colourless crystals (11%).

Mp.: 320–328 °C (dec.); IR (KBr): 3295 cm^{-1} and 3147 cm^{-1} (NH), 3067 cm^{-1} (CH arom.), 1709 cm^{-1} and 1641 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 7.28–7.32 (m, 2H, ar-H), 7.56–7.59 (m, 1H, ar-H), 7.62–7.65 (m, 2H, ar-H), 7.94–7.97 (m, 2H, ar-H), 8.23–8.26 (m, 1H, ar-H), 8.70 (d, 1H, $J = 3.2$ Hz, ar-H), 10.69 (s, 1H, NH), 10.78 (s, 1H, NH), 12.36 (d, 1H, $J = 2.2$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 112.6, 121.1, 122.7, 123.6, 128.6 (2C), 129.3 (2C), 138.4 (tert. C); 112.4, 125.6, 130.9, 136.4, 136.8, 163.4, 164.4, 182.1 (quat. C); $C_{17}H_{12}ClN_3O_3$ (341.75); calcd. C 59.75, H 3.54, N 12.30; found C 59.68, H 3.53, N 12.07; HPLC-purity: 99.9% at 254 nm and 99.7% at 280 nm, $t_N = 3.23$ min, $t_M = 1.02$ min (ACN/H_2O; 40:60), λ_{max}: 245 nm and 330 nm.

*5.1.8.7. 2-(1H-Indol-3-yl)-N′-(4-iodobenzoyl)-2-oxoacetohydrazide (**7g**).* Preparation according to General Procedure B. Reaction time: 3.5 h. Recrystallization from ethanol 96%/toluene (5:1) yielded light yellow powder (40%).

Mp.: 345–348 °C (dec.); IR (KBr): 3338 cm^{-1}, 3294 cm^{-1} and 3147 cm^{-1} (NH), 1709 cm^{-1} and 1635 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 7.27–7.32 (m, 2H, ar-H), 7.55–7.59 (m, 1H, ar-H), 7.70–7.72 (m, 2H, ar-H), 7.92–7.95 (m, 2H, ar-H), 8.22–8.25 (m, 1H, ar-H), 8.68 (d, 1H, $J = 3.2$ Hz, ar-H), 10.62 (s, 1H, NH), 10.71 (s, 1H, NH), 12.30 (d, 1H, $J = 2.7$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 112.6, 121.1, 122.6, 123.5, 129.3 (2C), 137.4 (2C), 138.3 (tert. C); 99.6, 112.5, 125.6, 131.7, 136.4, 163.4, 164.8, 182.1 (quat. C); $C_{17}H_{12}IN_3O_3$ (433.20); calcd. C 47.13, H 2.79, N 9.70; found C 47.20, H 2.66, N 9.41; HPLC-purity: 99.9% at 254 nm and 99.7% at 280 nm, $t_N = 4.15$ min, $t_M = 1.04$ min (ACN/H_2O; 40:60), λ_{max}: 257 nm and 330 nm.

*5.1.8.8. N′-(2-Fluorobenzoyl)-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7h**).* Preparation according to General Procedure B. Reaction time: 5 h. Recrystallization from ethanol 96% yielded yellow-green crystals (32%).

Mp.: 271–275 °C (dec.); IR (KBr): 3376 cm^{-1}, 3298 cm^{-1} and 3145 cm^{-1} (NH), 1709 cm^{-1} and 1639 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 7.27–7.32 (m, 2H, ar-H), 7.34–7.38 (m, 2H, ar-H), 7.55–7.64 (m, 2H, ar-H), 7.70 (ddd, 1H, $J = 2.7/7.1/7.1$ Hz, ar-H), 8.22–8.26 (m, 1H, ar-H), 8.70 (d, 1H, $J = 3.2$ Hz, ar-H), 10.39 (s, 1H, NH), 10.76 (s, 1H, NH), 12.33 (d, 1H, $J = 3.0$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 112.6, 116.3 (d, $^{2}J_{C,F} = 22$ Hz, C–CF), 121.2, 122.6, 123.6, 124.5 (d, $^{3}J_{C,F} = 3.4$ Hz, C–C–CF), 130.1 (d, $^{4}J_{C,F} = 2.8$ Hz, C–C–C–CF), 133.1 (d, $^{3}J_{C,F} = 9.0$ Hz, C–C–CF), 138.4 (tert. C); 112.5, 122.0 (d, $^{2}J_{C,F} = 14$ Hz, C–CF), 125.7, 136.4, 159.2 (d, $^{1}J_{C,F} = 250$ Hz, CF), 163.0, 163.2, 182.1 (quat. C); $C_{17}H_{12}FN_3O_3$ (325.29); calcd. C 62.77, H 3.72, N 12.92; found C 62.71, H 3.70, N 12.77; HPLC-purity: 99.9% at 254 nm and 99.8% at 280 nm, $t_N = 2.95$ min, $t_M = 1.03$ min (ACN/H_2O; 35:65), λ_{max}: 254 nm, 266 nm and 330 nm.

*5.1.8.9. N′-[4-(2,5-Dimethyl-1H-pyrrol-1-yl)benzoyl]-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7i**).* Preparation according to General Procedure B. Reaction time: 24 h. Recrystallization from ethanol 96% yielded orange powder (53%).

Mp.: 279–282 °C (dec.); IR (KBr): 3295 cm^{-1} and 3148 cm^{-1} (NH), 2921 cm^{-1} (CH aliph.), 1709 cm^{-1} and 1636 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 2.02 (s, 6H, CH_3), 5.85 (s, 2H, pyrrole-H), 7.27–7.33 (m, 2H, ar-H), 7.43–7.46 (m, 2H, ar-H), 7.56–7.60 (m, 1H, ar-H), 8.04–8.08 (m, 2H, ar-H), 8.23–8.27 (m, 1H, ar-H), 8.71 (d, 1H, $J = 3.1$ Hz, ar-H), 10.66 (s, 1H, NH), 10.76 (s, 1H, NH), 12.32 (d, 1H, $J = 3.0$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 12.8 (2xCH_3); 106.5 (2C), 112.6, 121.2, 122.7, 123.6, 128.0 (2C), 128.5 (2C), 138.4 (tert. C); 112.6, 125.7, 127.5 (2C), 131.3, 136.5, 141.4, 163.6, 164.8, 182.2 (quat. C); $C_{23}H_{20}N_4O_3$ (400.43); calcd. C 68.99, H 5.03, N 13.99; found C 68.74, H 4.85, N 13.80; HPLC-purity: 96.1% at 254 nm and 96.1% at 280 nm, $t_N = 2.68$ min, $t_M = 1.01$ min (ACN/H_2O; 50:50), λ_{max}: 267 nm and 328 nm.

*5.1.8.10. 2-(1H-Indol-3-yl)-N′-[4-(5-methyl-1,2,4-oxadiazol-3-yl) benzoyl]-2-oxoacetohydrazide (**7j**).* Preparation according to General Procedure B. Reaction time: 26 h. Recrystallization from ethanol 96%/toluene (1:1) yielded yellow powder (50%).

Mp.: 312–317 °C (dec.); IR (KBr): 3302 cm^{-1} and 3146 cm^{-1} (NH), 1708 cm^{-1} and 1647 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.71 (s, 3H, CH_3), 7.28–7.33 (m, 2H, ar-H), 7.56–7.59 (m, 1H, ar-H), 8.10–8.11 (m, 2H, ar-H), 8.16–8.17 (m, 2H, ar-H), 8.24–8.26 (m, 1H, ar-H), 8.72 (d, 1H, $J = 3.0$ Hz, ar-H), 10.77 (s, 1H, NH), 10.82 (s, 1H, NH), 12.36 (d, 1H, $J = 2.6$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 12.0 (CH_3); 112.6, 121.1, 122.7, 123.6, 127.1 (2C), 128.3 (2C), 138.4 (tert. C); 112.4, 125.6, 129.2, 134.6, 136.4, 163.4, 164.7, 166.9, 177.8, 182.1 (quat. C); $C_{20}H_{15}N_5O_4$ (389.36); calcd. C 61.69, H 3.88, N 17.99; found C 59.27, H 3.60, N 16.70; MS (EI): m/z (%) = 389 $[M]^{+\cdot}$ (4); HRMS (EI): m/z $[M]^{+\cdot}$ calcd. 389.11243; found 389.11181; HPLC-purity: 99.9% at 254 nm and 99.9% at 280 nm, $t_N = 3.50$ min, $t_M = 1.03$ min (ACN/H_2O; 35:65), λ_{max}: 256 nm and 330 nm.

*5.1.8.11. 2-(1H-Indol-3-yl)-N′-{4-[(6-methylpyrazin-2-yl)oxy] benzoyl}-2-oxoacetohydrazide (**7k**).* Preparation according to General Procedure B. Reaction time: 3 h. Recrystallization from ethanol 96% yielded yellow powder (8%).

Mp.: 273–275 °C (dec.); IR (KBr): 3288 cm^{-1} (NH), 1707 cm^{-1} and 1637 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.37 (s, 3H, CH_3), 7.28–7.32 (m, 2H, ar-H), 7.34–7.36 (m, 2H, ar-H), 7.56–7.59 (m, 1H, ar-H), 7.99–8.02 (m, 2H, ar-H), 8.23–8.25 (m, 1H, ar-H), 8.35 (s, 1H, pyrazine-H), 8.40 (s, 1H, pyrazine-H), 8.71 (d, 1H, $J = 3.1$ Hz, ar-H), 10.60 (s, 1H, NH), 10.75 (s, 1H, NH), 12.35 (d, 1H, $J = 2.9$ Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 20.5 (CH_3); 112.6, 120.7 (2C), 121.1, 122.6, 123.6, 129.3 (2C), 132.3, 138.4, 138.9 (tert. C); 112.5, 125.6, 128.6, 136.4, 151.1, 156.1, 158.1, 163.6, 164.7, 182.2 (quat. C); $C_{22}H_{17}N_5O_4$ (415.40); MS (EI): m/z (%) = 415 $[M]^{+\cdot}$ (6); HRMS (EI): m/z $[M]^{+\cdot}$ calcd. 415.12805; found 415.12737; HPLC-purity: 97.3% at 254 nm and 97.8% at 280 nm, $t_N = 3.60$ min, $t_M = 1.03$ min (ACN/H_2O; 35:65), λ_{max}: 252 nm, 303 nm and 329 nm.

*5.1.8.12. N′-({2′-[(4-Fluorobenzyl)oxy][1,1′-biphenyl]-4-yl}carbonyl)-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7l**).* Preparation according to General Procedure B. Reaction time: 24 h. Recrystallization from ethanol 96%/toluene (1:3) yielded yellow powder (24%).

Mp.: 227–231 °C (dec.); IR (KBr): 3247 cm^{-1} (NH), 1701 cm^{-1} and 1659 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 5.15 (s, 2H, OCH_2), 7.10 (ddd, 1H, $J = 1.0/7.5/7.5$ Hz, ar-H), 7.19–7.23 (m, 2H, ar-H), 7.25 (dd, 1H, $J = 0.9/8.7$ Hz, ar-H), 7.28–7.33

(m, 2H, ar-H), 7.38–7.41 (m, 2H, ar-H), 7.44–7.47 (m, 2H, ar-H), 7.56–7.59 (m, 1H, ar-H), 7.69–7.70 (m, 2H, ar-H), 7.95–7.96 (m, 2H, ar-H), 8.23–8.26 (m, 1H, ar-H), 8.72 (d, 1H, J = 2.3 Hz, ar-H), 10.59 (s, 1H, NH), 10.74 (s, 1H, NH), 12.35 (s, 1H, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 68.9 (OCH_2); 112.6, 113.2, 115.1 (d, 2C, $^2J_{C,F}$ = 21 Hz, C–CF), 121.1, 121.2, 122.6 (2C), 123.6, 127.1 (2C), 129.3 (2C), 129.4 (d, 2C, $^3J_{C,F}$ = 8.0 Hz, C–C–CF), 130.5, 138.4 (tert. C); 112.5, 125.6, 129.0, 130.5, 133.1 (d, $^4J_{C,F}$ = 3.0 Hz, C–C–C–CF), 136.4, 141.6, 154.9, 161.5 (d, $^1J_{C,F}$ = 244 Hz, CF), 163.6, 165.2, 182.3 (quat. C); $C_{30}H_{22}FN_3O_4$ (507.51); calcd. C 71.00, H 4.37, N 8.28; found C 71.05, H 4.36, N 8.32; HPLC-purity: 98.4% at 254 nm and 98.2% at 280 nm, t_N = 2.91 min, t_M = 1.08 min (ACN/H_2O; 60:40), λ_{max}: 267 nm and 303 nm.

*5.1.8.13. 2-(1H-Indol-3-yl)-2-oxo-N′-[(2′-{[3-(trifluoromethyl)phenoxy]methyl}[1,1′-biphenyl]-4-yl)carbonyl]acetohydrazide (**7m**).* Preparation according to General Procedure B. Reaction time: 3 h. Recrystallization from ethanol 96% yielded light yellow powder (37%).

Mp.: 221–228 °C (dec.); IR (KBr): 3355 cm^{-1} and 3310 cm^{-1} (NH), 1707 cm^{-1} and 1639 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 5.08 (s, 2H, OCH_2), 7.21–7.23 (m, 2H, ar-H), 7.28–7.32 (m, 3H, ar-H), 7.41–7.42 (m, 1H, ar-H), 7.48–7.53 (m, 3H, ar-H), 7.57–7.58 (m, 1H, ar-H), 7.59–7.60 (m, 2H, ar-H), 7.66–7.68 (m, 1H, ar-H), 7.98–8.00 (m, 2H, ar-H), 8.23–8.26 (m, 1H, ar-H), 8.71 (d, 1H, J = 3.1 Hz, ar-H), 10.63 (s, 1H, NH), 10.75 (s, 1H, NH), 12.35 (d, 1H, J = 2.7 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 68.0 (OCH_2); 111.2 (q, $^3J_{C,F}$ = 3.8 Hz, C–C–CF_3), 112.6, 117.3 (q, $^3J_{C,F}$ = 3.9 Hz, C–C–CF_3), 118.8, 121.1, 122.7, 123.6, 127.4 (2C), 128.1, 128.7, 129.1 (2C), 129.9, 130.1, 130.7, 138.4 (tert. C); 112.5, 123.7 (q, $^1J_{C,F}$ = 272 Hz, CF_3), 125.6, 130.3 (q, $^2J_{C,F}$ = 32 Hz, C–CF_3), 131.0, 133.2, 136.4, 140.8, 143.4, 158.2, 163.6, 165.0, 182.2 (quat. C); $C_{31}H_{22}F_3N_3O_4$ (557.52); calcd. C 66.78, H 3.98, N 7.54; found C 66.85, H 3.85, N 7.26; HPLC-purity: 99.1% at 254 nm and 98.8% at 280 nm, t_N = 4.96 min, t_M = 1.09 min (ACN/H_2O + 0.05% HCOOH; 60:40), λ_{max}: 260 nm and 328 nm.

*5.1.8.14. 2-(1H-Indol-3-yl)-2-oxo-N′-[(3′-{[3-(trifluoromethyl)phenoxy]methyl}[1,1′-biphenyl]-4-yl)carbonyl]acetohydrazide (**7n**).* Preparation according to General Procedure B. Reaction time: 4 h. Recrystallization from ethanol 96%/toluene (2:1) yielded light yellow powder (43%).

Mp.: 275–277 °C (dec.); IR (KBr): 3346 cm^{-1}, 3287 cm^{-1} and 3227 cm^{-1} (NH), 2961 cm^{-1} and 2905 cm^{-1} (CH aliph.), 1699 cm^{-1} and 1676 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 5.30 (s, 2H, OCH_2), 7.30–7.33 (m, 3H, ar-H), 7.37–7.40 (m, 2H, ar-H), 7.53–7.59 (m, 4H, ar-H), 7.77 (ddd, 1H, J = 2.0/6.7/6.7 Hz, ar-H), 7.87–7.89 (m, 2H, ar-H), 7.91 (m, 1H, ar-H), 8.05–8.06 (m, 2H, ar-H), 8.24–8.27 (m, 1H, ar-H), 8.73 (s, 1H, ar-H), 10.65 (s, 1H, NH), 10.76 (s, 1H, NH), 12.35 (s, 1H, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 69.4 (OCH_2); 111.3 (q, $^3J_{C,F}$ = 3.8 Hz, C–C–CF_3), 112.6, 117.2 (q, $^3J_{C,F}$ = 3.6 Hz, C–C–CF_3), 119.0, 121.1, 122.7, 123.6, 126.4, 126.5, 126.7 (2C), 127.6, 128.1 (2C), 129.2, 130.7, 138.4 (tert. C); 112.5, 123.9 (q, $^1J_{C,F}$ = 272 Hz, CF_3), 125.6, 130.2 (q, $^2J_{C,F}$ = 32 Hz, C–CF_3), 131.1, 136.4, 137.3, 139.1, 143.0, 158.5, 163.6, 165.0, 182.3 (quat. C); $C_{31}H_{22}F_3N_3O_4$ (557.52); calcd. C 66.78, H 3.98, N 7.54; found C 66.62, H 3.94, N 7.42; HPLC-purity: 99.4% at 254 nm and 99.4% at 280 nm, t_N = 5.24 min, t_M = 1.09 min (ACN/H_2O+0.05% HCOOH; 60:40), λ_{max}: 272 nm and 327 nm.

*5.1.8.15. N′-({3′-[(4-tert-Butyl-2-methylphenoxy)methyl][1,1″-biphenyl]-4-yl}carbonyl)-2-(1H-indol-3-yl)-2-oxoacetohydrazide (**7o**).* Preparation according to General Procedure B. Reaction time: 3 h. Double recrystallization from ethanol 96% yielded yellow powder (7%).

Mp.: 256–261 °C (dec.); IR (KBr): 3308 cm^{-1} (NH), 3150 cm^{-1} (CH arom.), 2956 cm^{-1} and 2866 cm^{-1} (CH aliph.), 1709 cm^{-1} and 1638 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400.1 MHz): δ (ppm) = 1.25 (s, 9H, CH_3), 2.23 (s, 3H, CH_3), 5.19 (s, 2H, OCH_2), 6.95 (d, 1H, J = 8.5 Hz, ar-H), 7.14 (dd, 1H, J = 2.5/8.5 Hz, ar-H), 7.19 (d, 1H, J = 2.2 Hz, ar-H), 7.27–7.33 (m, 2H, ar-H), 7.51–7.54 (m, 2H, ar-H), 7.56–7.60 (m, 1H, ar-H), 7.73 (ddd, 1H, J = 2.2/6.6/6.6 Hz, ar-H), 7.85–7.87 (m, 3H, ar-H), 8.04–8.06 (m, 2H, ar-H), 8.23–8.27 (m, 1H, ar-H), 8.73 (d, 1H, J = 3.3 Hz, ar-H), 10.63 (s, 1H, NH), 10.73 (s, 1H, NH), 12.34 (d, 1H, J = 3.3 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 16.4 (CH_3), 31.3 (3xCH_3), 69.0 (OCH_2); 111.3, 112.6, 121.2, 122.7, 123.2, 123.6, 125.7, 125.8, 126.7 (2C), 127.0, 127.5, 128.2 (2C), 129.2, 138.5 (tert. C); 33.6, 112.7, 125.2, 126.2, 131.2, 136.5, 138.6, 139.1, 142.6, 143.2, 154.1, 163.6, 165.1, 182.3 (quat. C); $C_{35}H_{33}N_3O_4$ (559.65); calcd. C 75.11, H 5.94, N 7.51; found C 74.85, H 5.75, N 7.35; HPLC-purity: 99.1% at 254 nm and 99.3% at 280 nm, t_N = 3.00 min, t_M = 1.08 min (ACN/H_2O; 72:25), λ_{max}: 272 nm and 327 nm.

*5.1.8.16. 2-(1H-Indol-3-yl)-2-oxo-N′-[(3′-{[4-(2-methoxyethyl)phenoxy]methyl}[1,1′-biphenyl]-4-yl)carbonyl]acetohydrazide (**7p**).* Preparation according to General Procedure B. Reaction time: 5 h. Recrystallization from ethanol 96%/toluene (2:1) yielded yellow powder (16%).

Mp.: 251–255 °C; IR (KBr): 3305 cm^{-1} (NH), 2925 cm^{-1} and 2860 cm^{-1} (CH aliph.), 1708 cm^{-1} and 1639 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.73 (t, 2H, J = 7.0 Hz, OCH_2-CH_2Ar), 3.23 (s, 3H, OCH_3), 3.48 (t, 2H, J = 7.0 Hz, OCH_2CH_2Ar), 5.17 (s, 2H, OCH_2), 6.95–6.98 (m, 2H, ar-H), 7.14–7.17 (m, 2H, ar-H), 7.29–7.33 (m, 2H, ar-H), 7.50 (ddd, 1H, J = 1.4/7.5/7.5 Hz, ar-H), 7.53 (dd, 1H, J = 7.7/7.7 Hz, ar-H), 7.57–7.60 (m, 1H, ar-H), 7.74 (ddd, 1H, J = 1.5/7.3/7.3 Hz, ar-H), 7.85 (m, 1H, ar-H), 7.86–7.88 (m, 2H, ar-H), 8.04–8.06 (m, 2H, ar-H), 8.24–8.27 (m, 1H, ar-H), 8.73 (d, 1H, J = 3.2 Hz, ar-H), 10.65 (s, 1H, NH), 10.76 (s, 1H, NH), 12.36 (d, 1H, J = 2.9 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 34.4 (OCH_2CH_2Ar), 57.7 (OCH_3), 68.9 (OCH_2), 72.9 (OCH_2CH_2Ar); 112.6, 114.5 (2C), 121.1, 122.7, 123.6, 126.1, 126.3, 126.7 (2C), 127.4, 128.1 (2C), 129.1, 129.7 (2C), 138.4 (tert. C); 112.5, 125.6, 131.0, 131.1, 136.4, 138.1, 139.0, 143.1, 156.6, 163.6, 165.1, 182.3 (quat. C); $C_{33}H_{29}N_3O_5$ (547.60); calcd. C 72.38, H 5.34, N 7.67; found C 72.11, H 5.26, N 7.76; HPLC-purity: 98.4% at 254 nm and 98.3% at 280 nm, t_N = 4.06 min, t_M = 1.09 min (ACN/H_2O + 0.05% HCOOH; 60:40), λ_{max}: 272 nm and 327 nm.

*5.1.8.17. N-{[4′-({2-[2-(1H-Indol-3-yl)-2-oxoacetyl]hydrazino}carbonyl)[1,1′-biphenyl]-3-yl]methyl}methanesulfonamide (**7q**).* Preparation according to General Procedure B. Reaction time: 3 h. Recrystallization from ethanol 96% yielded yellow powder (14%).

Mp.: 274–280 °C (dec.); IR (KBr): 3305 cm^{-1} (NH), 1709 cm^{-1} and 1641 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 600.1 MHz): δ (ppm) = 2.90 (s, 3H, CH_3), 4.27 (d, 2H, J = 6.1 Hz, CH_2), 7.29–7.32 (m, 2H, ar-H), 7.41 (d, 1H, J = 7.5 Hz, ar-H), 7.50 (dd, 1H, J = 7.5/7.5 Hz, ar-H), 7.57–7.59 (m, 1H, ar-H), 7.65 (t, 1H, J = 6.2 Hz, NH), 7.70 (d, 1H, J = 7.8 Hz, ar-H), 7.76 (m, 1H, ar-H), 7.85–7.87 (m, 2H, ar-H), 8.05–8.06 (m, 2H, ar-H), 8.24–8.26 (m, 1H, ar-H), 8.73 (d, 1H, J = 3.2 Hz, ar-H), 10.65 (s, 1H, NH), 10.76 (s, 1H, NH), 12.35 (d, 1H, J = 2.9 Hz, indole-NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 39.8 (CH_3), 45.9 (CH_2); 112.6, 121.1, 122.7, 123.6, 125.7, 126.1, 126.6 (2C), 127.4, 128.1 (2C), 129.1, 138.4 (tert. C); 112.5, 125.6, 131.0, 136.4, 138.9, 139.0, 143.2, 163.6, 165.0, 182.3 (quat. C); $C_{25}H_{22}N_4O_5S$ (490.53); calcd. C 61.21, H 4.52, N 11.42; found C 60.96, H 4.56, N 11.47; HPLC-purity: 96.8% at 254 nm and 98.3% at 280 nm, t_N = 3.81 min, t_M = 1.03 min (ACN/H_2O + 0.05% HCOOH; 40:60), λ_{max}: 272 nm and 327 nm.

5.2. Biological assays

5.2.1. In vitro kinase assays

A radiometric protein kinase assay (^{33}PanQinase® Activity Assay) was used for measuring the activity of the protein kinases. All assays were performed with a BeckmanCoulter/Sagian robotic system and 96-well FlashPlatesTM from Perkin Elmer (Boston, MA, USA) in a 50 μL reaction volume. The reaction cocktail was pipetted in 4 steps in the following order: 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/10 μL of enzyme solution (premixed). The assay for all enzymes contained 60 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-^{33}P]-ATP (approx. 5×10^5 cpm per well). The following substrates were used [substrate amount in square brackets given in ng/50 μL]: GSK3(14–27) [2000] for AKT1; $Poly(Glu,Tyr)_{4:1}$ [125] for FAK, IGF-1R; SRC, VEGF-R2; $Poly(Glu,Tyr)_{4:1}$ [125] or $Poly(Ala,Glu,Lys,Tyr)_{6:2.5:1}$ [125] for INS-R; $Poly(Glu,Tyr)_{4:1}$ [250] for AXL; RBER-CHKtide [2000] for ARK5 and PRK1; RBER-CHKtide [2000] or Casein [1000] for PLK1; poly(Ala,Glu,Lys,Tyr)6:2:5:1 [125] for MET; p38-alpha-KA [2000] for SAK. The reaction cocktails were incubated at 30 °C for 80 min. The reaction was stopped by adding 50 μL of 2% (v/v) phosphoric acid. Plates were aspirated and washed two times with 200 μL of 0.9% (w/v) NaCl. Incorporation of ^{33}P was determined with a microplate scintillation counter (Microbeta Trilux, Wallac). For calculation of IC_{50} values, data were generated for comparison without enzyme in the presence of substrate ("low control"). Moreover, data with enzyme but without inhibitor were generated ("high control"). The difference between high and low control was taken as 100% enzyme activity. The residual activities for each concentration and the compound IC_{50} values were calculated using Quattro Workflow V2.x (Quattro Research GmbH, Munich, Germany; www.quattroresearch.com/). The fitting model for the IC_{50} determinations was "Sigmoidal response (variable slope)" with parameters "top" fixed at 100% and "bottom" at 0%. The fitting method used was a least squares fit. In case of solubility problems, outliers in the higher concentration range were removed to generate more realistic sigmoidal curves for IC_{50} calculation. As parameter for assay quality, the Z′-factor [32] was calculated for the low and high controls of each plate. Assay plates were repeated in case the Z′-factor dropped below 0.4.

5.2.2. In vitro cell line screening

The in vitro cell line screening was performed at the American National Cancer Institute (Bethesda, U.S.A.) [33]. In brief, approx. 60 human tumour cell lines are cultivated in RPMI 1640 medium containing 5% foetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96 well microtiter plates with plating densities ranging from 5000 to 40,000 cells/well and are then incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h. After 24 h, representatives of cell lines are fixed with TCA to determine the cell density at the time of drug addition (Tz). Stock solutions of test compounds in DMSO (400-fold of the final maximum test concentration) are diluted with growth medium containing 50 μg/mL gentamicin. 100 μL aliquots of the test compound dilution are added to the microtiter wells already containing the cancer cells and 100 μL of medium. The plates are incubated for 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. Cells are fixed *in situ* by addition of 50 μL of cold 50% (w/v) TCA and incubated for 60 min at 4 °C. After discarding the supernatant, the plates are washed five times with water and air dried. 100 μL 0.4% sulforhodamine B (SRB) solution in 1% acetic acid is added. After 10 min, the cavities are washed five times with 1% acetic acid and the plates are air dried. Bound SRB is solubilized with 10 mM trizma base, and the absorbance at 515 nm is measured on a plate reader. The percentage of growth is calculated using the different absorbance measurements [time zero (Tz), control growth (C), and growth in the presence of drug (Ti)]. The growth inhibition 50% (GI_{50}) is the concentration for a Ti value satisfying the equation $[(Ti-Tz)/(C-Tz)] \times 100 = 50$.

Further details of the test method have been published [26,34,35].

5.3. Docking

Ligands were prepared for the docking using HyperChem 8.0.4 (Hypercube Inc., Gainesville, Florida) by energy minimization (MM+ force field, RMS gradient 0.1 kcal/Å mol). Docking was carried out using the docking engines ArgusDock (parameters: grid resolution 0.4; ligand: flexible; docking precision: normal; augmented root node: extend root node by 1 extra torsion) and GADock (parameters: grid resolution 0.4; ligand: flexible, population size: 100, max. generations: 1000, mutation rate: 0.8; crossover rate: 0.8; elitism: 5), as implemented in ArgusLab (ArgusLab 4.0.1, Mark A. Thompson, Planaria Software LLC, Seattle, WA, http://www.arguslab.com). ArgusLab requires a standard pdb-file for both receptor and ligand as input. Atomic charges are assigned internally by the program without involvement of the user. The resulting docking poses were scored using AScore as implemented in ArgusLab. Validation of the docking methods was carried out by redocking the cocrystallized ligand. Visualization was accomplished with Chimera 1.4 [36].

Acknowledgements

Funding by a grant of the European Commission (Contract No LSHB-CT-2004-503467; to S.S., F.T., C.S., M.H.G.K., and C.K.) is gratefully acknowledged. The authors thank the National Cancer Institute for evaluating compound **7o** in the NCI's in vitro cell line screening project (IVCLSP).

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