European Journal of Medicinal Chemistry 46 (2011) 1331–1342

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

2-Acylamino-5-chlorobenzophenones with enhanced selectivity towards malaria parasites

Swetlana Heinrich [a], Mirko Altenkämper [a], Benjamin Bechem [a], Johann Perruchon [a], Regina Ortmann [a], Hans-Martin Dahse [b], Yulin Wang [c], Michael Lanzer [c], Martin Schlitzer [a,*]

[a] Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, Marburg 35032, Germany
[b] Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e. V. Hans-Knöll-Institut, Beutenbergstrasse 11a, 07745 Jena, Germany
[c] Institut für Parasitologie, Universität Heidelberg, Im Neuenheimer feld 24, Heidelberg 69120, Germany

ARTICLE INFO

Article history:
Received 19 August 2010
Received in revised form
21 January 2011
Accepted 26 January 2011
Available online 3 February 2011

Keywords:
Antimalarial agents
Plasmodium falciparum
Benzophenone derivatives
QSAR
Enhanced selectivity

ABSTRACT

Previously we described a series of 5-acylaminobenzophenones with considerable antimalarial activity. Unfortunately, most compounds also displayed high cytotoxicity resulting in low selectivity towards malaria parasites. Through the replacement of the 5-acylamino moiety by simple chlorine and further modifications of the 2-acylamino residue we could obtain inhibitors with improved selectivity towards malaria parasites combined with an acceptable reduction of antimalarial activity.

1. Introduction

Almost half of the world's population, especially in the developing countries is affected by malaria. The relatively few drugs available to fight this disease are continuously threatened by the development of resistance [1,2]. The urgent need for the development of novel agents against malaria which preferable should act through hitherto unexploited mechanisms of action is undisputed.

Recently, we described 2,5-diaminobenzophenones carrying acyl or sulfonyl residues at the 5-amino group with significant antimalarial activity [3]. However, compounds also displayed considerable cytotoxicity and therefore unsatisfactory selectivity towards malaria parasites. Best representative in terms of activity and selectivity was the di-isopropyl urea derivative **1** with an IC_{50} of 110 nM and a selectivity index (CC_{50}/IC_{50} (*Plasmodium falciparum*)) of 36. In comparison to the parent non-acylated or non-sulfonylated 5-amino derivative **2** the N-modified compounds were up to 70fold more active (Fig. 1). Acylation of the 5-amino group changes two molecular properties: first, the electron-donating 5-substituent is converted into an electron-withdrawing and, second, a more or less spacious lipophilic substituent is added. Often more lipophilic compounds also display enhanced cytotoxicity. We therefore speculated that the replacement of the 5-acylamino moiety could reduce cytotoxicity while possibly antimalarial activity could be preserved. We chose the readily available 2-amino-5-chlorobenzophenone (**3**) as the basic scaffold of this series. We extensively varied the acyl residue at the 2-amino group to explore the influence of this moiety on antimalarial activity as well as the selectivity towards the parasites.

2. Results and discussion

2.1. Chemistry

Synthesis (Scheme 1) of the target compounds is following the route established [3,4] for this type of compounds. Briefly, commercially available 2-amino-5-chlorobenzophenone (**3**) was acylated with appropriate α-chloro or α-bromo acyl chlorides. Then, the α-halogene was replaced by different amines to obtain compounds **5**, **9**, **10** and **11**. In case of the amino acid derivatives **6**–**8** acylation was carried out with N-boc-protected amino acids using phosphorous oxychloride activation in pyridine. The resulting boc-protected amino acid derivatives **6** were deprotected by HCl in

* Corresponding author. Tel.: +49 6421 28 25698; fax: +49 6421 28 25953.
E-mail address: schlitzer@staff.uni-marburg.de (M. Schlitzer).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.01.056

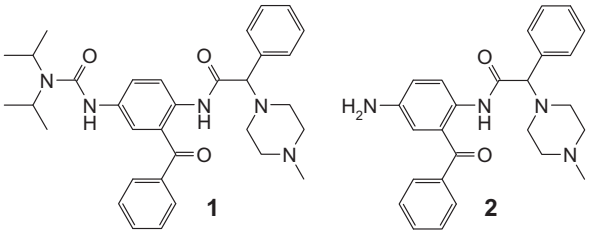
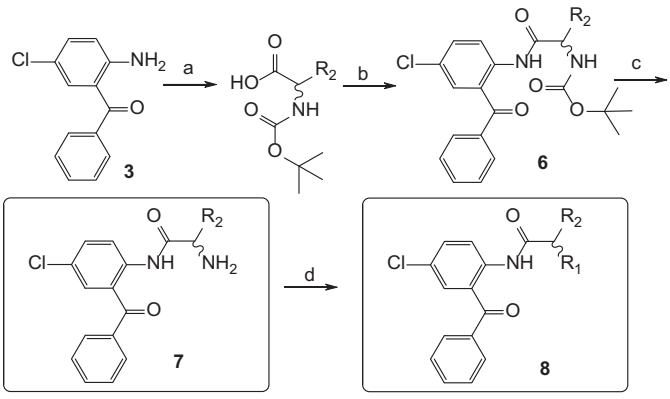


Fig. 1. **1** di-isopropyl urea derivative IC_{50} = 110 nM, **2** 5-amino derivative IC_{50} = 6.5 μM.

dioxane to obtain the amino acid derivatives **7** as hydrochlorides. Carbamoylation of **7** with methylpiperazine carbamoyl chloride yielded the urea-amino acid derivatives **8** (Scheme 2).

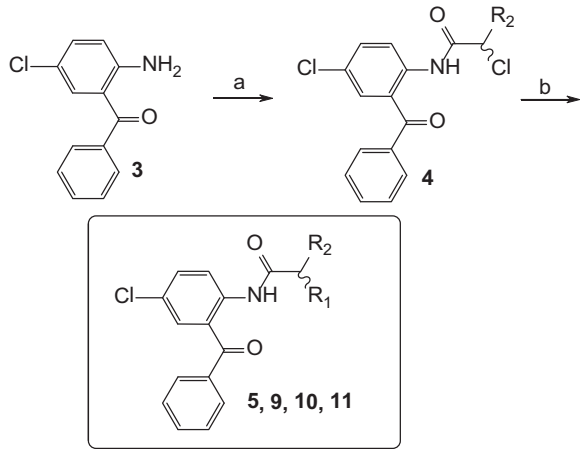


Scheme 2. Synthesis of chlorobenzophenone derivatives: a) b) Boc-protec-ted amino acids, phosphorylchloride, pyridine, −15 °C to rt, 0.5 h; c) HCl (4M) in dioxane, rt 2 h; d) 4-methylpiperazine-1-carbonylchloride, dichloromethane.

2.2. Antimalarial activity

In vitro antiplasmodial activity of the compounds was determined using a standard cell proliferation assay in which synchronized blood stage cultures of the *P. falciparum* clone Dd2 were incubated with different concentrations of the compound for 72 h before the half maximal growth inhibition coefficient (IC_{50}-value) was determined. In parallel, the IC_{50}-value of the Dd2 strain for the established antimalarial drugs chloroquine and quinine (137 ± 11 nM and 284 ± 18 nM, respectively) were determined. The chloroquine and quinine IC_{50}-values were consistent with published data [5].

The methylpiperazinyl derivative **5a** is the direct 5-chloro analogue of the series of 5-acylamino-substituted benzophenones we described previously [3]. Its antimalarial activity with an IC_{50} value of 3.54 μM falls well within the range of activity of most of the 5-acylamino-benzophenones [3]. Replacement of the *N*-methyl group by an ethyl residue (**5b**) did not change antimalarial activity (IC_{50} = 3.62 μM). Further elongation of the *N*-alkyl residue reduced activity (**5c**; IC_{50} = 6.23 μM). More spacious residues like phenyl (**5d**), nitrophenyl (**5e**) and benzyl (**5f**) let to inactive compounds. Replacement of piperazinyl's methyl substituent by formyl (**5g**) did not alter antimalarial activity as did not the introduction of an ethoxycarbonyl moiety (**5h**). The more spacious tert-butyloxycarbonyl residue produced a less active derivative (**5i**; IC_{50} = 8.21 μM). In contrast, addition of a hydroxyl group to the terminal carbon of the ethyl residue of **5b** improved antimalarial activity 10fold. The hydroxyethylpiperazinyl derivative **5j** inhibited the growth of cultured malaria parasites with an IC_{50} of 330 nM. Due to the higher antimalarial activity of **5j** and an acceptable cytotoxicity, an improved selectivity index of 150 was obtained for this compound. Replacement of the piperazine heterocycle by piperidine (**5k**) or piperidone (**5l**) reduced antimalarial activity 2–3fold. Since in case of the piperazine-based compound pair **5a**/**5j** a hydroxyethyl residue had considerably improved antimalarial activity, we also added this residue to the 4-position of the piperidine. However in this case, this modification further reduced antimalarial activity (**5m**; IC_{50} = 15 μM). Replacement of the methylpiperazinyl residue by morpholinyl (**5n**), thiomorpholinyl (**5o**) or S-oxo-thiomorpholinyl (**5p**) resulted in less active compounds, while the imidazolyl derivative **5q** was nearly as active as the methylpiperazinyl derivative **5a**.

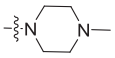
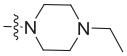
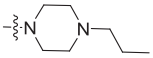
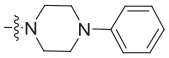
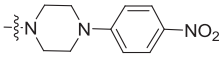
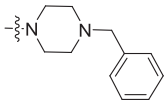
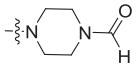
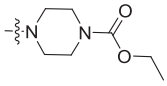
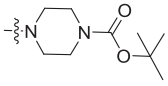
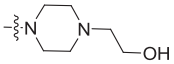
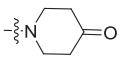
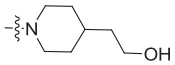
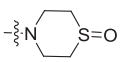


Scheme 1. Synthesis of chlorobenzophenone derivatives: a) α-chloro or α-bromo acyl chlorides, toluene, reflux; b) different amines, acetonitrile, reflux.

In the next series, we replaced the nitrogen-containing heterocycle by a simple amino group resulting in the phenylglycine derivatives **7a**-**c**. The racemic phenylglycine derivative **7a** showed an IC_{50} of 6.71 μM and therefore, an activity comparable to the heterocylic derivatives where the single nitrogen is directly connected to the α-position of the acyl residue. In case of the phenylglycine derivatives a marked difference in activity is seen with the stereoisomers. While the IC_{50} values obtained for the racemate and the R-enantiomer where comparable, the S-enantiomer did not show any activity at 16 μM. A boc-protective group on the amino group attenuated the stereo differentiation. Both enantiomers (**6a**,**b**) are of comparable activity. Replacement of the phenylglycine in **7a** by racemic glycine and serine resulted in inactive compounds (**7d**, **e**), while the phenylalanine (**7f**) (IC_{50} = 14.11 μM) and tryptophane (**7g**) (IC_{50} = 12.13 μM) derivatives showed some activity although both were less active than the R-phenylglycine derivative (**7b**). Since considerable activity has only recorded with compounds having a second amino group, we focussed our attention on this type of compounds. First, we added a *N*-methylpiperazinylcarbonyl moiety to the amino group of the R- and S-phenylglycine derivative. Again the R-enatiomer **8a** of the resulting ureas **8** was more active than the S-enatiomer **8b**. However, the difference was not that pronounced as with the unsubstituted phenylglycine derivatives with IC_{50} values of 1.35 μM and 4.21 μM, respectively. Replacement of the piperazine heterocycle by two open chain diamines of different length resulted in two compounds (**9a**, **b**) being slightly more active (IC_{50}'s 2.20 and 1.57 μM) than the methylpiperazinyl derivative **5a**.

Highest activity was obtained so far with the hydroxyethyl-substituted piperazinyl compound **5j**. We speculated that the terminal hydroxyl group could form hydrogen bonds to the putative target acting as a hydrogen bond donor or acceptor. Therefore,

Table 1
Structure and antimalarial activity of target compounds.

Cmpd.	R^1	R^2	IC_{50} *P. falciparum* (μm)	CC_{50} (μM)[a]	SI[b]
5a			3.54 ± 1.86	63	18
5b			3.62 ± 1.76	76	21
5c			6.23 ± 0.96	38	6
5d			>20	>98	5
5e			>20	>90	5
5f			>20	>118	6
5g			3.77 ± 0.73	50	13
5h			4.26 ± 0.83	64	15
5i			8.21 ± 1.64	>94	11
5j			0.33 ± 0.11	49	150
5k			10.23 ± 2.62	63	6
5l			7.43 ± 1.22	27	4
5m			15.00 ± 0. 98	19	1
5n			6.72 ± 1.03	39	6
5o			11.47 ± 1.49	>111	10
5p			9.02 ± 1.02	71	8

(*continued on next page*)

Table 1 (*continued*).

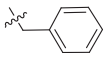
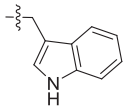
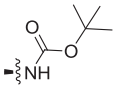
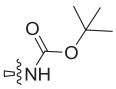
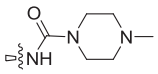
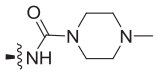
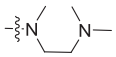
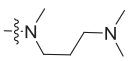
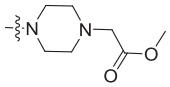
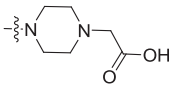
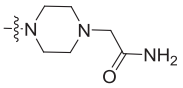
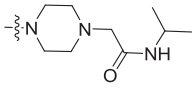
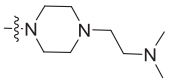
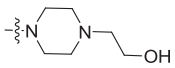
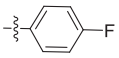
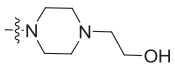
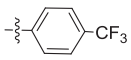
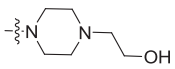
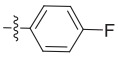
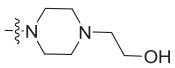
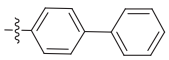
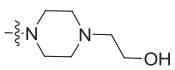
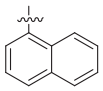
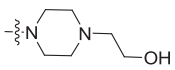
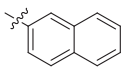
Cmpd.	R^1	R^2	IC $_{50}$ *P. falciparum* (μm)	CC_{50} (μM)[a]	SI[b]
5q			4.51 ± 0.89	55	12
7a			6.71 ± 2.90	37	6
7b			9.25 ± 1.44	12	1
7c			>16	43	3
7d			>20	128	6 > 20
7e			>20	nd	nd
7f			14.11 ± 3.46	38	3
7g			12.13 ± 3.81	6	0.5
6a			6.18 ± 1.90	>108	17
6b			8.47 ± 1.19	>108	13
8a			1.35 ± 0.83	60	45
8b			4.21 ± 0.63	72	17
9a			2.20 ± 0.33	45	21
9b			1.57 ± 0.21	79	50
10a			>3	>99	33
10b			>3	>102	34

Table 1 (*continued*).

Cmpd.	R^1	R^2	IC $_{50}$ *P. falciparum* (μm)	CC_{50} (μM)[a]	SI[b]
10c			3.32 ± 0.45	65	22
10d			0.98 ± 0.35	40	41
10e			2.56 ± 0.72	39	15
11a			0.84 ± 0.22	38	45
11b			0.60 ± 0.21	17	28
11c			0.56 ± 0.17	17	31
11d			0.28 ± 0.11	16	59
11e			0.95 ± 0.27	17	18
11f			0.18 ± 0.09	17	94

nd = not determine.
[a] Cytotoxicity (Hela cells).
[b] SI = selectivity index = CC_{50} (HeLa)/IC_{50} (*P.falciparum*).

we placed several polar functions capable to act as hydrogen bond acceptors or as hydrogen bond donor/acceptors. The compounds **10a-e** failed in showing antimalarial activity superior to **5j**. Therefore, we kept the hydroxyethyl piperazinyl moiety and varied the phenyl residue. All six compounds **11a-f** displayed submicromolar antimalarial activity with the biphenylyl (**11d**) (IC_{50} = 280 nM) and the 2-naphthyl derivative (**11f**) (IC_{50} = 180 nM) being slightly more active than the phenyl derivative **5j** (IC_{50} = 330 nM).

2.3. Cytotoxicity

Cytotoxicity of selected compounds was evaluated against HeLa cells. Viability of the cells was determined after a 72 h incubation period using methylene blue staining and photometric evaluation (Table 1). Most compounds showed cytotoxicity against HeLa cells in the two digit micromolar range. There is no obvious correlation between cytotoxicity and antimalarial activity indicating a different mechanism of antimalarial and cytotoxic action. Two of the compounds with highest antimalarial activity **5j** and **11f** show selectivity indices of 150 and 94, respectively, the first being an acceptable value.

3. Conclusion

In our preceding paper we described a series of 5-acylaminobenzophenones with considerable antimalarial activity. There value was however reduced by high cytotoxicity and therefore low selectivity of most compounds. We speculated that it might possible to separate antimalarial from unwanted cytotoxic activity. Through replacement of the 5-acylamino moiety by a simple chlorine and modification or the 2-acylamino residue we obtained inhibitors which were slightly less active against cultured malaria parasites but showed improved selectivity against the parasites. The selectivity index could be improved 4fold from 36 to 150. Because of these results we are encouraged to continue our investigations with this class of antimalarials.

4. Experimental

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Jeol Lambda 500 delta, a Jeol JNM-GX-400, a Jeol Eclipse 500 and a Jeol Eclipse

400 spectrometer. Mass spectra were obtained with a Vacuum Generator VG 7070H using a Vector 1 data acquisition system from Teknivent, an AutoSpec mass spectrometer from Micromass, an API 2000 LC-MS-MS system of PE SCIEX using Analyst 1.2 of Applied Biosystems/MDS SCIEX and on a MStation JMS 700 from Jeol using Jeol Mass Data System MS-MP9021D 2.30. IR spectra were recorded on a Nicolet 510PFTIR spectrometer, a Jasco FT/IR 410 FTIR, a Perkin Elmer Paragon 1000 FTIR or a Bruker alpha-P spectrometer. Microanalyses were obtained with a CH analyzer according to Dr. Salzer from Labormatic, a Hewlett–Packard CHN analyzer type 185, and a Vario EL from Elementar. Melting points were obtained with a Reichert Austria microscope and are uncorrected. Column chromatography was carried out using silica gel 60 (0.062–0.200 mm) from Machery-Nagel and silica gel 60 (0.040–0.063 mm) from Merck.

4.1.1. General procedure 1: activation of various acids as acid chlorides and reaction with aromatic amines

The various carboxylic acids were dissolved in dichloromethane and 0.2 mL oxalylchloride per mmol acid was added. The mixture was stirred for 2 h and the volatiles were evaporated in vacuo. The resulting acyl chlorides were dissolved in dioxane (approx. 30 mL) and added to a solution of the appropriate aromatic amine in hot toluene (approx. 50 mL). The mixtures were heated under reflux for 2 h. After the solvent was removed in vacuo, the crude products were purified by recrystallization, or chromatographed on silica gel.

4.1.2. General procedure 2: Substitution of the halogene of 2-chloro-(phenyl)acetylchloride derivates with amines

The 2-chloro-(phenyl)acetylchloride derivative was dissolved in freshly distilled acetonitrile, 3 equivalents of the amine were added and the mixture was heated under reflux for 24–48 h. After removing the acetonitrile under reduced pressure, the obtained solid was dissolved in ethyl acetate and was purified either by column chromatography or by washing with a saturated solution of K_2CO_3, drying over Na_2SO_4 and removing the solvent under reduced pressure. In case further purification was neccessary, the crude product was recrystallized, or chromatographed on silica gel.

4.1.3. General procedure 3: Deprotection of N-boc groups

Boc-protecting compounds were dissolved in a solution of HCl (4 m) in dioxane and were stirred at room temperature for 2 h. Then, the solvent was removed in vacuo to obtain the hydrochloric salt precipitates.

4.1.4. General procedure 4: acylation of amines with Boc-protected amino acids

Protected amino acids and primary amines were dissolved in dry pyridine. The solution was cooled to −15 °C and phosphoryl chloride was added dropwise with vigorous stirring. The reaction being complete after a total of 0.5 h. The mixture was then quenched with crushed ice/water (100 mL) and was extracted three times with ethylacetate. The combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4. Then the solvent was removed under reduced pressure. In case further purification was neccessary, the crude product was recrystallized, or chromatographed on silica gel.

*4.1.4.1. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-methyl-piperazinyl)-2-phenylacetamide (**5a**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-methylpiperazine (1.5 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate) to give a light yellow crystalline solid: yield 1.28 g (57%). Mp 122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.27 (s, 3H), 2.51 (s, br, 8H), 3.94 (s, 1H), 7.25–7.31 (m, 3H), 7.36–7.38 (m, 2H), 7.45–7.48 (m, 2H), 7.52–7.55 (m, 2H), 7.64 (m, 1H), 7.77–7.79 (m, 2H), 8.55 (m, 1H), 11.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 45.83, 54.79, 76.92, 123.08, 126.11, 127.46, 128.33, 128.60, 128.71, 128.92, 130.04, 131.91, 133.06, 133.34, 135.38, 137.78, 137.97, 171.12, 197.06; IR (KBr): ν (cm^{-1}): = 3178, 2938, 2798, 1692, 1639, 1595, 1565, 1504, 1446, 1394, 1307, 1281, 1245, 1179, 1163, 1141, 1119, 1099, 1078, 1019, 1011, 946, 878, 862, 820, 762, 736, 700, 648, 537; MS (EI, 70 eV): *m/z* 188 (100), 190 (23), 406 (12), 447 (15) $[M]^+$, 448 (5) $[M+1]^+$; (EI-HRMS): calcd: 447.1714, found: 447.1748.

*4.1.4.2. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-ethyl-1-piperazinyl)-2-phenylacetamide (**5b**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-ethylpiperazine (1.7 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate) to give a light brown crystalline solid: yield 1.29 g (56%). Mp 113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 1.03 (t, 3H, *J* = 7.2 Hz), 2.39–2.43 (q, 2H, *J* = 7.2 Hz), 2.47–2.62 (m, 8H), 3.96 (s, 1H), 7.25–7.30 (m, 3H), 7.34–7.37 (m, 2H), 7.45–7.47 (m, 2H), 7.51–7.55 (m, 2H), 7.63–7.66 (m, 1H), 7.77–7.79 (m, 2H), 8.54–8.56 (m, 1H), 11.59 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 11.90, 52.09, 52.44, 76.65, 123.25, 126.35, 127.63, 128.46, 128.76, 128.85, 129.15, 130.23, 132.01, 133.23, 133.47, 135.58, 137.93, 138.11, 171.14, 196.96; IR (KBr): ν (cm^{-1}) = 3855, 3745, 3676, 3630, 3184, 2966, 2818, 1695, 1654, 1636, 1596, 1560, 1501, 1445, 1395, 1310, 1280, 1243, 1155, 1017, 948, 859, 762, 737, 726, 700, 647, 536; MS (EI, 70 eV): *m/z* 84 (9), 91 (15), 202 (100), 204 (23), 461 (9) $[M]^+$, 462 (3) $[M+1]^+$; MS (EI-HRMS) calcd: 461.1870, found: 461.1873.

*4.1.4.3. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-propyl-1-piperazinyl)-2-phenylacetamide (**5c**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol), *N*-propyl piperazine dihydrobromide (4.4 g, 15.0 mmol) and sodium hydrogencarbonate (2.5 g, 30.0 mmol). Purification: the crude compound was dissolved in a minimal amount of dichloromethane and then precipitated by dropwise addition of n-pentane to give a light brown crystalline solid.: yield 1.38 mg (58%). Mp 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 0.87–0.92 (m, 3H), 1.47–1.58 (m, 2H), 2.28–2.71 (m, 10H), 3.98 (s, 1H), 7.27–7.28 (m, 3H), 7.34–7.36 (m, 2H), 7.46–7.48 (m, 2H), 7.52–7.55 (m, 2H), 7.63–7.67 (m, 1H), 7.76–7.78 (m, 2H), 8.55–8.58 (m, 1H), 11.62 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 11.85, 19.55, 52.71, 60.24, 77.20, 123.04, 126.05, 127.48, 128.37, 128.59, 128.73, 128.93, 130.02, 131.92, 133.07, 133.39, 135.23, 137.78, 137.96, 170.95, 197.29; IR (KBr): ν (cm^{-1}) = 3200, 2956, 2821,1691, 1643, 1597, 1560, 1501, 1445, 1394, 1309, 1279, 1241, 1156, 1099, 1011, 943, 802, 763, 736, 699, 648; MS (FAB): *m/z* 476 (100) $[M + H]^+$, 477 (32) $[M]^+$, 478 (37) $[M + H]^+$; MS (FAB-HRMS) calcd: 476.2105, found: 476.2113.

*4.1.4.4. (R,S)-N-(2-benzoyl-5-chlorophenyl)-2-phenyl-2-(4-phenyl-piperazin-1-yl)acetamide (**5d**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-phenylpiperazine (2.4 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate) to give a yellow crystalline solid: yield 1.30 g (51%); Mp 102 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.46–2.57 (m, 4H), 3.14–3.19 (m, 4H), 3.90 (s, 1H), 6.70–6.78 (m, 3H), 7.12–7.13 (m, 2H), 7.29–7.34 (m, 3H), 7.39–7.43 (m. 2H), 7.46–7.53 (m, 4H), 7.61–7.65 (m, 1H), 7.72–7.75 (m, 2H), 8.57 (m, 1H), 11.71 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 48.82, 51.53, 77.00, 115.95, 119.62, 123.03, 127.50, 128.45, 128.59, 128.79, 128.94, 129.08, 130.05, 131.07, 131.65, 132.05, 133.04, 133.40, 135.30, 137.72, 138.02, 170.99, 197.13; IR (KBr): ν (cm^{-1}) = 3855, 3745, 3676, 3630, 2826, 1735, 1696, 1648, 1598,

1570, 1497, 1449, 1395, 1282, 1238, 1140, 1013, 945, 761, 698; MS (FAB): *m/z* 509 (100) $[M]^+$, 510 (36) $[M + H]^+$, 511 (38) $[M]^+$, 512 (12) $[M + H]^+$; MS (FAB-HRMS) calcd: 510.1948, found: 510.1958.

*4.1.4.5. (R,S)-N-(2-benzoyl-4-chlorphenyl)-2-[4-(4-nitrophenyl)-1-piperazinyl]-2-phenylacetamide (**5e**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and 1-(4-nitrophenyl)piperazine (3.1 g, 15.0 mmol). While cooling down a white solid was removed. The solvent was evaporated and the compound was purified with column chromatography (silica gel, ethyl acetate/isohexane 1:1) and dried under vacuum to afford an orange solid: yield 2.08 g (75%). Mp 113 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.64 (s, br, 4H), 3.54 (s, br, 4H), 4.06 (s, 1H), 6.75–6.22 (m, 2H), 7.30–7.38 (m, 3H), 7.39–7.44 (m, 2H), 7.49–7.55 (m, 4H), 7.63–7.67 (m, 1H), 7.72–7.75 (m, 2H), 8.08–8.12 (m, 2H), 8.50 (m, 1H), 11.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C (ppm): 46.77, 50.97, 77.21, 112.69, 123.08, 125.80, 125.91, 127.77, 128.63, 128.77, 128.96, 129.98, 130.47, 132.27, 133.14, 133.62, 137.72, 137.94, 138.59, 139.87, 142.82, 154.63, 197.46; IR (KBr): ν (cm^{-1}) = 3745, 1700, 1696, 1685, 1654, 1648, 1597,1570, 1560, 1507, 1448, 1395, 1324, 1283, 1243, 1115, 1009, 945, 928, 829, 753, 700, 668; MS (EI, 70 eV): *m/z* 91 (24), 105 (13), 165 (8), 175 (21), 231 (9), 257 (8), 266 (15), 280 (6), 296 (100), 297 (87), 298 (17), 554 (9) $[M]^+$, 555 (3) $[M+1]^+$, 556 (3) $[M]^+$, 557 (1) $[M+1]^+$; MS (EI-HRMS) calcd: 554.1721, found: 554.1677.

*4.1.4.6. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-benzyl-1-piperazinyl)-2-phenylacetamide (**5f**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-benzylpiperazine (2.6 g, 15.0 mmol). Purification: the crude compound was dissolved in a minimal amount of dichloromethane and then precipitated by dropwise addition of *n*-pentane to give a white cristalline solid: yield 1.97 g (75%). Mp 157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.53 (s, br, 8H), 3.49 (d, 1H, *J* = 13.3 Hz), 3.53 (d, 1H, *J* = 13.3 Hz), 3.96 (s, 1H), 7.27–7.36 (m, 10H), 7.45–7.48 (m, 2H), 7.53–7.57 (m, 2H), 7.64–7.69 (m, 1H), 7.77–7.80 (m, 2H), 8.53–8.56 (m, 1H), 11.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 2.75, 62.82, 76.72, 123.08, 126.22, 127.09, 127.46, 128.18, 128.28, 128.59, 128.65, 128.98, 129.27, 129.68,130.08, 131.81, 133.06,133.28, 135.38, 137.75, 137.88, 171.17, 196.97; IR (KBr): ν (cm^{-1}) = 3745, 2928, 2798, 2711, 2632, 2503, 2478, 1701, 1691, 1654, 1640, 1590, 1566, 1496, 1461, 1452, 1401, 1364, 1345, 1307, 1247, 1142, 1124, 1089, 1050, 1028, 1004, 938, 798, 743, 698, 489, 463, 430; MS (EI, 70 eV): *m/z* 77 (11), 91 (100), 92 (11), 105 (22), 118 (10), 134 (13), 146 (16),175 (12), 266 (57), 523 (12) $[M]^+$, 524 (5) $[M+1]^+$; MS (EI-HRMS) calcd: 523.2027, found: 523.2005; MS (FAB): *m/z* 524 (100) $[M + H]^+$, 525 (37) $[M]^+$, 526 (39) $[M + H]^+$; MS (FAB-HRMS) calcd: 524.2105, found: 524.2133.

*4.1.4.7. (R,S)-N-(2-benzoyl-4-chlorphenyl)-2-[4-formyl-1-piperazinyl]-2-phenylacetamide (**5g**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-piperazincarbaldehyde (1.7 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate) to give a white crystalline solid: yield 1.27 g (55%). Mp 150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.40–2.56 (m, 4H), 3.50 (t, 2H, *J* = 5.1 Hz), 3.62–3.71 (m, 2H), 4.05 (s, 1H), 7.28–7.37 (m, 5H), 7.48–7.56 (m, 4H), 7.64–7.67 (m, 1H), 7.73–7.76 (m, 2H), 7.97 (s, 1H), 8.59 (m, 1H), 11.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 39.82, 45.47, 50.97, 51.93, 76.65, 123.08, 125.71, 127.74, 128.75, 128.97, 129.09, 130.06, 132.45, 133.24, 133.77, 134.63, 135.21, 137.86, 138.17, 160.71, 170.50, 197.65; IR (KBr): ν (cm^{-1}) = 3180, 1689, 1660, 1636, 1565, 1503, 1443, 1393, 1317, 1304, 1279, 1244, 1156, 1007, 706; MS (EI, 70 eV): *m/z* 105 (11), 175 (14), 203 (100), 204 (62), 461 (2) $[M]^+$; MS (ESI): *m/z* 462 (100) $[M + H]^+$; MS (ESI-HRMS) calcd: 462.1584, found: 462.1623.

*4.1.4.8. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[4-(ethoxycarbonyl)-1-piperazinyl]-2-phenylacetamide (**5h**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenyl-acetamide (1.9 g, 5.0 mmol) and *N*-carbethoxypiperazine (2.4 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate/isohexane 2:3) to give a yellow solid: yield 2.10 g (83%). Mp 77 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.22 (t, 3H, *J* = 7.1 Hz), 2.36–2.50 (m, 4H), 3.58 (s, br, 4H), 4.01 (s, 1H), 4.09 (q, 2H, *J* = 7.1 Hz), 7.28–7.37 (m, 5H), 7.47–7.56 (m, 4H), 7.63–7.67 (m, 1H), 7.74–7.78 (m, 2H), 8.59 (m, 1H), 11.81 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 14.72, 43.55, 51.36, 61.41, 77.03, 123.08, 125.82, 127.63, 128.58, 128.71, 128.87, 129.12, 130.07, 132.34, 133.15, 133.64, 134.95, 137.89, 138.19, 155.46, 170.85, 197.44; IR (KBr): ν (cm^{-1}) = 3244, 2978, 2831, 1698, 1645, 1596, 1568, 1499, 1431, 1395, 1302, 1282, 1244, 1178, 1157, 1119, 1033, 1012, 945, 763, 701; MS (ESI): *m/z* 91 (10), 105 (17), 175 (22), 219 (25), 231 (10), 245 (8), 246 (6), 247 (100), 248 (94), 249 (26), 257 (6), 504 (3), 505 (3) $[M]^+$, 506 (1) $[M + H]^+$, 507 (1) $[M]^+$; MS (ESI-HRMS) calcd: 506.1847, found: 506.1858.

*4.1.4.9. tert-Butyl-4-(2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**5i**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-boc-piperazine (2.8 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate/isohexane 2:3) to give a yellowish solid: yield 437 mg (18%). Mp 86 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.42 (m, 9H), 2.32–2.49 (m, 4H), 3.49–3.56 (m, 4H), 3.99 (s, 1H), 7.27–7.38 (m, 5H), 7.46–7.56 (m, 4H), 7.63–7.67 (m, 1H), 7.74–7.77 (m, 2H), 8.59 (m, 1H), 11.79 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 28.39, 51.38, 76.86, 79.63, 122.99, 125.76, 127.51, 128.45, 128.60, 128.76, 129.00, 129.97, 132.21, 133.02, 133.52, 135.00, 137.82, 138.10, 154.59, 170.83, 197.33; IR (KBr): ν (cm^{-1}) = 2975, 2928, 1696, 1648, 1596, 1569, 1500, 1453, 1422, 1395, 1365, 1283, 1247, 117, 1135, 1003, 944, 874, 833, 761, 701, 654; MS (EI, 70 eV): *m/z* 42 (3), 55 (3), 56 (4), 105 (9), 175 (19), 219 (83), 220 (27), 221 (3), 257 (7), 275 (7), 276 (66), 277 (9), 534 (1) $[M]^+$, 535 (1) $[M+1]^+$, 536 (0.3) $[M]^+$; MS (ESI): *m/z* 478 (22), 534 (100) $[M + H]^+$, 1067 $[2 (M + H)]^+$; MS (ESI-HRMS) calcd: 534.2160, found: 534.2134.

*4.1.4.10. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[4-(2-hydroxyethyl)-piperazinyl]-2-phenylacetamide (**5j**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-(2-hydroxyethyl)piperazine (2.0 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate/isohexane 3:2, then ethylacetate) to give a light brown crystalline solid: yield 1.29 g (54%). Mp 157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.45–2.70 (m, 8H), 3.58–3.60 (t, 2H, *J* = 5.2 Hz), 3.99 (s, 1H), 7.26–7.32 (m, 3H), 7.35–7.37 (m, 2H), 7.46–7.49 (m, 2H), 7.52–7.55 (m, 2H), 7.63–7.67 (m, 1H), 7.76–7.78 (m, 2H), 8.55–8.57 (m, 1H), 11.64; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 52.26, 57.43, 59.26, 77.00, 123.14, 125.85, 125.99, 127.62, 128.72, 128.88, 129.01, 130.09, 132.17, 133.19, 133.60, 135.23, 137.89, 138.10, 170.86, 197.27; IR (KBr): ν (cm^{-1}) = 3290, 2945, 2830, 1696, 1647, 1594, 1567, 1501, 1451, 1395, 1283, 1251, 1179, 1157, 1135, 1071, 1012, 950, 878, 825, 800, 746, 731, 700; MS (FAB): *m/z* 478 (100) $[M + H]^+$, 479 (31) $[M]^+$, 480 (37) $[M + H]^+$; MS (FAB-HRMS) calcd: 478.1897, found: 478.1895.

*4.1.4.11. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[1-piperidyl]-2-phenylacetamide (**5k**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and piperidine (1.3 g, 15.0 mmol). Purification:

chromatography (silicagel, ethylacetate/isohexane 1:1) to give a yellowish solid: yield 1.25 g (57%). Mp 116 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.42 (s, br, 2H), 1.61–1.66 (m, 4H), 2.26–2.47 (m, 4H), 3.96 (s, 1H), 7.27–7.36 (m, 3H), 7.34–7.36 (m, 2H), 7.46–7.47 (m, 1H), 7.59 (dm, 3H), 7.79 (m, 2H), 8.57 (m, 2H), 11.65 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 24.31, 25.77, 52.77, 77.49, 123.06, 126.33, 127.28, 128.02, 128.47, 128.53, 129.09, 129.99, 131.75, 132.93, 133.16, 135.60, 137.83, 138.00, 171.75, 196.86; IR (KBr): ν (cm^{-1}) = 3183, 2933, 1693, 1642, 1597, 1564, 1505, 1445, 1393, 1308, 1280, 1243, 1179, 1156, 1101, 945, 880, 861, 809, 800, 762, 735, 700, 673, 661, 643, 537, 454; MS (EI, 70 eV): *m/z* 84 (3), 91 (7), 56 (4), 105 (6), 106 (5), 172 (4), 173 (3), 174 (100), 175 (63), 230 (3), 231 (4), 257 (3), 430 (6), 432 (2) $[M]^+$, 433 (1) $[M+1]^+$, 434 (0.2) $[M]^+$; MS (ESI): *m/z* 433 (100) $[M + H]^+$, MS (ESI-HRMS); calcd: 433.1683, found: 433.1706.

*4.1.4.12. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[4-oxopiperidin-1-yl]-2-phenylacetamide (**5l**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) und 4-piperidone hydrochloride hydrate (2.5 g, 15.0 mmol). For the release of 4-piperidone sodium hydrogencarbonate was added. Molecular sieves was added to remove the water. After the addition of *n*-pentane a white solid precipitated. The compound was purified with column chromatography (silica gel, ethyl acetate/isohexane 2:3) and dried under vacuum to afford a yellow solid: yield 350 mg (66%). Mp 95 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.59 (s, 4H), 2.72 (s, br, 2H), 2.80 (s, br, 2H), 4.18 (s, 1H), 7.26–7.47 (m, 5H), 7.46–7.55 (m, 4H), 7.63–7.67 (m, 1H), 7.74–7.75 (m, 2H), 8.61 (m, 1H), 11.99 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 41.14, 51.12, 75.90, 122.96, 125.64, 127.53, 127.62, 128.65, 128.83, 128.87, 129.99, 132.33, 133.14, 133.67, 135.14, 137.76, 138.16, 166.06, 192.99, 203.68; IR (KBr) ν (cm^{-1}) = 3241, 1696, 1646, 1569, 1501, 1448, 1395, 1283, 1246, 699; MS (EI, 70 eV): *m/z* 105 (55), 106 (63), 118 (61), 189 (100), 230 (44), 231 (64), 304 (58), 319 (70), 347 (72), 349 (30), 446 (4) $[M]^+$, 447 (1) $[M+1]^+$, 448 (1) $[M]^+$; MS (ESI): *m/z* 337 (8), 447 (100) $[M]^+$; MS (ESI-HRMS) calcd: 447.1475, found: 447.1467.

*4.1.4.13. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-(2-hydroxyethyl)-piperidin-1-yl)-2-phenylacetamide (**5m**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (496 mg, 1.3 mmol) und 4-piperidinethanol (500 mg, 3.9 mmol) and triethylamine (0.6 mL, 4.3 mmol). Purification: chromatography (silicagel, ethylacetate/isohexane 1:1) to give a yellow solid: yield 508 mg (76%). Mp 103 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.35–1.59 (m, 8H), 1.77 (m, 1H), 2.19 (m, 1H), 2.70 (d, 1H, J = 10.5 Hz), 2.95 (d, 1H, J = 11.7 Hz), 3.65 (dd, 2H, J = 5.5, 6.4 Hz), 3.97 (s, 1H), 7.27–7.38 (m, 5H), 7.46–7.49 (m, 2H), 7.54 (m, 2H), 7.65 (m, 1H), 7.78 (m, 2H), 8.57 (m, 1H), 11.63 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 31.91, 32.02, 32.17, 39.14, 50.16, 53.79, 60.41, 77.37, 122.96, 126.28, 127.30, 128.06, 128.47, 128.52, 129.01, 130.00, 131.65, 133.00, 133.15, 135.50, 137.69, 137.87, 171.62, 196.83; IR (KBr): ν (cm^{-1}) = 2924, 1692, 1641, 1563, 1502, 1393, 1278, 1241, 1156, 945, 736, 702, 647; MS (EI, 70: eV) *m/z* 91 (10), 105 (14), 128 (5), 180 (5), 216 (6), 218 (100), 219 (72), 220 (11), 257 (11), 258 (7), 259 (5), 476 (2) $[M]^+$, 477 (1) $[M + H]^+$; MS (EI-HRMS) calcd: 476.1867, found: 476. 1880.

*4.1.4.14. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-morpholino-2-phenylacetylamide (**5n**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and morpholine (1.3 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate/n-pentane 1:3) to give a yellowish solid: yield 1.88 g (86%). Mp 121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.42–2.54 (m, 4H), 3.79 (m, 4H), 3.96 (s, 1H), 7.27–7.34 (m, 3H), 7.36–7.40 (m, 2H), 7.47–7.57 (m, 4H), 7.63–7.67 (m, 1H), 7.76–7.79 (m, 2H), 8.55–8.57 (m, 1H), 11.74 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 52.14, 66.82, 77.39, 123.13, 125.98, 127.62, 128.56, 128.71, 128.87, 129.07, 130.10, 132.18, 133.17, 133.55, 135.05, 137.86, 138.10, 170.88, 197.34; IR (KBr): ν (cm^{-1}) = 2850, 2833, 1685, 1644, 1599, 1577, 1565, 1500, 1453, 1444, 1390, 1321, 1309, 1290, 1275, 1243, 1181, 1158, 1130, 1112, 1068, 1031, 1015, 946, 924, 878, 860; MS (EI, 70 eV): *m/z* 91 (12), 105 (24), 106 (3), 118 (6), 174 (7), 176 (100), 177 (71), 178 (11), 230 (4), 231 (8), 257 (4), 434 (1) $[M]^+$, 435 (1) $[^3M + 1]^+$, 436 (1) $[M]^+$; MS (ESI): *m/z* 435 (100) $[M + H]^+$; MS (ESI-HRMS) calcd: 435.1475, found: 435.1486.

*4.1.4.15. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-phenyl-2-thiomorpholin-oacetamide (**5o**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol), thiomorpholine (1.4 mL, 15.0 mmol) and triethylamine (2.1 mL, 15.0 mmol). The product crystallized from the reaction mixture and after drying in vacuo, afford a light brow solid; yield 1.24 g (54%). Mp 70 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 2.45–2.88 (m, 8H), 4.09 (s, 1H), 7.27–7.39 (m, 5H), 7.46–7.57 (m, 4H), 7.62–7.68 (m, 1H), 7.74–7.82 (m, 2H), 8.60 (m, 1H), 11.75 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 27.82, 53.62, 77.29, 122.96, 125.91, 127.50, 128.38, 128.61, 128.69, 129.23, 130.45, 132.09, 133.07, 133.45, 134.61, 137.81, 138.96, 170.97,197.19; IR (KBr): ν (cm^{-1}) = 2828, 1700, 1646, 1595, 1568, 1499, 1446, 1395, 1283, 1247, 1179, 1158, 1104, 953, 942, 804, 760, 734, 700, 675, 646; MS (EI, 70 eV): *m/z* 105 (5), 136 (2), 164 (8), 192 (100), 193 (34), 194 (13), 195 (2), 230 (2), 231 (2), 257 (3), 258 (2), 448 (2), 450 (0.8) $[M]^+$, 451 (0.2) $[M+1]^+$; MS (ESI): *m/z* 192 (4), 451 (100) $[M + H]^+$, 901 (3) $[2 (M + H)]^+$; MS (ESI-HRMS) calcd: 451.1247, found: 451.1256.

*4.1.4.16. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(1-oxo-4-thiomorpholin-4-yl)-2-phenylacetamide (**5p**).* (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-phenyl-2-thio-morpholino-acetamide (881 mg, 2.0 mmol) and potassium peroxomonosulfate (2.0 g, 7.3 mmol) were dissolved in 15 mL THF/15 mL Ethanol/10 mL H_2O and stirred overnight at rt. Then the solvent was removed in vacuo. The resulting residue was extracted with ethylacetate and dried over $MgSO_4$. The compound was purified with column chromatography (silica gel, ethyl acetate/isohexane 1:1 (remove unwanted fractions) and then pure ethanol) to afford a yellow solid: yield 161 mg (17%). Mp 82 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.64–2.70 (m, 1H), 2.79–2.99 (m, 4H), 3.05–3.17 (m, 2H), 3.28–3.36 (m, 1H), 4.19 (s, 1H, CH), 7.30–7.38 (m, 3H), 7.48–7.57 (m, 4H), 7.60–7.70 (m, 2H), 7.73–7.79 (m, 2H), 8.39 (m, 1H), 8.63 (m, 1H), 11.95 (s, 1H); IR (KBr): ν (cm^{-1}) = 2923, 1735, 1718, 1701, 1695, 1685, 1676, 1654, 1648, 1617, 1596, 1577, 1570, 1560, 1554, 1540, 1534, 1507, 1459, 1448, 1396, 1282, 1249, 1159, 1059, 1031, 949, 807, 737, 701; MS (EI, 70 eV): *m/z* 105 (58), 132 (49), 180 (31), 190 (31), 192 (84), 193 (30), 208 (100), 209 (52), 258 (84), 260 (41), 448 (30), 466 (56) $[M]^+$, 467 (16) $[M+1]^+$, 468 (23) $[M]^+$, 469 (7) $[M+1]^+$; MS (ESI): *m/z* 467 (100) $[M + H]^+$, 489 (23) $[M + Na]^+$, 933 (14) $[2 (M + H)]^+$; MS (EI-HRMS) calcd: 466.1118, found: 466.1099.

*4.1.4.17. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[1H-imidazol-1-yl]-2-phenylacetamide (**5q**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and imidazole (1.0 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate/isohexane 1:1) to give a orange solid: yield 937 mg (45%). Mp 61 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 6.01 (s, 1H), 7.10 (m, 2H), 7.40–7.43 (m, 5H), 7.48–7.57 (m, 4H), 7.60–7.66 (m, 3H), 7.72 (s, 1H), 8.60 (m, 1H), 11.09 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 65.89, 119.11, 123.05, 124.96, 128.21, 128.52, 128.58, 129.45, 129.48, 129.67, 129.86,

132.89, 133.11, 133.98, 134.07, 137.13, 137.37, 137.88, 166.67, 198.16; IR (KBr): ν (cm^{-1}) = 2924, 1700, 1578, 1506, 1394, 1283, 1245, 1077, 947, 808, 696, 664; MS (EI, 70 eV): *m/z* 103 (24), 105 (43), 157 (80), 158 (100), 159 (66), 180 (66), 182 (29), 258 (93), 259 (36), 260 (61), 415 (21) $[M]^+$, 416 (6) $[M+1]^+$, 417 (7) $[M]^+$, 418 (2) $[M+1]^+$; MS (EI-HRMS) calcd: 415.1088, found: 415.1108.

*4.1.4.18. (R)-tert-butyl-2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethylcarbamate (**6a**).* According to general procedure 4 from 2-amino-5-chlorobenzophenone (1.2 g, 5.0 mmol) and Boc-*l*-α-phenylglycine (1.3 g, 5.0 mmol) were dissolved in pyridine (~12 mL) and stirred with phosphorylchloride (0.5 mL). Purification: recrystallization from ethanol to give a light brown solid: yield 1.56 mg (67%). Mp 132 °C; ^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.40 (s, 9H), 5.28 (s, 1H), 5.70 (s, 1H), 7.29–7.33 (m, 1H), 7.37 (m, 2H), 7.45–7.51 (m, 6H), 7.60–7.67 (m, 3H), 8.61 (m, 1H), 11.19 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 28.26, 56.89, 73.84, 122.89, 124.67, 127.42, 127.72, 128.60, 128.74, 129.31, 129.90, 132.86, 132.96, 134.06, 137.50, 137.84, 138.67, 151.34, 169.29, 198.02; IR (KBr): ν (cm^{-1}) = 3367, 2977, 1691, 1654, 1636, 1580, 1500, 1458, 1396, 1368, 1316, 1287, 1251, 1169, 1102, 1048, 1024, 947, 843, 812, 764, 741, 719, 701, 670; MS (EI, 70 eV): *m/z* 105 (55), 106 (100), 150 (54), 206 (37), 231 (34), 241 (28), 243 (30), 257 (37), 258 (38), 345 (47), 388 (54), 390 (34), 464 (1) $[M]^+$,466 (1) $[M]^+$; MS (EI-HRMS) calcd: 464.1503, found: 464.1497.

*4.1.4.19. (R)-tert-butyl-2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethylcarbamate (**6b**).* According to general procedure 4 from 2-amino-5-chlorobenzophenone (4.6 g, 20.0 mmol) and boc-*d*-phenylglycine (5.0 g, 20.0 mmol). Purification: recrystallization from ethanol to give a white light brown solid: yield 3.92 mg (42%). Mp 132 °C; ^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.41 (s, 9H), 5.28 (s, 1H), 5.71 (s, 1H), 7.29–7.33 (m, 1H), 7.35–7.38 (m, 2H), 7.45–7.51 (m, 6H), 7.60–7.66 (m, 3H), 8.61 (m, 1H), 11.19 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 28.21, 59.56, 80.02, 122.89, 124.69, 127.43, 127.72, 128.60, 128.74, 129.31, 129.90, 132.85, 132.97, 134.05, 137.47, 137.85, 138.68, 168.25, 169.15, 198.17; IR (KBr): ν (cm^{-1}) = 3367, 2977, 1691, 1654, 1636, 1580, 1499, 1458, 1396, 1368, 1316, 1287, 1251, 1169, 1102, 1048, 1024, 947, 843, 764, 719, 701, 670; MS (EI, 70 eV): *m/z* 105 (45), 106 (100), 150 (39), 206 (32), 230 (26), 231 (33), 241 (26), 243 (32), 258 (27), 345 (59), 346 (28), 390 (47), 464 (1) $[M]^+$, 465 (0.2), $[M+1]^+$, 466 (0.7) $[M]^+$; MS (EI-HRMS) calcd: 464.1503, found: 464.1490.

*4.1.4.20. (R,S)-N-(2-benzoyl-4-chlorphenyl)-phenylglycinamide hydrochloride (**7a**).* According to general procedure 3 from (*R,S*)-*N*-(2-benzoyl-4-chlorphenyl)-*N*-tert-butoxycarbonyl-phenylglycinamide (464 mg, 1 mmol) with a solution of HCl (4 m) in dioxane (20 mL). Yellow solid: yield 212 mg (53%). Mp 210 °C; ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 5.22 (s, 1H, CH), 7.33–7.38 (m, 5H), 7.43 (t, 2H, *J* = 7.9 Hz), 7.56–7.59 (m, 1H), 7.63–7.68 (m, 5H), 8.75 (s, 3H), 11.22 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 56.20, 125.94, 128.58, 128.91, 129.34, 129.45, 129.58, 129.79, 130.24, 131.76, 133.39, 133.62, 133.71, 134.23, 136.52, 166.45, 193.11; IR (KBr): ν (cm^{-1}) = 3427, 2854, 2605, 1701, 1638, 1597, 1579,1498, 1396, 1309, 1288, 1255, 1119, 956, 763, 695; MS (ESI): *m/z* 365 (100), $[M + H]^+$; MS (EI-HRMS) calcd: 365.1051, found: 365.1079.

*4.1.4.21. (R)-N-(2-benzoyl-4-chlorophenyl)-2-phenylglycinamide hydrochloride (**7b**).* According to general procedure 3 from (*R*)-*N*-(2-benzoyl-4-chlorophenyl)-2-*N*-*tert*-butoxycarbonylphenylglycinamide (929 mg, 2.0 mmol) with a solution of HCl (4 M) in dioxane (40 mL). Purification: recrystallization from ethanol to give a yellow solid: yield 531 mg (66%). Mp 170 °C; ^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 5.20 (s, 1H), 7.31–7.45 (m, 8H), 7.55–7.69 (m, 5H), 8.61–8.89 (m, 3H), 11.14 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 66.22, 125.25, 127.84, 128.22, 128.57, 128.66, 128.79, 128.98, 129.52, 131.02, 132.55, 133.01, 133.05, 133.48, 135.79, 165.65, 192.41; IR (KBr): ν (cm^{-1}) = 2924, 2677, 1700, 1685, 1636, 1597, 1499, 1396, 1307, 1286, 1251, 1158, 953, 761, 696, 669, 646; MS (EI, 70 eV): *m/z* 106 (73), 107 (45), 230 (76), 231 (82), 232 (57), 233 (43), 241 (66), 243 (76), 45 (100), 346 (71), 347 (66), 364 (9) $[M^+]$, 365 (3) $[M+1]^+$, 366 (2) $[M]^+$, 367 (0.4) $[M+1]^+$; MS (ESI): *m/z* 365 (100) $[M + H]^+$, 729 (28) $[2M + H]^+$; MS (ESI-HRMS) calcd: 365.1057, found: 365.1092.

*4.1.4.22. (S)-N-(2-benzoyl-4-chlorophenyl)-2-phenylglycinamide hydrochloride (**7c**).* According to general procedure 3 from (*S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-*N*′-*tert*-butoxycarbonylphenyl glycinamide (929 mg, 2.0 mmol) with a solution of HCl (4 M) in dioxane (40 mL). Brown solid: yield 700 mg (87%). Mp 170 °C; ^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 5.15 (s, 1H), 7.31–7.48 (m, 6H), 7.54–7.62 (m, 4H), 7.65–7.69 (m, 3H), 8.63–8.77 (m, 3H), 10.98 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 55.71, 120.53, 126.37, 128.62, 128.99, 129.37, 129.43, 129.55, 130.32, 131.82, 133.73, 134.08, 136.46, 137.84, 139.09, 165.78, 196.86; IR (KBr): ν (cm^{-1}) = 2924, 2677, 1700, 1685, 1636, 1597, 1499, 1396, 1307, 1286, 1251, 1158, 953, 761, 696, 669, 646; MS (EI, 70 eV): *m/z* 86 (41), 106 (72), 107 (44), 230 (69), 231 (75), 232 (42), 241 (63), 243 (72), 258 (43), 345 (100), 346 (57), 347 (52), 364 (4) $[M]^+$, 365 (1) $[M+1]^+$, 366 (2) $[M]^+$; MS (ESI): *m/z* 347 (24), 365 (100) $[M + H]^+$; MS (ESI-HRMS) calcd: 365.1057, found: 365.1061.

*4.1.4.23. (R,S)-N-(2-benzoyl-4-chlorphenyl)-alaninamide hydrochloride (**7d**).* According to general procedure 3 from (*R,S*)-*N*-(2-benzoyl-4-chlorphenyl)-*N*-tert-butoxycarbonylalaninamid (1.5g, 3.7 mmol) with a solution of HCl (4 m) in dioxane (10 mL). Yellow solid: yield 1184 mg (94%). Mp 165 °C; ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 0.94 (d, 3H, *J* = 7.0 Hz), 3.93 (q, 1H, *J* = 6.9 Hz,), 7.42 (d, 1H, *J* = 2.5 Hz), 7.48–7.53 (m, 3H), 7.62–7.66 (m, 1H), 7.67–7.70 (m, 3H), 8.25 (s, 3H, NH_3^+),11.06 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 15.74, 48.11, 125.86, 128.37, 128.82, 129.14, 129.65, 131.26, 133.06, 133.57, 136.12, 168.03, 192.68; IR (KBr): ν (cm^{-1}) = 3308, 3187, 2969, 2693, 2499, 1691, 1604, 1535, 1477, 1448, 1388, 1274, 1234, 1121, 1000, 962, 826, 750, 698, 641; MS (EI, 70 eV): *m/z* 88 (15), 105 (31), 154 (18), 180 (24), 195 (12), 205 (11), 207 (15), 214 (25), 230 (92), 241 (96), 243 (97), 255 (22), 259 (74) 268 (15), 283 (100), 303 (23), $[M + HCl]^+$.

*4.1.4.24. (R,S)-N-(2-benzoyl-4-chlorphenyl)-serinamide hydrochloride (**7e**).* According to general procedure 3 from (*R,S*)-*N*-(2-benzoyl-4-chlorphenyl)-*N*-*tert*-butoxycarbonyl-O-*tert*-butyl-serin-amide (0.5 g, 1.1 mmol) with a solution of HCl (4 m) in dioxane (15 mL). Yellow solid: yield 284 mg (80%). ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 2.08 (s, 1H), 3.11–3.22 (m,1H), 3.91–4.01 (m, 1H), 5.46 (t, 1H, *J* = 4.6 Hz), 7.39–7.73 (m, 8H), 8.22 (s, 3H), 11.06 (s, 1H), ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 59.53, 73.63, 125.75, 128.31, 128.91, 129.29, 129.60, 131.36, 133.06, 133.24, 133.36, 133.55, 133.73, 136.12, 165.65, 193.03; IR (KBr): ν (cm^{-1}) = 3195, 2923, 1738, 1654, 1596, 1523, 1396,1288, 1254, 1119, 953, 871, 830, 741, 700; MS (EI, 70 eV): *m/z* 59 (42), 77 (11), 86 (33), 88 (40), 101 (10), 105 (33), 116 (53), 154 (9), 214 (10), 227 (12), 231 (94), 241 (40), 243 (24), 258 (13), 272 (42), 288 (35), 300 (19), 318 (2), $[M + HCl]^+$; MS (EI-HRMS) calcd: 319.0844, found: 319.0875.

*4.1.4.25. (R,S)-N-(2-benzoyl-4-chlorphenyl)-phenylalaninamide hydrochloride (**7f**).* According to general procedure 3 from (*R,S*)-*N*-(2-benzoyl-4-chlorphenyl)-*N*-tert-butoxycarbonylphenylalaninamide (1.5 g, 3.1 mmol) with a solution of HCl (4 m) in dioxane (10 mL). Yellow solid: yield 1271 mg (98%). Mp 205 °C; ^1H NMR (500 MHz,

DMSO-d_6) δ_H (ppm) = 2.55 (dd, 1H, J = 14.3, 4.8 Hz), 2.73 (dd, 1H. J = 14.3, 4.8 Hz), 4.21–4.25 (m, 1H, J = 4.6 Hz), 7.21–7.24 (m, 1H), 7.26–7.29 (m, 4H), 7.41 (d, 1H, J = 2.5 Hz), 7.51–7.56 (m, 3H), 7.61–7.65 (m, 1H), 7.69 (dd, 1H, J = 7.0 Hz), 7.73–7.75 (m, 2H), 8.22 (s, 3H), 11.35 (s, 1H);^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 35.86, 53.68, 125.79, 127.09, 128.35, 128.43, 128.74, 128.93, 129.43, 129.74, 131.12, 133.06 133.33, 133.54, 134.62, 136.14, 166.92, 192.74; IR (KBr): ν (cm^{-1}) = 3239, 3050, 2801, 2678, 2604, 1700, 1632, 1595, 1578, 1516, 1487, 1392, 1308, 1295, 1254, 1392, 1255, 1159, 1118, 978, 951, 833, 752, 701, 654; MS (EI, 70 eV): m/z 91 (12), 105 (13), 120 (28), 131 (18), 166 (11), 180 (10), 206 (15), 230 (14), 241 (92), 243 (75), 257 (16), 269 (100), 271 (89), 332 (11), 360 (91), 378 (2), $[M + HCl]^+$; MS (EI-HRMS) calcd: 379.1208, found: 379.1224.

*4.1.4.26. (R,S)-N-(2-benzoyl-4-chlorphenyl)-tryptophanamide hydrochloride (**7g**).* According to general procedure 3 from (*R,S*)-*N*-(2-benzoyl-4-chlorphenyl)-*N*-tert-butoxycarbonyltryptophanamide (0.5 g, 1 mmol) with a solution of HCl (4 M) in dioxane (20 mL). Yellow solid: yield 403 mg (89%). Mp 227 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 2.65–2.81 (m, 2H), 4.08–4.20 (m, 1H), 6.97–7.01 (m, 1H), 7.06–7.10 (m, 1H), 7.20 (d, 1H, J = 2.4 Hz), 7.33–7.36 (m, 1H), 7.43 (d, 1H, J = 2.5 Hz), 7.51–7.57 (m, 3H), 7.59–7.64 (m, 1H), 7.68–7.77 (m, 4H), 8.12 (s, 3H), 11.03 (s, 1H), 11.25 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 26.27, 52.84, 106.45, 111.31, 118.34, 118.56, 121.09, 124.95, 125.77, 126.81, 128.34, 128.86, 129.05, 129.77, 131.27, 133.06, 133.47, 133.56, 136.16, 136.21, 167.34, 192.77; IR (KBr): ν (cm^{-1}) = 3400, 2954, 2180, 1711, 1644, 1524, 1327,1296, 1246, 976, 743, 703; MS (ESI): m/z 418 (100), $[M + H]^+$; MS (EI-HRMS) calcd: 418.1317, found: 418.1295.

*4.1.4.27. (R)-N-(2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethyl)-4-methylpiperazin-1-carbamide (**8a**).* From (*R*)-*N*-(2-benzoyl-4-chlorophenyl)-2-phenyl-glycinamide hydrochloride (402 mg, 1.0 mmol) and 4-methyl-1-piperazine carbonylchloride (199 mg, 1.0 mmol), under addition of triethylamine. The glycineamide derivative was solved under ice cooling. Triethylamine and the piperazine derivative were slowly added and stirred vigorously and then slowly warm up to room temperature. After stirring for 72 h the solvent was removed in vacuo. The remaining solid was solved in a small amount of dichloromethane and washed with water. The collected organic phases were dried over anhydrous sodium sulfate and the solvent evaporated off in vacuo. Purification with column chromatography (silica gel, ethyl acetate/methanol 3:1) afford a yellow-orange solid: yield 140 mg (28%). Mp 79 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.27 (s, 3H), 2.30–2.43 (m, 4H), 3.39–3.56 (m, 4H), 5.48 (d, 1H, J = 5.1 Hz), 5.54 (d, 1H, J = 5.1 Hz), 7.29–7.33 (m, 1H), 7.34–7.39 (m, 2H), 7.46–7.52 (m, 6H), 7.59–7.68 (m, 3H), 8.63 (m, 1H), 11.26 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 43.66, 46.04, 54.54, 60.48, 122.70, 124.5, 127.56, 128.50, 128.62, 129.25, 129.82, 132.56, 132.76, 132.84, 133.93, 137.80, 137.84, 138.64, 156.37, 170.13, 198.11; IR (KBr): ν (cm^{-1}) = 3307, 2937, 2796, 1700, 1641, 1597, 1577, 1497, 1396, 1289, 1244, 1176, 1158, 1002, 947, 762, 699; MS (ESI): m/z 260 (18), 491 (100) $[M + H]^+$, 981 (12) $[2(M + H)]^+$; MS (ESI-HRMS) calcd: 491.1850, found: 491.1863.

*4.1.4.28. (R)-N-(2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethyl)-4-methylpiperazin-1-carboxamide (**8b**).* From (*R*)-*N*-(2-benzoyl-4-chlorophenyl)-2-phenyl-glycinamide hydrochloride (402 mg, 1.0 mmol) and 4-methyl-1-piperazine carbonylchloride (199 mg, 1.0 mmol), under addition of triethylamine. The glycine amide derivative was solved under ice cooling. Triethylamine and the piperazine derivative were slowly added and stirred vigorously and slowly warmed up to room temperature. After stirring for 72 h the solvent was removed in vacuo. The remaining solid was solved in a small amount of dichloromethane and washed with water. The collected organic phases were dried over anhydrous sodium sulfate and evaporated off in vacuo to afford a yellow-orange solid: yield 255 mg (52%). Mp 75 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.31 (s, 3H), 2.38–2.57 (m, 4H), 3.49–3.59 (m, 4H), 5.47 (d, 1H, J = 5.1 Hz), 5.62 (d, 1H, J = 5.1 Hz), 7.27–7.32 (m, 1H), 7.34–7.37 (m, 2H), 7.40–7.52 (m, 6H), 7.59–7.65 (m, 3H), 8.61 (m, 1H), 11.26 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 43.49, 45.84, 50.96, 54.01, 122.94, 124.85, 127.81, 127.85, 128.75, 128.88, 129.46, 130.03, 132.99, 133.11, 134.19, 137.90, 138.01, 138.80, 156.55, 170.31, 198.41; IR (KBr): ν (cm^{-1}) = 3314, 1700, 1696, 1685, 1636, 1597, 1577, 1560, 1507, 1448, 1396, 1289, 1254, 1176, 1158, 1002, 947, 737, 700, 652; MS (FAB): m/z 175 (5), 200 (21), 232 (30), 260 (26), 307 (18), 347 (11), 491 (62) $[M + H]^+$; MS (FAB-HRMS) calcd: 491.1850, found: 491.1888M + H]$^+$.

*4.1.4.29. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[(2-dimethyl-aminoethyl)-methylamino]-2-phenylacetamide (**9a**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N,N,N′*-trimethylethylendiamine (1.4 mL, 15.0 mmol) and triethylamine (2.1 mL, 15.0 mmol). The compound was purified with column chromatography (silica gel, ethyl acetate/isohexane 1:1 (remove unwanted fractions) and then pure ethanol) to afford a white solid: yield 584 mg (26%). Mp 107 °C; ^{1}H NMR (500 MHz,$CDCl_3$) δ_H (ppm) = 2.07 (s, 6H), 2.22 (s, 3H), 2.40–2.56 (m, 4H), 4.11 (s, 1H), 7.27–7.32 (m, 3H), 7.35–7.39 (m, 2H), 7.46–7.55 (m, 4H), 7.61–7.66 (m, 1H), 7.76–7.79 (m, 2H), 8.51–8.54 (m, 1H), 11.58 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 40.55, 45.84, 53.55, 57.53, 76.70, 123.23, 126.63, 127.60, 128.25, 128.63, 129.21, 130.10, 131.94, 133.07, 133.32, 135.79, 137.94, 137.97, 171.63, 197.09; IR (KBr): ν (cm^{-1}) = 3280, 2952, 2813, 2763, 1694, 1645, 1596, 1568, 1500, 1453, 1395, 1278, 1252, 1158, 1118, 1095, 1038, 947, 851, 806, 766, 735, 703, 671, 652, 634; MS (ESI): m/z 450 (100) $[M + H]^+$, 899 (3) $[2M + H]^+$; MS (ESI-HRMS) calcd: 450.1948, found: 450.1924.

*4.1.4.30. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[(3-(dimethylamino)-propyl)methyl-amino]-2-phenylacetamide (**9b**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N,N,N′*-trimethyl-1,3-propandiamine (1.7 g, 15.0 mmol). The compound was purified with column chromatography (silica gel, ethyl acetate/isohexane 1:1 (remove unwanted fractions) and then pure ethanol). After drying under vacuo, the solid was precipitated (dichloromethane/n-pentane) to afford a white-yellow solid: yield 873 mg (38%). Mp 101 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.87–2.09 (m, 2H), 2.22 (s, 3H), 2.41–2.48 (m, 2H), 2.51 (s, 6H), 2.81–3.26 (m, 2H), 4.08 (s, 1H), 7.27–7.34 (m, 5H), 7.46–7.55 (m, 4H), 7.61–7.67 (m, 1H), 7.68–7.72 (m, 2H), 8.57–8.61 (m, 1H), 11.66 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 23.16, 39.62, 43.69, 52.22, 56.53, 77.21, 122.95, 125.47, 127.48, 128.41, 128.66, 128.69, 129.09, 129.84, 132.40, 133.13, 133.82, 135.11, 137.99, 138.26, 171.10, 197.97; IR (KBr): ν (cm^{-1}) = 3249, 2952, 2821, 2777, 1691, 1644, 1596,1566, 1501, 1449, 1395, 1305, 1278, 1250, 1159, 1128, 1096, 1081, 1034, 948, 854, 812, 762, 737, 699, 671, 654, 636; MS (EI, 70 eV): m/z 57 (51), 85 (32), 91 (30), 105 (39), 115 (59), 118 (24), 120 (67), 134 (100), 135 (32), 205 (52), 404 (27), 463 (26) $[M]^+$, 464 (8) $[M+1]^+$, 465 (9) $[M]^+$, 466 (3) $[M+1]^+$; MS (EI-HRMS) calc.: 463.2027, found: 463.2052.

*4.1.4.31. (R,S)-Methyl-2-(4-(2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethyl)piperazin-1-yl) acetate (**10a**).* (*R,S*)-N-(2-benzoyl-4-chlorophenyl)-2-phenyl-2-(piperazin-1-yl)-acetamide (2.7 g, 5.3 mmol) and $NaHCO_3$ (1.6 g, 18.6 mmol) were suspended in acetone. To the stirring mixture methyl-2-chloroacetate (0.5 mL, 5.8 mmol) was added dropwise over a 3 min period. The reaction

mixture was refluxed for 22 h. Then the suspension was filtered and the residue was washed with acetone. After concentrating the filtrate, the solvent was removed in vacuo to obtain the crude product. Purification: recrystallization from acetone to give a yellow solid: yield 1790 mg (67%). Mp 140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.58 (m, br, 4H), 2.70 (m, br, 4H), 3.24 (s, 2H), 3.70 (s, 3H), 4.00 (s, 1H), 7.27–7.33 (m, 3H), 7.35–7.37 (m, 2H), 7.46–7.49 (m, 2H), 7.52–7.56 (m, 2H), 7.64–7.68 (m, 1H), 7.76–7.79 (m, 2H *H*-Aryl), 8.56–8.58 (m, 1H), 11.66 (s, br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 51.08, 51.75, 52.86, 59.21, 76.73, 123.03, 126.05, 127.54, 128.31, 128.61, 128.73, 129.05, 130.07, 132.07, 133.08, 133.42, 135.15, 137.89, 138.06, 170.53, 171.07, 197.12; IR (ATR): ν (cm^{-1}) = 3223, 2831, 1756, 1688, 1653, 1569, 1504, 1396, 1281, 1245, 1161, 1019, 948, 880, 808, 739, 457; MS (ESI): *m*/*z* 506 (100) $[M + H]^{+;}$ MS (EI-HRMS) calcd: 505.1768, found: 505.1758.

*4.1.4.32. (R,S)-2-(4-(2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethyl)piperazin-1-yl)acetic acid (**10b**).* A stirred solution of (*R,S*)-Methyl-2-(4-(2-(2-benzoyl-4-chlorophenylamino)-2-oxo-1-phenylethyl)piperazin-1-yl)-acetate (0.8 g, 1.5 mmol) in THF/MeOH (3:1) was treated with 1.5 equiv of a 1m solution of LiOH in H_2O. The resulting solution was stirred at rt for 24h. The reaction mixture was then poured into 1 N HCl and extracted with ethylacetate. The organic layer was separated, dried over $NaSO_4$, and the solvents were removed in vacuo. Purification: chromatography silicagel, dichlormethane/methanol 20:1, to give a light yellow solid: yield 420 mg (57%). Mp 131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.67–2.70 (m, 1H), 2.79–2.82 (m, 1H), 3.00–3.03 (m, 1H), 3.21–3.27 (m, 1H), 3.57–3.65 (m, br, 2H), 3.74–3.86 (m, br, 2H), 3.90 (s, 2H), 4.21 (s, 1H), 7.32–7.38 (m, br, 5H), 7.51–7.59 (m, br, 4H),7.67–7.74 (m, br, 3H), 8.62 (d, 1H, *J* = 8.8 Hz,), 11.97 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 43.32, 46.73, 63.72, 64.13, 65.85, 75.06, 122.73, 125.04, 127.93, 128.81, 128.94, 129.14, 129.24, 130.06, 132.94, 133.54, 133.75, 134.25, 137.64, 138.02, 166.01, 169.47, 198.68; IR (ATR): ν (cm^{-1}) = 3294, 2245, 1688, 1595, 1571, 1496, 1449, 1285, 1249, 1153, 1001, 902, 724, 699, 643, 427; MS (ESI): *m*/*z* 492 (100) $[M + H]^+$; MS (EI-HRMS) calcd: 491.1612, found: 491.1620.

*4.1.4.33. (R,S)-2-(4-(2-amino-2-oxoethyl)piperazin-1-yl)-N-(2-benzoyl-4-chlorophenyl)-2-phenylacetamide (**10c**).* (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-phenyl-2-(piperazin-1-yl)-acetamide (0.7 g, 1.5 mmol) and $NaHCO_3$ (0.4 g, 4.5 mmol) were dissolved in methanol. After adding 2-bromoacetamide (0.3g, 2.1 mmol) dropwise over a period of 2 min, the reaction mixture was refluxed for 16 h. The solvent was removed via rotary evaporation. Purification: chromatography silicagel, ethylacetate/methanol 6:0.1, to give a light yellow solid: yield 571 mg (72%). Mp 205 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.52 (m, br, 4H), 2.66 (m, br, 4H), 3.24 (s, 2H), 4.00 (s, 1H), 5.65 (s, br, 1H), 7.00 (s, br, 1H), 7.30–7.34 (m, 5H), 7.46–7.56 (m, 4H), 7.64–7.68 (m, 1H), 7.76–7.78 (m, 2H), 8.56 (d, 1H, *J* = 8.8 Hz), 11.68 (s, br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 51.42, 53.21, 61.20, 76.76, 123.05, 125.95, 127.57, 128.45, 128.67, 128.78, 129.08, 130.06, 132.05, 133.13, 133.46, 135.06, 137.75, 137.92, 170.85, 173.12, 197.15; IR (ATR): ν (cm^{-1}) = 3490, 3369, 2815, 1695, 1645, 1505, 1298, 1282, 1251, 943, 724, 697, 686, 649; MS (EI, 70 eV): *m*/*z* 56 (5), 77 (6), 91 (10), 104 (10), 232(100), 257 (3) 490 (3) $[M]^+$; MS (EI-HRMS) calcd: 490.1772, found: 490.1754.

*4.1.4.34. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-[4-(2-(isopropylamino)-2-oxoethyl)-piperazin-1-yl]-2-phenylacetamide (**10d**).* According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-chloro-2-phenylacetamide (1.9 g, 5.0 mmol) and *N*-(isopropyl)-1-piperazinacetamide (2.8 g, 15.0 mmol). Purification: chromatography (silicagel, ethylacetate/isohexane 2:3, then ethylacetate) to give a yellow crystalline solid: yield 1.41 g (53%). Mp 78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 1.10 (t, 6H, *J* = 6.7 Hz), 2.52 (s, br, 4H), 2.61 (s, br, 4H), 2.97 (s, 2H), 3.98 (s, 1H), 4.01–4.13 (m, 1H), 6.85 (s, br, 1H), 7.28–7.33 (m, 3H), 7.36–7.39 (m, 2H), 7.46–7.49 (m, 2H), 7.51–7.56 (m, 2H), 7.63–7.67 (m, 1H), 7.75–7.78 (m, 2H), 8.56 (m, 1H), 11.67 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 22.78, 40.63, 53.15, 61.42, 76.82, 122.99, 125.93, 127.55, 128.46, 128.66, 128.80, 128.94, 130.27, 132.08, 133.16, 133.42, 135.21, 137.69, 137.97, 170.89, 184.96, 197.15; IR (KBr): ν (cm^{-1}) = 3284, 3062, 2968, 2825, 1674, 1596, 1569, 1502, 1396, 1282, 1246, 1157, 1131, 879, 831, 803, 763, 729, 701, 652; MS (FAB): *m*/*z* 533 (100) $[M + H]^+$, 534 (35) $[M]^+$, 535 (38) $[M + H]^+$; MS (FAB-HRMS) calcd: 533.2319, found: 533.2310.

*4.1.4.35. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-(2-(dimethylamino) ethyl)piperazin-1-yl)phenylacetamide (**10e**).* (*R,S*)-*N*-(2-benzoyl-4-chlorophenyl)-2-phenyl-2-(piperazin-1-yl)-acetamide (0.6 g, 1.4 mmol) and $NaHCO_3$ (0.4 g, 4.8 mmol) were suspended in Propanol/Methanol (1:1). To the stirring mixture 2-chloro-*N*,*N*-dimethylethanamine (0.2 g, 1.6 mmol) was added dropwise over a 3 min period. The reaction mixture was subsequently refluxed for 48h. The solvent was removed via rotary evaporation. Purification: chromatography silicagel, dichlormethane/methanol/NH_3 940:50:10, to give a light yellow solid: yield 89 mg (13%). Mp 108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) = 2.56 (s, 6H), 2.41–2.58 (m, br, 12H), 3.96 (s, 1H), 7.27–7.37 (m, 5H), 7.45–7.55 (m, 4H), 7.63–7.68 (m, 1H), 7.75–7.79 (m, 2H), 8.54–8.57 (m, 1H), 11.62 (s, br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) = 45.75, 53.26, 56.12, 56.63, 76.87, 123.09, 126.15, 127.45, 128.31, 128.55, 128.66, 129.05, 130.08, 131.87, 133.07, 133.38, 135.24, 137.76, 137.92, 171.12, 196.07; IR (ATR): ν (cm^{-1}) = 3187, 2921, 2821, 1688, 1640, 1501, 1446, 1277, 1240, 944, 698, 647, 453, 411; MS (ESI): *m*/*z* 505 (100), $[M + H]^+$; MS (EI-HRMS) calcd: 505.2398, found: 505.2370.

*4.1.4.36. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-fluorophenyl)-2-(4-(2-hydroxyethyl)piperazin-1-yl)acetamide (**11a**).* According to general procedure 2 from *N*-(2-benzoyl-4-chlorophenyl)-2-bromo-2-4-(fluorophenyl)-acetamide (2.6 g, 5.8 mmol) und *N*-(2-hydroxyethyl)piperazine (2.1 mL, 17.4 mmol) and triethylamine (1.6 mL, 11.6 mmol). Purification: chromatography (silicagel, acetone) to give a light yellow solid: yield 2459 mg (85%). Mp 155 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) = 2.50–2.58 (m, br, 6H), 2.62–2.65 (m, 4H), 3.57 (t, 2H, *J* = 5.4 Hz), 3.99 (s, 1H), 6.97–7.03 (m, 2H), 7.32–7.37 (m, 2H), 7.46–7.56 (m, 4H), 7.63–7.69 (m, 1H), 7.52–7.78 (m, 2H), 8.56 (d, 1H, *J* = 9.7 Hz), 11.64 (s, br, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ_C (ppm) = 52.55, 57.72, 59.11, 75.92, 115.5,3 115.84, 123.05, 126.03, 127.63, 128.64, 130.05, 130.52, 130.63, 130.94, 132.03, 133.12, 133.45, 137.72, 137.91, 170.72, 197.24; IR (ATR): ν (cm^{-1}) = 3181, 3116, 2945, 2809, 1688, 1639, 1566, 1502, 1244, 1227, 1156, 946, 691, 644, 407; MS (ESI): *m*/*z* 496 (100) $[M + H]^+$; MS (EI-HRMS) calcd: 496.1802, found: 496.1803.

*4.1.4.37. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-(4-trifluoro-methyl)phenyl-acetamide (**11b**).* According to general procedure 2 from *N*-(2-benzoyl-4-chlorophenyl)-2-bromo-2-(4-(trifluoromethyl)phenyl)acetamide (2.3 g, 4.6 mmol) und *N*-(2-hydroxyethyl)piperazine (1.7 mL, 13.8 mmol) and triethylamine (1.3 mL, 9.2 mmol) Purification: chromatography (silicagel, acetone) to give a light yellow solid: yield 1590 mg (63%). Mp 162 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.52–2.58 (m, br, 6H), 2.65 (s, br, 4H), 3.57 (t, 2H, *J* = 5.4 Hz), 4.09 (s, 1H), 7.48–7.59 (m, br, 8H), 7.64–7.68 (m, 1H), 7.77 (dd, 2H, *J* = 8.2 Hz, *J* = 1.2 Hz), 8.55 (d, 1H, *J* = 8.9 Hz), 11.69 (s, br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 52.47, 57.73, 59.02, 76.1, 123.03, 125.64, 126.03, 127.84, 128.65, 129.45, 130.06, 132.16, 133.17, 133.52, 137.74, 139.04, 170.62, 197.22; IR (ATR): ν (cm^{-1}) = 3237, 2944, 2882, 2820, 1688, 1646, 1497, 1322, 1159, 1113,

1066, 1013, 700, 381; MS (ESI): *m/z* 546 (100), $[M + H]^+$; MS (EI-HRMS) calcd: 546.1810, found: 546.1771.

*4.1.4.38. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-bromophenyl)-2-(4-(2-hydroxyethyl)piperazin-1-yl)-acetamide (**11c**).* According to general procedure 2 from *N*-(2-benzoyl-4-chlorophenyl)-2-bromo-2-(4-bromophenyl)-acetamid (3.0g, 6.0 mmol) and *N*-(2-hydroxyethyl)piperazine (2.2 mL, 18 mmol) and triethylamine (1.7 mL, 12mmol). Purification: chromatography (silicagel, acetone) to give a yellow solid: yield 2386 mg (71%). Mp 178 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.55–2.62 (m, br, 6H), 2.69 (s, br, 4H), 3.61 (t, 2H, J = 5.3 Hz), 3.98 (s, 1H), 7.25 (d, 2H, J = 8.9 Hz), 7.45 (d, 2H, J = 8.5 Hz), 7.48–7.50 (m, 2H), 7.54 (t, 2H, J = 7.7 Hz), 7.64–7.68 (m, 1H), 7.76 (dd, 2H, J = 8.2 Hz, J = 1.2 Hz), 8.54–8.56 (m, 1H), 11.65 (s, br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 52.53, 57.64, 59.13, 76.16, 122.53, 123.04, 125.92, 127.75, 128.64, 130.04, 130.65, 131.94, 132.12, 133.12, 133.52, 134.12, 137.73, 137.83, 170.34, 197.32; IR (ATR): ν (cm^{-1}) = 3485, 3175, 2808, 1682, 1645, 1501, 1288, 1255, 1013, 832, 736, 698, 456, 405; MS (ESI): *m/z* 558 (100), $[M + H]^+$; MS (EI-HRMS) calcd: 556.1029, found: 556.1003.

*4.1.4.39. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(biphenyl-4-yl)-2-(4-(2-hydroxyethyl)piperazin-1-yl)acetamide (**11d**).* According to general procedure 2 from *N*-(2-benzoyl-4-chlorophenyl)-2-(biphenyl-4-yl)-2-bromo-acetamide (613 mg, 1.2 mmol) und *N*-(2-hydroxyethyl)piperazine (0.5 mL, 3.6 mmol) and triethylamine (0.3 mL, 2.4 mmol). Purification: chromatography (silicagel, acetone) to give a light yellow solid: yield 398 mg (60%). Mp 147 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) = 2.50–2.59 (m, br, 6H), 2.63–2.65 (m, br, 4H), 3.58 (t, 2H, J = 5.3 Hz), 4.04 (s, 1H), 7.30–7.36 (m, 1H), 7.39–7.57 (m, br, 12H), 7.63–7.69 (m, 1H), 7.77 (d, 2H, J = 1.5 Hz), 8.60 (d, 1H, J = 9.6 Hz), 11.68 (s, br, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ_C (ppm) = 52.65, 57.73, 59.12, 77.44, 123.13, 126.15, 127.06, 127.44, 127.54, 128.66, 128.73, 129.33, 130.03, 132.04, 133.02, 133.42, 134.23, 137.84, 138.05, 140.53, 141.32, 171.03, 197.12; IR (ATR): ν (cm^{-1}) = 3193, 2942, 2819, 1693, 1640, 1499, 1485, 1277, 1237, 1011, 737, 691, 383; MS (ESI): *m/z* 554 (100), $[M + H]^+$; MS (EI-HRMS) calcd: 554.2213, found: 554.2210.

*4.1.4.40. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-(naphtalen-1-yl)-acetamide (**11e**).* According to general procedure 2 from *N*-(2-benzoyl-4-chlorophenyl)-2-bromo-2-(naphtalen-1-yl)acetamide (1.4 g, 2.9 mmol) und *N*-(2-hydroxyethyl)piperazine (1.1 mL, 8.7 mmol) and triethylamine (0.8 mL, 5.8 mmol). Purification: chromatography (silicagel, acetone) to give a light yellow solid: yield 955 mg (62%). Mp 97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) = 2.61–2.69 (m, br, 6H), 2.71 (s, br, 4H), 2.96 (s, br, 1H), 3.60 (t, 2H, J = 5.3 Hz), 4.86 (s, 1H), 7.39–7.57 (m, br, 7H), 7.64–7.69 (m, 2H), 7.75–7.85 (m, br, 4H), 8.40 (d, 1H, J = 8.2 Hz), 8.56 (d, 1H, J = 8.8 Hz), 11.74 (s, br, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ_C (ppm) = 52.33, 57.13, 58.84, 77.03, 122.62, 123.33, 124.94, 125.43, 126.22, 127.12, 128.23, 128.43, 128.54, 129.52, 131.33, 131.64, 131.84, 132.66, 133.07, 133.74, 137.43, 137.65, 170.53, 196.05; IR (ATR): ν (cm^{-1}) = 3245, 2941, 2818, 1691, 1647, 1494, 1394, 1282, 1245, 949, 794, 776, 700, 412; MS (ESI): *m/z* 528.04 $[M + H]^+$; MS (EI-HRMS) calcd: 528.2076, found: 528.2054.

*4.1.4.41. (R,S)-N-(2-benzoyl-4-chlorophenyl)-2-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-(naphtalen-2-yl)-acetamide (**11f**).* According to general procedure 2 from *N*-(2-benzoyl-4-chlorophenyl)-2-bromo-2-(naphtalen-2-yl)acetamide (3.0 g, 6.3 mmol) und *N*-(2-hydroxyethyl)piperazine (2.3 mL, 18.9 mmol) and triethylamine (0.9 mL, 6.3 mmol). Purification: chromatography (silicagel, acetone) to give a light yellow solid: yield 2876 mg (86%). Mp 84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) = 2.57–2.63 (m, br, 6H), 2.67–2.69 (s, br, 4H), 2.79 (s, br, 1H), 3.59 (t, 2H, J = 5.3 Hz), 4.16 (s, 1H), 7.44–7.57 (m, br, 7H), 7.64–7.70 (m, 1H), 7.76–7.82 (m, br, 5H), 7.85 (s, 1H), 8.55 (d, 1H, J = 8.8 Hz), 11.72 (s, br, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ_C (ppm) = 52.66, 57.63, 59.22, 77.42, 123.13, 125.73, 126.12, 126.33, 127.63, 128.02, 128.51, 128.63, 128.74, 130.04, 132.05, 132.83, 133.12, 133.22, 133.33, 133.43, 137.86, 137.98,170.81, 197.22; IR (ATR): ν (cm^{-1}) = 3240, 2819, 1693, 1644, 1494, 1281, 1243, 1011, 947, 744, 699, 477; MS (ESI): *m/z* 528 (100) $[M + H]^+$; MS (EI-HRMS) calcd: 528.2079, found: 528.2054.

4.2. Antimalarial activity

The multi-drug resistant *P. falciparum* clone Dd2 [6] was maintained in continuous culture as described [7] and synchronized using the sorbitol method [8]. Drug assays based on [^{3}H]-hypoxanthine incorporation were done [9] and the percent inhibition was determined as described [10]. Each assay was repeated at least three independent times and the mean ± SEM was calculated. IC_{50} values were calculated from the sigmoidal dose-response curves using a Hill function (Sigma Plot, SPSS).

4.3. Cytotoxic assay

HeLa (DSM ACC 57) cells were grown in RPMI 1640 culture medium (GIBCO BRL 21875-034) supplemented with 25 μg/mL gentamicin sulfate (BioWhittaker 17-528Z), and 10% heat inactivated fetal bovine serum (GIBCO BRL 10500-064) at 37 °C in high density polyethylene flasks (NUNC 156340). The test substances were dissolved in DMSO (10 mg/ml) before being diluted in the cell culture medium (1:200). The adherent HeLa cells were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA (Biochrom KG L2163). For each experiment approximately 10,000 cells were seeded with 0.1 mL RPMI 1640 (GIBCO BRL 21875-034), containing 25 μg/mL gentamicin sulfate (BioWhittaker 17-528Z), but without HEPES, per well of the 96-well microplates (NUNC 167008). For the cytotoxic assay HeLa cells were preincubated for 48 h without the test substances. The dilutions of the test substances were carried out carefully on the monolayers of HeLa cells after the preincubation time. The HeLa cells were further incubated for 72 h at 37 °C in a humidified atmosphere and 5% CO_2. The adherent HeLa cells were fixed with 25% glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gently washing the stain was eluted with 0.2 ml of 0.33 M HCl per well. The optical densities were measured at 660 nm in SUNRISE microplate reader (TECAN). For data analysis the Magellan software (TECAN) was used.

References

[1] M. Schlitzer, Chem. Med. Chem. 2 (2007) 944–949.
[2] M. Schlitzer, Arch. Pharm. 341 (2008) 149–163.
[3] M. Altenkämper, B. Bechem, J. Perruchon, S. Heinrich, A. Mädel, R. Ortmann, H.M. Dahse, E. Freunscht, Y. Wang, J. Rath, A. Stich, M. Hitzler, P. Chiba, M. Lanzer, M. Schlitzer, Bioorg. Med. Chem. 17 (2009) 7690–7697.
[4] K. Kohring, J. Wiesner, M. Altenkämper, J. Sakowski, K. Silber, A. Hillebrecht, P. Haebel, H.-M. Dahse, R. Ortmann, H. Jomaa, G. Klebe, M. Schlitzer, Chem. Med. Chem. 3 (2008) 1217–1231.
[5] C.P. Sanchez, A. Rotmann, W.D. Stein, M. Lanzer, Mol. Microbiol. 70 (2008) 786.
[6] T.E. Wellems, L.J. Panton, I.Y. Gluzman, V.E. Rosario, R.W. Gwadz, A. Walker-Jonah, D.J. Krogstad, Nature 345 (1990) 253–255.
[7] W. Trager, J.B. Jensen, Science 193 (1976) 673–675.
[8] C. Lambros, J.P. Vanderberg, J. Parasitol. 65 (1979) 418–420.
[9] R.E. Desjardins, C.J. Canfield, J.D. Haynes, J.D. Chulay, Antimicrob. Agents Chemother. 16 (1979) 710–718.
[10] M.T. Ferding, R.A. Cooper, J. Mu, B. Deng, D.A. Joy, X.Z. Su, T.E. Wellems, Mol. Microbiol. 52 (2004) 985–997.