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Antimalarial and antitrypanosomal activity of a series of amide and sulfonamide derivatives of a 2,5-diaminobenzophenone

Mirko Altenkämper [a], Benjamin Bechem [a], Johann Perruchon [a], Swetlana Heinrich [a], Andrea Mädel [a], Regina Ortmann [a], Hans-Martin Dahse [b], Ellen Freunscht [c], Yulin Wang [c], Jennifer Rath [d], August Stich [d], Manuela Hitzler [e], Peter Chiba [e], Michael Lanzer [c], Martin Schlitzer [a,*]

[a] Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, 35032 Marburg, Germany
[b] Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e.V. Hans-Knöll-Institut, Beutenbergstrasse 11a, 07745 Jena, Germany
[c] Institut für Parastitologie, Universität Heidelberg, Im Neuenheimer Feld 24, 69120 Heidelberg, Germany
[d] Tropenmedizinische Abteilung, Missionsärztliche Klinik GmbH, Salvatorstr. 7, 97074 Würzburg, Germany
[e] Center for Physiology, Pathophysiology and Immunology, Institute of Medical Chemistry, Medical University of Vienna, Austria

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ABSTRACT

Here, we describe a series of readily obtainable benzophenone derivatives with antimalarial and antitrypanosomal activity. The most active compounds display submicromolar activity against *Plasmodium falciparum*. Micromolar activity is obtained against *Trypanosoma brucei*. Main problem of the compounds is low selectivity. However, there are indications that separation of antimalarial and cytotoxic activity might by possible. In addition, some compounds inhibit human ABC transporter with nanomolar activity.

1. Introduction

Malaria is one of the most important infectious diseases affecting almost half of the world's population, especially in the developing countries. The amatory for the fight against malaria is rather limited and continuously threatened by the development of resistance.[1,2] Although far less people are affected by African sleeping sickness, here the situation is even worse. Untreated this disease leads inevitably to death. Currently, there are only five drugs available for the treatment. Their use is limited by severe drug toxicity and resistance.[3–5] Therefore, there is an urgent need for the development of novel agents against these diseases preferably with hitherto unexploited mechanisms of action.

We have previously investigated the class of 2,5-diaminobenzophenones as farnesyltransferase inhibitors with antimalarial and antitrypanosomal activity.[6–8] Some of these compounds had an additional weak inhibitory effect on human ABC transporters (unpublished results). This effect was more pronounced, when the arylfurylacryloyl residue of our farnesyltransferase inhibiting benzophenones has been replaced by simple aryloyl residues, which in a related series of compounds significantly reduced farnesyltransferase inhibitory activity.[9]

Here, we investigated the effect of our novel benzophenone derivatives on the growth of cultured *Plasmodium falciparum* and *Trypanosoma brucei* parasites as well as their ability to inhibit multidrug resistance proteins.

2. Results and discussion

2.1. Chemistry

Synthesis (Scheme 1) of the target compounds followed the route described previously[6] for this type of compounds. It started with the acylation of commercially available 2-amino-5-nitrobenzophenone (**1**) which was acylated using α-chlorophenylacetic acid chloride or α-chloroacetic acid chloride yielding the acyl derivatives **2a** and **2b**. After replacement of the chlorine by *N*-methylpiperazine, the nitro group of the resulting amino acid derivative **3** was reduced to obtain the amino derivative **4**. The sequence is completed by the acylation of the amino group yielding the target compounds **5** and **6**, respectively.

* Corresponding author. Tel.: +49 6421 28 25896.
E-mail address: schlitzer@staff.uni-marburg.de (M. Schlitzer).

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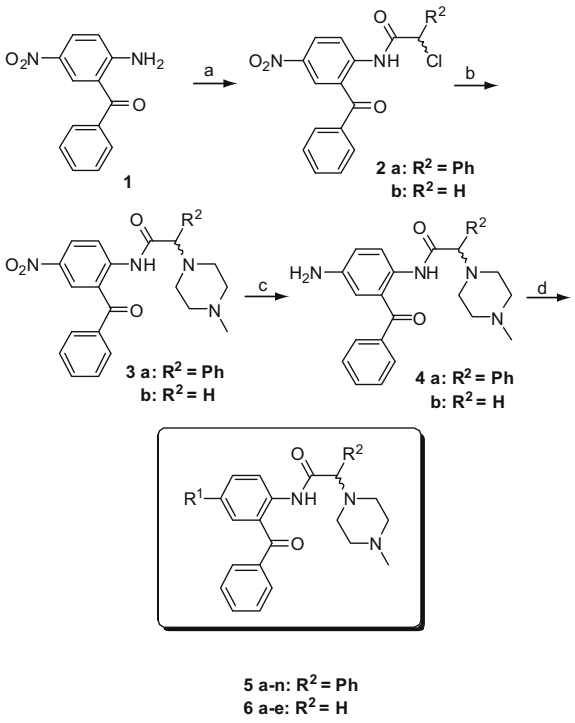

Scheme 1. Synthesis of acylbenzophenone derivatives: (a) 2-chloro-2-phenylacetyl chloride or 2-chloroacetyl chloride, toluene, reflux; (b) 1-methylpiperazine, acetonitrile, reflux; (c) $SnCl_2$ (dihydrate), ethyl acetate, reflux; (d) acyl chloride or carbamoyl chloride, toluene, reflux.

2.2. Antimalarial activity

We first investigated the in vitro antiplasmodial activity of the compounds, 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. IC_{50} values ranging from 110 ± 20 nM (compound **5n**) to >16 μM (compounds **5m**, **6b**, and **6c**) were obtained (Table 1). In parallel, we investigated the IC_{50} value of the Dd2 strain for the established antimalarial drugs chloroquine and quinine (137 ± 11 nM and 284 ± 18 nM, respectively). The chloroquine and quinine IC_{50} values were consistent with published data.[10]

In the series of the alkylacyl derivatives antimalarial activity increase slightly with increasing size of the alkyl residue from 9.72 μM of the acetyl derivative **5a** to 3.04 μM of the pivaloyl derivative **5c**. Replacement of the alkyl residues by phenyl resulted in a slightly more active derivative **5d** (IC_{50} = 1.61 μM), while further insertion of a methylene and a ethylene spacer (**5e** and **5f**, respectively) did not further increased antimalarial activity as did the replacement of the phenyl moiety by a 3-pyridyl residue (**5g**). When the carbonyl group was replaced by a sulfonyl moiety (compounds **5h–5j**), no significant influence on antimalarial activity could be recorded. However, submicromolar activity was obtained with the naphthyl derivatives **5k** and **5l**. Interestingly, the position of connection between the naphthyl residue and the carbonyl makes a notable difference in antimalarial activity. While the 1-naphthyl derivative **5k** displays an IC_{50} of 930 nM, the 2-naphthyl isomer **5l** is nearly fourfold more active (IC_{50} = 240 nM). This marked dependence of antimalarial activity on the precise orientation of the naphthyl residue argues for well-defined geometric requirements in the putative target structure. The same holds true for the pair of urea derivatives **5m** and **5n**. While the 4-methylpiperidyl derivative **5m** is virtually inactive (IC_{50} >16 μM), the di-isopropyl derivative **5n** displays with an IC_{50} value of 110 nM the highest antimalarial activity in this series. This value falls within the range of the reference compounds and is well below the threshold of 1 μM. Compounds with in vitro activities below this threshold are normally regarded as being worth further investigations.

To further explore structure activity relationships, the phenyl residue at the acyl moiety on the 2-amino group has been omitted in compounds **6a–6d**. In general, this markedly reduced antimalarial activity resulting in several inactive compounds.

2.3. Antitrypanosomal activity

In vitro antitrypanosomal activity of the compounds was determined against the *Trypanosoma brucei brucei* TC 221 laboratory strain, cultured under standard conditions.[11] Parasite viability was assessed using the Alamar Blue assay. In living parasites, the dye is reduced by redox equivalents stemming from the glycolytic pathway. A linear correlation has been found to exist between the fluorescence signal of the dye and the concentration of living trypanosomes.[12]

Activity against *T. brucei brucei* followed mainly the trends seen in antimalarial activity, but did not reach submicromolar levels (Table 1). However, in vitro activities of several compounds (**5d**–**5f**, **5j**–**5l**, and **6e**) are similar to that of nifurtimox (IC_{50} = 3.4 μM),[13] which has originally been developed and used against American Trypanosomias (Chaga's Disease), but now is also evaluated in combination with other antitrypanosomal drugs for the treatment of sleeping sickness[14] although it is in vitro markedly less active than currently used antitrypanosomal agents (IC_{50}'s: melarsoprol 2.6 nM, pentamidine 2.9 nM, suramin 310 nM).[13]

2.4. Cytotoxicity assay

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).

Major problem of these compounds is the high cytotoxicity and accordingly the low selectivity toward the malaria parasites. However, there is one exemption which is the most active compound, the di-isopropyl urea derivative **5n**, which because of its high antimalarial activity displays some selectivity toward malaria parasites. The selectivity index of this compound of 36 is still far to low but it indicates that it might be possible to further separate antimalarial from cytotoxic activity.

2.5. Inhibition of P-glycoproteins

ATP-dependent multidrug efflux pumps have been recognized as a major cause for the failure of anticancer therapy but its role in restricting the effectiveness of antimicrobial therapy is also increasingly recognized.[15] Multidrug efflux pumps use the energy provided by ATP-binding and hydrolysis to prevent toxic compounds from entering cells. In its functional form ATP-binding cassette (ABC) transporters consist of two nucleotide binding domains and two membrane spanning substrate (solute) binding domains. Because of the structural similarity of the benzophenones with known inhibitors of multidrug resistance transporters we have tested our compounds for possible activity against these transporters using the daunomycin efflux assay.

The daunomycin efflux assay[16] is a direct and accurate functional method to measure inhibition of PGP-mediated transmembrane transport. The resistant human T-lymphoblast cell line CCRF VCR 1000 overexpressing the ABCB1 transporter was used in these studies. The time dependent decrease in mean cellular fluorescence is determined in the presence of various concentra-

Table 1
Structure and antiparasitic activity of target compounds **5** and **6**

Cmpd	R^1/R^2	IC_{50} *P. falciparum* (μM)	CC_{50}[a] (μM)	SI[b]	IC_{50} *T. brucei brucei*[c] (μM)	EC_{50}[d] (μM)
5a	MeCONH/Ph	9.72 ± 1.16	105	11	9.05 ± 2.69 6.59 ± 1.11	6.29 ± 1.11
5b	EtCONH/Ph	7.19 ± 1.21	84	12	16.73 ± 2.00 24.62 ± 7.02	3.30 ± 0.74
5c	*t*-BuCONH/Ph	3.04 ± 0.31	45	15	11.42 ± 0.25 4.67 ± 1.61	0.39 ± 0.04
5d	PhCONH/Ph	1.61 ± 0.28	16	10	3.28 ± 0.13 3.28 ± 0.24	0.24 ± 0.19
5e	Ph-CH_2–CONH/Ph	2.00 ± 0.26	16	8	3.64 ± 0.65 3.25 ± 0.10	0.36 ± 0.12
5f	Ph-$(CH_2)_2$–CONH/Ph	1.80 ± 0.26	8	4	3.27 ± 1.18 3.02 ± 0.21	0.06 ± 0.01
5g	3-Pyridyl-CONH/Ph	2.26 ± 0.56	37	16	5.06 ± 2.25 7.02 ± 1.73	5.12 ± 1.45
5h	$MeSO_2NH$/Ph	10.62 ± 3.27	99	9	5.58 ± 2.66 5.84 ± 2.60	15.52 ± 1.37
5i	$PhSO_2NH$/Ph	0.91 ± 0.46	26	29	5.38 ± 0.78 2.92 ± 1.18	0.89 ± 0.36
5j	$(PhSO_2)_2NH$/Ph	1.24 ± 0.72	12	10	3.01 ± 0.61 3.12 ± 0.06	0.04 ± 0.01
5k	1-Naphthyl-CONH/Ph	0.93 ± 0.16	4	4	1.93 ± 0.33 2.68 ± 0.18	0.23 ± 0.04
5l	2-Naphthyl-CONH/Ph	0.24 ± 0.05	4	17	1.98 ± 0.88 2.64 ± 0.35	0.04 ± 0.01
5m	*N*-Methyl-1-piperidyl CONH/Ph	>16	nd	nd	nd	4.97 ± 2.63
5n	$(iPr)_2NCONH$/Ph	0.11 ± 0.02	4	36	nd	0.46 ± 0.13
6a	MeCONH/H	9.45 ± 0.34	53	6	23.05 ± 7.12 22.91 ± 4.56	nd
6b	EtCONH/H	>16	>122	>8	53.43 ± 18.05 71.49 ± 14.07	nd
6c	PhCONH/H	>16	>109	>7	49.43 ± 3.59 83.18 ± 17.94	nd
6d	Ph-$(CH_2)_2$–CONH/H	6.72 ± 0.23	80	12	13.92 ± 0.65 21.89 ± 1.19	2.23 ± 0.23
6e	2-Naphthyl-CONH/H	2.01 ± 0.30	9	5	0.81 ± 0.33 2.01 ± 0.44	0.48 ± 0.05

[a] Cytotoxicity (HeLa cells).
[b] SI = selectivity index = CC_{50} (HeLa)/IC_{50} (*P. falciparum*).
[c] 48 h/72 h.
[d] EC_{50} refers to daunomycin efflux-test, nd = not determined.

tions of modifier and the first-order rate constants (V_{max}/K_m) are calculated by nonlinear regression analysis. A correction for simple diffusion is achieved by subtracting the efflux rates observed in the parental line. EC_{50} values of modifiers are calculated from dose–response curves of V_{max}/K_m versus modifier concentration. These studies were performed according to previously published methods,[17,18] slightly adjusted to our requirements.[19]

Activity of the benzophenones against the ABCB1 transporter (Table 1) ranges between 15.5 μM for the methylsulfonyl derivative **5h** and 40 nM for the 2-naphthoyl derivative **5l** and the bis-(phenylsulfonyl) derivative **5j** with several other derivatives being also in the submicromolar range. Two general trends can be outlined. Sulfonamides are less active than the corresponding carbonamides and more spacious lipophilic acyl residues tend to produce higher activity in the daunorubicin efflux assay.

An analogue of human ABC transporters is the *P. falciparum* P-glycoprotein homologue Pgh-1 (or multidrug resistance protein 1 (*Pf*MDR 1)), which is located in the membrane of the digestive vacuole and has been shown to transport solutes, including antimalarial drugs, into the digestive vacuole.[12,20] Copy number of the *pfmdr1* gen and polymorphism alters parasite sensitivity to arylaminoalcohols.[21] Furthermore, the theory has been put forward that Pgh-1 might be the target of quinine and other arylaminoalcohols.[12] At present, we have no data suggesting that our compounds interfere with Pgh-1. At micromolar concentrations, none of the compounds inhibited Pgh-1-mediated accumulation of fluorochromes into the parasite's digestive vacuole (data not shown). So, currently we do not have firm clue on the mechanism of action of these compounds against malaria parasites.

3. Conclusion

In the series of readily obtainable benzophenone derivatives described here we saw mainly micromolar activity against *P. falciparum* and *T. brucei brucei*. However, two compounds of this series displayed considerable antimalarial activity with IC_{50} values of 240 and 110 nM, respectively, which merit further attention to this class of compounds. Although selectivity is generally low and even with the most active compound far from being satisfactory, this compound demonstrates that it has not necessary be a fruitless attempt to try to dissociate antimalarial from cytotoxic activity.

Further investigations will be directed toward the improvement of antimalarial activity with concurrent reduction of cytotoxicity and thereby enhancement of selectivity against malaria parasites.

4. Experimental

4.1. Chemistry

^1H 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 FT-IR or a Brucker 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) 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.

4.1.2. General procedure 2: substitution of the halogen of 2-chloro-(phenyl)alkylcarboxylic acid derivatives with amines

The 2-chloro-(phenyl)alkylcarboxylic acid derivative was dissolved in freshly distilled acetonitrile, 3 equiv 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 necessary, the crude product was recrystallized, or chromatographed on silica gel.

4.1.3. General procedure 3: reduction of aromatic nitro compounds

Aromatic nitro compounds were dissolved in ethyl acetate (50–100 mL) and $SnCl_2 \times 2H_2O$ (1.125 g per mmol nitro compound) was added. The mixture was heated under reflux for 2 h. Then, $NaHCO_3$-solution was added until pH 7–8 was reached and the organic layer was separated. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with brine and dried over $MgSO_4$. Then, the solvent was removed in vacuo to obtain the crude products.

4.1.3.1. (*R,S*)-*N*-(2-Benzoyl-4-nitrophenyl)-2-chloro-2-phenylacetamide (2a). According to general procedure 1 from (*R,S*)-2-chloro-2-phenylacetyl chloride (1.46 mL, 10.0 mmol) and 2-amino-5-nitrobenzophenone (2.42 g, 10.0 mmol). Purification: recrystallization from ethanol to give a yellowish solid: yield 3.63 g (92%). Mp 134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 5.52 (s, 1H), 7.38–7.43 (m, 3H), 7.55–7.59 (m, 4H), 7.70 (m, 1H), 7.73–7.75 (m, 2H), 8.42 (m, 1H), 8.52 (m, 1H), 8.86 (m, 1H), 12.12 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 62.11, 121.50, 123.26, 127.75, 128.76, 128.87, 128.94, 129.10, 129.45, 129.95, 133.59, 135.85, 137.10, 142.02, 144.96, 167.46, 197.68; IR (KBr): ν = 3113, 2960, 2362, 1841, 1718, 1690, 1642, 1616, 1596, 1581, 1535, 1509, 1350, 1277, 1249, 1152, 742; MS (ESI): *m*/*z* 417 (100) [^{35}Cl, M+Na]$^+$, 419 (35) [^{37}Cl, M+Na]$^+$; HRMS (ESI): calcd: 417.0618, found: 417.0615.

4.1.3.2. *N*-(2-Benzoyl-4-nitrophenyl)-2-chloroacetamide (2b). According to general procedure 1 from 2-chloroacetyl chloride (0.80 mL, 10.0 mmol) and 2-amino-5-nitrobenzophenone (2.42 g, 10.0 mmol). Purification: recrystallization from ethanol to give a white crystalline solid: yield 3.06 g (96%). Mp 176 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 4.25 (s, 2H), 7.53–7.59 (m, 2H), 7.66–7.72 (m, 1H), 7.72–7.77 (m, 2H), 8.44–8.46 (m, 1H), 8.51 (m, 1H), 8.91 (m, 1H), 11.92 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.05, 121.62, 123.55, 128.60, 128.86, 128.87, 129.98, 133.67, 137.04, 142.14, 144.52, 165.96, 197.49; IR (KBr): ν = 3241, 1689, 1641, 1617, 1597, 1587, 1549, 1511, 1447, 1414, 1400, 1345, 1320, 1305, 1284, 1225, 1172, 1126, 1093, 1080, 969, 915, 855, 805, 762, 702, 654; MS (ESI): *m*/*z* 288 (8), 341 (82) [^{35}Cl, M+Na]$^+$, 659 (100) [2 (^{35}Cl, M+Na)]$^+$; HRMS (ESI): calcd: 341.0305, found: 341.0302 [^{35}Cl, M+Na]$^+$.

4.1.3.3. (*R,S*)-*N*-(2-Benzoyl-4-nitrophenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (3a). According to general procedure 2 from (*R,S*)-*N*-(2-benzoyl-4-nitrophenyl)-2-chloro-2-phenylacetamide (1.97 g, 5.0 mmol) and *N*-methylpiperazine (1.66 mL, 15.0 mmol). Purification: column-chromatography, silica gel, ethyl acetate/isohexane (1:3): yield 1.54 g (67%). Mp 101 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.32 (s, 3H), 2.59 (s, 8H), 4.04 (s, 1H), 7.29–7.42 (m, 5H), 7.55–7.61 (m, 3H), 7.71 (m, 1H), 7.79–7.82 (m, 2H), 8.37 (m, 1H), 8.46 (m, 1H), 12.13 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.60, 54.62, 76.80, 121.40, 123.79, 127.89, 128.16, 128.56, 128.61, 128.87, 128.90, 130.00, 133.57, 134.67, 137.27, 141.46, 145.03, 171.72, 196.74; IR (KBr): ν = 2936, 2797, 1700, 1647, 1577, 1494, 1336, 1275, 1256, 1135, 1092, 1008, 697, 648; MS (ESI): *m*/*z* 189 (23), 459 (100) [^{35}*Cl*, M+H]$^+$; HRMS (ESI): calcd: 459.2032, found: 459.2031.

4.1.3.4. *N*-(2-Benzoyl-4-nitrophenyl)-2-(4-methyl-1-piperazinyl)-acetamide (3b). According to general procedure 2 from *N*-(2-benzoyl-4-nitrophenyl)-2-chloroacetamide (2.9 g, 9.0 mmol) and *N*-methylpiperazine (3.0 mL, 27.0 mmol). Purification: precipitation from dichloromethane solution with *n*-pentane: yield 1.99 g (58%). Mp 178 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.49 (s, 3H), 2.79 (s, br, 8H), 3.25 (s, 2H), 7.51–7.59 (m, 2H), 7.67–7.72 (m, 1H), 7.74–7.77 (m, 2H), 8.39–8.45 (m, 2H), 8.93–8.97 (m, 1H), 11.94 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.46, 52.71, 54.48, 61.96, 121.36, 124.02, 127.98, 128.48, 128.84, 130.02, 133.60, 137.20, 141.54, 144.54, 170.52, 196.31; IR (KBr): ν = 3426, 3203, 2937, 2820, 1696, 1653, 1609, 1597, 1577, 1560, 1522, 1499, 1449, 1414, 1374, 1341, 1279, 1255, 1172, 1140, 1096, 1011, 985, 966, 917, 874; MS (EI): *m*/*z* 33 (22), 41 (22), 58 (28), 70 (54), 71 (24), 86 (23), 98 (21), 99 (29), 113 (100), 114 (58), 157 (62), 382 (58) [M]$^+$, 383 (13) [M+1]$^+$; HRMS (ESI): calcd: 382.1641, found: 382.1673.

4.1.3.5. (*R,S*)-*N*-(4-Amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (4a). According to general procedure 3 from (*R,S*)-*N*-(2-benzoyl-4-nitrophenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (1.10 g, 2.5 mmol) and tin(II) chloride (2.80 g, 12.3 mmol). Purification: column-chromatography, silica gel, ethyl acetate/methanol (1:1): yield 907 mg (86%). Mp 148 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 2.14 (s, 3H), 2.17–2.25 (m, 4H), 2.27–2.40 (m, 4H), 3.83 (s, 1H), 5.21 (s, 2H), 6.33 (m, 1H), 6.74 (m, 1H), 7.25–7.31 (m, 5H), 7.51–7.58 (m, 3H), 7.63–7.67 (m, 1H), 7.72 (m, 2H), 10.38 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 45.60, 54.62, 75.15, 117.20, 121.89, 123.79, 123.85, 125.87, 127.89, 128.14, 128.36, 128.73, 129.54, 132.63, 136.35, 137.64, 144.97, 168.90, 196.70; IR (KBr): ν = 3455, 3361, 3274, 1671, 1632, 1606, 1595, 1577, 1512, 1446, 1434, 1329, 1289, 1251, 1178, 1158, 1139, 1010, 964, 868, 700; MS (EI): *m*/*z* 70 (13), 91 (12), 105 (25), 189 (100), 190 (59), 195 (21), 211 (33), 212 (42), 224 (18), 238 (13), 239 (18), 240 (21), 271 (7), 328 (7), 330 (10), 428 (30) [M]$^+$; HRMS (EI): calcd: 428.2212, found: 428.2213.

4.1.3.6. *N*-(4-Amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)acetamide (4b). According to general procedure 3 from *N*-(2-benzoyl-4-nitrophenyl)-2-(4-methyl-1-piperazinyl)acetamide (2.0 g, 5.0 mmol) and tin(II) chloride (5.6 g, 26.0 mmol). Purification: column-chromatography, silica gel, ethyl acetate/methanol (3:2): yield 605 mg (34%). Mp 74 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.36 (s, 3H), 2.60 (s, br, 8H), 3.10 (s, 2H), 3.64 (s, br, 2H), 6.77 (m, 1H), 6.87–6.91 (m, 1H), 7.46–7.49 (m, 2H), 7.57–7.60 (m, 1H), 7.77–7.80 (m, 2H), 8.27–8.29 (m, 1H), 10.93 (s, 1H,) ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.88, 53.39, 54.82, 62.11, 117.70, 119.67, 123.68, 127.33, 128.28, 130.03, 130.11, 132.60, 138.21, 141.53, 169.28, 197.43; IR (KBr): ν (cm^{-1}): = 3429, 2937, 2816, 1670, 1596, 1577, 1616, 1437, 1374, 1328, 1290, 1245, 1169, 1012, 975, 830, 746, 707, 479; MS (EI, 70 eV): *m*/*z* 70 (45), 99 (21), 113 (90), 114 (53), 212 (81), 225 (24), 282 (25), 295 (47), 296 (24), 309 (22), 352 (100) $[M]^+$, 353 (29) $[M+1]^+$; MS (EI-HRMS): calcd: 352.1899, found: 352.1910.

4.1.3.7. (*R,S*)-*N*-(4-Acetylamino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (5a). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and acetic acid chloride (0.09 g, 1.2 mmol). Purification: column-chromatography, silica gel, ethyl acetate/methanol (1:2): yield 132 mg (28%). Mp 85 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) 2.09 (s, 3H), 2.29 (s, 3H), 2.53 (s, br, 8H), 3.94 (s, 1H), 7.27 (s, 1H), 7.25–7.31 (m, 2H), 7.34–7.39 (m, 3H), 7.43 (m, 1H), 7.49–7.53 (m, 2H), 7.59–7.63 (m, 1H), 7.80–7.83 (m, 2H), 7.90 (m, 1H), 8.48 (m, 1H), 11.56 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 24.31, 45.65, 54.67, 76.84, 122.33, 124.16, 124.77, 125.31, 125.39, 128.26, 128.40, 128.66, 128.84, 130.11, 132.38, 132.78, 135.47, 137.95, 168.30, 170.90, 197.80; IR (KBr): ν = 3276, 2933, 2846, 2669, 1550, 1508, 1453, 1402, 1292, 1140, 1009, 702; MS (EI): *m*/*z* 70 (6), 91 (8), 105 (6), 146 (4), 189 (100), 190 (43), 211 (6), 212 (7), 254 (14), 280 (10), 372 (5), 470 (14) $[M]^+$; HRMS (EI): calcd: 470.2317, found: 470.2320.

4.1.3.8. (*R,S*)-*N*-(2-Benzoyl-4-propanoylaminophenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5b). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and propionyl chloride (0.11 g, 1.2 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether (4:1): yield 300 mg (62%). Mp 80 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.18 (t, 3H), 2.29 (s, 3H), 2.31 (q, 2H), 2.53 (s, br, 8H), 3.94 (s, 1H), 7.24–7.29 (m, 3H), 7.29 (s, 1H), 7.36–7.40 (m, 2H), 7.45 (m, 1H), 7.51 (m, 2H), 7.62 (m, 1H), 7.82 (m, 2H), 7.91 (m, 1H), 8.47 (m, 1H), 11.51 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 9.37, 30.09, 45.70, 54.69, 76.78, 122.33, 123.72, 124.52, 125.77, 128.20, 128.29, 128.55, 128.87, 130.03, 132.78, 133.17, 134.61, 135.35, 137.80, 170.96, 172.38, 197.58; IR (KBr): ν = 3276, 3062, 2939, 2804, 1669, 1548, 1506, 1453, 1403, 1291, 1243, 1009; MS (EI): *m*/*z* 70 (16), 91 (20), 98 (12), 105 (10), 146 (8), 187 (9), 190 (72), 211 (12), 216 (11), 238 (22), 268 (24), 394 (15), 386 (10), 484 (49) $[M]^+$; HRMS (EI): calcd: 484.2474, found: 484.2477.

4.1.3.9. (*R,S*)-*N*-(2-Benzoyl-4-pivaloylaminophenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5c). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.30 g, 0.7 mmol) and pivalic acid chloride (0.11 mL, 0.8 mmol). Purification: column-chromatography, silica gel, ethyl acetate/methanol (1:2): yield 215 mg (60%). Mp 90 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) 1.27 (s, 9H), 2.29 (s, 3H), 2.52 (s, br, 8H), 3.94 (s, 1H), 7.26 (s, 1H), 7.27–7.30 (m, 3H), 7.34–7.39 (m, 2H), 7.47–7.49 (m, 1H), 7.50–7.55 (m, 2H), 7.60–7.63 (m, 1H), 7.82–7.85 (m, 2H), 7.87–7.89 (m, 1H), 8.49 (m, 1H), 11.44 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 27.56, 39.52, 45.58, 54.61, 76.86, 122.31, 123.75, 124.93, 125.69, 128.23, 128.48, 128.65, 128.91, 130.22, 132.63, 132.96, 135.33, 135.55, 137.96, 170.76, 176.55, 197.69; IR (KBr): ν = 3340, 2967, 2801, 1685, 1534, 1507, 1400, 1291, 1241, 1162, 702; MS (EI): *m*/*z* 56 (18), 91 (15), 105 (8), 189 (100), 290 (71), 211 (14), 296 (38), 322 (20), 414 (12), 512 (39) $[M]^+$; HRMS (EI): calcd: 512.2787, found: 512.2784.

4.1.3.10. (*R,S*)-*N*-(2-Benzoyl-4-benzoylaminophenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5d). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and benzoyl chloride (0.17 g, 1.2 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether/isohexane (8:2:1): yield 474 mg (89%). Mp 135 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) 2.42 (s, 3H), 2.62 (s, br, 4H), 2.74 (s, br, 4H), 3.98 (s, 1H), 7.27–7.32 (m, 3H), 7.35–7.38 (m, 2H), 7.42–7.46 (m, 2H), 7.50–7.55 (m, 3H), 7.59–7.64 (m, 2H), 7.80–7.85 (m, 4H), 8.01 (s, 1H), 8.05–8.10 (m, 1H), 8.54 (m, 1H), 11.64 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.05, 54.39, 76.49, 122.34, 124.66, 125.22, 125.49, 127.06, 128.48, 128.76, 128.81, 128.87, 129.05, 130.16, 132.03, 132.62, 132.98, 134.36, 135.26, 135.77, 138.04, 165.73, 170.54, 198.12; IR (KBr): ν = 3282, 2937, 2804, 1654, 1596, 1507, 1401, 1290, 1139, 1009, 980, 703; MS (EI): *m*/*z* 70 (12), 77 (5), 91 (17), 105 (39), 106 (8), 146 (6), 187 (6), 189 (100), 190 (72), 191 (10), 211 (7), 212 (4), 216 (8), 316 (34), 317 (9), 342 (33), 343 (15), 434 (13), 530 (10), 532 (31) $[M]^+$; HRMS (EI): calcd: 532.2474, found: 532.2478.

4.1.3.11. (*R,S*)-*N*-(2-Benzoyl-4-phenylacetylaminophenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5e). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and phenyl acetyl chloride (0.2 mL, 1.5 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether (4:1): yield 233 mg (43%). Mp 190 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.27 (s, 3H), 2.49 (s, br, 8H), 3.65 (s, 2H), 3.92 (s, 1H), 7.28 (s, 1H), 7.21–7.43 (m, 11H), 7.50 (m, 2H), 7.61 (m, 1H), 7.80 (m, 2H), 7.92 (m, 1H), 8.43 (m, 1H), 11.49 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 44.53, 45.78, 54.75, 76.91, 122.22, 123.92, 124.66, 125.51, 127.70, 128.22, 128.41, 128.63, 128.92, 129.21, 129.44, 130.20, 132.19, 132.93, 134.09, 135.51, 135.55, 137.88, 169.12, 170.93, 197.61; IR (KBr): ν = 3328, 3029, 2938, 2838, 2795, 1695, 1658, 1545, 1504, 1453, 1400, 1322, 1288, 1247, 1139, 1013, 846, 700; MS (EI): *m*/*z* 70 (10), 91 (44), 105 (10), 119 (11), 136 (20), 189 (100), 190 (70), 212 (23), 238 (24), 330 (25), 356 (16), 428 (17), 546 (11) $[M]^+$; HRMS (EI): calcd: 546.2631, found: 546.2650 $[M]^+$.

4.1.3.12. (*R,S*)-*N*-[2-Benzoyl-4-(3-phenylpropionyl)aminophenyl]-2-(4-methylpiperazin-1-yl)-2-phenylacetamide (5f). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and 3-phenylpropionic acid chloride (0.2 mL, 1.5 mmol). Purification: column-chromatography, silica gel, methanol/ethyl acetate (1:4): yield 393 mg (70%). Mp 65 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.32 (s, 3H), 2.58 (t, 2H, $^3J_{H-H}$ = 7.6 Hz), 2.61 (s, br, 8H), 2.98 (t, 2H, $^3J_{H-H}$ = 7.6 H), 3.94 (s, 1H), 7.13–7.24 (m, 4H), 7.24–7.28 (m, 4H), 7.29 (s, 1H), 7.32–7.34 (m, 1H), 7.35–7.38 (m, 2H), 7.52 (m, 2H), 7.62–7.64 (m, 1H), 7.74–7.83 (m, 3H), 8.44 (m, 1H), 11.55 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 31.48, 39.27, 45.27, 54.39, 77.20, 122.30, 124.24, 124.99, 125.29, 126.42, 128.30, 128.33, 128.43, 128.63, 128.70, 128.86, 130.14, 132.30, 132.90, 135.43, 135.47, 137.98, 140.43, 170.42, 170.80, 197.80; IR (KBr): ν = 3274, 3061, 3027, 2937, 2823, 1667, 1506, 1454, 1403, 1290, 1140, 1076, 1010, 749, 699; MS (EI): *m*/*z* 91 (12), 105 (6), 189 (100), 190 (44), 212 (11), 238 (12), 344 (12), 560 (9) $[M]^+$; HRMS (EI): calcd: 560.2787, found: 560.2785 $[M]^+$.

4.1.3.13. (*R,S*)-*N*-(2-Benzoyl-4-nicotinoylaminophenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5g). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and nicotinic acid chloride hydrochloride (0.27 g, 1.5 mmol) under addition of triethylamine (0.3 mL, 2.0 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether/isohexane (8:2:2): yield 350 mg (66%). Mp 95 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.29 (s, 3H), 2.52 (s, br, 8H), 3.93 (s, 1H), 7.25–7.29 (m, 3H), 7.32–7.39 (m, 3H), 7.50 (m, 2H), 7.58–7.62 (m, 2H), 7.81–7.85 (m, 2H), 7.93 (m, 1H), 8.14 (m, 1H), 8.45 (s, 1H), 8.46 (m, 1H), 8.69–8.72 (m, 1H), 9.03–9.07 (m, 1H), 11.58 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.72, 54.72, 76.84, 122.51, 123.56, 124.71, 125.56, 125.64, 128.32, 128.47, 128.68, 128.91, 130.16, 130.22, 132.22, 132.99, 135.33, 135.42, 135.87, 137.87, 148.04, 152.52, 163.91, 171.15, 197.59; IR (KBr): ν = 3259, 2938, 2804, 1673, 1545, 1506, 1452, 1402, 1288, 1139, 1010, 729, 702; MS (EI): *m/z* 70 (14), 91 (21), 106 (39), 189 (100), 190 (69), 211 (16), 317 (52), 343 (58), 435 (19), 533 (25) $[M]^+$; HRMS (EI): calcd: 533.2427, found: 533.2434 $[M]^+$.

4.1.3.14. (*R,S*)-*N*-[2-Benzoyl-4-(methylsulfamoyl)phenyl]-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5h). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and methylsulfonyl chloride (0.17 g, 1.5 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether (2:1): yield 368 mg (73%). Mp 95 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.28 (s, 3H), 2.51 (s, br, 8H), 2.91 (s, 3H), 3.95 (s, 1H), 7.25–7.34 (m, 6H), 7.27 (s, 1H), 7.46 (m, 1H), 7.48–7.52 (m, 2H), 7.61–7.63 (m, 1H), 7.77–7.81 (m, 2H), 8.53 (m, 1H), 11.57 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 39.45, 45.75, 54.73, 76.81, 122.96, 126.00, 126.47, 127.07, 128.32, 128.54, 128.69, 128.91, 130.15, 130.81, 133.17, 135.32, 137.09, 137.61, 171.18, 197.21; IR (KBr): ν = 3242, 2937, 2807, 1685, 1508, 1448, 1396, 1322, 1289, 1236, 1151, 985, 702; MS (EI): *m/z* 70 (17), 91 (22), 98 (11), 146 (10), 189 (100), 190 (72), 211 (23), 216 (12), 237 (12), 316 (15), 408 (7), 506 (24) $[M]^+$; HRMS (EI): calcd: 506.1988, found: 506.1999 $[M]^+$.

4.1.3.15. (*R,S*)-*N*-[2-Benzoyl-4-(phenylsulfamoyl)phenyl]-2-(4-methylpiperazin-1-yl)-2-phenylacetylamide (5i). Similar to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) diluted in dichloromethane and cooled down to 0 °C. Benzenesulfonyl chloride (0.1 mL, 1.0 mmol) was added and stirred for further 3 h. After that the reaction mixture was washed with saturated aqueous sodiumhydrogencarbonate solution (20 mL) and saturated aqueous sodiumchloride solution (20 mL). The combined organic phases were dried over anhydrous sodiumsulfate. The solvent was removed in vacuo. Purification: column-chromatography, silica gel, methanol/ethyl acetate (1:2): yield 100 mg (18%). Mp 73 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.30 (s, 3H), 2.53 (s, br, 8H), 3.94 (s, 1H), 7.14 (m, 1H), 7.27 (s, 1H), 7.28–7.30 (m, 4H), 7.33–7.36 (m, 2H), 7.38–7.48 (m, 4H), 7.57 (m, 1H), 7.58–7.64 (m, 3H), 7.67–7.70 (m, 2H), 8.45 (m, 1H), 11.60 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.58, 54.61, 76.85, 113.75, 122.53, 125.34, 126.93, 127.13, 128.00, 128.28, 128.46, 128.65, 128.70, 128.89, 129.16, 130.73, 132.94, 135.31, 136.97, 137.73, 139.06, 171.00, 197.35; IR (KBr): ν = 3231, 3061, 2937, 2825, 1685, 1646, 1596, 1578, 1512, 1448, 1320, 1290, 1164, 1091, 1010, 984, 721, 690, 583; MS (EI): *m/z* 70 (18), 91 (24), 98 (14), 105 (16), 146 (11), 189 (100), 190 (70), 211 (16), 216 (15), 219 (14), 237 (22), 352 (10), 378 (34), 470 (12), 568 (21) $[M]^+$; HRMS (EI): calcd: 568.2144, found: 568.2141 $[M]^+$.

4.1.3.16. (*R,S*)-*N*-[2-Benzoyl-4-[*N*-(phenylsulfonyl)phenyl-sulfamoyl]phenyl]-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5j). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and benzene sulfonyl chloride (0.2 mL, 2.0 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether (2:1): yield 300 mg (72%). Mp 175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.32 (s, 3H), 2.57 (s, br, 8H), 3.98 (s, 1H), 7.11 (m, 1H), 7.27–7.33 (m, 4H), 7.40 (m, 4H), 7.47 (m, 2H), 7.48–7.54 (m, 4H), 7.64–7.72 (m, 4H), 7.90 (m, 3H), 8.66 (m, 1H), 11.96 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.72, 54.71, 77.16, 121.87, 124.54, 127.21, 127.53, 128.43, 128.46, 128.51, 128.70, 128.79, 128.87, 129.13, 129.16, 130.03, 132.96, 132.98, 133.09, 134.10, 135.26, 135.98, 136.44, 137.60, 141.29, 171.44, 197.00; IR (KBr): ν = 3202, 3059, 2935, 2796, 1692, 1508, 1448, 1385, 1285, 1260, 1170, 936, 881, 723; MS (EI): *m/z* 77 (19), 91 (11), 105 (11), 142 (10), 189 (100), 190 (68), 211 (30), 219 (11), 237 (18), 351 (13), 378 (24), 470 (9); MS (ESI): 569 (20), 709 (100) $[M+H]^+$, 1417 (8) $[2\ (M+H)]^+$; *m/z* HRMS (ESI): calcd: 709.2155, found: 709.2146 $[M+H]^+$.

4.1.3.17. (*R,S*)-*N*-(2-Benzoyl-4-(1-naphthoylamino)phenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5k). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and 1-naphthoyl chloride (0.19 g, 1.2 mmol). Purification: column-chromatography, silica gel, methanol/ethyl acetate (2:1): yield 430 mg (74%). Mp 100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) 2.29 (s, 3H), 2.52 (s, br, 8H), 3.91 (s, 1H), 7.28–7.32 (m, 3H), 7.36–7.39 (m, 2H), 7.41–7.45 (m, 1H), 7.50–7.55 (m, 4H), 7.58–7.68 (m, 3H), 7.84–7.92 (m, 4H), 7.94 (s, 1H), 8.05–8.10 (m, 1H), 8.25–8.29 (m, 1H), 8.54 (m, 1H), 11.58 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.59, 54.66, 60.35, 76.80, 122.39, 124.12, 124.58, 125.04, 125.12, 125.20, 125.43, 126.55, 127.34, 128.28, 128.39, 128.45, 128.66, 128.89, 129.99, 130.16, 131.14, 132.70, 132.90, 133.66, 133.87, 135.46, 135.71, 137.95, 167.54, 170.83, 197.74; IR (KBr): ν = 3258, 3059, 2937, 2800, 1649, 1503, 1450, 1404, 1288, 1139, 1009, 781, 702; MS (EI): *m/z* 70 (15), 91 (21), 98 (12), 99 (12), 127 (33), 146 (10), 155 (64), 156 (27), 172 (13), 188 (13), 189 (100), 190 (73), 191 (15), 216 (12), 366 (64), 367 (20), 392 (40), 393 (20), 484 (15), 485 (7), 582 (23) $[M]^+$; HRMS (EI): calcd: 582.2631, found: 582.2633.

4.1.3.18. (*R,S*)-*N*-(2-Benzoyl-4-[2-naphthoylamino)phenyl]-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (5l). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.43 g, 1.0 mmol) and 2-naphthoyl chloride (0.29 g, 1.5 mmol). Purification: column-chromatography, silica gel, methanol/ethyl acetate (1:5): yield 340 mg (58%). Mp 110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.31 (s, 3H), 2.54 (s, br, 8H), 3.90 (s, 1H), 7.23–7.29 (m, 3H), 7.31–7.36 (m, 2H), 7.44–7.56 (m, 5H), 7.61 (m, 1H), 7.76–7.87 (m, 6H), 8.07–8.12 (m, 1H), 8.30 (m 1H), 8.48 (m, 1H), 8.52 (s, 1H), 11.59 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.47, 54.54, 76.91, 122.36, 123.53, 124.53, 125.38, 125.47, 126.86, 127.72, 127.75, 127.90, 128.26, 128.41, 128.55, 128.64, 128.87, 128.89, 130.13, 131.57, 132.46, 132.83, 132.86, 134.81, 135.44, 135.53, 137.95, 165.90, 170.92, 197.76; IR (KBr): ν = 3377, 3059, 2936, 2799, 1648, 1597, 1539, 1505, 1401, 1319, 1288, 1239, 1138, 1010, 775, 702; MS (EI): *m/z* 70 (20), 91 (27), 98 (19), 105 (11), 127 (32), 146 (14), 154 (64), 189 (100), 190 (76), 216 (16), 366 (70), 392 (63), 484 (35), 582 (38) $[M]^+$; HRMS (EI): calcd: 582.2631, found: 582.2644 $[M]^+$.

4.1.3.19. (*R,S*)-*N*-[2-Benzoyl-4-(*N*′,*N*′-diisopropylureido)phenyl]-2-(4-methylpiperazin-1-yl)-2-phenylacetamide (5m). According to general procedure 1 from (*R,S*)-*N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-2-phenylacetamide (**4a**) (0.30 g, 0.7 mmol) and *N,N*-diisopropylcarbamoyl chloride (0.17 g, 1.1 mmol).

Purification: column-chromatography, silica gel, methanol/ethyl acetate (1:1): yield 138 mg (36%). Mp 150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.21 (d, 12H, $^3J_{H-H}$ = 6.6 Hz), 2.10 (s, 3H), 2.24 (s, br, 8H), 3.78 (s, 1H), 3.88 (q, 2H, $^3J_{H-H}$ = 6.5 Hz), 7.25–7.35 (m, 4H), 7.54–7.58 (m, 4H), 7.65–7.70 (m, 2H), 7.73–7.78 (m, 2H), 7.82 (m, 1H), 8.19 (s, 1H), 10.68 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 21.02, 45.85, 54.88, 59.63, 75.01, 121.43, 122.22, 123.52, 126.74, 127.76, 128.12, 128.44, 128.75, 129.72, 130.52, 132.82, 135.93, 136.65, 137.38, 153.52, 169.28, 196.28; IR (KBr): ν = 3307, 2967, 2934, 2798, 1666, 1597, 1505, 1452, 1319, 1292, 1237, 1142, 1010, 702; MS (EI): *m*/*z* 70 (29), 86 (72), 87 (34), 91 (18), 101 (46), 105 (42), 128 (35), 146 (8), 189 (100), 190 (76), 238 (28), 264 (71), 296 (12), 365 (18), 454 (17), 555 (5) $[M]^+$; HRMS (EI): calcd: 555.3209, found: 555.3208 $[M]^+$.

4.1.3.20. *N*-(4-Acetylamino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)acetamide (6a). According to general procedure 1 from *N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)acetamide (**4b**) (353 mg, 1.0 mmol) and acetic acid chloride (0.9 g, 1.2 mmol). Purification: column-chromatography, silica gel, ethyl acetate/methanol (1:2): yield 296 mg (75%). Mp 165 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) 2.11 (s, 3H), 2.33 (s, 3H), 2.57 (s, br, 8H), 3.13 (s, 2H), 7.48 (m, 4H), 7.58 (m, 1H), 7.80 (m, 2H), 7.85 (m, 1H), 8.56 (m, 1H), 11.30 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 24.33, 45.97, 53.48, 54.82, 62.20, 122.45, 123.79, 124.52, 125.89, 128.35, 130.20, 132.58, 132.85, 134.95, 137.88, 168.35, 169.91, 197.06; IR (KBr): ν (cm^{-1}) = 3370, 2950, 2811, 1667, 1653, 1640, 1594, 1558, 1539, 1506, 1446, 1401, 1329, 1279, 1015, 831, 707; MS (EI, 70 eV): *m*/*z* 70 (37), 99 (29), 113 (71), 140 (33), 254 (55), 267 (41), 337 (54), 351 (41), 394 (100) $[M]^+$; MS (EI-HRMS): calcd: 394.2005, found: 394.2019.

4.1.3.21. *N*-(2-Benzoyl-4-propanoylaminophenyl)-2-(4-methyl-piperazin-1-yl)acetamide (6b). According to general procedure 1 from *N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)-acetamide (**4b**) (353 mg, 1.0 mmol) and propionyl chloride (0.1 g, 1.2 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether (4:1): yield 317 mg (78%). Mp 120 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 1.19 (t, 3H, $^3J_{H-H}$ = 7.5 Hz), 2.33 (q, 2H, $^3J_{H-H}$ = 7.5 Hz), 2.57 (s, br, 8H), 3.12 (s, 2H), 7.36 (m, 4H), 7.50 (m, 1H), 7.80 (m, 2H), 7.86 (m, 1H), 8.51 (m, 1H), 11.26 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 9.48, 30.49, 45.93, 53.45, 54.79, 62.18, 122.47, 123.48, 124.38, 126.00, 128.38, 130.21, 132.69, 132.88, 134.78, 137.87, 169.85, 171.98, 197.07; IR (KBr): ν (cm^{-1}) = 2937, 2811, 1668, 1596, 1546, 1508, 1460, 1401, 1294, 1013, 835, 706; MS (EI, 70 eV): *m*/*z* 43 (28), 57 (42), 70 (34), 84 (32), 113 (100), 205 (52), 268 (47), 322 (30), 351 (30), 408 (78) $[M]^+$; MS (EI-HRMS): calcd: 408.2161, found: 408.2160.

4.1.3.22. *N*-(2-Benzoyl-4-benzoylaminophenyl)-2-(4-methyl-piperazin-1-yl)-2-phenylacetamide (6c). According to general procedure 1 from *N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)acetamide (**4b**) (353 mg, 1.0 mmol) and benzoyl chloride (0.9 g, 1.2 mmol). Purification: column-chromatography, silica gel, methanol/diethyl ether/isohexane (8:2:1): yield 289 mg (63%). Mp 185 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 2.34 (s, 3H), 2.59 (s, br, 8H), 3.14 (s, 2H), 7.50 (m, 5H), 7.60 (m, 1H), 7.68 (m, 1H), 7.83 (m, 4H), 8.00 (m, 1H), 8.59 (m, 1H), 11.34 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.93, 53.44, 54.80, 62.18, 122.53, 124.02, 125.00, 125.99, 127.04, 128.42, 128.74, 130.23, 131.98, 132.65, 132.90, 134.46, 135.18, 137.88, 165.73, 169.92, 197.06; IR (KBr): ν (cm^{-1}): = 3312, 2930, 2797, 1689, 1668, 1616, 1596, 1513, 1445, 1396, 1332, 1293, 1253, 1166, 1013, 895, 836, 703; MS (EI, 70 eV): *m*/*z* 70 (40), 105 (42), 113 (80), 316 (78), 329 (30), 400 (51), 414 (35), 456 (6) $[M]^+$, 457 (100) $[M+1]^+$; MS (EI-HRMS): calcd: 456.2161, found: 456.2165.

4.1.3.23. *N*-(2-Benzoyl-4-phenylacetylaminophenyl)-2-(4-methylpiperazin-1-yl)-2-phenylacetamide (6d). According to general procedure 1 from *N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)acetamide (**4b**) (176 mg, 0.5 mmol) and 3-phenylpropionic acid chloride (0.1 mL, 0.8 mmol). Purification: column-chromatography, silica gel, methanol; yield 94 mg (39%). Mp 57 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm) = 2.51–2.60 (m, 8H), 2.79–2.90 (m, 3H), 3.08 (s, 2H), 7.14–7.29 (m, 5H), 7.54–7.58 (m, 2H), 7.66–7.84 (m, 5H), 8.09–8.14 (m, 1H), 10.10 (m 1H), 10.77 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm) = 31.29, 35.80, 38.42, 51.14, 53.88, 61.12, 120.01, 122.31, 123.16, 123.80, 126.5, 128.77, 128.84, 128.87, 129.06, 130.28, 133.42, 138.09, 141.45, 141.65, 171.07, 174.26, 197.06; IR (KBr): ν (cm^{-1}): = 3425, 1685, 1507, 1403, 1289, 701; MS (EI, 70 eV): *m*/*z* 70 (35), 113 (78), 114 (61), 344 (75), 357 (35), 427 (52), 428 (45), 441 (36), 482 (27), 484 (100) $[M]^+$, 485 (35) $[M+1]^+$; MS (EI-HRMS): calcd: 484.2474, found: 484.2475.

4.1.3.24. *N*-[2-Benzoyl-4-(2-naphthoylamino)phenyl]-2-(4-methylpiperazin-1-yl)-2-phenylacetamide (6e). According to general procedure 1 from *N*-(4-amino-2-benzoylphenyl)-2-(4-methyl-1-piperazinyl)acetamide (**4b**) (176 mg, 0.5 mmol) and 2-naphthoyl chloride (142 mg, 0.8 mmol). Purification: column-chromatography, silica gel, methanol/ethyl acetate (1:3): yield 121 mg (48%). Mp 68 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm) = 2.35 (s, 3H), 2.63 (s, br, 8H), 3.13 (s, 2H), 7.47–7.59 (m, 5H), 7.66–7.72 (m, 1H), 7.80–7.91 (m, 6H), 8.03 (m, 1H), 8.09 (m, 1H), 8.33 (m, 1H), 8.61 (m 1H), 11.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) = 45.45, 52.96, 54.45, 62.06, 120.49, 123.44, 124.16, 125.09, 125.83, 127.00, 127.67, 127.81, 128.02, 128.46, 128.75, 128.94, 130.29, 131.63, 132.56, 132.69, 132.94, 134.91, 135.33, 137.95, 165.77, 169.79, 197.25; IR (KBr): ν (cm^{-1}): = 3855, 3752, 3745, 3676, 3425, 1735, 1701, 1685, 1654, 1648, 1560, 1507, 1400, 1284; MS (EI, 70 eV): *m*/*z* 70 (40), 113 (100), 114 (60), 155 (46), 366 (74), 367 (24), 379 (24), 449 (36), 450 (28), 504 (25), 506 (82) $[M]^+$, 507 (31) $[M+1]^+$; MS (EI-HRMS): calcd: 506.2318, found: 506.2289.

4.2. Antimalarial activity

The multidrug resistant *P. falciparum* clone Dd2[22] was maintained in continuous culture as described[23] and synchronized using the sorbitol method.[24] Drug assays based on [^{3}H]-hypoxanthine incorporation were done[25] and the percent inhibition was determined as described.[26] 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 (SigmaPlot, SPSS).

4.3. Antitrypanosomal activity

4.3.1. Parasite culture

Trypomastigote forms of *T. brucei brucei* laboratory strain TC 221 were cultured in Baltz medium according to standard conditions.[11]

4.3.2. In vitro cytotoxicity assays

The test compounds were dissolved in DMSO or 0.1 M NaOH. A defined number of parasites (10^4 trypanosomes per mL were exposed in test chambers of 96-well plates to various concentrations of the test substances in a final volume of 200 μL. Positive (trypanosomes in culture medium) and negative controls (test substance without trypanosomes) were run with each plate. The plates were then incubated at 37 °C in an atmosphere of 5% CO_2 for a total time period of 72 h. A reading was done at 48 h. The effect of test substances was quantified in ED_{50} values by linear interpolation[27] of three different measurements. The activity of the test substances

was measured by light absorption in an MR 700 Microplate Reader at a wave length of 550 nm with a reference wave length of 630 nm, using the Alamar Blue®.[12]

4.4. 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.

4.5. Inhibition of P-glycoproteins

4.5.1. Cell line

The ABCB1 overexpressing human T-lymphoblast cell line CCRF-CEM vcr1000 was provided by V. Gekeler (Byk Gulden, Konstanz, Germany). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum under standard culture conditions. The resistant cell line was cultured in the presence of 1000 ng/mL vincristine. The selective agent was washed out one week before experiments.

4.5.2. Daunomycin zero *trans*-efflux studies

Cells were pelleted, the supernatant was removed and the cells were resuspended at a density of 1×10^6/mL in RPMI1640 medium containing 10% fetal calf serum and 3 μM daunomycin. Cell suspensions were incubated at 37 °C for 30 min. Tubes were chilled on ice and pelleted at 500 g. Supernatant were removed and cells were washed once with ice-cold medium at a density of 1×10^6 cells/mL. Subsequently, cells were resuspended in medium prewarmed to 37 °C, containing either no compound or various concentrations of the compound.

Eight concentrations (serial dilution of 1:2, 5) were tested for each substance. After 1–4 min, aliquots of the incubation mixture were transferred to tubes containing an equal volume of ice-cold stop solution (medium plus 100 μM verapamil). Samples were measured within 1 h on a FACScalibur flow cytometer (BD Biosciences, Heidelberg, Germany). Five thousand viable cells were gated and accumulated for the determination of mean fluorescence values.

4.5.3. Steady state drug accumulation protocol

Cells were pelleted, the supernatant was removed and the cells were resuspended at a density of 1×10^6/mL in RPMI1640 medium containing 10% fetal bovine serum and 3 μM daunomycin. Eight different concentrations of the compound were added to aliquots of the cell suspension and one aliquot was prepared without compound. Mixtures were incubated for 30 min at 37 °C. Tubes were chilled on ice and washed once with ice-cold medium at a density of 1×10^6/mL. Cell pellets were resuspended in ice-cold stop solution and were measured within 1 h on a FACScalibur flow cytometer (BD Biosciences, Heidelberg, Germany). Five thousand viable cells were gated and accumulated for the determination of mean fluorescence values.

4.5.4. Calculation of EC_{50} values

For efflux experiments, first-order rate constants were calculated from the time depended linear decrease in mean fluorescence by nonlinear regression analysis. EC_{50} values were determined from dose–response curves of modifier concentration versus initial efflux rates. EC_{50} values from steady state accumulation experiments were obtained by dose–response curves of daunomycin steady state levels versus modifier concentration using a hyperbolic curve fitting procedure. At least two independently performed experiments were used to calculate the average EC_{50} value of a compound.

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