

Article

Novel 2-Phenoxyanilide Congeners Derived from a Hit Structure of the TCAMS: Synthesis and Evaluation of Their *in Vitro* Activity against *Plasmodium falciparum*

Thomas Weidner ^{1,†}, Abed Nasereddin ^{2,†}, Lutz Preu ¹, Johann Grünefeld ¹, Ron Dzikowski ² and Conrad Kunick ^{1,3,*}

¹ Institut für Medizinische und Pharmazeutische Chemie, Technische Universität Braunschweig, Beethovenstraße 55, 38106 Braunschweig, Germany; t.weidner@tu-braunschweig.de (T.W.); l.preu@tu-braunschweig.de (L.P.); j.gruenefeld@tu-braunschweig.de (J.G.)

² Department of Microbiology and Molecular Genetics, IMRIC, The Kuvim Center for the Study of Infectious and Tropical Diseases, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel; abedn@ekmd.huji.ac.il (A.N.); rond@ekmd.huji.ac.il (R.D.)

³ Center of Pharmaceutical Engineering (PVZ), Technische Universität Braunschweig, Franz-Liszt-Straße 35A, 38106 Braunschweig, Germany

* Correspondence: c.kunick@tu-braunschweig.de; Tel.: +49-531-391-2754; Fax: +49-531-391-2799

† These authors contributed equally to this work.

Academic Editor: Derek J. McPhee

Received: 18 December 2015 ; Accepted: 2 February 2016 ; Published: 17 February 2016

Abstract: The Tres Cantos Antimalarial Compound Set (TCAMS) is a publicly available compound library which contains 13533 hit structures with confirmed activity against *Plasmodium falciparum*, the infective agent responsible for malaria tropica. The TCAMS provides a variety of starting points for the investigation of new antiplasmodial drug leads. One of the promising compounds is TCMDC-137332, which seemed to be a good starting point due to its antiplasmodial potency and its predicted physicochemical properties. Several new analogues based on a 2-phenoxyanilide scaffold were synthesized by standard amide coupling reactions and were fully characterized regarding their identity and purity by spectroscopic and chromatographic methods. Furthermore, the results of the biological evaluation of all congeners against *Plasmodium falciparum* NF54 strains are presented. The findings of our *in vitro* screening could not confirm the presumed nanomolar antiplasmodial activity of TCMDC-137332 and its derivatives.

Keywords: diarylether; luciferase; Malaria; 2-phenoxyanilide; *Plasmodium falciparum*; TCAMS; TCMDC-137332

1. Introduction

Malaria is still one of the most severe infectious diseases. Approximately 3.2 billion people are at risk of being infected. Despite a decreasing number of mortal cases in the last decade due to better vector control and artemisinin-based combination therapy (ACT), still 584,000 deaths caused by malaria infection were reported in 2013 [1]. Although there are effective chemotherapeutics for the treatment of malaria, the discovery of new drugs is important due to the increasing resistances against available drugs [2,3]. A good strategy to circumvent the problem of resistance is to develop compounds acting with new mechanisms of action. Hence there is an urgent need for compounds with novel chemotypes which differ from scaffolds of existing drugs.

Major sources for drug discovery are phenotypic screening libraries, providing a huge number of new starting points. In the last few years extensive libraries with compounds showing antimalarial activity have been published by Novartis [4], St. Jude's Children Research Hospital [5], the Medical Research Council Technology [6] and GlaxoSmithKline (GSK) [7]. The latter was derived from a high throughput screening (HTS) of nearly 2 million substances of the GSK corporate collection. 13,533 of these, known as the Tres Cantos Antimalarial Compound Set (TCAMS), were active against the malaria parasite *Plasmodium falciparum* and inhibited the parasite growth by at least 80% at 2 μ M concentration. *In silico* clustering and filtering of the TCAMS set performed by Calderon *et al.* resulted in 552 compounds which were declared as "quality starting points from the TCAMS" [8]. Since then, a few of the compound classes have been further examined, e.g., cyclopropylcarboxamides [9], 2-amino-1-phenylethanols [10], aminohydantoines [11] or carbamoyltriazoles [12].

One of the 552 promising compounds is TCMDC-137332 (1), which has an estimated IC₅₀ value of 7 nM and therefore seemed to be one of the most potent structures of this collection [7,8]. We decided to further investigate this compound which matches nearly all criteria of the Lipinski rules for orally available drugs [13] (Figure 1) and is structurally dissimilar to currently known antimalarials [14].

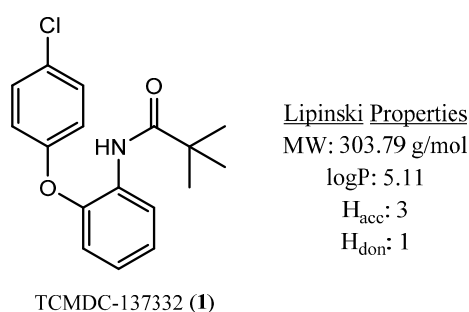


Figure 1. Molecular structure of TCMDC-137332 and its properties concerning Lipinski's rules for orally available drugs; chemicalize.org was used for calculation of logP [15].

2. Results and Discussion

2.1. Chemistry

To proof its activity, we resynthesized TCMDC-137332. We also prepared a series of congeners, all having a 2-phenoxyanilide scaffold (Figure 2). The first group of ten compounds was substituted with chlorine in para position of the phenoxy residue, like in the scaffold of TCMDC-137332 (1–10). Another equivalent series was synthesized, in which the chlorine was replaced by a more polar methoxy group (11–20).

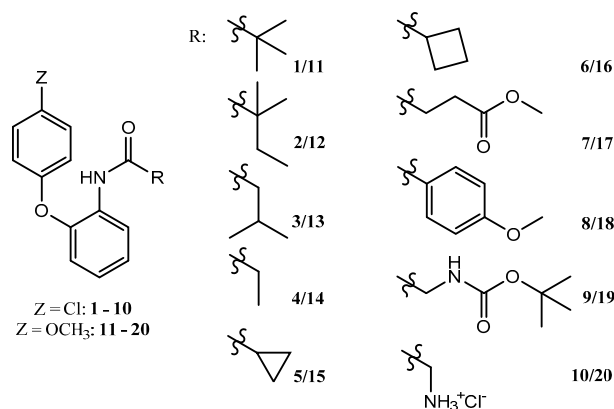
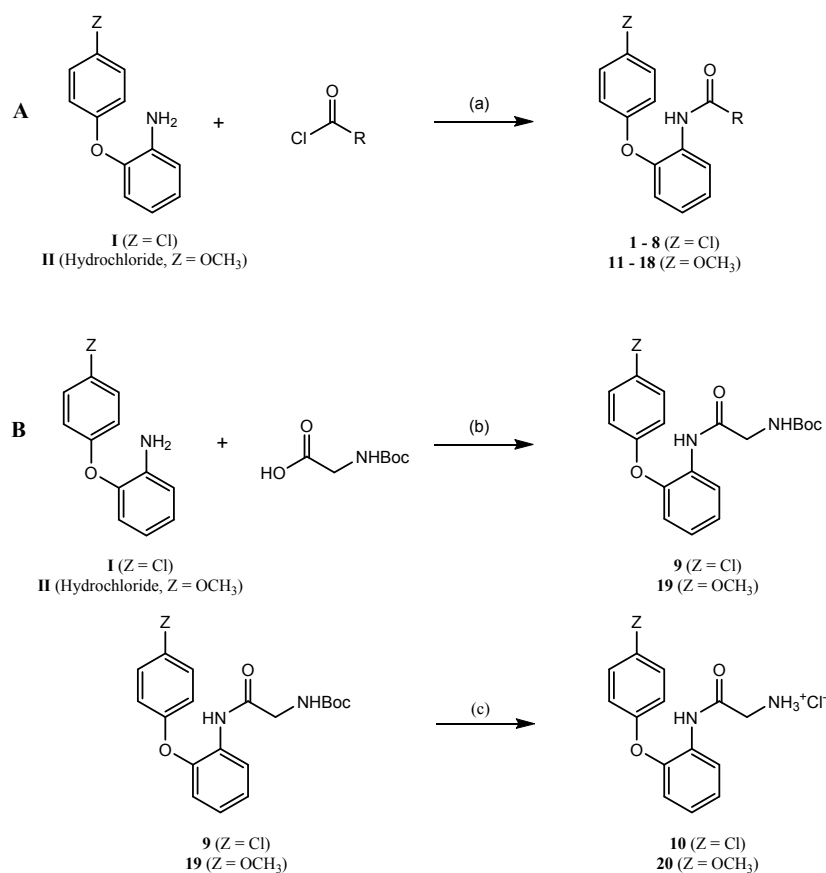


Figure 2. Structures of TCMDC-137332 (1) and its congeners (2–19).

The synthesis of phenoxyanilides **1–8** and **11–18** was achieved by standard amide coupling reactions of commercially available 2-(4-chlorophenoxy)aniline **I** or 2-(4-methoxyphenoxy)aniline hydrochloride **II** with acid chlorides in presence of triethylamine, as outlined in Scheme 1A. Compounds **9** and **19** were synthesized by the reaction of 2-(4-substituted)phenoxyanilines and Boc-protected glycine in the presence of PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) together with DIPEA (Diisopropylethylamine). Cleavage of the protecting group was accomplished by treatment of **9** and **19**, respectively, with trifluoroacetic acid and subsequent precipitation of the hydrochloride salts **10** and **20** with a hydrochloric acid solution in propan-2-ol (Scheme 1B). The compounds were synthesized in satisfactory to excellent yields (53%–93%) and the purity (determined with elemental analyses and HPLC) of most of the products was sufficient for biological evaluation even before the final purification step (recrystallization, flash chromatography).













Scheme 1. (A) Synthesis of 2-phenoxyanilides **1–8** and **11–18**. Reaction conditions: (a) triethylamine, toluene, 0 °C → rt. Residues R: see Figure 2; (B) Synthesis of 2-phenoxyanilides **9**, **10**, **19** and **20**. Reaction conditions: (b) PyBOB, DIPEA, dichloromethane, 0 °C → rt; (c) 1. Trifluoroacetic acid, dichloromethane, rt, 2. Hydrochloric acid (37%)/propan-2-ol (1:1).

2.2. Calculations of Rule-of-Five-properties

We determined the properties concerning Lipinski's Rule of Five (RO5) for orally active drugs for all derivatives. The RO5 can be a useful reference to predict the quality of a structure to be an orally available drug-like compound [13,16], which was outlined by Calderon *et al.* for TCMDC-137332 [8]. Considering the molecular weight (<380 g/mol), the amount of H-bond acceptors (≤6) and H-bond donors (≤3), the values of every compound in this series comply with the proposed values of Lipinski *et al.* [13]. Estimation of lipophilicity has been carried out using chemicalize.org for calculation of logP [15]. According to the RO5 the logP should have a maximum value of 5. The calculated logP compared to TCMDC-137332 was reduced by replacement of the *tert*-butyl residue by smaller residues,

cyclic aliphatic residues like cyclopropyl- or cyclobutyl-groups, and more polar groups. Introducing a methoxy group instead of the chlorine residue also reduced the calculated logP value. Some examples of the calculated octanol/water partition coefficients are outlined in Table 1.

Table 1. Calculated logP values, the number of H-bond acceptors (H_{acc}) and donors (H_{don}) and the molecular weight (MW) of selected compounds.

ID	Z	R	logP	H_{acc}	H_{don}	MW
1	Cl		5.11	3	1	303.79
11	OCH ₃		4.35	4	1	299.37
4	Cl		4.02	3	1	275.73
14	OCH ₃		3.25	4	1	271.32
5	Cl		4.10	3	1	287.74
15	OCH ₃		3.33	4	1	283.33
7	Cl		3.29	5	1	333.77
17	OCH ₃		2.52	6	1	329.35
10	Cl		2.39	3	3	276.72
20	OCH ₃		1.63	4	3	272.30

2.3. In Vitro Assay

After the full spectroscopic characterization of all congeners and validation of their purity by elemental analyses and chromatographic methods *in vitro* assays were carried out using *P. falciparum* NF54-*luc* erythrocyte stage parasites expressing luciferase. The derivatives 1–20 were examined in 3 μ M concentration performing viability assays measuring the bioluminescence in a luciferase assay system as described earlier [17–20]. A first screening, in which the cultures were incubated for 48 h, gave surprisingly low inhibition rates of the parasite growth (see Figure 3). With 22% reduction of the parasite viability, 5 was the most effective compound in this screening, followed by 13 and 15 with about 18% inhibition. The resynthesized TCMDC-137332 (1) failed to show any antiparasmodial activity, just as compounds 10 and 20 which contain polar aminomethyl residues. Due to the unexpected outcome of the first screening session, we performed another assay in which the cultures were incubated for a longer period (96 h), to identify slow acting compounds. However, the prolongation of the incubation time did not improve the inhibitory effect of the derivatives on the parasite growth significantly, the only exception being compound 16, which diminished the proliferation of *P. falciparum* NF54 by 31%. Compared to the first screening, 16 showed a 3-fold better inhibition rate, indicating that it is probably a rather slow acting inhibitor. However, the results of most of the derivatives were slightly inferior compared to the first screening. The assumed high antiparasmodial activity of TCMDC-137332 (1) could not be confirmed by these assays.

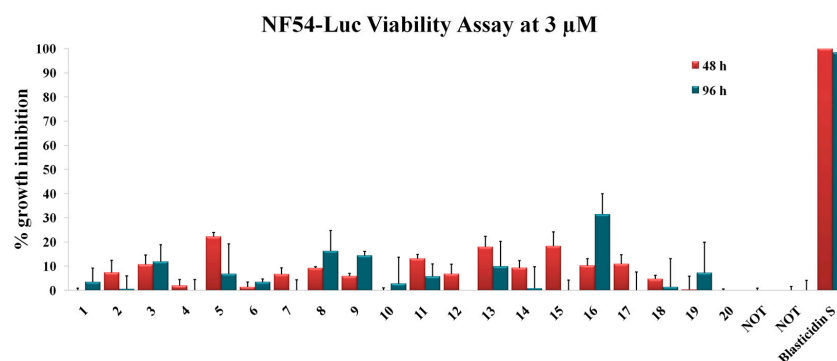


Figure 3. Results of the *in vitro* assay of derivatives 1–20 in 3 μ M concentration against *P. falciparum* NF54-*Luc* parasite line with 48 h (red) and 96 h (blue) incubation time. NOT: no treatment; Blasticidin S: antibiotic used as antiparasmodial standard.

3. Experimental Section

3.1. Apparatus and Materials

Starting materials were purchased from the suppliers indicated below and were used without further purification. Pivaloyl chloride was from Merck-Schuchardt (Hohenbrunn, Germany), 2,2-dimethylbutyryl chloride, 3-methylbutyryl chloride and 4-methoxybenzoyl chloride were from Alfa-Aesar (Karlsruhe, Germany), propyl chloride, cyclopropanecarbonyl chloride, cyclobutanecarbonyl chloride and methyl succinyl chloride were from Acros (Geel, Belgium), Boc-glycine was from Aldrich (Steinheim, Germany), 2-(4-chlorophenoxy)aniline was from Enamine (Monmouth Jct., NJ, USA), 2-(4-methoxyphenoxy)aniline hydrochloride was from Fluorochem (Hadfield, UK). Toluene and dichloromethane were dried by published methods before usage [21]. Melting points were determined on an electric variable heater (Electrothermal IA9200, Bibby Scientific, Stone, UK) in open glass capillaries and are uncorrected. IR spectra were recorded as KBr disks on a Thermo Nicolet FT-IR 200 (Thermo Nicolet, Madison, WI, USA). $^1\text{H-NMR}$ spectra and $^{13}\text{C-NMR}$ spectra were recorded on BrukerAvance DRX-400 and BrukerAvance II-600 instruments (Bruker Corporation, Billerica, MA, USA) (at the NMR laboratories of the Chemical Institutes of the Technische Universität Braunschweig). Chemical shifts were recorded as δ values in ppm and are referenced to an internal standard tetramethylsilane. Signals in ^{13}C spectra were assigned based on the result of ^{13}C DEPT135 experiments. Elemental analyses were determined on a CE Instruments FlashEA 1112 elemental analyzer (Thermo Quest, San Jose, CA, USA). Mass spectra were recorded on a Finnigan-MAT 95 (Thermo Finnigan MAT, Bremen, Germany). Accurate measurements were conducted according to the peak match method using perfluorokerosene (PFK) as an internal mass reference. (EI) MS: ionization energy 70 eV (Department of Mass Spectrometry of the Chemical Institutes of the Technische Universität Braunschweig). TLC: Polygram Sil G/UV₂₅₄ (Macherey-Nagel, Düren, Germany), 40 mm \times 80 mm, visualization by UV illumination (254 and 366 nm). Purity was determined by HPLC using isocratic and gradient elution performed on Merck Hitachi Elite LaChrom systems (Hitachi High Technologies Inc., San Jose, CA, USA): pump L-2130, autosampler L-2200, diode array detector L-2450 (isocratic elution) or UV detector L-2400 (gradient elution), organizer box L-2000; column, Merck LiChroCART 125-4, LiChrosphere 100, RP 18, 5 μm (Merck, Darmstadt, Germany); flow rate 1.000 mL/min; detection wavelength: 254 and 280 nm (isocratic elution) and 254 nm (gradient elution); AUC, % method; time of detection 15 min (isocratic elution) or 20 min (gradient elution), retention time (t_{R}); dead time (t_{M}) related to DMSO. For isocratic runs, mixtures of ACN and water or mixtures of ACN and buffer were used. For all gradient runs, mixtures of ACN and water were used (gradient: 0–2 min: 10% ACN, 2–12 min: 10% \rightarrow 90% ACN, linear, 12–20 min 90% ACN). Preparation of $\text{H}_2\text{O} + (\text{Et}_3\text{NH})_2\text{SO}_4$ buffer (pH 2.7) for isocratic HPLC: triethylamine (20.0 mL) and sodium hydroxide (242 mg) were dissolved in water to 1 L. The solution was adjusted to pH 2.7 by addition of sulfuric acid. All compounds which were biologically tested were of >95% purity. Absorption maxima (λ_{max}) were extracted from the spectra recorded by the DAD in the HPLC peak maxima in isocratic runs (software, EZ Chrom Elite Client/server, version 3.1.3., Scientific Software Inc., Pleasanton, CA, USA).

3.2. Chemical Synthesis and Characterization of Compounds 1–20

General Procedure for the Synthesis of Compounds 1–8

To a stirred and cooled solution of 2-(4-chlorophenoxy)aniline (330 mg, 1.50 mmol) and triethylamine (230 μL , 1.65 mmol) in toluene (5 mL), the appropriate acid chloride (pivaloyl chloride, 2,2-dimethylbutyryl chloride, 3-methylbutyryl chloride, propyl chloride, cyclopropanecarbonyl chloride, cyclobutanecarbonyl chloride, methyl succinyl chloride, 4-methoxybenzoyl chloride) (1.65 mmol) was added. Subsequently the reaction mixture was allowed to warm to room temperature. The progress of the reaction was monitored by TLC. After 2–9 h the reaction mixture was extracted with a saturated sodium hydrogen carbonate solution, with a hydrogen chloride solution (10%), with

brine and finally with water. Afterwards the organic solution was dried over sodium sulfate and evaporated under reduced pressure. The residue was further purified by recrystallization or column chromatography over silica gel.

N-[2-(4-Chlorophenoxy)phenyl]-2,2-dimethylpropanamide (1): Crystallization from ethanol (70%) yielded slightly brown needles (277 mg, 0.92 mmol, 61%); m.p.: 99–100 °C ; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3336 (br, N-H), 1663 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆) δ [ppm] = 1.05 (s, 9H, C(CH₃)₃), 6.80–7.01 (m, 2H, arom. H), 6.99–7.15 (m, 1H, arom. H), 7.15–7.32 (m, 2H, arom. H), 7.27–7.48 (m, 2H, arom. H), 7.56–7.78 (m, 1H, arom. H), 8.72 (s, 1H, NH); ¹³C-NMR: (101 MHz, DMSO-*d*₆) δ [ppm] = 26.95 (3C, CH₃), 118.40 (2C), 120.46, 124.63, 126.13, 126.62, 129.49 (2C) (CH), 38.12, 126.38, 130.28, 147.84, 155.91 (C), 176.13 (C=O); C₁₇H₁₈ClNO₂ (303.78): calcd. C 67.21, H 5.97, N 4.61, found C 67.13, H 6.00, N 4.51; EI-MS: *m/z* (%): 303.1 [M]⁺ (28), 176.1 [M – 127]⁺ (100); HPLC: 99.7% at 254 nm, 99.9% at 280 nm; t_R = 7.42 min, t_M(DMSO) = 1.06 min (ACN/H₂O 60:40), λ_{\max} [nm] = 230, 275; HPLC-gradient: 99.4%, t_R = 13.62 min, t_M(DMSO) = 1.28 min.

N-[2-(4-Chlorophenoxy)phenyl]-2,2-dimethylbutanamide (2): Crystallization from methanol/water (33:10) yielded a beige solid (332 mg, 1.05 mmol, 70%); m.p.: 69–70 °C ; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3316 (br, N-H), 1657 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆) δ [ppm] = 0.67 (t, *J* = 7.4 Hz, 3H, CH₃), 1.01 (s, 6H, C(CH₃)₂), 1.47 (q, *J* = 7.4 Hz, 2H, CH₂), 6.87–6.96 (m, 2H, arom. H), 7.03–7.13 (m, 1H, arom. H), 7.17–7.27 (m, 2H, arom. H), 7.34–7.43 (m, 2H, arom. H), 7.59–7.68 (m, 1H, arom. H), 8.70 (s, 1H, NH); ¹³C-NMR: (101 MHz, DMSO-*d*₆) δ [ppm] = 8.82, 24.54 (2C) (CH₃), 32.82 (CH₂) 118.49 (2C), 120.46, 124.63, 126.16, 126.74, 129.55 (2C) (CH), 42.42, 126.43, 130.29, 147.98, 155.99 (C), 175.48 (C=O); C₁₈H₂₀ClNO₂ (317.81): calcd. C 68.03, H 6.34, N 4.41, found C 68.09, H 6.22, N 4.28; EI-MS: *m/z* (%): 317.1 [M]⁺ (28), 190.1 [M – 127]⁺ (100); HPLC: 98.7% at 254 nm, 99.0% at 280 nm; t_R = 9.82 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 232, 275; HPLC-Gradient: 97.3%, t_R = 14.17 min, t_M(DMSO) = 1.28 min.

N-[2-(4-Chlorophenoxy)phenyl]-3-methylbutanamide (3): Purification by column chromatography (dichloromethane/methanol 200:1) yielded a slightly yellow solid (418 mg, 1.38 mmol, 92%); m.p.: 69–71 °C ; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3291 (m, N-H), 1655 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 0.81 (d, *J* = 6.7 Hz, 6H, CH₃), 1.92 (hept, *J* = 6.8 Hz, 1H, CH(CH₃)₂), 2.14 (d, *J* = 7.2 Hz, 2H, CH₂), 6.79–7.08 (m, 3H, arom. H), 7.08–7.25 (m, 2H, arom. H), 7.28–7.51 (m, 2H, arom. H), 7.88 (dd, *J* = 7.6 Hz, 2.2 Hz, 1H, arom. H), 9.38 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ [ppm] = 22.10 (2C, CH₃), 44.93 (CH₂), 25.60, 119.28 (2C), 119.83, 124.34, 124.87, 125.25, 129.56 (2C) (CH), 126.67, 130.18, 147.04, 155.98 (C), 170.89 (C=O); C₁₇H₁₈ClNO₂ (303.78): calcd. C 67.21, H 5.97, N 4.61, found C 66.95, H 6.02, N 4.39; EI-MS: *m/z* (%): 303.1 [M]⁺ (16), 219.0 [M – 84]⁺ (100); HPLC: 98.1% at 254 nm, 99.0% at 280 nm; t_R = 6.03 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 249; HPLC-gradient: 95.6%, t_R = 13.23 min, t_M(DMSO) = 1.28 min.

N-[2-(4-Chlorophenoxy)phenyl]propanamide (4): Purification by column chromatography (petroleum ether/ethyl acetate 4:1) yielded a colorless solid (265 mg, 0.96 mmol, 63%); m.p.: 99–100 °C ; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3330 (br, N-H), 1675 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆) δ [ppm] = 0.98 (t, *J* = 7.6 Hz, 3H, CH₃), 2.29 (q, *J* = 7.6 Hz, 2H, CH₂), 6.93–7.02 (m, 3H, arom. H), 7.08–7.22 (m, 2H, arom. H), 7.37–7.46 (m, 2H, arom. H), 7.91–7.98 (m, 1H, arom. H), 9.39 (s, 1H, NH); ¹³C-NMR: (101 MHz, DMSO-*d*₆): δ [ppm] = 9.65 (CH₃), 29.03 (CH₂), 119.53 (2C), 124.24, 124.43, 125.02, 129.59 (2C) (CH), 126.78, 130.19, 146.93, 155.85 (C), 172.31 (C=O), one signal missing in ¹³C-NMR; C₁₅H₁₄ClNO₂ (275.73): calcd. C 65.34, H 5.12, N 5.08, found C 65.33, N 4.98, H 5.02; EI-MS: *m/z* (%): 275.1 [M]⁺ (18), 219.0 [M – 56]⁺ (100); HPLC: 98.9% at 254 nm, 99.4% at 280 nm; t_R = 3.95 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 248, 274; HPLC-gradient: 96.0%, t_R = 12.23 min, t_M(DMSO) = 1.28 min.

N-[2-(4-Chlorophenoxy)phenyl]cyclopropanecarboxamide (5): Crystallization from ethanol (70%) yielded beige needles (387 mg, 1.34 mmol, 90%); m.p.: 100–102 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3371, 3332 (m, N-H), 1663 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆) δ [ppm] = 0.67–0.77 (m, 4H, CH₂), 1.95 (td, *J* = 7.5 Hz, 3.9 Hz, 1H, CH), 6.94 (dd, *J* = 7.5 Hz, 2.1 Hz, 1H, arom. H), 6.96–7.03 (m, 2H, arom. H), 7.06–7.26 (m, 2H, arom. H), 7.37–7.41 (m, 2H, arom. H), 7.96 (dd, *J* = 7.7 Hz, *J* = 2.1 Hz, 1H, arom. H), 9.71 (s, 1H, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ [ppm] = 7.16 (2C, CH₂), 13.86, 119.14, 119.70 (2C), 124.01, 124.73, 129.55 (2C) (CH), 126.82, 130.09, 146.66, 155.71 (C), 171.99 (C=O); C₁₆H₁₄ClNO₂ (287.74): calcd. C 66.79, H 4.90, N 4.87, found C 66.96, H 4.92, N 4.77; EI-MS: *m/z* (%): 287.1 [M]⁺ (21), 219.0 [M – 68]⁺ (100); HPLC: 98.4% at 254 nm, 98.3% at 280 nm; *t*_R = 4.40 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 241; HPLC-gradient: 97.5%, *t*_R = 12.57 min, *t*_M(DMSO) = 1.28 min.

N-[2-(4-Chlorophenoxy)phenyl]cyclobutanecarboxamide (6): Purification by column chromatography (toluene/petroleum ether 10:1 → 20:1) yielded a slightly brown solid (246, 0.82 mmol, 53%); m.p.: 88–89 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3433 (br, N-H), 1657 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆) δ [ppm] = 1.66–2.14 (m, 6H, CH₂), 3.19–3.33 (m, 1H, CH), 6.90–7.03 (m, 3H, arom. H), 7.12–7.20 (m, 2H, arom. H), 7.36–7.45 (m, 2H, arom. H), 7.91 (dd, *J* = 7.4 Hz, 2.1 Hz, 1H, arom. H), 9.22 (s, 1H, NH); ¹³C-NMR: (101 MHz, DMSO-*d*₆) δ [ppm] = 17.65, 24.47 (2C) (CH₂), 38.88, 119.28 (2C), 119.78, 124.36, 124.74, 125.18, 129.55 (2C) (CH), 126.68, 130.18, 147.00, 155.87 (C), 173.09 (C=O); C₁₇H₁₆ClNO₂ (301.77): calcd. C 67.66, H 5.34, N 4.64, found C 67.81, H 5.43, N 4.55; EI-MS: *m/z* (%): 301.1 [M]⁺ (20), 219.0 [M – 82]⁺ (100); HPLC: 99.4% at 254 nm, 99.8% at 280 nm; *t*_R = 5.86 min; *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 229; HPLC-gradient: 98.4%, *t*_R = 13.15 min; *t*_M(DMSO) = 1.28 min.

Methyl 4-[[2-(4-chlorophenoxy)phenyl]amino]-4-oxobutanoate (7): Crystallization from methanol yielded a beige solid (278 mg, 0.83 mmol, 56%); m.p.: 109–110 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3341 (m, N-H), 1721 (s, C=O, ester) 1687 (s, C=O, amide); ¹H-NMR: (400 MHz, CDCl₃) δ [ppm] = 2.35–2.97 (m, 4H, CH₂), 3.67 (s, 3H, OCH₃), 6.83 (dd, *J* = 8.1 Hz, 1.4 Hz, 1H, arom. H), 6.93–7.05 (m, 3H, arom. H), 7.12 (td, *J* = 7.8 Hz, 1.5 Hz, 1H, arom. H), 7.28–7.38 (m, 2H, arom. H), 7.91 (s, 1H, NH), 8.41 (d, *J* = 7.8 Hz, 1H, arom. H); ¹³C-NMR (101 MHz, CDCl₃) δ [ppm] = 51.93 (CH₃) 29.15, 32.27 (CH₂), 117.77, 119.84 (2C), 121.14, 124.10, 124.44, 129.95 (2C) (CH), 128.98, 129.78, 145.22, 155.13 (C), 169.69 (C=O), 173.23 (C=O); C₁₇H₁₆ClNO₄ (333.77): calcd. C 61.18, H 4.83, N 4.20, found: C 61.15, H 4.83, N 4.21; EI-MS: *m/z* (%): 333.1 [M]⁺ (12), 174.1 [M – 159]⁺ (100); HPLC: 99.3% at 254 nm, 99.7% at 280 nm; *t*_R = 3.44 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 228; HPLC-gradient: 98.5%, *t*_R = 11.99 min, *t*_M(DMSO) = 1.28 min.

N-(2-(4-Chlorophenoxy)phenyl)-4-methoxybenzamide (8): Crystallization from methanol yielded colorless needles (346mg, 0.98 mol, 65%); m.p.: 101–102 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3420 (m, N-H), 1660 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆) δ [ppm] = 3.81 (s, 3H, OCH₃), 6.89–7.13 (m, 5H, arom. H), 7.17–7.30 (m, 2H, arom. H), 7.30–7.48 (m, 2H, arom. H), 7.65–7.76 (m, 1H, arom. H), 7.75–7.99 (m, 2H, arom. H), 9.69 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ [ppm] = 55.29 (CH₃), 113.44 (2C), 119.36 (2C), 119.69, 124.15, 126.32, 126.83, 129.38 (2C), 129.48 (2C) (CH), 126.25, 126.64, 129.79, 149.02, 155.71, 161.80 (C), 164.69 (C=O); C₂₀H₁₆ClNO₃ (353.80): calcd. C 67.90, H 4.56, N 3.96, found: C 67.78, H 4.43, N 3.95; EI-MS: *m/z* (%): 353.1 [M]⁺ (8), 135.0 [M – 218]⁺ (100); HPLC: 99.7% at 254 nm, 99.8% at 280 nm; *t*_R = 6.37 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 266; HPLC-gradient: 99.0%, *t*_R = 13.37 min, *t*_M(DMSO) = 1.28 min.

General Procedure for the Synthesis of Compounds 11–18

To a stirred and cooled solution of 2-(4-methoxyphenoxy)aniline hydrochloride (1.50 mmol) and triethylamine (4.00 mmol) in toluene (5–8 mL), the appropriate acid chloride (pivaloyl chloride, 2,2-dimethylbutyryl chloride, 3-methylbutyryl chloride, propyl chloride, cyclopropanecarbonyl chloride, cyclobutanecarbonyl chloride, methyl succinyl chloride, 4-methoxybenzoyl chloride) (1.65 mmol) was added. Thereafter the reaction mixture was allowed to warm to room temperature. The progress of the reaction was monitored by TLC. After 4–24 h an aqueous work-up was performed

similar to that of compounds 1–8. The resulting residue was further purified by recrystallization or column chromatography over silica gel.

N-[2-(4-Methoxyphenoxy)phenyl]-2,2-dimethylpropanamide (**11**): Crystallization from ethanol (70%) yielded a colorless solid (401 mg, 1.34 mmol, 89%); m.p.: 70–71 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3317 (m, N-H), 1661 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 1.11 (s, 9H, C(CH₃)₃), 3.72 (s, 3H, OCH₃), 6.85–6.90 (m, 1H, arom. H), 6.92 (s, 4H, arom. H), 7.06–7.16 (m, 2H, arom. H), 7.69–7.78 (m, 1H, arom. H), 8.62 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆): δ [ppm] = 27.06 (3C), 55.41 (CH₃), 114.86 (2C), 118.35, 119.09 (2C), 123.25, 125.06, 125.40 (CH), 38.90, 129.48, 148.96, 149.97, 155.27 (C), 176.10 (C=O); C₁₈H₂₁NO₃ (299.37): calcd. C 72.22, H 7.07, N 4.68, found C 72.21, H 7.25, N 4.73; EI-MS: *m/z* (%): 299.1 [M]⁺ (48), 176.1 [M – 123]⁺ (100); HPLC: 99.4% at 254 nm, 99.4% at 280 nm; *t*_R = 5.21 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 248; HPLC-Gradient: 99.3%, *t*_R = 12.88 min, *t*_M(DMSO) = 1.28 min.

N-[2-(4-Methoxyphenoxy)phenyl]-2,2-dimethylbutanamide (**12**): Crystallization from ethanol (70%) yielded a slightly yellow solid (415 mg, 1.32 mmol, 88%); m.p.: 71–72 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3433 (m, N-H), 1674 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 0.72 (t, *J* = 7.4 Hz, 3H, CH₃), 1.07 (s, 6H, C(CH₃)₂), 1.51 (q, *J* = 7.4 Hz, 2H, CH₂), 3.72 (s, 3H, OCH₃), 6.83–6.92 (m, 1H, arom. H), 6.92 (s, 4H, arom. H), 7.05–7.18 (m, 2H, arom. H), 7.66–7.74 (m, 1H, arom. H), 8.60 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆): δ [ppm] = 8.90, 32.96 (2C), 55.42 (CH₃), 24.61 (CH₂), 114.90 (2C), 118.31, 119.17 (2C), 123.21, 125.34, 125.50 (CH), 42.56, 129.43, 149.21, 150.00, 155.28 (C), 175.42 (C=O); C₁₉H₂₃NO₃ (313.40): calcd. C 72.84, H 7.40, N 4.47, found C 72.72, H 7.45, N 4.52; EI-MS: *m/z* (%): 313.1 [M]⁺ (51), 190.1 [M – 123]⁺ (100); HPLC: 98.0% at 254 nm, 98.3% at 280 nm; *t*_R = 6.69 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 228; HPLC-gradient: 98.1%, *t*_R = 13.43 min, *t*_M(DMSO) = 1.28 min.

N-[2-(4-Methoxyphenoxy)phenyl]-3-methylbutanamide (**13**): Purification by column chromatography (petroleum ether/ethyl acetate 4:1) yielded a colorless oil (303 mg, 1.01 mmol, 67%); IR (NaCl): $\bar{\nu}$ [cm⁻¹] = 3426 (m, N-H), 3317 (m, br, N-H), 1679 (s, C=O); ¹H-NMR: (600 MHz, DMSO-*d*₆): δ [ppm] = 0.87 (d, *J* = 6.7 Hz, 6H, CH₂), 1.99 (hept, *J* = 6.8 Hz, 1H, CH), 2.21 (d, *J* = 7.2 Hz, 2H, CH₂), 3.73 (s, 3H, OCH₃), 6.72–6.83 (m, 1H, arom. H), 6.89–7.01 (m, 4H, arom. H), 7.01–7.10 (m, 2H, arom. H), 7.77–8.09 (m, 1H, arom. H), 9.35 (s, 1H, NH); ¹³C-NMR (151 MHz, DMSO-*d*₆): δ [ppm] = 22.12 (2C), 55.32 (CH₃), 44.94 (CH₂) 25.60, 114.81 (2C), 117.42, 120.01 (2C), 122.71, 123.95, 124.65 (CH), 129.16, 148.63, 149.70, 155.35 (C), 170.87 (C=O); C₁₈H₂₁NO₃ (299.37): calcd. C 72.22, H 7.07, N 4.68, found C 71.88, H 7.33, N 4.52; EI-MS: *m/z* (%): 299.1 [M]⁺ (48), 176.1 [M – 123]⁺ (100); HPLC: 98.5% at 254 nm, 98.4% at 280 nm; *t*_R = 4.37 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{\max} [nm] = 248; HPLC-gradient: 98.1%, *t*_R = 12.47 min, *t*_M(DMSO) = 1.28 min.

N-[2-(4-Methoxyphenoxy)phenyl]propanamide (**14**): Crystallization from ethanol (70%) yielded colorless crystals (379 mg, 1.40 mmol, 93%); m.p.: 107–109 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3299 (m, br, N-H), 1651 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 1.02 (t, *J* = 7.6 Hz, 3H, CH₃), 2.35 (q, *J* = 7.5 Hz, 2H, CH₂), 3.74 (s, 3H, OCH₃), 6.70–6.79 (m, 1H, arom. H), 6.90–7.09 (m, 6H, arom. H), 7.87–8.10 (m, 1H, arom. H), 9.33 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆): δ [ppm] = 9.68, 55.39 (CH₃), 29.09 (CH₂), 114.89 (2C), 117.23, 120.24 (2C), 122.70, 123.53, 124.45 (CH), 129.26, 148.49, 149.64, 155.48 (C), 172.28 (C=O); C₁₆H₁₇NO₃ (271.32): calcd. C 70.83, H 6.32, N 5.16, found C 70.70, H 6.37, N 5.12; EI-MS: *m/z* (%): 271.1 [M]⁺ (54), 215.1 [M – 59]⁺ (100); HPLC: 98.6% at 254 nm, 97.9% at 280 nm; *t*_R = 4.89 min, *t*_M(DMSO) = 1.06 min (ACN/H₂O = 50:50), λ_{\max} [nm] = 239; HPLC-gradient: 97.5%, *t*_R = 11.41 min, *t*_M(DMSO) = 1.28 min.

N-[2-(4-Methoxyphenoxy)phenyl]cyclopropanecarboxamide (**15**): Crystallization from ethanol (70%) yielded a colorless solid (360 mg, 1.27 mmol, 85%); m.p.: 138–139 °C; IR (KBr): $\bar{\nu}$ [cm⁻¹] = 3312 (m, br, N-H), 1660 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 0.65–0.87 (m, 4H, CH₂), 1.95–2.14 (m, 1H, CH), 3.75 (s, 3H, OCH₃), 6.64–6.81 (m, 1H, arom. H), 6.88–7.06 (m, 6H, arom. H), 7.88–8.05 (m, 1H, arom. H), 9.69 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆): δ [ppm] = 55.39 (CH₃), 7.25 (2C,

CH₂), 14.02, 114.93 (2C), 116.93, 120.47 (2C), 122.57, 123.38, 124.32 (CH), 129.21, 148.38, 149.54, 155.56 (C), 172.04 (C=O); C₁₇H₁₇NO₃ (283.33): calcd. C 72.07, H 6.05, N 4.94, found C 71.86, H 6.20, N 4.93; EI-MS: *m/z* (%): 283.1 [M]⁺ (43), 215.1 [M – 68]⁺ (100); HPLC: 99.2% at 254 nm, 99.4% at 280 nm; t_R = 5.79 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 50:50), λ_{max} [nm] = 247; HPLC-gradient: 98.6%, t_R = 11.73 min, t_M(DMSO) = 1.28 min.

N-[2-(4-Methoxyphenoxy)phenyl]cyclobutanecarboxamide (**16**): Crystallization from ethanol (70%) yielded a colorless solid (378 mg, 1.27 mmol, 85%); m.p.: 72–74 °C; IR (KBr): ν̄ [cm⁻¹] = 3294 (m, br, N-H), 1665 (s, C=O); ¹H-NMR: (400 MHz, CDCl₃): δ [ppm] = 1.82–2.07 (m, 2H, CH₂), 2.13–2.27 (m, 2H, CH₂), 2.28–2.45 (m, 2H, CH₂), 3.08–3.29 (m, 1H, CH), 3.81 (s, 3H, OCH₃), 6.74 (dd, *J* = 8.1 Hz, 1.4 Hz, 1H, arom. H), 6.84–7.01 (m, 5H, arom. H), 7.06 (td, *J* = 7.8 Hz, 1.4 Hz, 1H, arom. H), 7.71 (s, 1H, NH), 8.47 (dd, *J* = 8.1 Hz, 1.6 Hz, 1H, arom. H); ¹³C-NMR (101 MHz, CDCl₃) δ [ppm] = 55.67 (CH₃) 18.02, 25.32 (2C) (CH₂), 41.10, 114.99 (2C), 116.31, 120.29 (2C), 120.51, 123.35, 123.54 (CH) 129.29, 146.55, 149.48, 156.21 (C), 173.22 (C=O); C₁₇H₁₇NO₃ (283.33): calcd. C 72.07, H 6.05, N 4.94, found C 71.86, H 6.20, N 4.93; EI-MS: *m/z* (%): 297.1 [M]⁺ (38) 215.1 [M – 82]⁺ (100); HPLC: 99.3% at 254 nm, 99.2% at 280 nm; t_R = 4.15 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{max} [nm] = 248; HPLC-Gradient: 98.5%, t_R = 12.93 min, t_M(DMSO) = 1.28 min.

Methyl 4-([2-(4-methoxyphenoxy)phenyl]amino)-4-oxobutanoate (**17**): Crystallization from methanol yielded slightly yellow crystals (347 mg, 1.05 mmol, 70%); m.p.: 82–83 °C; IR (KBr): ν̄ [cm⁻¹] = 3335 (m, br, N-H), 1721 (s, C=O, ester), 1682 (s, C=O, amide); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 2.55 (t, *J* = 6.8 Hz, 2H, CH₂), 2.66 (t, *J* = 6.8 Hz, 2H, CH₂), 3.58 (s, 3H, COOCH₃), 3.75 (s, 3H, OCH₃), 6.67–6.80 (m, 1H, arom. H), 6.86–7.12 (m, 6H, arom. H), 7.84–8.05 (m, 1H, arom. H), 9.52 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ [ppm] = 51.27, 55.39 (CH₃), 28.61, 30.62 (CH₂), 114.90 (2C), 117.00, 120.43 (2C), 122.60, 123.19, 124.41 (CH), 129.06, 148.39, 149.54, 155.54 (C), 170.19, 172.77 (C=O); C₁₈H₁₉NO₅ (329.35): calcd. C 65.64, H 5.82, N 4.25, found C 65.52, H 5.91, N 4.12; EI-MS: *m/z* (%): 329.1 [M]⁺ (32), 297.1 [M – 32]⁺ (100); HPLC: 98.0% at 254 nm, 98.2% at 280 nm; t_R = 4.39 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 50:50), λ_{max} [nm] = 245; HPLC-gradient: 97.4%, t_R = 11.20 min, t_M(DMSO) = 1.28 min.

N-(2-(4-Methoxyphenoxy)phenyl)-4-methoxybenzamide (**18**): Crystallization from methanol yielded colorless needles (344 mg, 0.98 mmol, 66%); m.p.: 88–89 °C; IR (KBr): ν̄ [cm⁻¹] = 3442 (m, N-H), 1674 (s, C=O); ¹H-NMR: (400 MHz, DMSO-*d*₆): δ [ppm] = 3.72 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 6.80–6.88 (m, 1H, arom. H), 6.90–6.96 (m, 2H, arom. H), 6.96–7.04 (m, 4H, arom. H), 7.06–7.20 (m, 2H, arom. H), 7.76 (dd, *J* = 7.5 Hz, 2.1 Hz, 1H, arom. H), 7.84–7.93 (m, 2H, arom. H), 9.61 (s, 1H, NH); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ [ppm] = 55.37 (2C, CH₃), 113.57 (2C), 114.88 (2C), 117.63, 120.08 (2C), 122.78, 125.89, 126.05, 129.45 (2C) (CH), 126.48, 128.93, 149.69, 150.56, 155.44, 161.87 (C), 164.68 (C=O); C₂₁H₁₉NO₄ (349.39): calcd. C 72.19, H 5.48, N 4.01, found C 72.01, H 5.46, N 3.90; EI-MS: *m/z* (%): 349.1 [M]⁺ (20), 135.0 [M – 214]⁺ (100); HPLC: 99.4% at 254 nm, 99.4% at 280 nm; t_R = 4.71 min, t_M(DMSO) = 1.06 min (ACN/H₂O = 60:40), λ_{max} [nm] = 268; HPLC-gradient: 98.8%, t_R = 12.78 min, t_M(DMSO) = 1.28 min.

tert-Butyl 2-([2-(4-chlorophenoxy)phenyl]amino)-2-oxoethylcarbamate (**9**)

A stirred solution of Boc-glycine (1.50 mmol), PyBOP (1.60 mmol) and DIPEA (3.50 mmol) in dichloromethane (10 mL) was cooled to 0 °C with an ice bath. To this solution 2-(4-chlorophenoxy)aniline (1.50 mmol), dissolved in a minimum amount of dichloromethane, was added dropwise. The reaction mixture was allowed to warm to room temperature and was then stirred for 24 h. Thereafter, an aqueous work-up was performed similar to that of compounds 1–8. The resulting oil was further purified by column chromatography over silica gel (dichloromethane/methanol 200:1) to give a slightly orange solid (358 mg, 0.95 mmol, 63%); m.p.: 50–51 °C; IR (KBr): ν̄ [cm⁻¹] = 3409 (m, N-H), 3342 (s, N-H), 1691 (br, s, C=O); ¹H-NMR: (600 MHz, DMSO-*d*₆): δ [ppm] = 1.34 (s, 9H, CH₃), 3.71 (d, *J* = 6.1 Hz, 2H, CH₂), 6.87–7.06 (m, 3H, arom. H),

7.05–7.30 (m, 3H, arom. H), 7.35–7.53 (m, 2H, arom. H), 8.12 (d, $J = 7.7$ Hz, 1H, NH), 9.33 (s, 1H, NH); ^{13}C -NMR (151 MHz, DMSO- d_6) δ [ppm] = 28.00 (3C, CH_3), 43.92 (CH_2), 118.82, 119.90 (2C), 122.35, 124.15, 124.63, 129.70 (2C) (CH), 78.17, 127.17, 129.64, 146.09, 155.47, 155.86, 168.51 (C); EI-MS: m/z (%): 376.1 $[\text{M}]^+$ (11), 219.1 $[\text{M} - 157]^+$ (100); HREI-MS: calcd. for $\text{C}_{19}\text{H}_{21}\text{ClN}_2\text{O}_4$ 376.11844, found 376.11803; HPLC: 96.3% at 254 nm, 96.3% at 280 nm; $t_R = 4.84$ min, $t_M(\text{DMSO}) = 1.06$ min (ACN/ $\text{H}_2\text{O} = 60:40$), λ_{max} [nm] = 230; HPLC-gradient: 95.8%, $t_R = 12.80$ min, $t_M(\text{DMSO}) = 1.28$ min.

tert-Butyl 2-[[2-(4-methoxyphenoxy)phenyl]amino]-2-oxoethylcarbamate (**19**)

A stirred solution of Boc-glycine (2.00 mmol), PyBOP (2.10 mmol) and DIPEA (5.00 mmol) in dichloromethane (10 mL) was cooled to 0 °C with an ice bath. To this solution, 2-(4-methoxyphenoxy)aniline hydrochloride (2.00 mmol), dissolved in dichloromethane (5 mL) together with DIPEA (2.00 mmol), was added dropwise. The reaction mixture was allowed to warm to room temperature and was then stirred for 20 h. Subsequently, an aqueous work-up was performed similar to that of compounds 1–8. The resulting oil was taken up in cold diethyl ether (10 mL) leading to precipitation of a beige solid, which was filtered off and discarded. The ether was evaporated under reduced pressure and the resulting residue was recrystallized from methanol/water (5:1) to give a slightly beige solid (495 mg, 1.33 mmol, 66%); m.p.: 92–93 °C; IR (KBr): $\bar{\nu}$ [cm^{-1}] = 3393 (w, N-H), 3313 (s, br, N-H), 1696 (s, C=O, carbamate), 1666 (s, C=O, amide); ^1H -NMR: (600 MHz, DMSO- d_6): δ [ppm] = 1.35 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.73–3.75 (m, 5H, CH_2 , OCH_3), 6.73 (dd, $J = 8.0$ Hz, 1.7 Hz, 1H, arom. H), 6.95–7.07 (m, 6H, arom. H), 7.24 (t, $J = 5.2$ Hz, 1H, arom. H), 8.13 (d, $J = 7.0$ Hz, 1H, NH), 9.30 (s, 1H, NH); ^{13}C -NMR (151 MHz, DMSO- d_6) δ [ppm] = 28.02 (3C), 55.33 (CH_3), 44.06 (CH_2), 114.92 (2C), 116.55, 120.52 (2C), 121.58, 122.67, 124.19 (CH), 78.22 128.63, 147.69, 149.16, 155.65, 155.90, 168.43 (C); $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_5$ (372.42): calcd. C 64.50, H 6.50, N 7.52, found C 64.10, H 6.68, N 7.61; EI-MS: m/z (%): 372.2 $[\text{M}]^+$ (18), 215.1 $[\text{M} - 157]^+$ (100); HPLC: 96.5% at 254 nm, 95.5% at 280 nm; $t_R = 3.41$ min, $t_M(\text{DMSO}) = 1.06$ min (ACN/ $\text{H}_2\text{O} = 60:40$), λ_{max} [nm] = 241; HPLC-gradient: 95.1%, $t_R = 11.99$ min, $t_M(\text{DMSO}) = 1.28$ min.

General Procedure for the Synthesis of Compounds 10 and 20

The reaction was performed under nitrogen atmosphere. The Boc-protected amine **9** (490 mg, 1.31 mmol) or **19** (250 mg, 0.67 mmol) was dissolved in dichloromethane (10 mL). The solution was cooled with an ice bath. To the well stirred solution 10 equivalents of trifluoroacetic acid (1.01 mL, 13.1 mmol or 0.516 mL, 6.70 mmol) were added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 24h. Afterwards, the solvent was evaporated under reduced pressure. The resulting residue was taken up with propan-2-ol (4–5 mL). Then a mixture of hydrochloric acid (37%) and propan-2-ol (1:1) was added dropwise until a precipitate was formed. Thereafter, diethyl ether (20–30 mL) was added and the mixture was refluxed for 2 h. After cooling to room temperature the formed precipitate was filtered off and dried under reduced pressure at 60 °C.

2-[[2-(4-Chlorophenoxy)phenyl]amino]-2-oxoethan-1-aminium chloride (**10**): Crystallization from diethyl ether/propan-2-ol yielded a beige solid (220 mg, 0.70 mmol, 54%); m.p.: 255–257 °C (decomp.); IR (KBr): $\bar{\nu}$ [cm^{-1}] = 3434 (m, br, N-H), 3124 (m, N-H), 1673 (s, C=O); ^1H -NMR: (600 MHz, DMSO- d_6): δ [ppm] = 3.78 (s, 2H, CH_2), 6.95 (dd, $J = 8.0$ Hz, 1.6 Hz, 1H, arom. H), 7.01–7.05 (m, 2H, arom. H), 7.13–7.21 (m, 2H, arom. H), 7.42–7.48 (m, 2H, arom. H), 8.03 (dd, $J = 8.0$ Hz, 1.9 Hz, 1H, arom. H), 8.14 (s, 3H, NH_3^+), 10.12 (s, 1H, NH); ^{13}C -NMR (151 MHz, DMSO- d_6) δ [ppm] = 40.91 (CH_2), 118.99, 120.12 (2C), 123.27, 124.12, 125.52, 129.72 (2C) (CH), 127.22, 128.90, 146.92, 155.45 (C), 165.48 (C=O); $\text{C}_{14}\text{H}_{13}\text{ClN}_2\text{O}_2 \cdot \text{HCl}$ (313.18): calcd. C 53.69, H 4.51, N 8.95, found C 53.89, H 4.51, N 8.57; EI-MS: m/z (%): 276.0 $[\text{M}_{\text{free base}}]^+$ (36), 219.0 $[\text{M} - 54]^+$ (100); HPLC: 99.9% 254 nm, 99.8% at 280 nm; $t_R = 3.41$ min, $t_M(\text{DMSO}) = 1.06$ min (ACN/Buffer = 30:70), λ_{max} [nm] = 231.

2-[[2-(4-Methoxyphenoxy)phenyl]amino]-2-oxoethan-1-aminium chloride (**20**): Crystallization from diethyl ether/propan-2-ol yielded a beige solid (189 mg, 0.61 mmol, 91%); m.p.: 249–250 °C (decomp.); IR

(KBr): $\bar{\nu}$ [cm⁻¹] = 3434 (m, br, N-H), 3114 (m, N-H), 1680 (s, C=O); ¹H-NMR: (600 MHz, DMSO-*d*₆): δ [ppm] = 3.76 (s, 2H, OCH₃), 3.84 (s, 2H, CH₂), 6.72–6.77 (m, 1H, arom. H), 6.95–7.04 (m, 4H, arom. H), 7.05–7.13 (m, 2H, arom. H), 7.88–8.14 (m, 1H, arom. H), 8.26 (s, 3H, NH₃⁺), 10.11 (s, 1H, NH); ¹³C-NMR (151 MHz, DMSO-*d*₆) δ [ppm] = 55.35 (CH₃), 40.85 (CH₂), 114.95 (2C), 116.75, 120.73 (2C), 122.61, 122.82, 125.16 (CH), 127.88, 148.66, 149.11, 155.69 (C), 165.28 (C=O); C₁₅H₁₆N₂O₃·HCl (308.76): calcd. C 58.35, H 5.55, N 9.07, found C 58.28, H 5.52, N 8.82; EI-MS: *m/z* (%): 272.1 [M_{free base}]⁺ (55), 200.1 [M – 72]⁺ (100); HPLC: 99.2% at 254 nm, 99.3% at 280 nm; t_R = 7.89 min, t_M(DMSO) = 1.06 min (ACN/Buffer = 20:80), λ_{\max} [nm] = 229.

3.2.1. Calculations of Lipinski Properties

Calculations of the octanol/water partition coefficients (logP) were performed using chemicalize.org [15]. The calculator plugins are based on Viswanadhan's fragmentation methods [22], the PHYSPROP[®] database [23] and an additional data set [24]. All three approaches were weighted equally for the calculations.

3.2.2. In Vitro Antimalarial Activity Assay

Erythrocytic stages of transgenic NF54-*luc P. falciparum* were used for the luciferase-based viability assay. These parasites constitutively express high levels of luciferase. The parasites were cultured as described earlier [19,25]. Firstly, the culture was dispensed in triplicate into white 96-well flat bottom plates (each well contains 250 μ L) (NUNC, Roskilde, Denmark) with parasitemia of 0.5%–1%. Then the cultures were incubated in the presence of a 3 μ M concentration of the test compounds for 48 h (37 °C, 90% N₂, 5% CO₂, and 5% O₂). Subsequently, 100 μ L RPMI1640 media was removed from each well and a 100 μ L volume of the Bright-Glo[®] substrate solution was added to each well. One of the cleavage products of the reaction is light, which was measured by a FLUOROSKAN FL luminometer (Thermo) machine, thereby detecting the amount of living parasites. The experiments were repeated with an incubation of 96 h. Untreated cultures were used as negative controls and to calculate the inhibition rate (0% inhibition of parasite growth). Blasticidin (Sigma-Aldrich, St. Louis, MO, USA), a drug used for *selection* of transfected parasites, was included as a positive control on each plate and gave >90% inhibition of parasite growth at 2 μ g/mL.

4. Conclusions

In conclusion, the previously published *N*-[2-(4-chlorophenoxy)phenyl]-2,2-dimethylpropanamide (TCMDC-137332, **1**) [7] and 19 derivatives were synthesized in satisfactory to excellent yields and were fully characterized regarding identity and purity. The predicted antimalarial activity of the herein examined hit structure of the TCAMS (**1**) was not confirmed in biological evaluation experiments. The growth of *Plasmodium falciparum* NF54 strains was not diminished in the expected nanomolar concentration by the 2-phenoxyanilide congeners. However, in a prolonged *in vitro* assay, in which cultures were incubated for 96 h, *N*-[2-(4-methoxyphenoxy)phenyl]cyclobutanecarboxamide (**16**) was identified to be a moderate inhibitor of the proliferation of *Plasmodium falciparum* NF54 strains.

Acknowledgments: The project was funded by the German Federal Ministry of Education and Research (BMBF BioDisc 7; 13GW0024). Support by the COST action CM1307 "Targeted chemotherapy towards diseases caused by endoparasites" (to Thomas Weidner and Conrad Kunick) is gratefully acknowledged.

Author Contributions: Thomas Weidner: experimental synthetic work, synthesis planning, chemoinformatics, instrumental analysis, writing of manuscript; Abed Nasereddin and Ron Dzikowski: biological evaluation; Lutz Preu: NMR interpretation, chemoinformatics; Johann Grunefeld: synthesis planning; Conrad Kunick: synthesis planning, literature search, writing of manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. *WHO: World Malaria Report 2014*, Available online: http://apps.who.int/iris/bitstream/10665/144852/2/9789241564830_eng.pdf?ua=1 (accessed on 26 January 2015).
2. Takala-Harrison, S.; Laufer, M.K. Antimalarial drug resistance in Africa: Key lessons for the future. *Ann. N. Y. Acad. Sci.* **2015**, *1342*, 62–67. [[CrossRef](#)] [[PubMed](#)]
3. Ashley, E.A.; Dhorda, M.; Fairhurst, R.M.; Amaratunga, C.; Lim, P.; Suon, S.; Sreng, S.; Anderson, J.M.; Mao, S.; Sam, B.; *et al.* Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **2014**, *371*, 411–423. [[CrossRef](#)] [[PubMed](#)]
4. Plouffe, D.; Brinker, A.; McNamara, C.; Henson, K.; Kato, N.; Kuhen, K.; Nagle, A.; Adrián, F.; Matzen, J.T.; Anderson, P.; *et al.* In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9059–9064. [[CrossRef](#)] [[PubMed](#)]
5. Guiguemde, W.A.; Shelat, A.A.; Bouck, D.; Duffy, S.; Crowther, G.J.; Davis, P.H.; Smithson, D.C.; Connelly, M.; Clark, J.; Zhu, F.; *et al.* Chemical genetics of *Plasmodium falciparum*. *Nature* **2010**, *465*, 311–315. [[CrossRef](#)] [[PubMed](#)]
6. Edlin, C.D.; Morgans, G.; Winks, S.; Duffy, S.; Avery, V.M.; Wittlin, S.; Waterson, D.; Burrows, J.; Bryans, J. Identification and *in vitro* ADME assessment of a series of novel anti-malarial agents suitable for hit-to-lead chemistry. *ACS Med. Chem. Lett.* **2012**, *3*, 570–573. [[CrossRef](#)] [[PubMed](#)]
7. Gamo, F.-J.; Sanz, L.M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J.-L.; Vanderwall, D.E.; Green, D.V.S.; Kumar, V.; Hasan, S.; *et al.* Thousands of chemical starting points for antimalarial lead identification. *Nature* **2010**, *465*, 305–310. [[CrossRef](#)] [[PubMed](#)]
8. Calderón, F.; Barros, D.; Bueno, J.M.; Coterón, J.M.; Fernández, E.; Gamo, F.J.; Lavandera, J.L.; León, M.L.; Macdonald Simon, J.F.; Mallo, A.; *et al.* An invitation to open innovation in malaria drug discovery: 47 quality starting points from the TCAMS. *ACS Med. Chem. Lett.* **2011**, *2*, 741–746. [[CrossRef](#)] [[PubMed](#)]
9. Rueda, L.; Castellote, I.; Castro-Pichel, J.; Chaparro, M.J.; de la Rosa, J.C.; Garcia-Perez, A.; Gordo, M.; Jimenez-Diaz, M.B.; Kessler, A.; Macdonald Simon, J.F.; *et al.* Cyclopropylcarboxamides: A new oral antimalarial series derived from the Tres Cantos Anti-Malarial Set (TCAMS). *ACS Med. Chem. Lett.* **2011**, *2*, 840–844. [[CrossRef](#)] [[PubMed](#)]
10. Chaparro, M.J.; Vidal, J.; Angulo-Barturen, I.; Bueno, J.M.; Burrows, J.; Cammack, N.; Castañeda, P.; Colmenarejo, G.; Coterón, J.M.; de Las Heras, L.; *et al.* Case study of small molecules as antimalarials: 2-Amino-1-phenylethanol (APE) derivatives. *ACS Med. Chem. Lett.* **2014**, *5*, 657–661. [[CrossRef](#)] [[PubMed](#)]
11. Meyers, M.J.; Tortorella, M.D.; Xu, J.; Qin, L.; He, Z.; Lang, X.; Zeng, W.; Xu, W.; Qin, L.; Prinsen, M.J.; *et al.* Evaluation of aminohydantoin as a novel class of antimalarial agents. *ACS Med. Chem. Lett.* **2014**, *5*, 89–93. [[CrossRef](#)] [[PubMed](#)]
12. McConville, M.; Fernández, J.; Angulo-Barturen, I.; Bahamontes-Rosa, N.; Ballell-Pages, L.; Castañeda, P.; de Cózar, C.; Crespo, B.; Guijarro, L.; Jiménez-Díaz, M.B.; *et al.* Carbamoyltriazoles, known serine protease inhibitors, are a potent new class of antimalarials. *J. Med. Chem.* **2015**, *58*, 6448–6455. [[CrossRef](#)] [[PubMed](#)]
13. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25. [[CrossRef](#)]
14. Schrader, F.C.; Barho, M.; Steiner, I.; Ortmann, R.; Schlitzer, M. The antimalarial pipeline—An update. *Int. J. Med. Microbiol.* **2012**, *302*, 165–171. [[CrossRef](#)] [[PubMed](#)]
15. Chemicalize.org was Used for Prediction of logP, December, 2015, Chemicalize.org and ChemAxon. Available online: <http://www.chemaxon.com> (accessed on 2 February 2016).
16. Wenlock, M.C.; Austin, R.P.; Barton, P.; Davis, A.M.; Leeson, P.D. A comparison of physiochemical property profiles of development and marketed oral drugs. *J. Med. Chem.* **2003**, *46*, 1250–1256. [[CrossRef](#)] [[PubMed](#)]
17. Salazar, E.; Bank, E.M.; Ramsey, N.; Hess, K.C.; Deitsch, K.W.; Levin, L.R.; Buck, J. Characterization of *Plasmodium falciparum* adenyl cyclase- β and its role in erythrocytic stage parasites. *PLoS ONE* **2012**, *7*, e39769. [[CrossRef](#)] [[PubMed](#)]
18. Dahan-Pasternak, N.; Nasereddin, A.; Kolevzon, N.; Pe'er, M.; Wong, W.; Shinder, V.; Turnbull, L.; Whitchurch, C.B.; Elbaum, M.; Gilberger, T.W.; *et al.* PfSec13 is an unusual chromatin-associated nucleoporin of *Plasmodium falciparum* that is essential for parasite proliferation in human erythrocytes. *J. Cell. Sci.* **2013**, *126*, 3055–3069. [[CrossRef](#)] [[PubMed](#)]

19. Fugel, W.; Oberholzer, A.E.; Gschloessl, B.; Dzikowski, R.; Pressburger, N.; Preu, L.; Pearl, L.H.; Baratte, B.; Ratin, M.; Okun, I.; *et al.* 3,6-Diamino-4-(2-halophenyl)-2-benzoylthieno[2,3-*b*]pyridine-5-carbonitriles are selective inhibitors of Plasmodium falciparum Glycogen Synthase Kinase-3. *J. Med. Chem.* **2013**, *56*, 264–275. [[CrossRef](#)] [[PubMed](#)]
20. Kolevzon, N.; Nasereddin, A.; Naik, S.; Yavin, E.; Dzikowski, R. Use of peptide nucleic acids to manipulate gene expression in the malaria parasite Plasmodium falciparum. *PLoS ONE* **2014**, *9*, e86802.
21. Armarego, W.L.F.; Chai, C.L.L. *Purification of Laboratory Chemicals*, 6th ed.; Elsevier/Butterworth-Heinemann: Amsterdam, The Netherlands; Boston, MA, USA, 2009.
22. Viswanadhan, V.N.; Ghose, A.K.; Revankar, G.R.; Robins, R.K. Atomic physicochemical parameters for three dimensional structure directed quantitative structure-activity relationships. 4. Additional parameters for hydrophobic and dispersive interactions and their application for an automated superposition of certain naturally occurring nucleoside antibiotics. *J. Chem. Inf. Model.* **1989**, *29*, 163–172.
23. PHYSPROP Database. Available online: <http://www.scinc.com/what-we-do/environmental/scientific-databases.html> (accessed on 8 October 2015).
24. Klopman, G.; Li, J.-Y.; Wang, S.; Dimayuga, M. Computer automated log P calculations based on an extended group contribution approach. *J. Chem. Inf. Model.* **1994**, *34*, 752–781. [[CrossRef](#)]
25. Dzikowski, R.; Frank, M.; Deitsch, K. Mutually exclusive expression of virulence genes by malaria parasites is regulated independently of antigen production. *PLoS Pathog.* **2006**, *2*, e22. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of the compounds are available from the authors.



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).