Department für Pharmazie[1] – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Germany and Biochemisches Institut der Justus-Liebig-Universität[2] Gießen, Germany

2-(Arylpropionylamino)- and 2-(arylacryloylamino)benzophenones: Farnesyltransferase inhibition and antimalarial activity

K. Fucik[1], K. Kettler[1], J. Wiesner[2], R. Ortmann[1], D. Unterreitmeier[1], J. Krauss[1], F. Bracher[1], H. Jomaa[2], M. Schlitzer[1]

Received November 19, 2003, accepted January 20, 2004

Prof. Dr. Martin Schlitzer, Department für Pharmazie, Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität, Butenandtstraße 5–13, D-81377 München
Martin.Schlitzer@cup.uni-muenchen.de

Pharmazie 59: 744–752 (2004)

Structural variation of the 2-acylamino moiety of some benzophenone farnesyltransferase inhibitors led to the *para*-trifluoromethylphenylpropionyl derivative with relatively low farnesyltransferase inhibition but considerable antimalarial activity and no cytotoxicity.

1. Introduction

Farnesyltransferase is involved in the post-translational modification of numerous proteins of which the majority has important functions in intracellular signal transduction. Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesyl pyrophosphate to the thiol of a cysteine side chain of the protein substrate. The cysteine residue is part of a characteristic carboxy-terminal consensus sequence, the so-called CAAX box (C, cysteine; A, amino acid with aliphatic side chain; X, serine or methionine) (Fu and Casey 1999; Wittinghofer and Waldmann 2000a, b; Bell 2000). Various inhibitors of the farnesyltransferase have been developed as potential cancer therapeutics. The compounds of several pharmaceutical companies are in advanced stages of clinical studies (Cox and Der 2002; Purcell and Donehower 2002).

In addition to mammals, farnesyltransferases were also identified in other eukaryotic organisms including pathogenic protozoa of the genera *Plasmodium* (Chakrabarti et al. 1998, 2002), *Trypanosoma* (Yokoyama et al. 1998, 2000; Bruckner et al. 2002), *Leishmania* (Bruckner et al. 2002) and *Toxoplasma* (Ibrahim et al. 2001). Therefore, inhibition of the farnesyltransferase has also been suggested as new strategy for the treatment of parasitic infections (Cox and Der 2002). The most important of these protozoa caused diseases is Malaria tropica caused by the infection with *Plasmodium falciparum*. Approximately 40% of the world population lives in areas with malaria risk, and 2 to 3 million people die each year from malaria. Because of the increasing spread of malaria parasites resistant to chloroquine and other commonly used antimalarials there is an urgent need for new therapeutics (Sachs and Malaney 2002; Ridley 2002).

We have developed a novel class of farnesyltransferase inhibitors based on a benzophenone scaffold (Schlitzer 2002). In course of our studies towards the establishment of structure activity relationships of this type of compounds we identified inhibitors **4a** and **5a** as promising lead structures especially because of their inhibition of yeast farnesyltransferase with IC_{50}-values of 40 nM and 5 nM, respectively (Kettler et al. 2003). In addition, the phenylpropionic acid derivative **4a** displayed an interesting *in vitro* antimalarial activity with an IC_{50} of 310 nM (Wiesner et al. 2003b). In the present study it was our objective to see how structural variations of phenylpropionic and the cinnamic acid residue at the 2-amino group of the benzophenone core influence farnesyltransferase inhibition as well as antimalarial activity.

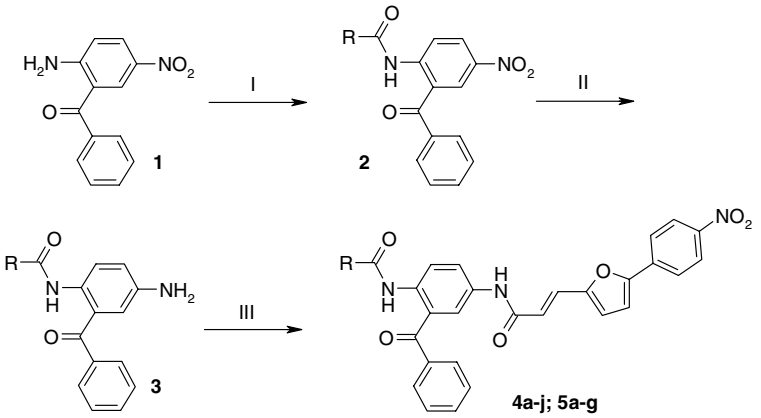
2. Investigations and results

Target compounds were prepared from the commercially available 2-amino-5-nitrobenzophenone **1**, which was first acylated at the 2-amino group by appropriate 3-arylpropionic acid chlorides and cinnamic acid chlorides, respectively (Scheme 1). Then, the 5-nitro group was reduced and the resulting amino function was acylated by 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (Böhm et al. 2001). *Para*-nitro and trifluoromethyl cinnamic acid were obtained from the corresponding benzaldehydes by Knoevenagel condensation. *Para*-, *meta*- and *ortho*-trifluoromethylphenylpropionic acid were prepared by catalytic hydrogenation of the appropriate cinnamic acid derivatives which in turn were obtained from the aldehydes. 4-Nitrophenylpropionic acid was obtained by nitration of phenylpropionic acid (Moloney et al. 1999).

Because of the reduction step involved in the synthesis according to Scheme 1, an alternative route had to be followed for the preparation of the nitro compounds **4k** and **5h** (Scheme 2). First, the 2-amino group of **1** was protected as trifluoroacetamide (**6**). After reduction of the 5-nitro group, the resulting amine **7** was acylated with 3-[5-(4-nitrophenyl)-2-furyl]arylic acid chloride. After removal of the protective group from **8** the resulting intermediate **9** (Kettler et al. 2003) was acylated by 4-nitrophenylpropionic acid chloride and 4-nitrocinnamic acid chloride, respectively.

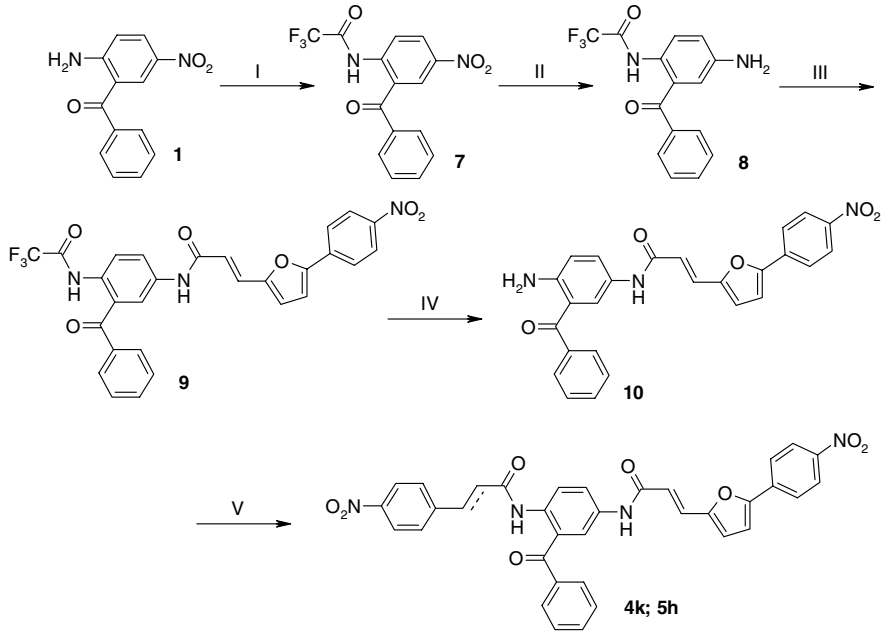
The farnesyltransferase inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described by Pompliano et al. (1992). The assay employs yeast farnesyltransferase (FTase) fused to glutathione *S*-

Scheme 1



(I) R−CO−Cl, toluene/dioxane, reflux, 2h; (II) $SnCl_2 \times 2\,H_2O$, EtOAc, reflux 2h; (III) 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride, toluene/dioxane, reflux, 2h

Scheme 2



(I) TFAA, DCM/pyridine, 0 °C, 2h; (II) $SnCl_2 \times 2H_2O$, EtOAc, reflux 2h; (III) 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride, toluene/dioxane, reflux, 2h; (IV) K_2CO_3, dioxane/H_2O, reflux, 3h; (V) $O_2N-C_6H_4-(CH_2)_2-COCl$ or $O_2N-C_6H_4-HC{=}CH-COCl$, toluene/dioxane, reflux, 2h

transferase at the N-terminus of the β-subunit (Del Villar et al. 1997). The heterologous expression of the farnesyltransferase genes from *P. falciparum* has not been achieved so far and, therefore, no recombinant enzyme is available for routine screening (Chakrabarti et al. 2002).

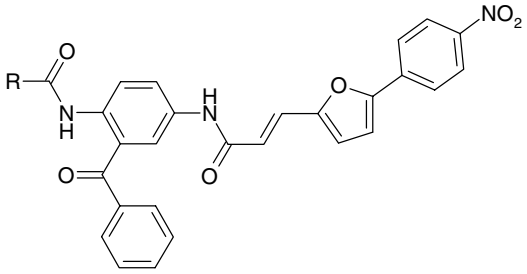
Farnesylpyrophosphate and the dansylated pentapeptide Ds-GlyCysValLeuSer were used as substrates. Upon farnesylation of the cysteine thiol, the dansyl residue is placed into a lipophilic environment. The resulting enhancement of fluorescence at 505 nm is used to monitor the enzyme reaction.

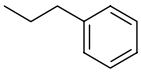
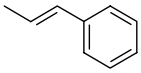
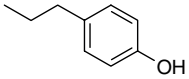
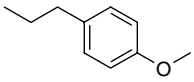
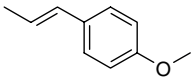
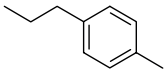
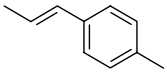
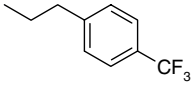
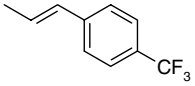
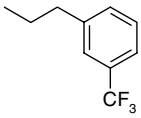
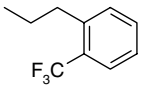
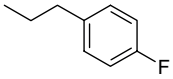
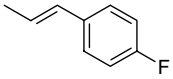
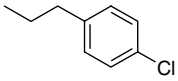
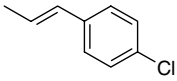
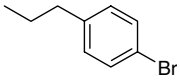
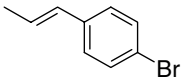
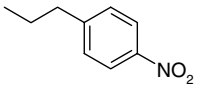
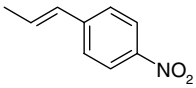
The farnesyltransferase inhibitory activities of compounds **4** and **5** are displayed in the Table. In the case of the phenylpropionyl derivatives **4** as well as with the cinnamoyl derivatives **5** all new compounds turned out to be considerably less active than the initial unsubstituted leads **4a** and **5a**. Clear structure activity relationships cannot be delineated.

Compounds **4** and **5** were assayed for their inhibitory activity against intraerythrocytic forms of *P. falciparum* strain Dd2 using a semi-automated microdilution assay (Desjardins et al. 1979; Trager and Jensen 1976; Ancelin et al. 1998). The growth of the parasites was monitored through the incorporation of tritium labeled hypoxanthine. Comparability of different experiments was ensured by concurrent assay of standard compounds. The Dd2 strain used for the inhibition assays is resistant to several commonly used anti-malarial drugs (chloroquine, cycloguanile and pyrimethamine) (Table).

Activity of the cinnamic acid substituted derivatives **5** against the intraerythrocytic forms of *P. falciparum* was

Table: Farnesyltransferase inhibition and anti-malarial activity[a, b] of compounds 4 and 5



Compd.	R	IC_{50} (nM) FTase	IC_{50} (nM) *P. falciparum*	Compd.	R	IC_{50} (nM) FTase	IC_{50} (nM) *P. falciparum*
4a		40	310	**5a**		5	2300
4b		215	3250				
4c		435	1300	**5b**		136	21000
4d		411	440	**5c**		150	8300
4e		1378	61	**5d**		224	20000
4f		300	625				
4g		900	1080				
4h		646	440	**5e**		184	27000
4i		231	125	**5f**		343	42000
4j		900	165	**5g**		169	42000
4k		178	1400	**5h**		71	10000

[a] Activity was assayed by measuring radioactive hypoxanthine uptake by the multi-resistant *P. falciparum* strain Dd2. IC_{50} values (nM) for standard antimalarials were: chloroquine, 170; pyrimethamine, 2500; cycloguanile, 2200; quinine, 380; lumefantrine, 30; artemisinin, 18

[b] Antimalarial activity of compounds **4a**–**k** has been published elsewhere (Wiesner et al. 2003b)

generally very low. This can be mainly attributed to the low solubility of this type of compounds in the culture medium which most probably prevents effective drug levels inside the parasite.

In case of the phenylpropionyl derivatives **4** antimalarial activity changes considerably with the substitution on the terminal phenyl residue. While the hydroxy- (**4b**), methoxy- (**4c**) and nitro- (**4k**) derivatives are markedly less active than the lead, the methyl- (**4d**) and the fluoro- (**4h**) derivatives are roughly equipotent to the lead **4a**. However, the chloro- (**4i**) and the bromo- (**4j**) derivatives are considerably more active while the trifluoromethyl derivative (**4e**) is the most active compound of this series displaying an IC_{50}-value of 61 nM which is already in an interesting range.

Shifting the trifluoromethyl group from the para- to the meta- (**4f**) or ortho- (**4g**) position resulted in an increasing reduction in activity.

The phenylpropionyl substituted derivatives were assayed for potential cytotoxic activity using the MTT-assay employing HL-60 cell line. In this test, the reduction of a tetrazolium bromide to the corresponding formazane is used to measure cell viability because the reaction occurs only in active mitochondria and therefore in living cells. Compounds were assayed in concentrations between 10^{-9} to 10^{-4} mol/l. Apart from low activity in the micromolar

range for the meta- and ortho-trifluoromethyl derivatives (**4f**, **g**), no cytotoxic activity was observed especially not for the most active antimalarial compound **4e**.

3. Discussion

Although the newly prepared cinnamoyl derivatives **5b–g** displayed fair farnesyltransferase activity their unexpected low solubility prevented any relevant antimalarial activity. Therefore, this particular substructure was no longer considered for further studies.

With respect to antimalarial activity structure activity relationships observed with the phenylpropionic acid derivatives **4** closely resemble those observed in a series of structurally closely related inhibitors having an arylacetic acid substructure at 2-amino group of the benzophenone core (Wiesner et al. 2003a). In both cases, good activity was found with the chloro- and the bromo-substituent while the para-trifluoromethyl derivatives are the most active compounds in the series. Correlation between farnesyltransferase inhibitory and antimalarial activity is generally bad (Fig.) but one has to keep in mind that the activity against an isolated enzyme is compared with the activity against an intracellular living complete organism. Furthermore, since farnesyltransferase of *P. falciparum* is not available for routine screening, FTase of a different species (yeast) has to be used. Although amino acid sequences regarding the enzymes active side are quite similar, there are some differences.

Our general experience is that good farnesyltransferase inhibitors not necessarily need to be active antimalarial agents (Wiesner et al. 2003c). From the present set of data this conclusion has to be modified in an important aspect. The bromo and the trifluoromethyl compound show that relatively week farnesyltransferase inhibitors can also be considerably active as antimalarials. This argues strongly for the possibility of the development of specific farnesyltransferase inhibitors with relative specificity against *P. falciparum*. One could argue that the activity of those compounds might orginate from another mechanism than farnesyltransferase inhibition. However, we have shown with structurally closely related compounds that they in fact inhibit the protein farnesylation in *P. falciparum* cultures (Wiesner et al. 2004).

In addition it is important to note that the most active antimalarial compound **4e** displayed no measurable cytotoxic activity in the MTT-assay.

In conclusion, the results presented encourage further development of benzophenone-based farnesyltransferase inhibitors as potential antimalarial agents.

4. Experimental

4.1. Preparation

^1H and ^{13}C NMR spectra were recorded on a Jeol Eclipse 400 and a Jeol Eclipse 500 spectrometer. Mass spectra were obtained with a PE Biosystems API 2000. IR spectra were recorded on a JASCO FT/IR-410 Fourier Transform Infrared Spectrometer. Microanalyses were obtained from an elementar vario el and were within $\pm$ 0.4% of the calculated values. Melting points were obtained with a Reichert Austria microscope and are uncorrected. Liquid chromatography was carried out using silica gel 60 from ICM Silitech. The preparation of compounds **4a** and **5a** has been described elsewhere (Kettler et al. 2003)

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 toluene and 0.1 mL $SOCl_2$ per mmol acid was added. The mixture was heated under reflux for 2 h and the volatiles were evaporated *in vacuo*. The resulting acyl chlorides were dissolved in toluene or dioxane (approx. 10 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. Then, the solvent was removed *in vacuo* and the crude products were purified by recrystallisation from ethanol.

4.1.2. General procedure 2: Reduction of aromatic nitro compounds

Aromatic nitro compounds **2** were dissolved in EtOAc (50–100 mL) and $SnCl_2 \times 2\,H_2O$ (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 two times with EtOAc. 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. Compounds **2a–o**

The compounds **2a–o** were prepared from 2-amino-5-nitrobenzophenone **1** and the appropriate 3-arylpropionic acid chloride or cinnamic acid chloride according to general procedure 1.

4.1.3.1. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-hydroxyphenyl)propionic acid amide (**2a**)

Yield 60%. ^1H NMR (CD_2Cl_2): δ (ppm) = 2.76 (t, ^3J = 8.0 Hz, 2 H, CH_2), 2.99 (t, ^3J = 8.0 Hz, 2 H, CH_2), 4.85 (s, 1 H, OH), 6.71 (m, 2 H, Ar-H), 7.10 (m, 2 H, Ar-H) 7.56 (m, 2 H, Ar-H), 7.69 (m, 3 H, Ar-H), 8.39 (dd, ^3J = 8.8 Hz, ^4J = 2.0 Hz, 1 H, Ar-H), 8.43 (d, ^4J = 2.0 Hz, 1 H, Ar-H), 8.89 (d, ^3J = 8.8 Hz, Ar-H), 11.00 (s, 1 H, NH).

4.1.3.2. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-methoxyphenyl)propionic acid amide (**2b**)

Yield 57%. ^1H NMR ($CDCl_3$): δ (ppm) = 2.76 (t, ^3J = 7.5 Hz, 2 H, CH_2), 3.03 (t, ^3J = 7.5 Hz, 2 H, CH_2), 3.71 (s, 3 H, OCH_3), 6.78 (m, 2 H, Ar-H),

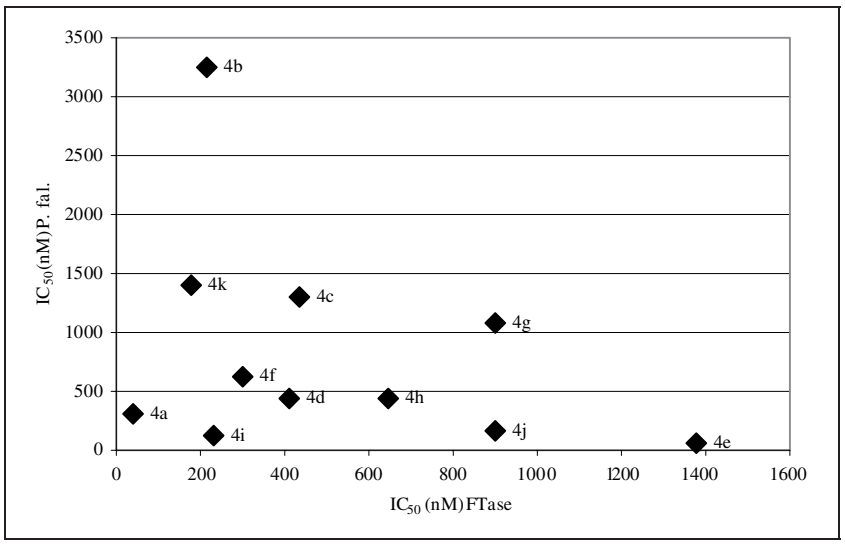


Fig.:
Correlation between farnesyltransferase inhibition and antimalarial activity for compounds **4a–k**

7.15 (m, 2 H, Ar-H), 7.55 (m, 2 H, Ar-H), 7.68 (m, 3 H, Ar-H), 8.41 (dd, $^3J = 9.5$ Hz, $^4J = 3.0$ Hz, 1 H, Ar-H), 8.46 (d, $^4J = 3.0$ Hz, 1 H, Ar-H), 8.91 (d, $^3J = 9.5$ Hz, 1 H, Ar-H), 11.07 (s, 1 H, NH).

4.1.3.3. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-methylphenyl)propionic acid amide (**2c**)

Yield 58%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.26 (s, 3 H, CH_3), 2.80 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 3.02 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 7.07 (m, 2 H, Ar-H), 7.12 (m, 2 H, Ar-H) 7.55 (m, 2 H, Ar-H), 7.68 (m, 3 H, Ar-H), 8.42 (dd, $^3J = 9.5$ Hz, $^4J = 3.0$ Hz, 1 H, Ar-H), 8.47 (d, $^4J = 3.0$ Hz, 1 H, Ar-H), 8.93 (d, $^3J = 9.5$ Hz, 1 H, Ar-H), 11.07 (s, 1 H, NH).

4.1.3.4. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-trifluoromethylphenyl)propionic acid amide (**2d**)

Purification: Column chromatography (dichloromethane:n-hexane 8 : 2); Yield 54%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.48 (t, $^3J = 7.9$ Hz, 2 H, CH_2), 2.73 (t, $^3J = 7.9$ Hz, 2 H, CH_2), 7.37 (m, 2 H, Ar-H), 7.54 (m, 2 H, Ar-H) 7.61 (m, 2 H, Ar-H), 7.67 (m, 1 H, Ar-H), 7.72 (m, 2 H, Ar-H), 7.81 (d, $^3J = 9.0$ Hz, 1 H, Ar-H), 8.16 (d, $^4J = 2.8$ Hz, 1 H, Ar-H), 8.44 (dd, $^3J = 9.0$ Hz, $^4J = 2.8$ Hz, 1 H, Ar-H), 10.60 (s, 1 H, NH).

4.1.3.5. *N*-(2-Benzoyl-4-nitrophenyl)-3-(3-trifluoromethylphenyl)propionic acid amide (**2e**).

Yield 39%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.51 (t, $^3J = 8.0$ Hz, 2 H, CH_2), 2.75 (t, $^3J = 8.0$ Hz, 2 H, CH_2), 7.45 (m, 2 H, Ar-H), 7.52 (m, 2 H, Ar-H) 7.54 (m, 2 H, Ar-H), 7.67 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.84 (d, $^3J = 9.0$ Hz, 1 H, Ar-H), 8.16 (d, $^4J = 2.8$ Hz, 1 H, Ar-H), 8.44 (dd, $^3J = 9.0$ Hz, $^4J = 2.8$ Hz, 1 H, Ar-H), 10.56 (s, 1 H, NH).

4.1.3.6. *N*-(2-Benzoyl-4-nitrophenyl)-3-(2-trifluoromethylphenyl)propionic acid amide (**2f**)

Yield 55%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.43 (t, $^3J = 7.6$ Hz, 2 H, CH_2), 2.78 (t, $^3J = 7.6$ Hz, 2 H, CH_2), 7.41 (m, 2 H, Ar-H), 7.53 (m, 2 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.71 (m, 2 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.81 (d, $^3J = 9.0$ Hz, 1 H, Ar-H), 8.19 (d, $^4J = 2.6$ Hz, 1 H, Ar-H), 8.45 (dd, $^3J = 9.0$ Hz, $^4J = 2.6$ Hz, 1 H, Ar-H), 10.59 (s, 1 H, NH).

4.1.3.7. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-fluorophenyl)propionic acid amide (**2g**)

Yield 61%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.79 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 3.06 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 6.94 (m, 2 H, Ar-H), 7.19 (m, 2 H, Ar-H) 7.56 (m, 2 H, Ar-H), 7.69 (m, 3 H, Ar-H), 8.41 (dd, $^3J = 9.5$ Hz, $^4J = 3.0$ Hz, 1 H, Ar-H), 8.46 (d, $^4J = 3.0$ Hz, 1 H, Ar-H), 8.90 (d, $^3J = 9.5$ Hz, 1 H, Ar-H), 11.08 (s, 1H, NH).

4.1.3.8. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-chlorophenyl)propionic acid amide (**2h**)

Yield 76%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.80 (t, $^3J = 7.7$ Hz, 2 H, CH_2), 3.05 (t, $^3J = 7.7$ Hz, 2 H, CH_2), 7.18 (m, 2 H, Ar-H), 7.23 (m, 2 H, Ar-H) 7.56 (m, 2 H, Ar-H), 7.68 (m, 3 H, Ar-H), 8.41 (dd, $^3J = 9.3$ Hz, $^4J = 2.7$ Hz, 1 H, Ar-H), 8.46 (d, $^4J = 2.7$ Hz, 1 H, Ar-H), 8.90 (d, $^3J = 9.3$ Hz, 1 H, Ar-H), 11.11 (s, 1 H, NH).

4.1.3.9. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-bromophenyl)propionic acid amide (**2i**)

Yield 67%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.80 (t, $^3J = 7.9$ Hz, 2 H, CH_2), 3.04 (t, $^3J = 7.9$ Hz, 2 H, CH_2), 7.13 (m, 2 H, Ar-H), 7.39 (m, 2 H, Ar-H) 7.58 (m, 2 H, Ar-H), 7.68 (m, 3 H, Ar-H), 8.41 (dd, $^3J = 9.3$ Hz, $^4J = 2.8$ Hz, 1 H, Ar-H), 8.47 (d, $^4J = 2.8$ Hz, 1 H, Ar-H), 8.90 (d, $^3J = 9.3$ Hz, 1 H, Ar-H), 11.12 (s, 1 H, NH).

4.1.3.10. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-methoxyphenyl)acrylic acid amide (**2j**)

Yield 54%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.84 (s, 3 H, OCH_3), 6.50 (d, $^3J = 15.5$ Hz, 1 H, =CH), 6.93 (m, 2 H, Ar-H), 7.54 (m, 2 H, Ar-H), 7.56 (m, 2 H, Ar-H), 7.67 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.78 (d, $^3J = 15.5$ Hz, 1 H, =CH), 8.45 (dd, $^3J = 9.4$ Hz, $^4J = 2.7$ Hz, 1 H, Ar-H), 8.52 (d, $^4J = 2.7$ Hz, 1 H, Ar-H), 9.08 (d, $^3J = 9.4$ Hz, 1 H, Ar-H), 11.41 (s, 1 H, NH).

4.1.3.11. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-methylphenyl)acrylic acid amide (**2k**)

Yield 52%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.39 (s, 3 H, CH_3), 6.59 (d, $^3J = 15.6$ Hz, 1 H, =CH), 7.24 (m, 2 H, Ar-H), 7.51 (m, 2 H, Ar-H) 7.57 (m, 2 H, Ar-H), 7.69 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.80 (d, $^3J = 15.6$ Hz, 1 H, =CH), 8.45 (dd, $^3J = 9.2$ Hz, $^4J = 2.6$ Hz, 1 H, Ar-H), 8.52 (d, $^4J = 2.6$ Hz, 1 H, Ar-H), 9.09 (d, J = 9.2 Hz, 1 H, Ar-H), 11.45 (s, 1 H, NH).

4.1.3.12. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-trifluoromethylphenyl)acrylic acid amide (**2l**)

Yield 58%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.90 (d, $^3J = 15.8$ Hz, 1 H, =CH), 7.56 (m, 2 H, Ar-H), 7.66 (d, $^3J = 15.8$ Hz, 1 H, =CH), 7.68 (m, 1 H, Ar-H), 7.79 (m, 2 H, Ar-H) 7.81 (m, 2 H, Ar-H), 7.82 (m, 2 H, Ar-H), 8.00 (d, $^3J = 9.0$ Hz, 1 H, Ar-H), 8.16 (d, $^4J = 2.8$ Hz, 1 H, Ar-H), 8.48 (dd, $^3J = 9.0$ Hz, $^4J = 2.8$ Hz, 1 H, Ar-H), 10.92 (s, 1 H, NH).

4.1.3.13. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-fluorophenyl)acrylic acid amide (**2m**)

Yield 79%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.56 (d, $^3J = 15.6$ Hz, 1 H, =CH), 7.13 (m, 2 H, Ar-H), 7.57 (m, 2 H, Ar-H) 7.68 (m, 2 H, Ar-H), 7.71 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.78 (d, $^3J = 15.6$ Hz, 1 H, =CH), 8.47 (dd, $^3J = 9.3$ Hz, $^4J = 2.8$ Hz, 1 H, Ar-H), 8.53 (d, $^4J = 2.8$ Hz, 1 H, Ar-H), 9.08 (d, $^3J = 9.3$ Hz, 1 H, Ar-HH), 11.46 (s, 1 H, NH).

4.1.3.14. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-chlorophenyl)acrylic acid amide (**2n**)

Yield 58%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.61 (d, $^3J = 15.7$ Hz, 1 H, =CH), 7.41 (m, 2 H, Ar-H), 7.53 (m, 2 H, Ar-H) 7.58 (m, 2 H, Ar-H), 7.69 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.77 (d, $^3J = 15.7$ Hz, 1 H, =CH), 8.49 (dd, $^3J = 9.3$ Hz, $^4J = 2.6$ Hz, 1 H, Ar-H), 8.53 (d, $^4J = 2.6$ Hz, 1 H, Ar-H), 9.08 (d, $^3J = 9.3$ Hz, 1 H, Ar-H), 11.49 (s, 1 H, NH).

4.1.3.15. *N*-(2-Benzoyl-4-nitrophenyl)-3-(4-bromophenyl)acrylic acid amide (**2o**)

Yield 68%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.62 (d, $^3J = 15.6$ Hz, 1 H, =CH), 7.45 (m, 2 H, Ar-H), 7.54 (m, 2 H, Ar-H) 7.57 (m, 2 H, Ar-H), 7.68 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 7.75 (d, $^3J = 15.6$ Hz, 1 H, =CH), 8.46 (dd, $^3J = 9.3$ Hz, $^4J = 2.6$ Hz, 1 H, Ar-H), 8.56 (d, $^4J = 2.6$ Hz, 1 H, Ar-H), 9.07 (d, $^3J = 9.3$ Hz, 1 H, Ar-H), 11.48 (s, 1 H, NH).

4.1.4. Compounds **3a–o**

The compounds **3a–o** were prepared from the aromatic nitro compounds **2a–o** according to general procedure 2.

4.1.4.1. *N*-(4-Amino-2-benzoylphenyl)-3-(4-hydroxyphenyl)propionic acid amide (**3a**)

Yield 100%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.63 (t, $^3J = 7.3$ Hz, 2 H, CH_2), 2.95 (t, $^3J = 7.3$ Hz, 2 H, CH_2), 3.64 (s, 2 H, NH_2), 5.65 (s, 1 H, OH), 6.68 (m, 2 H, Ar-H), 6.78 (d, $^4J = 2.7$ Hz, 1 H, Ar-H), 6.91 (dd, $^3J = 8.8$ Hz, $^4J = 2.7$ Hz, 1 H, Ar-H), 7.03 (m, 2H, Ar-H), 7.49 (m, 2 H, Ar-H), 7.57 (m, 1 H, Ar-H), 7.69 (m, 2 H, Ar-H), 8.29 (d, $^3J = 8.8$ Hz, 1 H, Ar-H), 10.19 (s, 1 H, NH).

4.1.4.2. *N*-(4-Amino-2-benzoylphenyl)-3-(4-methoxyphenyl)propionic acid amide (**3b**)

Yield 99%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.64 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 2.98 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 2.98 (s, 2 H, NH_2), 3.71 (s, 3 H, OCH_3), 6.78 (m, 2 H, Ar-H), 6.78 (d, $^4J = 3.0$ Hz, 1 H, Ar-H), 6.91 (dd, $^3J = 9.0$ Hz, $^3J = 3.0$ Hz, 1 H, Ar-H), 7.14 (m, 2 H, Ar-H), 7.48 (m, 2 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.69 (m, 2 H, Ar-H), 8.34 (d, $^3J = 9.0$ Hz, 1 H, Ar-H), 10.21 (s, 1 H, NH).

4.1.4.3. *N*-(4-Amino-2-benzoylphenyl)-3-(4-methylphenyl)propionic acid amid (**3c**)

Yield 91%. ^{1}H NMR ($CDCl_3$) δ (ppm) = 2.23 (s, 3 H, CH_3), 2.54 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 2.99 (t, $^3J = 7.5$ Hz, 2 H, CH_2), 3.61 (s, 2 H, NH_2), 6.78 (d, $^4J = 3.0$ Hz, 1 H, Ar-H), 6.91 (dd, $^3J = 9.0$ Hz, $^4J = 3.0$ Hz, 1 H, Ar-H), 7.05 (m, 2H, Ar-H), 7.10 (m, 2H, Ar-H), 7.48 (m, 2 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.70 (m, 2 H, Ar-H), 8.33 (d, $^3J = 9.0$ Hz, 1 H, Ar-H), 10.19 (s, 1 H, NH).

4.1.4.4. *N*-(4-amino-2-benzoylphenyl)-3-(4-trifluoromethylphenyl)propionic acid amide (**3d**)

Yield 92%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.23 (t, $^3J = 8.1$ Hz, 2 H, CH_2), 2.63 (t, $^3J = 8.1$ Hz, 2 H, CH_2), 5.22 (s, 2 H, NH_2), 6.59 (d, $^4J = 2.6$ Hz, 1 H, Ar-H), 6.73 (dd, $^3J = 8.5$ Hz, $^4J = 2.6$ Hz, 1 H, Ar-H), 7.05 (d, J = 8.5 Hz, 1 H, Ar-H), 7.31 (m, 2 H, Ar-H), 7.48 (m, 2 H, Ar-H), 7.58 (m, 3 H, Ar-H), 7.66 (m, 2 H, Ar-H), 9.50 (s, 1 H, NH).

4.1.4.5. *N*-(4-Amino-2-benzoylphenyl)-3-(3-trifluoromethylphenyl)propionic acid amide (**3e**)

Yield 92%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.23 (t, ^{3}J = 8.2 Hz, 2 H, CH_2), 2.66 (t, ^{3}J = 8.2 Hz, 2 H, CH_2), 5.22 (s, 2 H, NH_2), 6.60 (d, ^{4}J = 2.7 Hz, 1 H, Ar-H), 6.74 (dd, ^{3}J = 8.5 Hz, ^{4}J = 2.7 Hz, 1 H, Ar-H), 7.06 (d, ^{3}J = 9.0 Hz, 1 H, Ar-H), 7.40 (m, 2 H, Ar-H), 7.47 (m, 2 H, Ar-H) 7.49 (m, 2 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.66 (m, 2 H, Ar-H), 9.51 (s, 1 H, NH).

4.1.4.6. *N*-(4-amino-2-benzoylphenyl)-3-(2-trifluoromethylphenyl)propionic acid amide (**3f**)

Yield 77%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.18 (t, ^{3}J = 7.9 Hz, 2 H, CH_2), 2.69 (t, ^{3}J = 7.9 Hz, 2 H, CH_2), 5.26 (s, 2 H, NH_2), 6.62 (d, ^{4}J = 2.6 Hz, 1 H, Ar-H), 6.74 (dd, ^{3}J = 8.6 Hz, ^{4}J = 2.6 Hz, 1 H, Ar-H), 7.07 (d, ^{3}J = 8.6 Hz, 1 H, Ar-H), 7.34 (m, 2 H, Ar-H), 7.47 (m, 2 H, Ar-H) 7.59 (m, 3 H, Ar-H), 7.67 (m, 2 H, Ar-H), 9.54 (s, 1 H, NH).

4.1.4.7. *N*-(4-Amino-2-benzoylphenyl)-3-(4-fluorophenyl)propionic acid amide (**3g**)

Yield 94%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.65 (t, ^{3}J = 7.5 Hz, 2 H, CH_2), 3.01 (t, ^{3}J = 7.5 Hz, 2 H, CH_2), 3.61 (s, 2 H, NH_2), 6.79 (d, ^{4}J = 3.0 Hz, 1 H, Ar-H), 6.91 (m, 3 H, Ar-H), 7.17 (m, 2 H, Ar-H), 7.48 (m, 2 H, Ar-H), 7.60 (m, 1 H, Ar-H), 7.69 (m, 2 H, Ar-H), 8.31 (d, ^{3}J = 8.5 Hz, 1 H, Ar-H), 10.22 (s, 1 H, NH).

4.1.4.8. *N*-(4-Amino-2-benzoylphenyl)-3-(4-chlorophenyl)propionic acid amide (**3h**)

Yield 95%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.65 (t, ^{3}J = 8.0 Hz, 2 H, CH_2), 3.00 (t, ^{3}J = 8.0 Hz, 2 H, CH_2), 3.67 (s, 2 H, NH_2), 6.79 (d, ^{4}J = 2.8 Hz, 1H, Ar-H), 6.91 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.8 Hz, 1 H, Ar-H), 7.16 (m, 2 H, Ar-H), 7.18 (m, 2 H, Ar-H), 7.49 (m, 2 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.73 (m, 2 H, Ar-H), 8.31 (d, ^{3}J = 8.8 Hz, 1 H, Ar-H), 10.22 (s, 1 H, NH).

4.1.4.9. *N*-(4-Amino-2-benzoylphenyl)-3-(4-bromophenyl)propionic acid amide (**3i**)

Yield 92%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.65 (t, ^{3}J = 7.6 Hz, 2 H, CH_2), 2.99 (t, ^{3}J = 7.6 Hz, 2 H, CH_2), 3.62 (s, 2 H, NH_2), 6.79 (d, ^{4}J = 2.8 Hz, 1H, Ar-H), 6.91 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.8 Hz, 1 H, Ar-H), 7.10 (m, 2 H, Ar-H), 7.32 (m, 2 H, Ar-H), 7.48 (m, 2 H, Ar-H), 7.60 (m, 1 H, Ar-H), 7.69 (m, 2 H, Ar-H), 8.32 (d, ^{3}J = 8.8 Hz, 1 H, Ar-H), 10.24 (s, 1 H, NH).

4.1.4.10. *N*-(4-Amino-2-benzoylphenyl)-3-(4-methoxyphenyl)acrylic acid amide (**3j**)

Yield 90%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.63 (s, 2 H, NH_2), 3.84 (s, 3 H, OCH_3), 6.45 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 6.84 (d, ^{4}J = 2.8 Hz, 1 H, Ar-H), 6.91 (m, 2 H, Ar-H), 6.95 (dd, ^{3}J = 8.9 Hz, ^{4}J = 2.8 Hz, 1 H, Ar-H), 7.47 (m, 2 H, Ar-H), 7.51 (m, 2 H, Ar-H), 7.60 (m, 1 H, Ar-H), 7.67 (d, ^{3}J = 15.6 Hz, 1H, =CH), 7.73 (m, 2 H, Ar-H), 8.54 (d, ^{3}J = 8.9 Hz, 1 H, Ar-H), 10.60 (s, 1 H, NH).

4.1.4.11. *N*-(4-Amino-2-benzoylphenyl)-3-(4-methylphenyl)acrylic acid amide (**3k**)

Yield 99%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.37 (s, 3 H, CH_3), 3.64 (s, 2 H, NH_2), 6.53 (d, ^{3}J = 19.5 Hz, 1 H, =CH), 6.85 (d, ^{4}J = 2.7 Hz, 1 H, Ar-H), 6.96 (dd, ^{3}J = 9.0 Hz, ^{4}J = 2.7 Hz, 1 H, Ar-H), 7.18 (m, 2 H, Ar-H), 7.46 (m, 2 H, Ar-H), 7.49 (m, 2 H, Ar-H), 7.60 (m, 1 H, Ar-H), 7.64 (d, ^{3}J = 19.5Hz, 1 H, =CH), 7.75 (m, 2 H, Ar-H), 8.55 (d, ^{3}J = 9.0 Hz, 1 H, Ar-H), 10.64 (s, 1 H, NH).

4.1.4.12. *N*-(4-Amino-2-benzoylphenyl)-3-(4-trifluoromethylphenyl)acrylic acid amide (**3l**)

Yield 99%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.67 (s, 2 H, NH_2), 6.66 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 6.87 (d, ^{4}J = 2.8 Hz, 1 H, Ar-H), 6.97 (dd, ^{3}J = 9.0 Hz, ^{4}J = 2.8 Hz, 1 H, Ar-H), 7.50 (m, 2 H, Ar-H), 7.52 (m, 4 H, Ar-H), 7.64 (m, 1 H, Ar-H), 7.72 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 7.75 (m, 2 H, Ar-H), 8.53 (d, ^{3}J = 9.0 Hz, 1 H, Ar-H), 10.74 (s, 1 H, NH).

4.1.4.13. *N*-(4-Amino-2-benzoylphenyl)-3-(4-fluorophenyl)acrylic acid amide (**3m**)

Yield 99%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.70 (s, 2 H, NH_2), 6.50 (d, ^{3}J = 15.5 Hz, 1 H, =CH), 6.85 (d, ^{4}J = 2.8Hz, 1 H, Ar-H), 6.96 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.8 Hz, 1 H, Ar-H), 7.07 (m, 2 H, Ar-H), 7.49 (m, 4 H, Ar-H), 7.58 (m, 1 H, Ar-H), 7.65 (d, ^{3}J = 15.5 Hz, 1 H, =CH), 7.74 (m, 2 H, Ar-H), 8.54 (d, ^{3}J = 8.8 Hz, 1 H, Ar-H), 10.65 (s, 1 H, NH).

4.1.4.14. *N*-(4-Amino-2-benzoylphenyl)-3-(4-chlorophenyl)acrylic acid amide (**3n**)

Yield 97%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.68 (s, 2 H, NH_2), 6.55 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 6.86 (d, ^{4}J = 2.7 Hz, 1 H, Ar-H), 6.95 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.7 Hz, 1 H, Ar-H), 7.36 (m, 2 H, Ar-H), 7.48 (m, 4 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.65 (d, J = 15.6 Hz, 1 H, =CH), 7.73 (m, 2 H, Ar-H), 8.53 (d, ^{3}J = 8.8 Hz, 1 H, Ar-H), 10.67 (s, 1 H, NH).

4.1.4.15. *N*-(4-Amino-2-benzoylphenyl)-3-(4-bromophenyl)acrylic acid amide (**3o**)

Yield 77%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.67 (s, 2 H, NH_2), 6.57 (d, ^{3}J = 15.7 Hz, 1 H, =CH), 6.86 (d, ^{4}J = 2.7 Hz, 1 H, Ar-H), 6.96 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.7 Hz, 1 H, Ar-H), 7.42 (m, 2 H, Ar-H), 7.50 (m, 4 H, Ar-H), 7.59 (m, 1 H, Ar-H), 7.64 (d, ^{3}J = 15.7 Hz, 1 H, =CH), 7.73 (m, 2 H, Ar-H), 8.53 (d, ^{3}J = 8.8 Hz, 1 H, Ar-H), 10.68 (s, 1 H, NH).

4.1.5. Compounds **4b–j** *and* **5b–h**

The compounds **4b–j** and **5b–h** were prepared from 3-[5-(nitrophenyl)-2-furyl]acrylic acid chloride and the appropriate aromatic amines **3a–o** according to general procedure 1.

4.1.5.1. (*E*)-*N*-[3-Benzoyl-4-[3-(4-hydroxyphenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4b**).

Yield 38%; m.p. 224 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3378, 3367, 1666, 1623, 1597, 1546, 1514, 1402, 1333, 1245, 853, 797, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.22 (t, ^{3}J = 8.3 Hz, 2 H, CH_2), 2.47 (t, ^{3}J = 8.3 Hz, 2 H, CH_2), 6.63 (m, 2 H, Ar-H), 6.79 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 6.89 (m, 2 H, Ar-H), 7.06 (d, ^{3}J = 3.6 Hz, 1 H, Ar-H), 7.43 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 7.46 (m, 3 H, Ar-H), 7.52 (d, ^{3}J = 9.0 Hz, 1 H, Ar-H), 7.64 (m, 1 H, Ar-H), 7.69 (m, 2 H, Ar-H), 7.80 (d, ^{4}J = 2.3 Hz, 1 H, Ar-H), 7.90 (dd, ^{3}J = 9.0, ^{4}J = 2.3 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 9.11 (s, 1 H, OH), 9.94 (s, 1 H, NH), 10.48 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 30.14, 38.08 (2 CH_2), 113.29, 115.49, 117.80, 120.63 (5 Ar-C), 121.24 (=CH), 122.57, 124.90, 124.95, 125.00 (6 Ar-C), 127.20 (2 Ar-C and 1 =CH), 128.64, 129.34, 130.03, 131.43, 133.02, 135.61, 137.61, 146.87, 152.70, 152.87, 155.88 (15 Ar-C), 163.59, 171,29 (2 C=O), 195.23 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 602 (100) [M^+ + H], 537 (9), 469 (9), 454 (28), 316 (16), 295 (15), 242 (16), 228 (13), 218 (15), 159 (22), 122 (16).

4.1.5.2. (*E*)-*N*-[3-Benzoyl-4-[3-(4-methoxyphenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4c**)

Purification: column chromatography (ethylacetate:n-hexane 2 : 3); yield 14%; m.p. 200 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3381, 1677, 1628, 1594, 1550, 1510, 1331, 1244, 851, 799, 750. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.25 (t, ^{3}J = 8.6 Hz, 2 H, CH_2), 2.51 (t, ^{3}J = 8.6 Hz, 2 H, CH_2), 3.70 (s, 3 H, OCH_3), 6.81 (m, 3 H, Ar-H and =CH), 7.03 (m, 2 H, Ar-H), 7.07 (d, ^{3}J = 3.2 Hz, 1 H, Ar-H), 7.44 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 7.46 (d, ^{3}J = 3.2 Hz, 1 H, Ar-H), 7.53 (m, 2 H, Ar-H), 7.65 (m, 1 H, Ar-H), 7.71 (m, 3 H, Ar-H), 7.81 (d, ^{4}J = 2.4 Hz, 1 H, Ar-H), 7.91 (dd, ^{3}J = 8.4 Hz, ^{4}J = 3.2 Hz, 1 H, Ar-H), 8.02 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H) 9.97 (s, 1 H, NH), 10.50 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 30.09, 40.10 (2 CH_2), 55.40 (OCH_3), 113.29, 114.16, 117.82, 120.65 (5 Ar-C), 121.23 (=CH), 122.59, 124.96, 125.01 (5 Ar-C), 127.21 (=CH), 128.67, 129.46, 130.06, 132.04, 133.05, 133.25, 135.62, 136.00, 137.61, 146.87, 152.71, 152.88, 157.95 (18 Ar-C), 163.61, 170.95 (2 C=O), 195.26 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 616 (87) [M^+ + H], 594 (31), 577 (67), 482 (50), 477 (33), 460 (100), 242 (30).

4.1.5.3. (*E*)-*N*-[3-Benzoyl-4-[3-(4-methylphenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4d**)

Yield 9%; m.p. 206 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3378, 1677, 1628, 1596, 1546, 1510, 1401, 1331, 1245, 851, 799, 751.^{1}H NMR (DMSO-d_6): δ (ppm) = 2.26 (t, ^{3}J = 8.6 Hz, 2 H, CH_2), 2.51 (t, ^{3}J = 8.6 Hz, 2 H, CH_2), 3.34 (s, 3 H, CH_3), 6.79 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 6.99 (m, 2 H, Ar-H), 7.03 (m, 3 H, Ar-H), 7.43 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 7.50 (m, 3 H, Ar-H), 7.53 (d, ^{3}J = 8.4 Hz, 1 H, Ar-H), 7.65 (m, 1 H, Ar-H), 7.71 (m, 2 H, Ar-H), 7.81 (d, ^{4}J = 2.8 Hz, 1 H, Ar-H), 7.90 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.8 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H) 9.97 (s, 1 H, NH), 10.49 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 20.57 (CH_3), 30.10, 37.54 (2 CH_2), 112.79, 117.34, 120.72 (3 Ar-C), 120.73 (=CH), 122.08, 124.35, 124.45, 124.51, 126.72 (8 Ar-C), 127.89 (1 Ar-C and 1 =CH), 128.15, 128.78, 129.59, 131.52, 132.52, 134.71, 135.16, 137.11, 137.77, 146.34, 152.21, 152.38 (16 Ar-C), 163.11, 170.13 (2 C=O), 195.26 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 600 (100) [M^+ + H], 454 (31), 316 (24), 279 (29), 242 (41), 217 (16).

4.1.5.4. (*E*)-*N*-[3-Benzoyl-4-[3-(4-trifluoromethylphenyl)propionylamino]-phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4e**)

Yield 81%; m.p. 226 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3418, 3084, 2965, 1685, 1674, 1630, 1597, 1562, 1509, 1328, 1232, 1165, 1107, 850, 794, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.36 (t, ^3J = 7.9 Hz, 2 H, CH_2), 2.70 (t, ^3J = 7.9 Hz, 2 H, CH_2), 6.79 (d, ^3J = 15.6 Hz, 1 H, =CH), 7.05 (d, ^3J = 3.7 Hz, 1 H, Ar-H), 7.35 (m, 2 H, Ar-H), 7.42 (m, 2 H, Ar-H and =CH), 7.45 (d, ^3J = 8.7 Hz, 1 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.59 (m, 2 H, Ar-H), 7.66 (m, 1 H, Ar-H), 7.71 (m, 2 H, Ar-H), 7.81 (d, ^4J = 2.4 Hz, 1 H, Ar-H), 7.91 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 1 H, Ar-H), 8.02 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 9.99 (s, 1 H, NH), 10.48 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 30.72, 37.27 (2 CH_2), 113.42, 117.99, 120.67 (3 Ar-C), 121.30 (=CH), 122.63, 125.06 (3 Ar-C), 125.12 (q, ^1J = 272.8 Hz, CF_3), 125.13 (3 Ar-C), 125.65 (q, ^3J = 3.5 Hz, 2 C̲H-C-CF_3), 127.34 (=CH), 127.35 (q, ^2J = 30.0 Hz, C̲-CF_3), 128.79, 129.54, 130.21, 131.94, 132.13, 133.21, 135.75, 136.23, 137.65, 146.50, 146.96, 152.83, 152.98 (16 Ar-C), 163.73, 170.40, (2 C=O) 195.31 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 654 [M^+ + H] (61), 527 (33), 525 (54), 518 (30), 510 (36), 497 (100), 479 (30), 450 (21), 319 (23), 315 (15), 301 (7), 294 (22), 288 (15), 242 (75), 217 (9).

4.1.5.5. (*E*)-*N*-[3-Benzoyl-4-[3-(3-trifluoromethylphenyl)propionylamino]-phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4f**)

Yield 49%; m.p. 214 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3381, 3312, 3111, 1685, 1677, 1627, 1596, 1551, 1510, 1401, 1332, 1245, 1161, 1122, 852, 827, 799, 751.^{1}H NMR (DMSO-d_6): δ (ppm) = 2.36 (t, ^3J = 8.3 Hz, 2 H, CH_2), 2.72 (t, ^3J = 8.3 Hz, 2 H, CH_2), 6.79 (d, ^3J = 15.5 Hz, 1 H, =CH), 7.06 (d, ^3J = 3.7 Hz, 1 H, Ar-H), 7.43 (d, ^3J = 15.5 Hz, 1 H, =CH), 7.46 (m, 2 H, Ar-H), 7.47 (m, 2 H, Ar-H), 7.50 (m, 2 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.64 (m, 1 H, Ar-H), 7.70 (m, 2H, Ar-H), 7.80 (d, ^4J = 2.5 Hz, 1 H, Ar-H), 7.91 (dd, ^3J = 8.7, ^4J = 2.5 Hz, 1 H, Ar-H), 8.02 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.00 (s, 1 H, NH), 10.48 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 32.59, 39.50 (2 CH_2), 115.44, 120.00, 122.71 (3 Ar-C), 123.32 (=CH), 124.65 (1 Ar-C), 125.29 (q, ^3J = 3.5 Hz, 1 C̲H−C−CF_3), 126.85 (q, ^1J = 273.9 Hz, CF_3), 127.07, 127.14 (5 Ar-C), 127.25 (q, ^3J = 3.5 Hz, 1 C̲H−C−CF_3), 129.35 (=CH), 130.79 (2 Ar-C), 131.60 (q, ^2J = 31.3 Hz, C̲−CF_3), 132.22, 131.85, 134.00, 134.04, 135.00, 135.20, 137.74, 138.20, 139.66, 145.01, 148.97, 154.83, 154.99 (14 Ar-C), 165.74, 172.45, (2 C=O) 197.32 (Ph-C=O-Ph). MS (ESI−): m/z (%) = 653 [M^+] (11), 652 (13), 402 (14), 397 (9), 381 (9), 270 (9), 261 (43), 254 (13), 248 (11), 239 (12), 217 (21), 187 (21), 165 (35), 161 (26), 145 (13), 113 (100), 111 (88).

4.1.5.6. (*E*)-*N*-[3-Benzoyl-4-[3-(2-trifluoromethylphenyl)propionylamino]-phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4g**)

Yield 63%; m.p. 221 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3385, 1703, 1679, 1654, 1629, 1596, 1553, 1514, 1398, 1330, 1312, 1230, 1150, 1109, 853, 790, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.31 (t, ^3J = 8.4 Hz, 2 H, CH_2), 2.76 (t, ^3J = 8.4 Hz, 2 H, CH_2), 6.80 (d, ^3J = 15.5 Hz, 1 H, =CH), 7.06 (d, ^3J = 3.7 Hz, 1 H, Ar-H), 7.41 (d, ^3J = 15.5 Hz, 1 H, =CH), 7.42 (m, 2H, Ar-H), 7.46 (m, 2 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.58 (m, 1 H, Ar-H), 7.64 (m, 2 H, Ar-H), 7.70 (m, 2 H, Ar-H), 7.83 (d, ^4J = 2.4 Hz, 1 H, Ar-H), 7.94 (dd, ^3J = 8.7, ^4J = 2.4 Hz, 1 H, Ar-H), 8.03 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.00 (s, 1 H, NH), 10.50 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 26.75, 36.81 (2 CH_2), 112.59, 117.17, 119.83 (3 Ar-C), 120.46 (=CH), 121.81, 124.22 (3 Ar-C), 124.71 (q, ^1J = 177.0 Hz, CF_3), 124.30 (3 Ar-C), 125.37 (q, ^3J = 3.5 Hz, 1 C̲H−C−CF_3), 126.39, 126.52 (2 Ar-C), 126.78 (q, ^2J = 31.3 Hz, C̲−CF_3), 127.93, 129.28 (4 Ar-C), 130.79 (=CH), 131.02, 131.40, 132.31, 132.36, 134.89, 135.15, 136.80, 139.22, 146.11, 151.99, 152.13 (11 Ar-C), 162.90, 169.33 (2 C=O), 194.42 (Ph-C=O-Ph). MS (ESI-): m/z (%) = 653 [M^+] (9), 261 (14), 238 (8), 231 (13), 217 (21), 187 (15), 181 (16), 165 (25), 161 (35), 155 (21), 113 (100), 111 (59).

4.1.5.7. (*E*)-*N*-(3-Benzoyl-4-[3-(4-fluorophenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4h**)

Yield 99%, mp: 220 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3370, 1685, 1628, 1596, 1548, 1509, 1330, 1224, 851, 798, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.28 (t, ^3J = 8.4 Hz, 2 H, CH_2), 2.57 (t, ^3J = 8.4 Hz, 2 H, CH_2), 6.80 (d, ^3J = 15.4 Hz, 1 H, =CH), 7.01 (m, 2 H, Ar-H), 7.07 (d, ^3J = 3.6 Hz, 1 H, Ar-H), 7.15 (m, 2 H, Ar-H), 7.44 (d, ^3J = 15.4 Hz, 1 H, =CH), 7.45 (d, ^3J = 8.6 Hz, 1 H, Ar-H), 7.47 (d, ^3J = 3.6 Hz, 1 H, Ar-H), 7.53 (m, 2 H, Ar-H), 7.65 (m, 1 H, Ar-H), 7.71 (m, 2 H, Ar-H), 7.81 (d, ^4J = 2.0 Hz, 1 H, Ar-H), 7.90 (dd, ^3J = 8.6, ^4J = 2.0 Hz, 1 H, Ar-H), 8.02 (m, 2 H, Ar-H), 8.35 (m, 2 H, Ar-H), 9.98 (s, 1 H, NH), 10.50 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 29.59, 37.41 (2 CH_2), 112.80 (1 Ar-C), 114.87 (d, ^2J = 20.9 Hz, 2 C̲H−CF), 117.36, 120.11 (2 Ar-C), 120.72 (=CH), 122.05, 124.46, 124.51 (5 Ar-C), 126.73 (=CH), 128.17, 129.60, 129.78, 129.85, 131.44, 132.58, 135.16, 135.57, 137.01, 137.08, 146.36, 152.23, 152.38 (17 Ar-C), 161.35 (d, ^1J = 240.5 Hz, CF), 163.12, 169.98 (2 C=O), 194.74 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 604 (100) [M^+ + H], 557 (22), 454 (23), 333 [$C_{19}H_{13}N_2O_4^+$] (15), 316 (23), 305 (15), 279 (43), 261 (30), 242 (56), 217 (38), 195 (26), 130 (18).

4.1.5.8. *N*-[3-Benzoyl-4-[3-(4-chlorophenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4i**)

Yield 61%; mp: 199 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 1682, 1629, 1597, 1558, 1509, 1400, 1330, 1288, 1230, 852, 788, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.30 (t, ^3J = 8.3 Hz, 2 H, CH_2), 2.59 (t, ^3J = 8.3 Hz, 2 H, CH_2), 6.78 (d, ^3J = 15.6 Hz, 1 H, =CH), 7.06 (d, ^3J = 3.4 Hz, 1 H, Ar-H), 7.14 (m, 2 H, Ar-H), 7.28 (m, 2 H, Ar-H), 7.43 (d, ^3J = 15.6 Hz, 1 H, =CH), 7.45 (d, ^3J = 3.4 Hz, 1 H, Ar-H), 7.46 (d, ^3J = 8.7 Hz, 1 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.64 (m, 1 H, Ar-H), 7.70 (m, 2 H, Ar-H), 7.80 (d, ^4J = 2.4 Hz, 1 H, Ar-H), 7.91 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 9.97 (s, 1 H, NH), 10.48 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 29.64, 37.02 (2 CH_2), 112.74, 117.25, 120.12 (3 Ar-C), 120.71 (=CH), 122.05, 124.42, 124.45 (6 Ar-C), 126.69 (=CH), 128.08, 128.12, 129.53, 129.91, 130.45, 130.40, 131.44, 132.53, 135.11, 135.52, 137.06, 139.89, 146.34, 152.19, 152.35 (19 Ar-C), 163.09, 169.87 (2 C=O), 194.73 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 623 [^{37}Cl M^+ + H] (15), 622 [^{37}Cl M^+] (43), 621 [M^+ + H] (48), 620 [M^+] (100), 454 (28), 401 (9), 377 (11), 363 (9), 349 (13), 333 (24), 319 (11), 305 (30), 271 (16), 261 (17), 242 (7), 225 (10).

4.1.5.9. (*E*)-*N*-[3-Benzoyl-4-[3-(4-bromophenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4j**)

Yield 52%; m.p. 206 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3380, 1677, 1627, 1596, 1548, 1510, 1401, 1332, 1245, 1225, 852, 799, 751. ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.31 (t, ^3J = 7.7 Hz, 2H, CH_2), 2.56 (t, ^3J = 7.7 Hz, 2 H, CH_2), 6.78 (d, ^3J = 15.6 Hz, 1 H, =CH), 7.08 (m, 3 H, Ar-H), 7.41 (m, 2 H, Ar-H), 7.43 (d, ^3J = 15.6 Hz, 1 H, =CH), 7.45 (d, ^3J = 3.4 Hz, 1 H, Ar-H), 7.46 (d, ^3J = 8.7 Hz, 1 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.65 (m, 1 H, Ar-H), 7.70 (m, 2 H, Ar-H), 7.71 (d, ^4J = 2.4 Hz, 1 H, Ar-H), 7.90 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 1 H, Ar-H), 8.02 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 9.97 (s, 1 H, NH), 10.49 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 29.60, 36.82 (2 CH_2), 112.59, 116.95, 118.72, 120.12 (4 Ar-C), 120.71 (=CH), 122.11, 124.31, 126.59 (5 Ar-C), 127.93 (=CH), 128.23, 129.29, 129.42, 130.20, 130.82, 130.92, 131.07, 131.52, 135.00, 135.35, 137.35, 140.22, 146.31, 152.09, 152.29 (19 Ar-C), 162.99, 169.77 (2 C=O), 194.75 (Ph-C=O-Ph). MS (ESI): m/z (%) = 667 [^{81}Br M^+ + H] (43), 666 [^{81}Br M^+] (100), 665 [M^++H] (42), 664 [M^++] (85), 454 (28), 349, (28), 333 (28), 319 (21), 305 (29), 271 (17), 242 (16), 225 (11).

4.1.5.10. (*E*)-*N*-(3-Benzoyl-4-[3-(4-methoxyphenyl)acryloylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**5b**)

Yield 33%; m.p. 277 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3369, 3117, 1677, 1682, 1627, 1600, 1547, 1509, 1331, 1256, 1161, 853, 805, 753. ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.78 (s, 3 H, OCH_3), 6.55 (d, ^3J = 15.7 Hz, 1 H, =CH), 6.79 (d, ^3J = 15.5 Hz, 1 H, =CH), 6.97 (m, 2 H, Ar-H), 7.05 (d, ^3J = 3.6 Hz, 1 H, Ar-H), 7.37 (d, ^3J = 15.7 Hz, 1 H, =CH), 7.43 (d, ^3J = 15.5 Hz, 1 H, =CH), 7.44 (d, ^3J = 3.6 Hz, 1 H, Ar-H), 7.51 (m, 2 H, Ar-H), 7.53 (m, 2 H, Ar-H), 7.63 (d, ^3J = 8.8 Hz, 1 , Ar-H), 7.64 (m, 1 H, Ar-H), 7.78 (m, 2 H, Ar-H), 7.80 (d, ^4J = 2.5 Hz, 1 H, Ar-H), 7.92 (dd, ^3J = 8.8, ^4J = 2.5 Hz, 1 H, Ar-H), 8.02 (m, 2 H, Ar-H), 8.33 (m, 2 H, Ar-H) 10.20 (s, 1 H, NH), 10.48 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 55.70 (OCH_3), 113.25, 114.83, 117.75 (4 Ar-C), 119.43 (=CH), 120.75 (1 Ar-C), 121.23 (=CH), 122.54, 124.45, 124.93, 124.96, 127.18 (7 Ar-C), 127.60 (=CH), 128.64, 129.83, 130.23, 131.34, 132.38, 133.01, 135.67, 137.60 (12 Ar-C), 140.61 (=CH), 146.84, 152.69, 152.87, 161.05 (4 Ar-C), 163.59, 164.22 (2 C=O), 195.28 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 614 (100) [M^+ + H], 495 (17), 473 (26), 349 (16), 333 (21), 305 (23), 271 (13), 261 (30), 242 (16), 217 (16).

4.1.5.11. (*E*)-*N*-(3-Benzoyl-4-[3-(4-methylphenyl)acryloylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**5c**)

Yield 73%; m.p. 271 °C. IR (KBr): $\tilde{\nu}$ = 3380, 1683, 1623, 1596, 1546, 1509, 1399, 1332, 1162, 853, 807, 752. ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.33 (s, 3 H, CH_3), 6.62 (d, ^3J = 15.9 Hz, 1 H, =CH), 6.78 (d, ^3J = 15.9 Hz, 1 H, =CH), 7.03 (d, ^3J = 3.1 Hz, 1 H, Ar-H), 7.20 (m, 2 H, Ar-H), 7.36 (d, ^3J = 15.9 Hz, 1 H, =CH), 7.41 (d, ^3J = 15.9 Hz, 1 H, =CH), 7.49 (m, 3 H, Ar-H), 7.53 (m, 4 H, Ar-H), 7.63 (m, 3 H, Ar-H), 7.77 (dd, ^3J = 11.4, ^4J = 4.0 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.33 (m, 2 H, Ar-H) 10.23 (s, 1 H, NH), 10.49 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 21.59 (CH_3), 113.30 (1 Ar-C), 118.06 (=CH), 120.74, 121.05 (2 Ar-C), 121.35 (=CH), 122.62, 124.78, 125.11, 125.19 (6 Ar-C), 127.39 (=CH), 128.34, 128.85, 130.17, 130.43, 131.52, 132.30, 132.44, 133.26, 135.60, 135.77, 137.66, 140.25 (16 Ar-C), 140.97 (=CH), 147.01, 152.86, 153.02 (3 Ar-C), 163.77, 164.21 (2 C=O), 195.32 (Ph-C=O-Ph). MS (ESI−): m/z (%) = 596 (64) [M^+ + H], 441 (16), 391 (16), 367 (20), 333 (28), 300 (20), 284 (32), 272 (20), 260 (24), 255 (36), 231 (20), 187 (32), 165 (44), 147 (48), 111 (100).

4.1.5.12. (*E*)-*N*-[3-Benzoyl-4-[3-(4-trifluoromethylphenyl)acryloylamino]-phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**5d**)

Yield 47%; m.p. 246 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3388, 3292, 3100, 1683, 1671, 1628, 1599, 1402, 1322, 1286, 1330, 1286, 1234, 1196, 1071, 1127, 1097, 851, 831, 798, 752. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.71 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 6.79 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 7.07 (d, ^{3}J = 3.3 Hz, 1 H, Ar-H), 7.43 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 7.46 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 7.47 (d, ^{3}J = 3.3 Hz, 1 H, Ar-H), 7.54 (m, 2 H, Ar-H), 7.65 (m, 2 H, Ar-H), 7.78 (m, 4 H, Ar-H), 7.81 (m, 3 H, Ar-H), 7.95 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.4 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.38 (s, 1 H, NH), 10.53 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 112.80, 117.41, 120.05 (3 Ar-C), 120.62 (=CH), 121.95 (1 Ar-C), 122.68 (q, ^{1}J = 256.4 Hz, CF_3), 124.30 (1 Ar-C and 1 =CH), 124.49, 124.57 (4 Ar-C), 125.79 (q, ^{3}J = 3.9 Hz, 2 CH–C–CF_3), 126.80 (=CH), 128.25, 128.34, 129.84, 131.44 (8 Ar-C), 131.96 (q, ^{2}J = 31.8 Hz, C–CF_3), 132.76, 135.06, 135.15, 135.48, 136.96 (5 Ar-C), 138.63 (=CH), 146.37, 152.23, 152.39 (3 Ar-C), 163.03, 163.20 (2 C=O), 194.79 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 652 [M^+ + H] (100), 455 (13), 407 (6), 363 (6), 349 (11), 338 (6), 333 (11), 305 (9), 271 (16), 225 (9).

4.1.5.13. (*E*)-*N*-[3-Benzoyl-4-[3-(4-fluorophenyl)acryloylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]-acrylic acid amide (**5e**)

Yield 68%; m.p. 304 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3375, 1677, 1631, 1599, 1545, 1509, 1401, 1335, 1241, 1173, 854, 800, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.64 (d, ^{3}J = 15.8 Hz, 1 H, =CH), 6.79 (d, ^{3}J = 15.4 Hz, 1H, =CH), 7.06 (d, ^{3}J = 3.8 Hz, 1 H, Ar-H), 7.25 (m, 2 H, Ar-H), 7.41 (d, ^{3}J = 15.8 Hz, 1 H, =CH), 7.43 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 7.47 (d, ^{3}J = 3.8 Hz, 1 H, Ar-H), 7.53 (m, 2 H, Ar-H), 7.61 (m, 4 H, Ar-H), 7.78 (m, 2 H, Ar-H), 7.80 (d, ^{4}J = 2.2 Hz, 1 H, Ar-H), 7.93 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.2 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.29 (s, 1 H, NH), 10.51 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 112.75 (1 Ar-C), 115.83 (d, ^{2}J = 27.2 Hz, 2 CH–C–F), 117.26, 120.21 (2 Ar-C), 120.69, 121.34 (2 =CH), 122.01, 124.00, 124.08, 124.43 (7 Ar-C), 126.71 (=CH), 128.14, 129.79, 129.91, 131.13, 131.90, 132.55, 135.16, 135.34, 137.05 (13 Ar-C), 139.17 (1 =CH), 146.33, 152.21, 152.37 (3 Ar-C), 163.11, 163.35 (2 C=O), 163.50 (d, ^{1}J = 240.5 Hz, CF), 194.74 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 602 (100) [M^+ + H], 483 (24), 461 (47), 455 (28), 425 (16), 337 (30), 327 (16), 315 (40), 305 (28), 261 (30), 242 (96), 217 (67), 195 (23), 149 (28), 130 (29).

4.1.5.14. (*E*)-*N*-[3-Benzoyl-4-[3-(4-chlorophenyl)acryloylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]-acrylic acid amide (**5f**)

Yield 33%; m.p. 165 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3398, 2967, 1683, 1668, 1626, 1598, 1512, 1403, 1331, 1284, 1231, 1180, 851, 818, 752. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.70 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 6.79 (d, ^{3}J = 15.5 Hz, 1 H, =CH), 7.06 (d, ^{3}J = 3.5 Hz, 1 H, Ar-H), 7.40 (d, ^{3}J = 15.6 Hz, 1 H, =CH), 7.46 (m, 2 H, 1 Ar-H and 1 =CH), 7.48 (m, 2 H, Ar-H), 7.53 (m, 2 H, Ar-H), 7.64 (m, 4 H, Ar-H), 7.77 (m, 2 H, Ar-H), 7.80 (d, ^{4}J = 2.2 Hz, 1 H, Ar-H), 7.94 (dd, ^{3}J = 8.7 Hz, ^{4}J = 2.2 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.30 (s, 1 H, NH), 10.50 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6C): δ (ppm) = 112.87, 117.47, 120.12 (3 Ar-H), 120.71, 122.00 (2 =CH), 122.30, 124.22, 124.50, 124.58 (7 Ar-H), 126.80 (=CH), 128.25, 129.00, 129.45, 129.84, 131.30, 131.50, 132.68, 133.53, 134.24, 135.16, 135.48, 137.00 (16 Ar-C), 139.03 (=CH), 146.40, 152.26, 152.40 (3 Ar-C), 163.17, 163.27 (2 C=O), 194.71 (Ph–C=O–Ph). MS (ESI+): m/z (%) = 621 [^{37}Cl M^+ + H] (14), 620 [^{37}Cl M^+] (43), 619 [M^+ + H] (43), 618 [M^+] (100), 478 (9), 456 (13), 407 (11), 377 (11), 364 (11), 349 (21), 333 (30), 319 (13), 305 (21), 271 (20), 261 (21), 242 (28), 225 (13).

4.1.5.15. (*E*)-*N*-[3-Benzoyl-4-[3-(4-bromophenyl)acryloylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**5g**)

Yield 86%; m.p. 278 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3391, 1682, 1665, 1626, 1598, 1510, 1402, 1330, 1286, 1231, 1108, 1072, 851, 813, 752. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.71 (d, ^{3}J = 15.7 Hz, 1 H, =CH), 6.79 (d, ^{3}J = 15.5 Hz, 1 H, =CH), 7.07 (d, ^{3}J = 3.6 Hz, 1 H, Ar-H), 7.38 (d, ^{3}J = 15.7 Hz, 1 H, =CH), 7.43 (d, ^{3}J = 15.5 Hz, 1 H, =CH), 7.46 (d, ^{3}J = 3.6 Hz, 1 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.55 (m, 2 H, Ar-H), 7.64 (m, 4 H, Ar-H), 7.78 (m, 3 H, Ar-H), 7.94 (dd, ^{3}J = 9.0 Hz, ^{4}J = 2.2 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.29 (s, 1 H, NH), 10.50 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 112.90, 117.49, 120.10 (3 Ar-C), 120.71 (1 =CH and 1 Ar-C), 121.99 (=CH), 122.35, 124.22, 124.50, 124.59 (7 Ar-C), 126.80 (=CH), 128.26, 129.69, 129.83, 131.30, 131.48, 131.93, 132.69, 133.87, 135.16, 135.49, 137.00 (15 Ar-C), 139.12 (=CH), 146.40, 152.26, 152.40 (3 Ar-C), 163.17, 163.26 (2 C=O), 194.69 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 665 [^{81}Br M^+ + H] (50), 664 [^{81}Br M^+]$^{\cdot}$ (100), 663 [M^+ + H] (35), 662 [M^+] (86), 454 (28), 349, (28), 333 (28), 319 (21), 305 (29), 271 (17), 242 (16), 225 (11).

4.1.6. Compounds **4k** *and* **5h***

The compounds 4k and 5h were prepared from **10** and 4-nitrophenylpropionic acid chloride or 4-nitrocinnamic acid chloride according to general procedure 1.

4.1.6.1. (*E*)-*N*-[3-Benzoyl-4-[3-(4-nitrophenyl)propionylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**4k**)

Yield 82%; m.p. 269 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3378, 3114, 1691, 1666, 1628, 1596, 1545, 1516, 1344, 1331, 1247, 851, 797, 752. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.38 (t, ^{3}J = 7.3 Hz, 2 H, CH_2), 2.75 (t, ^{3}J = 7.3 Hz, 2 H, CH_2), 6.79 (d, ^{3}J = 15.0 Hz, 1 H, =CH), 6.07 (d, ^{3}J = 4.0 Hz, 1 H, Ar-H), 7.41 (d, ^{3}J = 9.0 Hz, 1 H, Ar-H), 7.44 (d, ^{3}J = 15.0 Hz, 1 H, =CH), 7.45 (m, 2 H, Ar-H), 7.46 (d, ^{3}J = 4.0 Hz, 1 H, Ar-H), 7.52 (m, 2 H, Ar-H), 7.64 (m, 1 H, Ar-H), 7.70 (m, 2 H, Ar-H), 7.80 (d, ^{4}J = 2.5 Hz, 1 H, Ar-H), 7.90 (dd, ^{3}J = 9.0, ^{4}J = 2.5 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.10 (m, 2H, Ar-H), 8.34 (m, 2H, Ar-H), 9.99 (s, 1 H, NH), 10.48 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 30.16, 36.37 (2 CH_2), 112.87, 117.45, 120.05 (3 Ar-C), 120.71 (=CH), 122.03, 123.39, 124.49, 124.56, 124.57 (8 Ar-C), 126.78 (=CH), 128.23, 129.48, 129.63, 131.30, 131.61, 132.67, 135.15, 135.65, 137.01, 145.89, 146.39, 149.40, 152.25, 152.39 (17 Ar-C), 163.15, 169.66 (2 C=O), 194.71 (Ph-C=O-Ph). MS (ESI+): m/z (%) = 631 (100) [M^+ + H], 455 (28), 393 (21), 349 (30), 333 (45), 319 (22), 305 (30), 271 (24), 242 (62).

4.1.6.2. (*E*)-*N*-(3-Benzoyl-4-[3-(4-nitrophenyl)acryloylamino]phenyl]-3-[5-(4-nitrophenyl)-2-furyl]-acrylsäureamid (**5h**)

Yield 81%; m.p. 339 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3383, 1685, 1627, 1598, 1550, 1515, 1497, 1403, 1337, 1249, 1171, 889, 851, 789, 751. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.79 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 6.90 (d, ^{3}J = 15.7 Hz, 1 H, =CH), 7.10 (d, ^{3}J = 3.5 Hz, 1 H, Ar-H), 7.18 (d, ^{3}J = 8.8 Hz, 1 H, Ar-H), 7.34 (d, ^{3}J = 15.4 Hz, 1 H, =CH), 7.45 (d, ^{3}J = 3.5 Hz, 1 H, Ar-H), 7.54 (m, 3 H, Ar-H), 7.65 (m, 2 H, Ar-H), 7.78 (m, 3 H, Ar-H), 7.80 (d, ^{4}J = 2.4 Hz, 1 H, Ar-H), 7.95 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.4 Hz, 1 H, Ar-H), 8.01 (m, 2 H, Ar-H), 8.24 (m, 2H, Ar-H), 8.34 (m, 2 H, Ar-H), 10.42 (s, 1 H, NH), 10.52 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 112.58, 117.16, 119.87 (3 Ar-C), 120.42 (=CH), 121.73, 123.81, 124.01, 124.21, 124.28 (8 Ar-C), 125.51, 126.53 (2 =CH), 127.98, 128.50, 129.57, 131.05, 132.44, 134.89, 135.38, 136.71 (11 Ar-C), 137.69 (=CH), 140.86, 146.12, 147.37, 151.99, 152.13 (6 Ar-C), 162.53, 162.92 (2 C=O), 194.46 (Ph-C=O-Ph). MS (ESI−): m/z (%) = 627 (8) [M^+ − H], 261 (14), 255 (7), 249 (20), 217 (6), 187 (11), 165 (10), 155 (16), 113 (100).

4.2. Enzyme preparation

Yeast farnesyltransferase was used as a fusionprotein to glutathione S-transferase at the N-terminus of the β-subunit. Farnesyltransferase was expressed in *Escherichia coli* DH5α grown in LB media containing ampicillin and chloramphenicol for co-expression of pGEX-DPR1 and pBC-RAM2 for farnesyltransferase production (Del Villar 1997). The enzyme was purified by standard procedures with glutathione-agarose beads for selective binding of the target protein.

4.3. Farnesyltransferase assay

The assay was conducted as described (Pompliano et al. 1992). Farnesylpyrophosphate (FPP) was obtained as a solution of the ammonium salt in methanol-10 mM aqueous NH_4Cl (7 : 3) from Sigma-Aldrich. Dansyl-Gly-CysValLeuSer (Ds-GCVLS) was custom synthesized by ZMBH, Heidelberg, Germany. The assay mixture (100 µL volume) contained 50 mM Tris/HCl pH 7.4, 5 mM $MgCl_2$, 10 µM $ZnCl_2$, 5 mM dithiothreitol (DTT), 7 µM Ds-GCVLS, 20 µM FPP and 5 nmol (approx.) yeast GST-farnesyltransferase and 1% of various concentrations of the test compounds dissolved in dimethylsulfoxide (DMSO). The progress of the enzyme reaction was followed by monitoring the enhancement of the fluorescence emission at 505 nm (excitation 340 nm). The reaction was started by addition of FPP and run in a Quartz cuvette thermostatted at 37 °C. Fluorescence emission was recorded with a Perkin Elmer LS50B spectrometer. IC_{50} values (concentrations resulting in 50% inhibition) were calculated from initial velocity of three independent measurements of four to five different concentrations of the respective inhibitor.

4.4. MTT assay

A solution of the substances in DMSO (1 µL, several concentrations between 10^{-9} to 10^{-4} mol/l) was incubated with 99 µL of a suspension of HL 60 cells (9×10^5 cells/mL) in RPMI 1640 medium (PAA Laboratories GmbH, Austria) with 10% FKS in 96 well plates for 24 h. Then, 10 µL of an MTT solution in PBS (5 mg/mL) were added and the plate was incubated for another 2 h.

The cells were quenched with 190 µL DMSO and after a few minutes, the plates were evaluated on a Dynatech MRX using wavelength of 570 nm, a reference wavelength of 630 nm (Mosmann 1983).

4.5. In vitro measurement of P. falciparum parasite growth inhibition

Compounds were tested by a semiautomated microdilution assay against intraerythrocytic forms of *P. falciparum* (Desjardins et al.1979). The *P. falciparum* strain Dd2 was cultivated by a modification of the method described by Trager and Jensen (1976). The culture medium consisted of RPMI 1640 supplemented with 10% human type 0^+ serum and 25 mM HEPES. Human type 0^+ erythrocytes served as host cells. The cultures were kept at 37 °C in an atmosphere of 5% O_2, 3% CO_2, and 92% N_2.

Drug testing was carried out in 96-well microtiter plates. The compounds were dissolved in DMSO (10 mM) and prediluted in complete culture medium (final DMSO concentrations $\leq 1\%$) (In order to avoid a loss of lipophilic test compounds by adsorbance to the plastic material used for the assay, complete culture medium containing erythrocytes was used to dilute the DMSO stock solutions). Infected erythrocytes (200 μL per well, with 2% hematocrit and 0.4% parasitemia, predominantly ring-stage parasites) were incubated in duplicate with a serial dilution of the drugs for 48 h (Ancelin 1998). After the addition of 0.8 μCi [^{3}H]-hypoxanthine in 50 μL medium per well, the plates were further incubated for 24 h. Cells were collected on glass fiber filters with a cell harvester (Micromate 196, Packard) and incorporated radioactivity measured using a β-counter (Matrix 9600, Packard).

Acknowledgement: The pGEX-DPR1 and pBC-RAM2 plasmids were kindly provided by Prof. F. Tamanoi (UCLA).

References

Ancelin ML, Calas M, Bompart J, Cordina G, Martin D, Bari MB, Jei T, Druilhe P, Vial HJ (1998) Antimalarial activity of 77 phospholipid polar head analogs: close correlation between inhibition of phospholipid metabolism and *in vitro Plasmodium falciparum* growth. Blood 91: 1426–1437.

Bell IM (2000) Inhibitors of protein prenylation 2000. Exp Opin Ther Patents 10: 1813–1831.

Böhm M, Mitsch A, Wißner P, Sattler I, Schlitzer M (2001) Exploration of novel aryl binding sites of farnesyltransferase using molecular modeling and benzophenone-based farnesyltransferase inhibitors. J Med Chem 44: 3117–3124.

Buckner FS, Yokoyama K, Nguyen L, Grewal A, Erdjument-Bromage H, Tempst P, Strickland CL, Xiao L, Van Voorhis WC, Gelb MH (2000) Cloning, heterologous expression, and distinct substrate specificity of protein farnesyltransferase from *Trypanosoma brucei*. J Biol Chem 275: 21870–21876.

Buckner FS, Eastman RT, Nepumuceno-Silva JL, Speelmon EC, Myler PJ, Van Voorhis WC, Yokoyama K (2002) Cloning, heterologous expression, and substrate specificities of protein farnesyltransferases from *Trypanosoma cruzi* and *Leishmania major*. Mol Biochem Parasitol 122: 181–188.

Chakrabarti D, Azam T, DelVecchio C, Qiu L, Park Y, Allen CM (1998) Protein prenyl transferase activities of *Plasmodium falciparum*. Mol Biochem Parasitol 94: 175–184.

Chakrabarti D, Da Silva T, Barger J, Paquette S, Patel H, Patterson S, Allen CM (2002) Protein farnesyltransferase and protein prenylation in *Plasmodium falciparum*. J Biol Chem 277: 42066–42073.

Cox AD, Der CJ (2002) Farnesyltransferase inhibitors: promises and realities. Curr Op Pharm 2: 388–393.

Del Villar K, Mitsuzawa H, Yang W, Sattler I, Tamanoi F (1997) Amino acid substitutions that convert the protein substrat specificity of farnesyltransferase to that of geranylgeranyltransferase type I. J Biol Chem 272: 680–687.

Desjardins RE, Canfield CJ, Haynes JD, Chulay JD (1979) Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother. 16: 710–718.

Fu H-W, Casey PJ (1999) Enzymology and biology of CaaX protein prenylation. Rec Prog Hormon Res 54: 315–343.

Ibrahim M, Azzouz N, Gerold P, Schwarz RT (2001) Identification and characterisation of *Toxoplasma gondii* protein farnesyltransferase. Int J Parasitol 31: 1489–1497.

Kettler K, Sakowski J, Silber K, Sattler I, Klebe G, Schlitzer M (2003) Non-thiol farnesyltransferase inhibitors: *N*-(4-acylamino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amides. Bioorg Med Chem 11: 1521–1530.

Moloney GP, Martin GR, Mathews N, Milne A, Hobbs H, Dodsworth S, Sang PY, Knight C, Williams M, Maxwell M, Glen RC (1999) Synthesis and serotonergic activity of substituted 2,*N*-benzylcarboxamido-5-(2-ethyl-1-dioxoimidazolidinyl)-*N*,*N*-dimethyltrypt-amine derivatives: novel antagonists for the vascular 5-HT_{1B}-like receptor. J Med Chem 42: 2504–2526.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J Immun Meth 65: 55–63.

Pompliano DL, Gomez RP, Anthony NJ (1992) Intramolecular fluorescece enhancement: a continuous assay of ras farnesyl: protein transferase. J Am Chem Soc 114: 7945–7946.

Purcell WT, Donehower RC (2002) Evolving therapies: Farnesyltransferase inhibitors. Curr Onc Rep 4: 29–36.

Ridley RG (2002) Medical need, scientific opportunity and the drive for antimalarial drugs. Nature 415: 686–693.

Sachs J, Malaney P (2002) The economic and social burden of malaria. Nature 415: 680–685.

Schlitzer M (2002) Structure based design of benzophenone-based non-thiol farnesyltransferase inhibitors. Curr Pharm Design 8: 1713–1722.

Trager W, Jensen JB (1976) Human malaria parasites in continous culture. Science 193: 673–675.

Wiesner J, Kettler K, Sakowski J, Ortmann R, Jomaa H, Schlitzer M (2003a) Structure-activity relationships of novel anti-malarial agents: 5. *N*-(4-Acylamino-3-benzoylphenyl)-[5-(4-nitrophenyl)-2-furyl]acrylic acid amides. Bioorg Med Chem Lett 13: 361–363.

Wiesner J, Fucik K, Kettler K, Sakowski J, Ortmann R, Jomaa H, Schlitzer M (2003b) Structure-activity relationships of novel anti-malarial agents: 6. *N*-(4-arylpropionylamino-3-benzoylphenyl)-[5-(4-nitrophenyl)-2-furyl]acrylic acid amides. Bioorg Med Chem Lett 13: 1539–1541.

Wiesner J, Ortmann R, Mitsch A, Wißner P, Sattler I, Jomaa H, Schlitzer M (2003c) Farnesyltransferase-inhibitors: 5-arylacryloylaminobenzophenones show anti-malarial activity. Pharmazie 58: 289–290.

Wiesner J, Kettler K, Sakowski J, Ortmann R, Katzin AM, Kimura EA, Silber K, Klebe G, Jomaa H, Schlitzer M (2004a) Farnesyltransferase-Inhibitoren hemmen das Wachstum von Malaria-Erregern *in vitro* und *in vivo*. Angew Chem, 116: 254–257

Wiesner J, Kettler K, Sakowski J, Ortmann R, Katzin AM, Kimura EA, Silber K, Klebe G, Jomaa H, Schlitzer M (2004a) Farnesyltransferase inhibitors inhibit the growth of malaria parasites in vitro and in vivo. Angew Chem Int Ed 43: 251–254.

Wittinghofer A, Waldmann H (2000a) Ras – ein molekularer Schalter bei der Tumorentstehung. Angew Chem 112: 4360–4383

Wittinghofer A, Waldmann H (2000b) Ras – A moelecular switch involved in tumor formation. Angew Chem Int Ed 39: 4192–4214.

Yokoyama K, Trobridge P, Buckner FS, Van Voorhis WC, Stuart KD, Gelb MH (1998) The effects of protein farnesyltransferase inhibitors on trypanosomatids: inhibition of protein farnesylation and cell growth. J Biol Chem 273: 26497–26505.