Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 93–101
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Short communication

Non-thiol farnesyltransferase inhibitors: *N*-(4-aminoacylamino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2 furyl]acrylic acid amides and their antimalarial activity

Katja Kettler [a], Jochen Wiesner [b], Katrin Silber [c], Peter Haebel [c], Regina Ortmann [a], Isabel Sattler [d], Hans-Martin Dahse [d], Hassan Jomaa [b], Gerhard Klebe [c], Martin Schlitzer [a,*]

[a] *Department für Pharmazie, Zentrum für Pharmaforschung, Ludwig-Maximilians Universität München, Butenandtstraße 5-13, 81377 München, Germany*
[b] *Biochemisches Institut der Universität Gießen, Friedrichstraße 24, 35249 Gießen, Germany*
[c] *Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, 35032 Marburg, Germany*
[d] *Hans-Knöll-Institut für Naturstoff-Forschung e. V., Beutenbergstraße 11, 07745 Jena, Germany*

Received 22 March 2004; received in revised form 1 September 2004; accepted 6 September 2004

Available online 25 November 2004

Abstract

Water solubility was previously found to be essential for in vivo-antimalarial activity of a novel type of benzophenone-based farnesyltransferase inhibitors. Introduction of a α-amino group into the phenylacetic acid substructure provided more soluble compounds with high farnesyltransferase inhibitory activity. The in vitro-antimalarial activity was detrimentally influenced by this structural modification.

Keywords: Non-thiol farnesyltransferase inhibitors; Structure–activity relationships; Antimalarial activity

1. Introduction

Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesylpyrophosphate (FPP) to the thiol of a cysteine side chain of proteins, which carry at the C-terminus the so-called CAAX-sequence. C represents a cysteine which side chain is farnesylated; A, amino acids which normally, but not necessarily, carry aliphatic side chains, and X mostly methionine or serine [1–3].

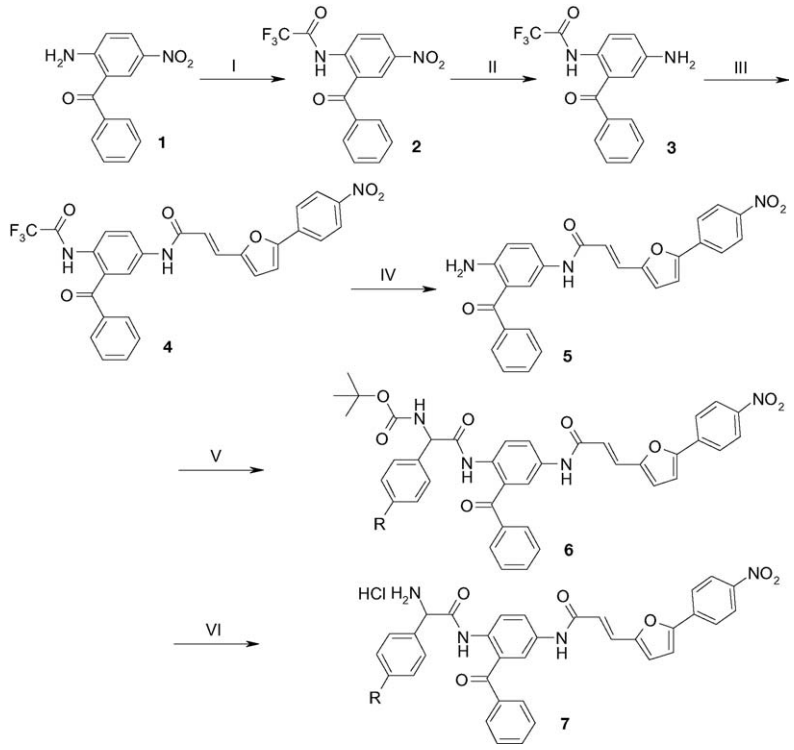
Farnesyltransferase inhibitors have been developed as novel anti-cancer agents. Several farnesyltransferase inhibitors are in advanced stages of clinical trials for the therapy of different types of cancer [4,5].

However, farnesyltransferase has also been identified in different parasites pathogenic to humans as for instance *Plasmodium falciparum* [6,7], different *Trypanosoma* species [8–10], *Leishmania major* [10] and *Toxoplasma gondii* [11]. Therefore, farnesyltransferase has been suggested as a novel target in the development of drugs directed against parasite infections [4]. *P. falciparum* is a target of particular importance since it is the causative agent of *Malaria tropica*, which is responsible for 300–500 million clinical cases every year, and 1–3 million deaths [12,13].

After farnesyltransferase inhibitors had already been tested in advanced clinical trials for the treatment of tumor diseases, the development of antiplasmodial farnesyltransferase inhibitors has now been generally accepted as a new strategy towards new drugs against malaria (Medicines for Malaria Venture—annual report 2003). Development of a variety of new antimalarial compounds directed against not-yet exploited molecular targets [14] is considered to be essential in order to face the increasing threat by multi-resistant *P. falciparum* strains as well as the parasite's ability to readily develop resistance against new drugs. It has been argued [15] that in clinical studies with cancer patients, the side effects of farnesyltransferase inhibitors were acceptable and, thus, should also be tolerable for antimalarial therapy. However, while the antiproliferative activity of human farne-

* Corresponding author. Tel.:+49 89 2180 77804; fax: +49 89 2180 79992.
E-mail address: martin.schlitzer@cup.uni-muenchen.de (M. Schlitzer).

0223-5234/$ - see front matter © 2004 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2004.09.019



Scheme 1. (I) TFAA, DCM/pyridine, 0 °C, 2 h; (II) $SnCl_2 \times 2H_2O$, EtOAc, reflux 2 h; (III) 3-[5(4-nitrophenyl)-2-furyl]acrylic acid chloride, toluene/dioxane, reflux, 2 h; (IV) K_2CO_3, dioxane/H_2O, reflux, 3 h; (V) *R*–C_6H_4–CH(NHCOO-*tert*-Bu)–COOH, $POCl_3$, pyridine, 0 °C, 0.5 h; (6) HCl (g), dioxane, RT, 1 h.

syltransferase inhibitors represents the desired therapeutic principle in cancer therapy, such an activity precludes the use in children and pregnant women, the main target populations in malaria therapy. Therefore, we feel that in the long-term perspective the development of parasite specific inhibitors is mandatory.

In previous work we have developed benzophenone-type non-thiol farnesyltransferase inhibitors with the nitrophenylfurylacryloyl residue at the 5-amino group, specifically designed to utilize an aryl binding site of farnesyltransferase [16]. Several of these benzophenone-based farnesyltransferase inhibitors (e.g. **8c**) were highly active against a multi-drug resistant *P. falciparum* strain in vitro [17–26]. However, even though possessing in vitro IC_{50}-values in the same range as some standard antimalarials, these compounds were completely inactive in a murine malaria model, presumably due to poor water solubility. Introducing a methyl piperazine residue resulted in soluble compounds (e.g. Schl-2171; Fig. 1) with comparably low activity against cultured *P. falciparum* parasites but representing the first farnesyltransferase inhibitors with in vivo-antimalarial activity described in the literature, as demonstrated by curing mice infected with the rodent malaria parasite *P. vinckei* [27]. In the present study we addressed the question whether the piperazinyl moiety of Schl-2171 can be replaced by a simple amino group (Fig. 1). The activity of the novel inhibitors was assayed against isolated farnesyltransferase and intraerythrocytic forms of *P. falciparum*.

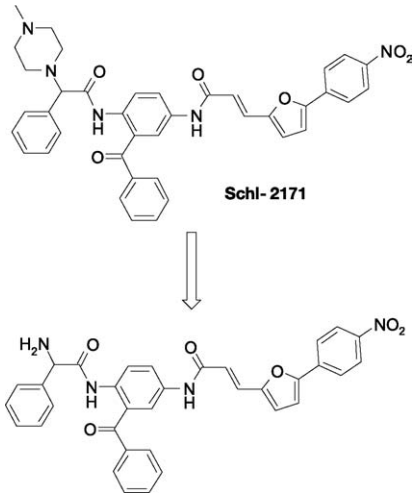


Fig. 1. The piperazinyl moiety of Schl-2171 was formally reduced to an amino group yielding aminoacyl-substituted benzophenones.

2. Results and discussion

2.1. Chemistry

The key step in the preparation of the target compounds **7** was the acylation of the 2-amino-5-acylaminobenzophenone **5**, which was prepared as described recently [28]. Briefly, the 2-amino group of **1** was protected as trifluoroacetamide **2**. After reduction of the 5-nitro group the resulting amine **3** was acylated with 3-[5-(4-nitrophenyl)-2-furyl]arylic acid chloride. After removal of the protective group from **4** the resulting intermediate **5** could be acylated by appropriate Boc-

protected phenylglycine derivatives, which were activated using phosphorous oxychloride in pyridine yielding the *N*-protected derivatives **6**. Acidic removal of the Boc group gave the target compounds **7** (Scheme 1).

2.2. Farnesyltransferase inhibition assay

The inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described by Pompliano et al. [29]. The assay employed yeast farnesyltransferase (FTase) fused to glutathione S-transferase at the N-terminus of the 9-subunit [30].

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

2.3. Inhibition of the growth of P. falciparum

Compounds **6** and **7** were assayed for their inhibitory activity against intraerythrocytic forms of the *P. falciparum* strains Dd2 using a semi-automated microdilution assay as described [31–33]. The growth of the parasites was monitored through the incorporation of tritiumlabeled hypoxanthine. The Dd2 strain is resistant to several commonly used antimalarial drugs (chloroquine, cycloguanile and pyrimethamine) (Table 1). The comparability of measurements of different series was granted by concurrent assay of standard compounds.

2.4. Flexible docking

The protein structure was taken from the PDB entry 1QBQ [34]. Ligands and solvent molecules were removed, but the zinc ion and farnesyldiphosphate were included as part of the protein. Docking was performed with AutoDock 3.0 [35,36]. Resulting ligand conformations with similar structures (rms deviation <1) were clustered together and represented by the conformer with the best docking energy.

2.5. Homology modeling

A homology model of *P. falciparum* farnesyltransferase was prepared to analyze the differences between mammalian and parasite enzymes. Sequences for alpha and beta subunits of rat (Q04631 and Q02293) and *P. falciparum* (Q8I503 and Q8IHP6) farnesyltransferase were retrieved from SWISS-Prot [37] and aligned with T-COFFEE [38]. Homology models were computed based on the alignments and the rat template structure (PDB entry: 1QBQ) using MODELLER [39].

2.6. Cytotoxicity assay

Cytotoxicity of selected compounds was evaluated against HeLa cells. Viability of the cells was determined after 72 h incubation period using methylene blue staining and photometric evaluation.

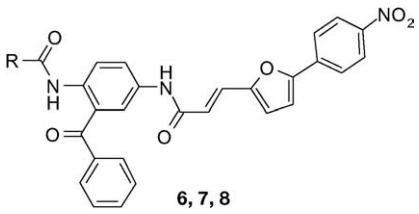
2.7. Structure–activity relationships

The underlying rationale for the design of piperazinyl-substituted benzophenones (e.g. Schl2171; Fig. 1) was the introduction of a functional group, which is charged at physiological pH in order to improve the water solubility of this type of compounds. These compounds represent the first farnesyltransferase inhibitors with in vivo-antimalarial activity described in the literature [27]. Here, we addressed the question of the effect of the addition of an amino group instead of a piperazinyl moiety to the α-position of the phenylacetic acid substructure of our benzophenone-based farnesyltransferase inhibitors as exemplified by compounds **8**.

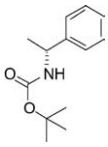
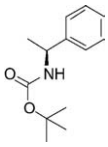
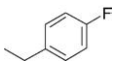
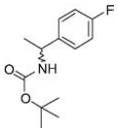
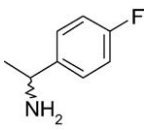
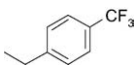
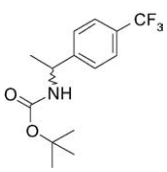
Amino acid derivatives **7** proved to be more water soluble than the parent compounds **8** (Table 1). Furthermore, these inhibitors displayed farnesyltransferase inhibitory activity, which is roughly in the same range as those of the parent compounds **8** (Table 1). Only the S-phenylglycine derivative **7a** is less active than the parent inhibitor **8a** and the R-enantiomer **7b**.

Flexible docking is ambiguous since for the same inhibitor several solutions are obtained with marked differences in the orientation of the aminoacyl substituent, but with very similar energy values. However, some possible insight is provided into the binding of the two enantiomers **7a** and **7b** (Fig. 2). Main feature of the binding of the amino acid derivatives is a hydrogen bond between the α-amino group and the hydroxyl of Ser 99β mediated by a structural water. In case of the more active R-enantiomer **7b**, this hydrogen bond is slightly shorter (3.3 Å) than with the less active S-enantiomer **7a** (3.6 Å). This difference is the only one revealed by flexible docking. Whether flexible docking is able to reveal all factors determining inhibitory activity, especially under the conditions described above, remains uncertain. Docking has been performed using the structure of rat farnesyltransferase. To address the question of structural differences between rat and *P. falciparum* farnesyltransferases a homology model was prepared for *P. falciparum* farnesyltransferase (Fig. 3). Although the sequences of rat and plasmodial farnesyltransferases show considerable differences, most active site residues are well conserved with a local sequence identity of 72%. Sequence variation is found at two opposite sides of the inhibitor binding site. The substitutions Tyr 166α to Phe, Met 193β to Val, Ala 129α to Ser, Pro 152β to Thr and Cys 95β to Ser are located in the region where the phenyl residue of the phenylglycine moiety is bound. The conservative nature of these substitutions and the fact that none of these residues is in direct contact with the inhibitor suggests similar inhibitor binding modes for rat and *P. falciparum* farnesyltransferase. The same holds true for the opposite binding site, where the most conservative exchanges Val 43β to Ile, Thr 44β to Asn and Gln 48β to Pro are too distant to affect inhibitor binding significantly. Only the

Table 1
Farnesyltransferase inhibitory activity and antimalarial activity [a] of compounds **6** and **7**. Farnesyltransferase inhibitory activity [28] and antimalarial activity [23] of parent compounds **8** lacking the α-substitution. Solubility of compounds **7** and **8a**

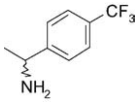


6, 7, 8

	R	FTase IC_{50}	Pfal IC_{50}	CC_{50}	Solubility in water	Solubility in buffer pH 7.4
8a		6 ± 3 nM	270 nM	>88 μM	<0.06 mM	<0.04 mM
6a		8 ± 2 nM	150 nM	>73 μM	–	–
7a		46 ± 3 nM	3200 nM	>80 μM	>1.5 mM	0.2 mM
6b		10 ± 2 nM	230 nM	>73 μM	–	–
7b		8 ± 2 nM	560 nM	>80 μM	>1.5 mM	0.2 mM
8c		15 ± 3 nM	230 nM	–	–	–
6c		100 ± 9 nM	200 nM	>73 μM	–	–
7c		25 ± 3 nM	950 nM	>78 μM	–	–
8d		26 ± 6 nM	47 nM	>57 μM	–	–
6d		8 ± 2 nM	625 nM	–	–	–

(continued on next page)

Table 1
(continued)

	R	FTase IC_{50}	Pfal IC_{50}	CC_{50}	Solubility in water	Solubility in buffer pH 7.4
7d		17 ± 4 nM	525 nM	–	>1.5 mM	0.08 mM

[a] IC_{50} values (nM) for standard antimalarials were: chloroquine, 170; pyrimethamine, 2500; cycloguanile, 2200; quinine, 380; lumefantrine, 30; artemisinin, 18.

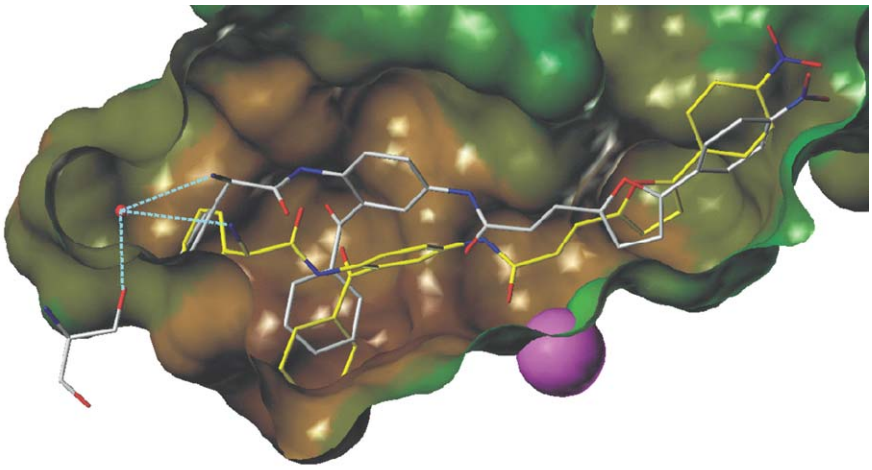


Fig. 2. Docking solutions for the *S*-phenylglycine derivative **7a** (yellow carbons) and *R*-phenylglycine derivative **7b** (white carbons). Both amino acid derivatives show a hydrogen bridge to Ser 99β mediated by a structural water (dotted lines), which is slightly longer for the less active R-enatiomer. Lipophilic (brown) and hydrophilic (green) properties of the active site are displayed on the Connolly surface. The structural zinc is represented by a violet sphere.

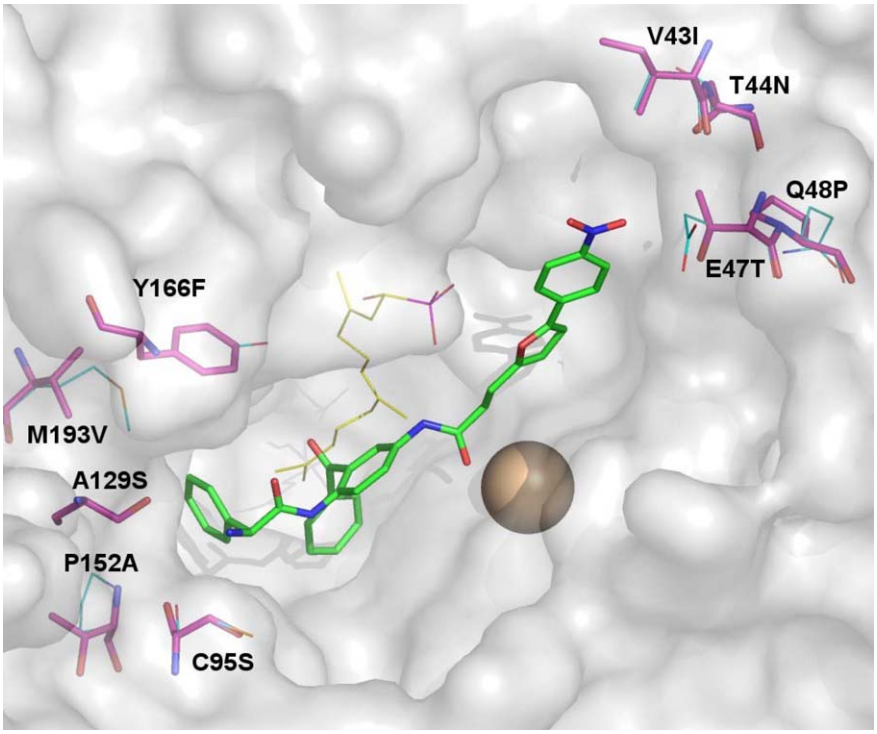


Fig. 3. Homology model for *P. falciparum* farnesyltransferase with inhibitor **7a** (green carbons). Amino acid residues which are different in rat and plasmodial farnesyltransferase are indicated (rat: light blue; Pfal: magenta). The structural zinc is shown as brown sphere.

replacement of the hydrogen bond acceptor Glu 47β by the hydrogen bond donor Thr might affect the binding of inhibitors carrying a terminal nitro group.

In summary, this homology model shows that the main conclusions concerning the binding of such benzophenone-based inhibitors derived from docking into the rat structure are also valid for the plasmodial farnesyltransferase. However, the homology model reveals some differences, which might be further exploited for inhibitor development. Interestingly, three of the four Boc-protected precursors (**6a**, **6b**, **6d**) also displayed high farnesyltransferase inhibitory activity. A striking difference in the structure–activity relationships is the lack of stereo-differentiation in case of the Boc-protected derivatives **6a** and **6b**, probably due to a different orientation of the acyl moiety. Unfortunately, no reliable docking solutions could be obtained for the Boc-derivatives due to the large number of rotatable bonds.

In contrast to the activity against isolated farnesyltransferase the antimalarial activity of the amino derivatives **7a–d** was significantly decreased compared to their parent compounds **8**. This effect was most prominent with the most active parent compound **8c**, where introduction of the amino group led to a 10-fold loss of activity, while only a 2.5-fold decrease was observed in case of **8b**, indicating that SARs deduced from the parent compounds are not necessarily relevant for the respective amino derivatives. Because of the overall low antimalarial activity, no in vivo experiments where performed.

Remarkably, with exception of the *para* trifluoromethyl phenylglycine derivative **6d**, the more lipophilic Boc-protected precursors **6a–c** were more active than the unprotected phenylglycine derivatives. Thus, one may speculate that the lower activity of the phenylglycine derivatives **7a–c** is in part attributable to a hindered membrane penetration of the more polar amino acid derivatives. However, a variety of established antimalarial compounds possesses amino groups, and even a mechanism for the accumulation of basic compounds in the parasite's food vacuole has been suggested. Nevertheless, membrane penetration seems to be a factor to be considered, since structural differences between mammalian and plasmodial farnesyltransferases as far as revealed by homology modeling, cannot account for the differences in farnesyltransferase inhibition and anitplasmodial activity.

When the antiplasmodial and the cytotoxic activity of the compounds is compared (Table 1), there is a striking difference between the IC_{50}-values against cultured parasites which are ranging between 150 and 3200 nM and the CC_{50}-values, which are >70–80 μM. This can be interpreted in that way that the farnesyltransferase inhibitors described here indeed display a certain degree of selectivity towards malarial parasites.

3. Conclusion

Introduction of a α-amino group into the phenylacetic acid substructure of our benzophenonebased farnesyltransferase inhibitors provided the desired compounds with better solubility preserving high farnesyltransferase inhibitory activity. However, antimalarial activity was detrimentally influenced by this structural modification. Although undesired, this finding is important since it shows that it takes more for an inhibitor to be an active antimalarial than to be a good farnesyltransferase inhibitor, providing argument for the development of specialized antimalarial farnesyltransferase inhibitors. Current work aims at the identification of alternative modifications resulting in favorable pharmacokinetic properties required for in vivo activity while preserving or even increasing the antimalarial activity of the parent benzophenone-based farnesyltransferase inhibitors.

4. Experimental

4.1. Synthesis

^{1}H-NMR spectra were recorded on a Jeol JMN-GX-400 and a Jeol JMN-LA-500 spectrometer. Mass spectra were obtained with a Vacuum Generator VG 7070 H using a Vector 1 data acquisition system from Teknivent or a AutoSpec mass spectrometer from Micromass. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. Microanalyses were obtained from a CH analyzer according to Dr. Salzer from Labormatic and from a Hewlett–Packard CHN analyzer type 185. Melting points were obtained with a Leitz microscope and are uncorrected. Column chromatography was carried out using silica gel 60 (0.062–0.200 mm) from Merck.

4.1.1. General procedure 1: acylation of **5** by Boc-protected amino acids

The various Boc-protected amino acids and equimolar amounts of *(E)-N*-(4-amino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide **(5)** [28] were dissolved in 3 ml pyridine per mmol amino acid. Then, the mixture was cooled to –15 °C and 0.1 ml $POCl_3$ per mmol amino acid were added dropwise. After 30 min at –15 °C, the mixture was poured into ice/water and extracted three to four times with EtOAc. The combined extracts were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and the solvent was evaporated to yield the crude product. The following compounds were prepared accordingly.

4.1.1.1. (E-,S)-N-*[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]*-N-*tert-butoxycarbonylphenylglycine amide* ***(6a)***. From Boc-L-phenylglycine (302 mg, 1.2 mmol) and *(E)-N*-(4-amino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (544 mg, 1.2 mmol). Yield: 585 mg (71%), m.p. 163 °C; IR (KBr) *ν* 3367, 1686, 1624, 1599, 1511, 1454, 1333, 1290, 1245, 1198, 1163, 1109, 1027, 967, 854, 796, 753, 696 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.34 (s, 9H), 5.19 (s, 1H), 6.74 (d, J = 16 Hz, 1H), 7.01–7.02 (m, 1H), 7.21–7.70 (m, 12H and d, J = 16 Hz,

1H), 7.82–7.84 (m, 2H), 7.88 (s, 1H), 7.97–8.00 (m, 2H), 8.30–8.32 (m, 2H), 10.37 (s, 1H), 10.48 (s, 1H); MS (FAB) *m/z* 686 (100) [M^+ + 1], 670 (2), 631 (23), 587 (90), 570 (18), 542 (8), 480 (63), 454 (63), 453 (38). Anal. ($C_{39}H_{34}N_4O_8$) C, H, N.

*4.1.1.2. (*E-,R*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-*N*-tert-butoxycarbonylphenylglycine amide (6b).* From Boc-D-phenylglycine (302 mg, 1.2 mmol) and *(E)-N*-(4-amino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (544 mg, 1.2 mmol). Yield: 461 mg (56%), m.p. 163 °C; IR (KBr) *v* 3369, 1676, 1622, 1599, 1512, 1402, 1333, 1289, 1244, 1199, 1162, 1109, 854, 753, 695 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.33 (s, 9H), 5.17 (s, 1H), 6.72 (d, J = 16 Hz, 1H), 7.00–7.01 (m, 1H), 7.24–7.69 (m, 12H and d, J = 16 Hz, 1H), 7.81–7.82 (m, 2H), 7.87 (s, 1H), 7.96–7.97 (m, 2H), 8.28–8.30 (m, 2H), 70.35 (s, 1H), 10.47 (s, 1H); MS (FAB) *m/z* 687 (46) [M^+ + 1], 631 (18), 587 (76), 570 (28), 542 (8), 408 (68), 454 (100), 453 (23); Anal. ($C_{39}H_{34}N_4O_8$) C, H, N.

*4.1.1.3. (*E-,R,S*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-*N*-tert-butoxycarbonyl-(4-fluorophenyl)glycine amide (6c).* From (*R*,*S*)-*tert*-butoxycarbonylamino(4-fluorophenyl)acetic acid (323 mg, 1.2 mmol) and (*E*)-*N*-(4-amino-3-benzoylphenyl)-3-[5(4-nitrophenyl)-2-furyl]acrylic acid amide (544 mg, 1.2 mmol). Yield: 571 mg (68%), m.p. 196 °C; IR (KBr) *v* 3414, 1685, 1627, 1600, 1510, 1334, 1292, 1163, 1110, 854 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.34 (s, 9H), 5.20 (s, 1H), 6.74 (d, J = 16 Hz, 1H), 7.01–7.10 (m, 3H), 7.33–7.50 (m, 6H and d, J = 16 Hz, 1H), 7.60–7.82 (m, 5H), 7.89 (s, 1H), 7.97–8.00 (m, 2H), 8.29–8.31 (m, 2H), 10.37 (s, 1H), 10.44 (s, 1H); MS (FAB) *m/z* 705 (100) [M^+ + 1], 649 (18), 605 (45), 588 (25), 480 (30), 454 (55), 453 (20); Anal. ($C_{39}H_{33}FN_4O_8$) C, H, N.

*4.1.1.4. (*E-,R,S*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-*N*-tert-butoxycarbonyl-(4-trifluoromethylphenyl)glycine amide (6d).* From (*R*,*S*)-*tert*-butoxycarbonylamino-(4-trifluoromethylphenyl)acetic acid (382 mg, 1.2 mmol) and (*E*)-*N*-(4-amino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (544 mg, 1.2 mmol). Yield: 562 mg (62%), m.p. 168 °C; IR (KBr) *v* 3314, 1691, 1627, 1599, 1513, 1403, 1333, 1314, 1247, 1157, 1109, 1037, 852, 752 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.38 (s, 9H), 5.56 (d, J = 8 Hz, 1H), 6.75 (d, J = 16 Hz, 1H), 7.04–7.05 (m, 1H), 7.40–7.59 (m, 6H and d, J = 16 Hz, 1H), 7.64–7.79 (m, 4H), 7.89–8.05 (m, 4H), 8.17 (d, J = 8 Hz, 1H), 8.33–8.35 (m, 2H), 8.64 (m, 1H), 10.51 (s, 1H), 10.94 (s, 1H); MS (FAB) *m/z* 755 (21) [M^+ + 1], 754 (20) [M^+], 655 (47), 454 (100), 438 (18), 353 (11), 264 (27), 242 (100), 196 (59); Anal. ($C_{40}H_{33}F_3N_4O_8$) C, H, N.

4.1.2. General procedure 2: Removal of the Boc-protective group

The Boc-protected compounds **6** were dissolved in 10 ml of 4 M HCl in dioxane. After stirring for 2 h, the solvent was removed in vacuo and the products were washed with acetone. The following compounds were prepared accordingly.

*4.1.2.1. (*E-,S*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]phenylglycine amide hydrochloride (7a).* From (*E*-,*S*)-*N*-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2furyl]acryloylamino]phenyl]-*N-tert*-butoxycarbonylphenylglycine amide **(6a)** (342 mg, 0.5 mmol). Yield: 195 mg (66%), m.p. 211 °C; IR (KBr) *v* 3374, 1698, 1625, 1598, 1554, 1511, 1403, 1332, 1291, 1247, 1180, 1107, 966, 852, 751, 695 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 5.17 (s, 1H), 6.80 (d, J = 16 Hz, 1H), 7.02–7.03 (m, 1H), 7.39–7.77 (m, 14H and d, J = 16 Hz, 1H), 7.91–8.01 (m, 2H), 8.30–8.32 (m, 2H), 8.69 (s, 3H), 10.58 (s, 1H), 10.75 (s, 1H); MS (ESI) *m/z* 587 (100) [M^+], 569 (16), 454 (4), 106 (5); Anal. ($C_{34}H_{26}N_4O_6$·HCl) C, H, N.

*4.1.2.2. (*E-,R*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]phenylglycine amide hydrochloride (7b).* From (*E*-,*R*)-*N*-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2furyl]acryloylamino]phenyl]-*N-tert*-butoxycarbonylphenylglycine amide **(6b)** (342 mg, 0.5 mmol). Yield: 205 mg (82%), m.p. 211 °C; IR (KBr) *v* 3368, 1699, 1625, 1598, 1550, 1510, 1402, 1332, 1292, 1246, 1181, 751, 695 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 5.16 (s, 1H), 6.80 (d, J = 16 Hz, 1H), 7.03–7.04 (m, 1H), 7.39–7.77 (m, 14H and d, J = 16 Hz, 1H), 7.91–8.01 (m, 2H), 8.30–8.33 (m, 2H), 8.68 (s, 3H), 10.56 (s, 1H), 10.72 (m, 1H); MS (ESI) *m/z* 587 (100) [M^+], 569 (17), 515 (4), 454 (4), 391 (4), 337 (4), 273 (6), 243 (3), 180 (3), 115 (27), 106 (9); Anal. ($C_{34}H_{26}N_4O_6$·HCl) C, H, N.

*4.1.2.3. (*E-,S*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-(4-fluorophenyl)glycine amide hydrochloride (7c).* From (*E*-,*S*)-*N*-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-*N-tert*-butoxycarbonyl-(4-fluorophenyl)glycine amide **(6c)** (342 mg, 0.5 mmol). Yield: 229 mg (75%), m.p. 211 °C; IR (KBr) *v* 3373, 1861, 1626, 1599, 1511, 1403, 1334, 1292, 1237, 1197, 1165, 1109, 852, 752 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 5.19 (s, 1H), 6.80 (d, J = 16 Hz, 1H), 7.01–7.21 (m, 3H), 7.39–7.78 (m, 10H and d, J = 16 Hz, 1H), 7.91–8.01 (m, 3H), 8.30–8.32 (m, 2H), 8.69 (s, 3H), 10.57 (s, 1H), 10.76 (s, 1H); MS (ESI) *m/z* 605 (100) [M^+], 588 (26), 525 (3), 515 (4), 481 (4), 454 (6), 437 (5), 393 (5), 349 (3), 271 (4), 186 (4), 123 (3); Anal. ($C_{34}H_{25}FN_4O_6$·HCl) C, H, N.

*4.1.2.4. (*E-,S*)-N-[2-benzoyl-4-[3-[5-(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-(4-trifluoro-methylphenyl)glycine amide hydrochloride (7d).* From (*E*-,*S*)-*N*-[2-benzoyl-4-[3-[5(4-nitrophenyl)-2-furyl]acryloylamino]phenyl]-*N-tert*-butoxycarbonyl-(4trifluoromethylphenyl)glycine amide **(6d)** (342 mg, 0.5 mmol). Yield: 120 mg (41%); m.p. 224 °C; IR (KBr) *v* 3371, 1698, 1678, 1624, 1599, 1547, 1510, 140, 1335, 1315, 1244, 1123, 1036, 852 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 5.14 (s, 1H), 6.84 (d, J = 16 Hz, 1H), 7.05 (m, 1H), 7.40

(d, J = 16 Hz, 1H), 7.43–7.51 (m, 3H), 7.60–7.71 (m, 6H), 7.74–7.77 (m, 1H), 7.83–7.87 (m, 2H), 7.98–8.02 (m, 3H), 8.32–8.34 (m, 2H), 9.00 (s, 3H), 10.46 (s, 1H), 10.75 (s, 1H); MS (FAB) *m/z* 654 (100) [M^+], 480 (10), 454 (41), 391 (10), 242 (62), 212 (26); Anal. ($C_{35}H_{25}F_3N_4O_6$·HCl) C, H, N.

4.1.3. Enzyme preparation

Yeast farnesyltransferase was used as a fusion protein to glutathione S-transferase at the Nterminus of the 9-subunit. Farnesyltransferase was expressed in *Escherichia coli* DH59 grown in LB media containing ampicillin and chloramphenicol for co-expression of pGEX-DPR1 and pBC-RAM2 for farnesyltransferase production [30]. The enzyme was purified by standard procedures with glutathione-agarose beads for selective binding of the target protein.

4.1.4. Farnesyltransferase assay

The assay was conducted as described [29]. FPP was obtained as a solution of the ammonium salt in methanol–10 mM aqueous NH_4Cl (7:3) from Sigma–Aldrich. Dansyl-GlyCysValLeuSer (Ds-GCVLS) was custom-synthesized by ZMBH, Heidelberg, Germany. The assay mixture (100 1 l volume) contained 50 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 10 1 M $ZnCl_2$, 5 mM dithiothreitol (DTT), 7 1 M Ds-GCVLS, 20 1 M 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 the enzyme and run in a Quartz cuvette thermostatted at 30 °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.

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

Compounds were tested by a semi-automated microdilution assay against intraerythrocytic forms of *P. falciparum* [31]. The *P. falciparum* strains Dd2 and 3D7 were cultivated by a modification of the method described by Trager and Jensen [32]. 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 ≤1%). 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 [33]. 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 was measured using a β-counter (Matrix 9600, Packard).

4.1.6. Cytotoxicity assay

HeLa (DSM ACC 57) cells were grown in RPMI 1640 culture medium (GIBCO BRL 21875034) supplemented with 25 µg ml^{-1} gentamicin sulfate (BioWhittaker 17-528Z), and 10% heat inactivated fetal bovine serum (GIBCO BRL 10500-064) at 37 °C in high density polyethylene flasks (NUNC 156340). The test substances are dissolved in DMSO (10 mg ml^{-1}) before being diluted in the cell culture medium (1:200). The adherent HeLa cells were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA (Biochrom KG L2163). For each experiment approximately 10,000 cells were seeded with 0.1 ml RPMI 1640 (GIBCO BRL 21875-034), containing 25 µg ml^{-1} gentamicin sulfate (BioWhittaker 17-528Z), but without HEPES per well of the 96-well microplates (NUNC 167008). For the cytotoxic assay HeLa cells were 48 h preincubated without the test substances. The dilutions of the test substances were carried out carefully on the monolayers of HeLa cells after the preincubation time. Cells of HeLa were incubated for 72 h at 37 °C in a humidified atmosphere and 5% CO_2. The adherent HeLa cells were fixed by 25% glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gently washing the stain was eluted by 0.2 ml of 0.33 N HCl in the wells. The optical densities were measured at 660 nm in SUNRISE microplate reader (TECAN). Comparisons of the different values were performed with software Magellan (TECAN).

4.2. Flexible docking

The protein structure was taken from the PDB entry 1QBQ [34]. Ligands and solvent molecules were removed, but the zinc ion and farnesyldiphosphate were included as part of the protein. For the use within AutoDock 3.0 [35,36], polar hydrogens were added with the PROTONATE utility from AMBER [40]. AMBER united atom force field charges were assigned [41], and solvation parameters were added using the ADDSOL utility from AutoDock 3.0. Ligand structures were built in mol2 format, Gasteiger partial atomic charges were assigned [42], and all bonds except for amides were kept rotatable. Docking runs were performed with the Lamarckian genetic algorithm included in AutoDock 3.0 [43], performing 50 independent runs per ligand, using an initial population of 50 randomly placed individuals, a maximum number of 1.5×10^6 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. Resulting ligand conformations that differ by less than 1 Å rmsd from each other were clustered together and were represented by the solution with the best docking energy.

Acknowledgements

The pGEX-DPR1 and pBC-RAM2 plasmids were kindly provided by Professor F. Tamanoi (UCLA). I.S. wishes to thank Professor Dr. S. Grabley for generous support and Ms. S. Egner for technical assistance.

References

[1] H.-W. Fu, P.J. Casey, Rec. Prog. Horm. Res. 54 (1999) 315–343.
[2] A. Wittinghofer, H. Waldmann, Angew. Chem. 112 (2000) 4360–4383, Angew. Chem. Int. Ed. Engl. 39 (2000) 4192–4214.
[3] I.M. Bell, Expert Opin. Ther. Pat. 10 (2000) 1813–1831.
[4] A.D. Cox, C.J. Der, Curr. Opin. Pharmacol. 2 (2002) 388–393.
[5] W.T. Purcell, R.C. Donehower, Curr. Oncol. Rep. 4 (2002) 29–36.
[6] D. Chakrabarti, T. Azam, C. DelVecchio, L. Qiu, Y. Park, C.M. Allen, Mol. Biochem. Parasitol. 94 (1998) 175–184.
[7] D. Chakrabarti, T. Da Silva, J. Barger, S. Paquette, H. Patel, S. Patterson, C.M. Allen, J. Biol. Chem. 277 (2002) 42066–42073.
[8] K. Yokoyama, P. Trobridge, F.S. Buckner, W.C. Van Voorhis, K.D. Stuart, M.H. Gelb, J. Biol. Chem. 273 (1998) 26497–26505.
[9] F.S. Buckner, K. Yokoyama, L. Nguyen, A. Grewal, H. Erdjument-Bromage, P. Tempst, C.L. Strickland, L. Xiao, W.C. Van Voorhis, M.H. Gelb, J. Biol. Chem. 275 (2000) 21870–21876.
[10] F.S. Buckner, R.T. Eastman, J.L. Nepumuceno-Silva, E.C. Speelmon, P.J. Myler, W.C. Van Voorhis, K. Yokoyama, Mol. Biochem. Parsitol. 122 (2002) 181–188.
[11] M. Ibrahim, N. Azzouz, P. Gerold, R.T. Schwarz, Int. J. Parasitol. 31 (2001) 1489–1497.
[12] J. Sachs, P. Malaney, Nature 415 (2002) 680–685.
[13] R.G. Ridley, Nature 415 (2002) 686–693.
[14] J. Wiesner, R. Ortmann, H. Jomaa, M. Schlitzer, Angew. Chem. 115 (2003) 5432–5451 Angew. Chem. Int. Ed. 42 (2003) 5274–5293.
[15] M.H. Gelb, W.C. Van Voorhis, F.S. Buckner, K. Yokoyama, R. Eastman, E.P. Carpenter, C. Panethymitaki, K.A. Brown, D.F. Smith, Mol. Biochem. Parasitol. 126 (2003) 155–163.
[16] M. Schlitzer, Curr. Pharm. Des. 8 (2002) 1713–1722.
[17] J. Wiesner, P. Wißner, H.-M. Dahse, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. 9 (2001) 785–792.
[18] J. Wiesner, A. Mitsch, P. Wißner, H. Jomaa, M. Schlitzer, Pharmazie 56 (2001) 443–444.
[19] J. Wiesner, A. Mitsch, P. Wißner, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. Lett. 11 (2001) 423–424.
[20] J. Wiesner, K. Kettler, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. Lett. 12 (2002) 543–545.
[21] J. Wiesner, A. Mitsch, P. Wißner, O. Krämer, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. Lett. 12 (2002) 2681–2683.
[22] J. Wiesner, R. Ortmann, A. Mitsch, P. Wißner, I. Sattler, H. Jomaa, M. Schlitzer, Pharmazie 58 (2003) 289–290.
[23] J. Wiesner, K. Kettler, J. Sakowski, R. Ortmann, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. Lett. 13 (2003) 361–363.
[24] J. Wiesner, K. Fucik, K. Kettler, J. Sakowski, R. Ortmann, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. Lett. 13 (2003) 1539–1541.
[25] J. Wiesner, A. Mitsch, H. Jomaa, M. Schlitzer, Bioorg. Med. Chem. Lett. 13 (2003) 2159–2161.
[26] J. Wiesner, A. Mitsch, M. Altenkämper, R. Ortmann, H. Jomaa, M. Schlitzer, Pharmazie 58 (2003) 854–856.
[27] J. Wiesner, K. Kettler, J. Sakowski, R. Ortmann, M.A. Katzin, E.A. Kimura, K. Silber, G. Klebe, H. Jomaa, M. Schlitzer, Angew. Chem. 116 (2004) 254–257 Angew. Chem. Int. Ed. 43 (2004) 251–254.
[28] K. Kettler, J. Sakowski, K. Silber, I. Sattler, G. Klebe, M. Schlitzer, Bioorg. Med. Chem. 11 (2003) 1521–1530.
[29] D.L. Pompliano, R.P. Gomez, N.J. Anthony, J. Am. Chem. Soc. 114 (1992) 7945–7946.
[30] K. Del Villar, H. Mitsuzawa, W. Yang, I. Sattler, F. Tamanoi, J. Biol. Chem. 272 (1997) 680–687.
[31] R.E. Desjardins, C.J. Canfield, J.D. Haynes, J.D. Chulay, Antimicrob. Agents Chemother. 16 (1979) 710–718.
[32] W. Trager, J.B. Jensen, Science 193 (1976) 673–675.
[33] M.L. Ancelin, M. Calas, J. Bompart, G. Cordina, D. Martin, M.B. Bari, et al., Blood 91 (1998) 1426–1437.
[34] C.L. Strickland, W.T. Windsor, R. Syto, L. Wang, R. Bond, R. Wu, et al., Biochemistry 37 (1998) 16601–16611.
[35] D.S. Goodsell, A.J. Olson, Proteins 8 (1990) 195–202.
[36] G.M. Morris, D.S. Goodsell, R. Huey, A.J. Olson, J. Comput. Aided Mol. Des. 10 (1996) 293–304.
[37] B. Boeckmann, A. Bairoch, R. Apweiler, M.-C. Blatter, A. Estreicher, E. Gasteiger, M.J. Martin, K. Michoud, C. O'Donovan, I. Phan, S. Pilbout, M. Schneider, Nucleic Acids Res. 31 (2003) 365–370.
[38] C. Notredame, D. Higgins, J. Heringa, J. Mol. Biol. 302 (2000) 205–217.
[39] M.A. Marti-Renom, A. Stuart, A. Fiser, R. Sánchez, F. Melo, A. Sali, Annu. Rev. Biophys. Biomol. Struct. 29 (2000) 291–325.
[40] D.A. Case, D.A. Pearlman, J.W. Caldwell, T.E. Cheatham III, J. Wang, W.S. Ross, C.L. Simmerling, T.A. Darden, K.M. Merz, R.V. Stanton, A.L. Cheng, J.J. Vincent, M. Crowley, V. Tsui, H. Gohlke, R.J. Radmer, Y. Duan, J. Pitera, I. Massova, G.L. Seibel, U.C. Singh, P.K. Weiner, P.A. Kollman, AMBER 7, University of California, San Francisco, 2002.
[41] S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Singh, C. Ghio, G. Alagona, et al., J. Am. Chem. Soc. 106 (1984) 765–784.
[42] J. Gasteiger, M. Marsili, Tetrahedron 36 (1980) 3219–3228.
[43] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, et al., J. Comput. Chem. 19 (1998) 1639–1662.