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# Non-thiol Farnesyltransferase Inhibitors: *N*-(4-Acylamino-3-benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic Acid Amides

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**Abstract**—We have designed the nitrophenylfurylacryl-substituted benzophenone **4f** as a non-thiol farnesyltransferase inhibitor utilizing a novel aryl binding site of farnesyltransferase. Variation of the 2-acylamino substituent at the benzophenone core structure of our initial lead **4f** yielded several non-thiol farnesyltransferase inhibitors with improved activity. These compounds display activity in the low nanomolar range.


## Introduction

In the past years, farnesyltransferase has become a major target in the development of potential anti-cancer drugs. Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesylpyrophosphate 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 whose side chain is farnesylated, A amino acids which normally, but not neccessarily, carry aliphatic side chains and X mostly methionine or serine.[1,2]

Although farnesyltransferase inhibitors have demonstrated their efficiency in various cancer cell culture assays, animal models and first clinical studies, it turned out that their mechanism of action is much more complicated than initially anticipated. Farnesyltransferase inhibitors display multiple effects as the inhibition of anchorage independent cell growth, reversal of the phenotype of cancer cells back to that of non-transformed cells, cell-cycle arrest and induction of apoptosis. It became obvious that these multiple effects cannot be attributed solely to the prevention of ras farnesylation. Several different farnesylated proteins as for instance ras, rhoB, and centromer binding proteins seem to be involved in the action of farnesyltransferase inhibitors. Although the exact mechanism of the antiproliferative effect of farnesyltransferase inhibitors remains to be determined, farnesyltransferase inhibitors are regarded as a major emerging strategy in cancer therapy.[3–12]

Most inhibitors described in literature are peptidomimetics resembling the CAAX-tetrapeptide recognition sequence of farnesylated proteins. The majority of these CAAX-peptidomimetics exhibit a free thiol group which is shown to coordinate the enzyme-bound zinc ion. However, free thiols are associated with several adverse drug effects[13] and, therefore, the development of farnesyltransferase inhibitors is clearly directed towards the so-called non-thiol farnesyltransferase inhibitors. The most frequently used replacements for cysteine are nitrogen-containing heterocycles. The ring nitrogen is supposed to coordinate to the enzyme-bound zinc similarly to the cysteine thiol group.[14] However, it has been shown that nitrogen heterocycles can be replaced by aryl residues lacking the ability to coordinate metal atoms without loosing too much of their farnesyltransferase inhibitory activity.[15,16] Therefore, the existence of at least one hitherto unknown aryl binding region in the farnesyltransferase's active site has been postulated.[17,18]

Using docking studies of model compounds of non-thiol farnesyltransferase inhibitors as well as GRID analysis

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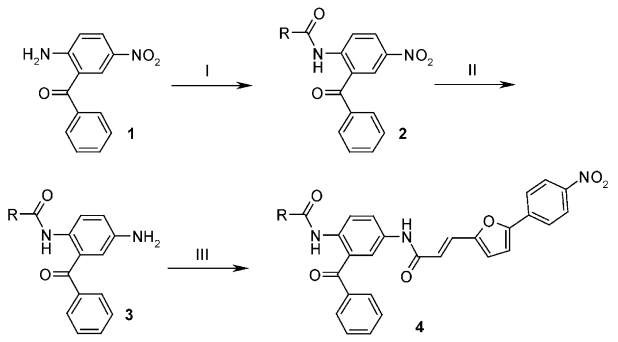


**Scheme 1.** (I) R-CO–Cl, toluene/dioxane, reflux, 2 h; (II) $SnCl_2 \times 2H_2O$, EtOAc, reflux 2 h; (III) 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride, toluene/dioxane, reflux, 2 h.

of farnesyltransferase's active site, we have identified two different aryl binding clefts in the farnesyltransferase's active site which we suggest to be the postulated aryl binding regions.[19]

We have designed the nitrophenylfurylacryl-substituted benzophenone **4f** as a non-thiol farnesyltransferase inhibitor utilizing one of these aryl binding sites.[19] In this study, we addressed the question how the replacement of the *p*-tolylacetyl residue at the 2-amino group of the benzophenone core structure of **4f** would influence farnesyltransferase inhibitory activity.

## Chemistry

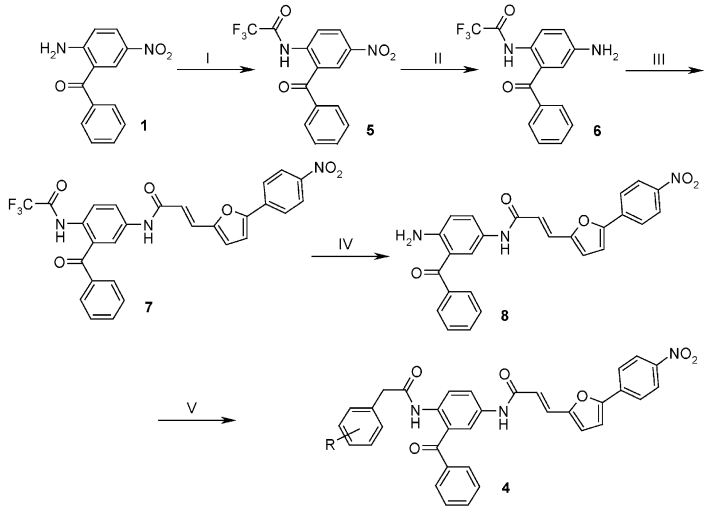
Synthesis of most of the target compounds **4a**–**x** was accomplished by acylation of appropriate 2-acylamino-5-aminobenzophenones **3** using 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Intermediates **3** were prepared as described previously[20] form 2-amino-5-nitrobenzophenone **1** (Scheme 1). However, since acylation of **1** by 2- or 4-trifluoromethylphenylacetic acid chloride failed and because of the reductions step involved in the synthesis according to Scheme 1, an alternative route had to be followed for the preparation of compounds **4m**–**o** (Scheme 2). First, the 2-amino group of **1** was protected as trifluoroacetamide (**5**). After reduction of the 5-nitro group, the resulting amine **6** was acylated with 3-[5-(4-nitrophenyl)-2-furyl]arylic acid chloride. After removal of the protective group from **7** the resulting intermediate **8** could by acylated by 2- and 4-trifluoromethylphenylacetic acid chloride and 4-nitrophenylacetic acid chloride, respectively, yielding compounds **4m**–**o**. The 4-fluoro compound **4i** was prepared in the same manner.

## Farnesyltransferase inhibition assay

The inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described by Pompliano.[21] The assay employed yeast farnesyltransferase (FTase) fused to Glutathione *S*-transferase at the N-terminus of the β-subunit.[22] Farnesylpyrophosphate 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 which results in an enhancement of fluorescence at 505 nm which is used to monitor the enzyme reaction.-Table 1

## Flexible docking

Flexible docking of selected compounds was performed using the program FlexX.[23] Based on the coordinates of the published crystal structure[24] of a ternary complex of farnesyltransferase, a farnesylpyrophosphate analogue and *N*-acetyl-Cys-Val-Ile-selenoMetOH (PDB-code 1QBQ), we have calculated the solvent accessible surface of the farnesyltransferase's active site using the program MOLCAD which is implemented in the molecular modeling software package SYBYL.[25] The nitrophenyl residue of the terminal biaryl moiety was placed into the region we have previously suggested to be the far aryl binding site.[19] Subsequently, the remaining fragments of the inhibitors were placed in the active site using the incremental construction algorithm of FlexX. The docking runs provided sets of solutions which were inspected according to their calculated energy score.



**Scheme 2.** (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-$C_6H_4$–$CH_2$–COCl, toluene/dioxane, reflux, 2 h.

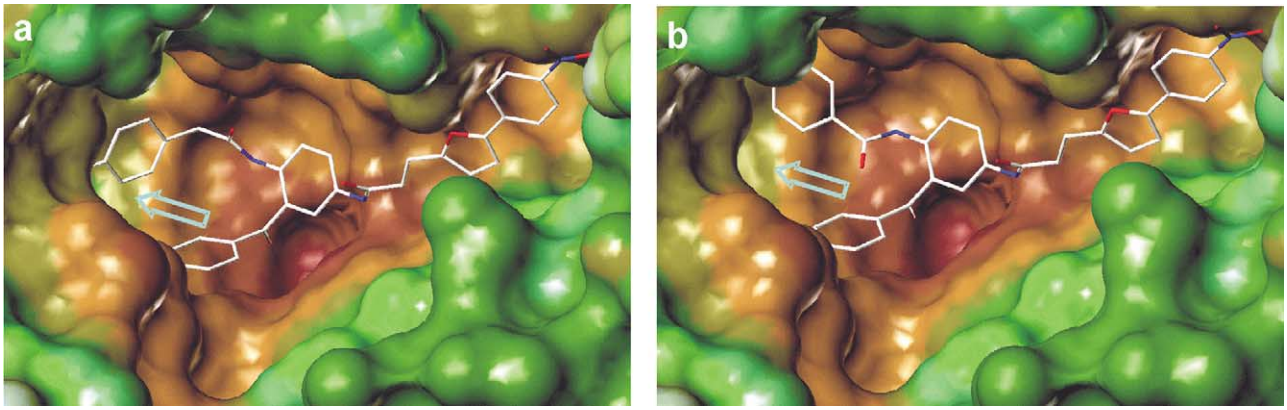



**Figure 1.** Docking solution of inhibitors **4f** (a) and **4a** (b) showing the terminal aryl of **4f** in a binding pocked (indicated by a blue arrow) while the phenyl residue of **4a** does not reach into this pocket.

Selected docking solutions were minimised with the MAB force field[26] and the resulting geometries were evaluated using the knowledge based scoring function DrugScore,[27] which was also used to locate areas of favoured binding of moieties with selected properties.

## Results and Discussion

Having developed compound **4f** as a novel non-thiol farnesyltransferase inhibitor which was specifically designed to make use of an aryl binding site of farnesyltransferase,[19] we then turned our attention towards the question how variations of the acyl substituent at the 2-amino group of the benzophenone core structure would influence the inhibitory activity of this class of compounds. Starting from the *p*-tolylacetic acid substituted lead **4f** we first turned to moieties with an unsubstituted phenyl residue and varied the distance between the amide carbonyl group and the terminal phenyl residue. Here it turned out that deviation from the original methylene spacer resulted in a considerable drop in inhibitory activity. While the unsubstituted phenylacetic acid compound **4b** is about 6-fold more active ($IC_{50}=6$ nM) than the original lead **4f** ($IC_{50}=35$ nM), the shorter benzoic acid compound **4a** ($IC_{50}=123$ nM) is markedly less active. This difference may be explained using flexible docking. Direct comparison of the docking solutions of the benzoic acid compound **4a** and the tolylacetic acid derivative **4f** shows the aryl residue of **4f** in a binding cleft indicated by a blue arrow in Fig. 1a while the phenyl residue of **4a** cannot reach into this cleft (Fig. 1b). This may be an explanation for the lower activity of the benzoic acid compound **4a**. As **4a** also the longer phenylpropionic acid compound **4c** ($IC_{50}=40$ nM) is less active than the lead **4f**. However, introduction of a *trans*-configurated double bond into the ethylene spacer of **4c** resulted in an inhibitor (**4d**) which was equipotent ($IC_{50}=5$ nM) to the phenylacetic acid derivative **4b**. The next step towards the establishment of systematic structure–activity relationships would have been the addition of substituents to the terminal phenyl residue of the phenylacetic acid derivative **4b** and the cinnamic acid derivative **4d**. We decided to focus our attention on the variation of the phenylacetic acid substructure and postpone the investigation of other cinnamic acid derivatives to a separate study.

Introduction of a *p*-methoxy (**4e**; $IC_{50}=35$ nM) or a *p*-methyl group (**4f**; $IC_{50}=35$ nM) resulted in a 6-fold reduction in activity in comparison to the unsubstituted phenylacetic acid derivative **4b**. Shifting the methyl group from the *para* to the *meta* (**4g**; $IC_{50}=58$ nM) and *ortho* position (**4h**; $IC_{50}=206$ nM), respectively, resulted in an increasing reduction of activity which is especially pronounced with the *ortho* derivative **4h**. Flexible docking might provide a rationale for this difference. Introduction of halogen substituents into the *para* position of the terminal phenyl residue resulted in all cases in a decreased activity in comparison to the unsubstituted derivative **4b**. This effect is at least pronounced with the fluoro derivative **4i** ($IC_{50}=15$ nM) what might be explained on the basis that the fluoro substituent is more similar to the hydrogen than the chloro or bromo substituent. These two substituents caused a considerable reduction in activity which is more pronounced with the chloro substituent (**4j**; $IC_{50}=102$ nM) than with the bromo substituent (**4k**; $IC_{50}=50$ nM). Surprisingly, shifting the bromo substituent from the *para* to the *ortho* position resulted in a more active inhibitor **4l**, ($IC_{50}=15$ nM). This result is in marked difference to the situation observed with the methyl substituents where the shift from the *para* to the *ortho* position caused a decrease in activity. Introduction of a trifluoromethyl group into the *para* position of the terminal phenyl resulted in an inhibitor **4m** which is slightly more potent ($IC_{50}=26$ nM) than the lead derivative **4f**. Again, shifting this substituent from the *para* to the *ortho* position resulted in an increase in activity (**4n**; $IC_{50}=14$ nM). These structure activity relationships are not easily understood. Especially disturbing is the different influence of the *ortho* position on the activity. In case of the methyl substituent this position strongly reduces activity, while in case of bromo or trifluoromethyl the *ortho* substituted derivatives are more active than their *para* analogoues. However, molecular modelling methods can provide some insight into these structure activity relationships. The first ranking FlexX docking results of

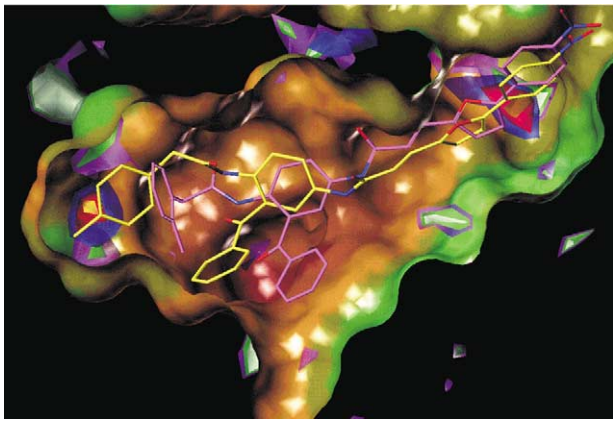


**Figure 2.** Comparison of the docking solutions of the *para* methyl substituted inhibitor **4f** (yellow) and the *ortho* methyl substituted inhibitor **4h** (margenta). Areas which according to DrugScore prevalently bind methyl are indicated by a blue-yellow-red cloud (increasing prevalence). The *para* methyl group of inhibitor **4f** (yellow) is located in such an area while the *ortho* methyl group of **4h** (margenta) is directed in no such region.

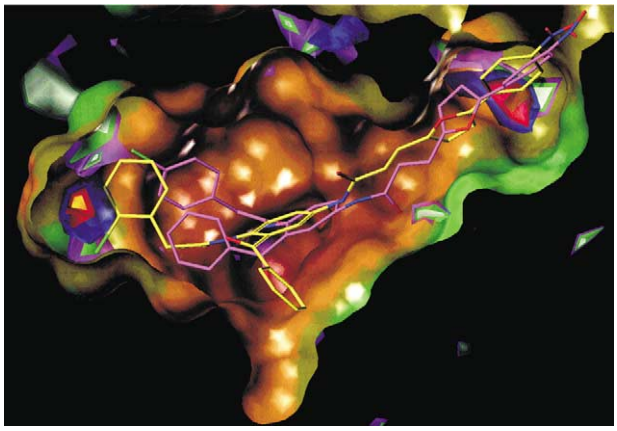


**Figure 3.** Comparison of the docking solutions of the *para* bromo substituted inhibitor **4k** (margenta) and the *ortho* bromo substituted inhibitor **4l** (yellow) Areas which according to DrugScore prevalently bind hydrogen bond acceptors are indicated by a violet-green-white cloud (increasing prevalence). It is clearly visible that the *ortho* bromo substituent points to such an area while the *para* bromo substituent cannot reach such an area.

inhibitors **4f**, **4h** (*para* and *ortho* methyl) and **4k**, **4l** (*para* and *ortho* bromo) were post-optimised with the MAB force field, minimising the ligands in the rigid binding pocket. Rescoring the resulting geometries of the methyl derivatives **4f** and **4h** with DrugScore clearly indicates the preference for the *para* derivative **4f**. As can be seen in Fig. 2 the *para* methyl group of inhibitor **4f** points into an area which favours binding of a methyl group indicated by a so called DrugScore hot spot. In contrast, the *ortho* methyl group of **4h** is not located in such an area what may explain its lower activity. In case of the bromo derivatives **4k** and **4l**, the *ortho* bromo substituent of **4l** is located in an area in which according to DrugScore binding of a hydrogen bond acceptor is favoured while the *para* substituent of **4k** cannot reach into such an area (Fig. 3). Since bromo as well as trifluoromethyl possess some weak hydrogen bond acceptor properties this may be the explanation for the prevalence of the *ortho* derivatives in this cases.

From all substituents placed into the *para* position the nitro group produced the largest decrease in activity (**4o**; $IC_{50} = 136$ nM) in comparison to the unsubstituted derivative **4b**. Introduction of an additional chloro substituent into the *ortho* position of the *p*-chloro derivative **4j** ($IC_{50} = 102$ nM) had no significant effect on the inhibitory activity (**4p**; $IC_{50} = 92$ nM).

Then, we turned our attention to bigger aromatic structures. Naphthyl residues connected via the 1- or 2-position directly to the amide carbonyl yielded compounds of medium activity (**4q**; $IC_{50} = 465$ nM; **4r**; $IC_{50} = 185$ nM). As seen with the phenyl derivatives **4a,b** introduction of a methylene spacer markedly improved activity. The 1- and 2-naphthylacetic acid derivatives (**4s**; $IC_{50} = 6$ nM; **4t**; $IC_{50} = 8$ nM) are as active as the phenylacetic acid derivative **4b**. Equal activity was found for the biphenylylacetic acid derivative **4u** ($IC_{50} = 7$ nM). Docking of this inhibitor (Fig. 4) provided an interesting solution with the biaryl moiety placed in a lipophilic chanal which was recently shown to bind the farnesyl residue the enzyme's reaction product, the farnesylated protein.[28]

Finally, we turned back to the ring-unsubstituted phenylacetic acid and introduced a substituent into the α-position. While the decrease observed with a methyl group in this position is small (**4v**; $IC_{50} = 16$ nM), this effect is pronounced with the bulkier phenyl group (**4w**; $IC_{50} = 660$ nM).

Although structure–activity relationships in this series of farnesyltransferase inhibitors are difficult to interpret, variation of the 2-acylamino substituent at the benzophenone core structure of our initial lead **4f** yielded several non-thiol farnesyltransferase inhibitors with improved activity. These compounds display activity in the low nanomolar range and will be used as starting points for further structural variation which led to inhibitors with improved properties as will be reported in due course.

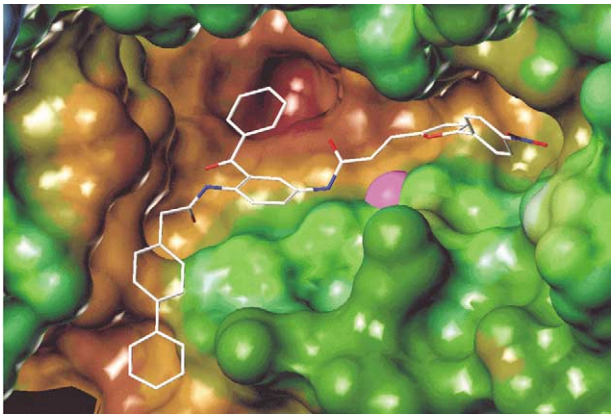
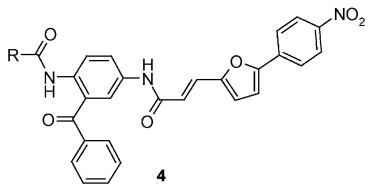
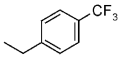
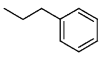
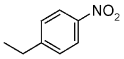
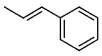
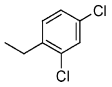
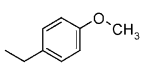
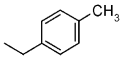
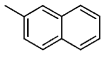
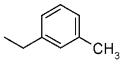
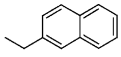
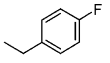
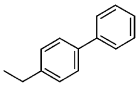
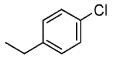
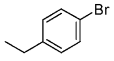
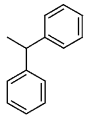


**Figure 4.** Docking solution of the biphenyl substituted inhibitor **4u** showing the biphenyl residue in a lipophilic chanal which normally habours the farnesyl residue of the farnesylated protein.

**Table 1.** Farnesyltransferase inhibitory activity of compounds **4a**–**x**



**4**

| Compd. | R | $IC_{50}$ (nM) | Compd. | R | $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **4a** |  | 123±19 | **4m** |  | 26±6 |
| **4b** |  | 6±3 | **4n** |  | 14±3 |
| **4c** |  | 40±4 | **4o** |  | 136±11 |
| **4d** |  | 5±2 | **4p** |  | 92±3 |
| **4e** |  | 35±1 | **4q** |  | 465±20 |
| **4f** |  | 35±5 | **4r** |  | 185±11 |
| **4g** |  | 58±5 | **4s** |  | 6±3 |
| **4h** |  | 206±27 | **4t** |  | 8±2 |
| **4i** |  | 15±3 | **4u** |  | 7±2 |
| **4j** |  | 102±39 | **4v** |  | 16±2 |
| **4k** |  | 50±11 | **4w** |  | 660±60 |
| **4l** |  | 15±4 | **4x** |  | 117±13 |

## Experimental

$^1$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 Generators VG 7070H 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. The preparation of following compounds has been described[20]: **2a-c**, **e-h**, **j**, **k**, **p**, **q**, **s-w**, **3a-c**, **e-h**, **j**, **k**, **p**, **q**, **s-w**, **5** and **6**.

### 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 to give the crude products.

### General procedure 2: reduction of aromatic nitro compounds

Aromatic nitro compounds **2** were dissolved in EtOAc (50–100 mL) and $SnCl_2\times2H_2O$ (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 $MgSO_4$.

***N*-3-Benzoyl-4-(benzoylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4a).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)benzoic acid amide (237 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 261 mg, (62%); mp: 252 °C. IR (KBr): $\nu = 3436$, 1628, 1599, 1548, 1510, 1334 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta = 6.78$ (d, $J = 16$ Hz, 1H,=CH), 7.03–7.04 (m, 1H, Ar), 7.42–7.49 (m, 3H, Ar and d, $J = 16$ Hz, 1H,=CH), 7.50–7.61 (m, 4H, Ar), 7.70–7.74 (m, 4H, Ar), 7.83–8.01 (m, 5H, Ar), 8.31–8.33 (m, 2H, Ar), 10.42 (s, 1H, –NH), 10.61 (s, 1H, –NH). MS (EI): $m/z = 557$ (33) [M$^+$], 316 (100), 105 (77), 242 (51), 91 (34). Anal. ($C_{33}H_{23}N_3O_6$): C, 71.09; H, 4.16; N, 7.54; found C, 71.17; H, 4.17; N, 7.52.

***N*-3-Benzoyl-4-(phenylacetylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4b).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)phenylacetic acid amide (248 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 307 mg, (72%); mp: 211 °C. IR (KBr): $\nu = 3383$, 1670, 1629, 1598, 1545, 1512, 1330 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta = 3.19$ (s, 2H, –$CH_2$), 6.75 (d, $J = 16$ Hz, 1H, = CH), 7.02–7.25 (m, 6H, Ar), 7.39–7.50 (m, 3H, Ar and d, $J = 16$ Hz, 1H,=CH), 7.60–7.69 (m, 4H, Ar), 7.87–8.00 (m, 4H, Ar), 8.30–8.32 (m, 2H, Ar), 10.05 (s, 1H, –NH), 10.36 (s, 1H, –NH). MS (EI): $m/z = 571$ (8) [M$^+$], 212 (100), 330 (81), 211 (48), 331 (24), 312 (23). Anal. ($C_{34}H_{25}N_3O_6$): C, 71.45; H, 4.41; N, 7.35; found C, 71.46; H, 4.42; N, 7.44.

***N*-3-Benzoyl-4-(3-phenylpropionyl)aminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4c).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-3-phenylpropionic acid amide (258 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 221 mg, (50%); mp: 204 °C. IR (KBr): $\nu = 3379$, 1598, 1511, 1402, 1332, 853, 798 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta = 2.31$ (s, 2H, $-CH_2$), 2.60 (s, 2H, $-CH_2$), 6.77 (d, $J = 16$ Hz, 1H,=CH), 7.04–7.14 (m, 6H, Ar), 7.40–7.52 (m, 4H, Ar and d, $J = 16$ Hz, 1H,=CH), 7.61–7.80 (m, 4H, Ar), 7.88–7.90 (m, 3H, Ar), 8.31–8.33 (m, 2H, Ar), 9.87 (s, 1H, –NH), 10.37 (s, 1H, –NH). MS (EI): $m/z = 585$ (8) [$M^+$], 212 (100), 344 (69), 211 (41), 213 (22), 345 (21). Anal. ($C_{35}H_{27}N_3O_6$): C, 71.79; H, 4.65; N, 7.18; found C, 71.88; H, 4.61; N, 7.19.

***N*-(2-Benzoyl-4-nitrophenyl)cinnamic acid amide (2d).** From cinnamic acide chloride (666 mg, 4.0 mmol) and 2-amino-5-nitrobenzophenone (969 mg, 4.0 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 698 mg (47%); mp: 145 °C. IR (KBr): $\nu = 3239$, 1627, 1579, 1502, 1344, 1276, 1149, 762 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta = 6.65$ (d, $J = 16$ Hz, 1H,=CH), 7.34–7.54 (m, 7H, Ar), 7.61–7.68 (m, 3H, Ar), 7.74 (d, $J = 16$ Hz, 1H,=CH), 8.38–8.47 (m, 2H, Ar), 9.01–9.03 (m, 1H, Ar), 11.41 (s, 1H, –NH). MS (EI): $m/z = 372$ (30)$M^+$, 131 (100), 103 (16), 131 (11).

***N*-(4-Amino-2-benzoylphenyl)cinnamic acid amide (3d).** From **2d** (633 mg, 1.7 mmol) according to general procedure 2. Yield: 535 mg (92%). $^1H$ NMR ($CDCl_3$): $\delta = 3.65$ (s, 2H, $-NH_2$), 6.56 (d, $J = 16$ Hz, 1H,=CH), 7.22–7.38 (m, 3H, Ar), 7.49–7.70 (m, 7H, Ar), 7.71 (d, $J = 16$ Hz, 1H,=CH), 8.38–8.47 (m, 2H, Ar), 9.01–9.03 (m, 1H, Ar), 11.41 (s, 1H, –NH).

***N*-3-Benzoyl-4-(cinnamoylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4d).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and 3d (257 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 247 mg (56%); mp: 265 °C. IR (KBr): $\nu = 3373$, 1559, 1335, 1243, 1171, 971, 854, 752 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta = 6.65$ (d, $J = 16$ Hz, 1H,=CH), 6.76 (d, $J = 16$ Hz, 1H,=CH), 7.01–7.12 (m, 1H, Ar), 7.32–7.40 (m, 10H, Ar), 7.40 (d, $J = 16$ Hz, 1H,=CH), 7.50 (d, $J = 16$ Hz, 1H,=CH), 7.53–8.07 (m, 6H, Ar), 8.30–8.32 (m, 2H, Ar), 10.71 (s, 1H, –NH), 10.39 (s, 1H, –NH). MS (EI): $m/z = 583$ (21) [$M^+$], 212 (100), 242 (95), 131 (88), 342 (73), 40 (28), 211 (21). Anal. ($C_{35}H_{25}N_3O_6$): C, 72.03; H, 4.32; N, 7.20; found C, 72.06; H, 4.41; N, 7.15.

***N*-3-Benzoyl-4-(4-methoxyphenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4e).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-4-methyloxyphenylacetic acid amide (360 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 500 mg (83%); mp: >250 °C. IR (KBr): $\nu = 3427$, 1665, 1631, 1598, 1551, 1507, 1334 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta = 3.33$ (s, 2H, $-CH_2$), 3.70 (s, 3H, $-CH_3$), 6.78 (m, 3H, Ar), 7.02 (m, 3H, Ar), 7.40 (m, 2H, Ar), 7.49 (m, 2H, Ar), 7.60 (m, 2H, Ar), 7.67 (m, 2H, Ar), 7.77 (m, 1H, Ar), 7.88 (m, 1H, Ar), 7.99 (m, 2H, Ar), 8.31 (m, 2H, Ar), 10.06 (s, 1H, –NH), 10.42 (s, 1H, NH). MS (EI): $m/z = 601$ (1) [$M^+$], 212 (100), 121 (64), 360 (53), 148 (34), 211 (32), 135 (31), 239 (25). Anal. ($C_{35}H_{27}N_3O_7$): C, 69.88; H, 4.52; N, 6.98; found C, 69.55; H, 4.69; N, 7.05.

***N*-3-Benzoyl-4-(3-methylphenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4g).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-3-methylphenylacetic acid amide (258 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 292 mg, (67%); mp: 222 °C. IR (KBr): $\nu = 3379$, 1597, 1510, 1330, 1108, 852, 752 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta = 2.27$ (s, 3H, $-CH_3$), 3.38 (s, 2H, $-CH_2$), 6.75 (d, $J = 16$ Hz, 1H, =CH), 6.90–7.13 (m, 5H, Ar), 7.40–7.47 (m, 4H, Ar), 7.48 (d, $J = 16$ Hz, 1H, =CH), 7.61–7.80 (m, 7H, Ar), 8.30–8.32 (m, 2H, Ar), 10.03 (s, 1H, –NH), 10.36 (s, 1H, –NH). MS (EI): $m/z = 585$ (58) [$M^+$], 242 (100), 212 (79), 344 (69), 453 (19), 345 (17), 243 (16). Anal. ($C_{35}H_{27}N_3O_6$): C, 71.79; H, 4.65; N, 7.18; found C, 71.69; H, 4.72; N, 7.48.

***N*-3-Benzoyl-4-(2-methylphenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4h).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-methylphenylacetic acid amide (258 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 303 mg, (69%); mp: 221 °C. IR (KBr): $\nu = 3361$, 1598, 1508, 1332, 1108, 853, 752 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta = 2.12$ (s, 3H, $-CH_3$), 3.43 (s, 2H, $-CH_2$), 6.72 (d, $J = 16$ Hz, 1H, =CH), 7.06–7.08 (m, 5H, Ar), 7.36–7.45 (m, 3H, Ar), 7.45 (d, $J = 16$ Hz, 1H, =CH), 7.59–7.75 (m, 5H, Ar), 7.85–7.95 (m, 3H, Ar), 8.20–8.29 (m, 2H, Ar), 9.98 (s, 1H, –NH), 10.33 (s, 1H, –NH). MS (EI): $m/z = 585$ (9) [$M^+$], 212 (100), 344 (82), 211 (38), 141 (37), 238 (27), 105 (24). Anal. ($C_{35}H_{27}N_3O_6$): C, 71.79; H, 4.65; N, 7.18; found C, 71.56; H, 4.79; N, 7.28.

***N*-3-Benzoyl-4-(trifluoroacetylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (7).** From N-(4-amino-2-benzoylphenyl)-trifluoracetamide (**6**)[xy] (0.616 g, 2.0 mmol) und 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (554 mg, 2.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 1002 mg (90%); mp: 244 °C. IR (KBr): $\nu = 3426$, 1626, 1599, 1520, 1332 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta = 6.74$ (d, $J = 16$ Hz, 1H, =CH), 7.06 (m, 1H, Ar), 7.38 (m, 2H, Ar), 7.47 (m, 2H, Ar and d, $J = 16$ Hz, 1H, =CH), 7.61 (m, 1H, Ar), 7.64 (m, 2H, Ar), 7.83 (m, 1H, Ar), 7.95 (m, 3H, Ar), 8.27 (m, 2H, Ar), 10.53 (s, 1H, –NH), 11.24 (s, 1H, –NH). MS (EI): $m/z = 549$ (24) [$M^+$], 242 (100), 243 (15), 196 (14), 550 (8), 308 (6), 139 (5), 212 (2).

***N*-4-Amino-3-benzoylphenyl-3-[5-(4-nitrophenyl)-2-furyl]-acrylic acid amide (8).** From 7 (559 mg, 1.0 mmol) according to general procedure 2. Yield: 385 mg (85%); mp: 257 °C. IR (KBr): $\nu = 3487$, 1622, 1598, 1550, 1511,

1330 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 6.70 (d, $J$ = 16 Hz, 1H,=CH), 6.86 (m, 2H, Ar), 6.95 (m, 1H, Ar), 7.32 (d, $J$ = 16 Hz, 1H, =CH), 7.37 (m, 1H, Ar), 7.51 (m, 2H, Ar), 7.58 (m, 3H, Ar), 7.67 (m, 1H, Ar), 7.71 (m, 1H, Ar), 7.96 (m, 2H, Ar), 8.29 (m, 2H, Ar), 9.95 (s, 1H, –NH). MS (EI): $m/z$ = 453 (100) [$M^+$], 212 (83), 242 (52), 454 (30), 211 (13), 213 (13), 243 (8), 455 (5).

***N*-3-Benzoyl-4-(4-fluorophenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4i).** From 4-flourophenylacetic acid chloride (93 mg, 0.6 mmol) and *N*-4-amino-3-benzoylphenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**8**) (272 mg, 0.6 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 260 mg (74%); mp: 212 °C. IR (KBr): ν = 3314, 1599, 1508, 1333 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 3.38 (s, 2H, $–CH_2$), 6.73 (d, $J$ = 16 Hz, 1H, =CH), 7.00–7.13 (m, 5H, Ar), 7.37–7.98 (m, 11H, Ar and d, $J$ = 16 Hz, 1H, =CH), 8.28–8.29 (m, 1H, Ar), 10.01 (s, 1H, –NH), 10.34 (s, 1H, –NH). MS (EI): $m/z$ = 589 (37) [$M^+$], 242 (100), 348 (87), 212 (67), 211 (36), 238 (24), 239 (17), 217 (17). Anal. ($C_{34}H_{24}FN_3O_6$): C, 69.27; H, 4.10; N, 7.13; found C, 69.31; H, 4.21; N, 6.94.

***N*-3-Benzoyl-4-(4-chlorophenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4j).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-4-chlorophenylacetic acid amide (274 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 277 mg, (61%); mp: 219 °C. IR (KBr): ν = 3386, 1599, 1511, 1333, 854, 753 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 3.42 (s, 2H, $–CH_2$), 6.71 (d, $J$ = 16 Hz, 1H, =CH), 7.03–7.29 (m, 5H, Ar), 7.40–7.50 (m, 3H, Ar and d, $J$ = 16 Hz, 1H, =CH), 7.56–7.68 (m, 4H, Ar), 7.78–8.00 (m, 4H, Ar), 8.31–8.33 (m, 2H, Ar), 10.05 (s, 1H, –NH), 10.38 (s, 1H, –NH). MS (EI): $m/z$ = 606 (7) [$M^+$], 605 (19) [$M^+$], 40 (100), 242 (98), 212 (77), 364 (58), 213 (38), 365 (35). Anal. ($C_{34}H_{24}ClN_3O_6$): C, 67.36; H, 3.99; N, 6.93; found C, 67.29; H, 4.04; N, 6.89.

***N*-3-Benzoyl-4-(4-bromophenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4k).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-4-bromophenylacetic acid amide (409 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 532 mg (82%); mp: 237 °C. IR (KBr): ν = 3384, 3302, 1680, 1628, 1597, 1553, 1511, 1332 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 3.38 (s, 2H, $–CH_2$), 6.77 (d, $J$ = 16 Hz, 1H, =CH), 7.04 (m, 3H, Ar), 7.40 (m, 4H, Ar), 7.47 (m, 2H, Ar), 7.55 (m, 1H, Ar), 7.60 (d, $J$ = 16 Hz, 1H, =CH), 7.66 (m, 2H, Ar), 7.77 (m, 1H, Ar), 7.88 (m, 1H, Ar), 7.99 (m, 2H, Ar), 8.30 (m, 2H, Ar), 10.05 (s, 1H, –NH), 10.37 (s, 1H, –NH). MS (EI): $m/z$ = 650 (4) [$M^+$], 212 (100), 242 (78), 408 (49), 211 (48), 410 (47), 238 (33), 239 (21). Anal. ($C_{34}H_{24}BrN_3O_6$): C, 62.78; H, 3.72; N, 6.46; found C, 62.41; H, 4.10; N, 6.44.

***N*-(2-Benzoyl-4-nitrophenyl)-2-bromophenylacetic acid amide (2l).** From (2-bromophenyl)acetic acid chloride (935 mg, 4.0 mmol) and 2-amino-5-nitrobenzophenone (969 mg, 4.0 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 1032 mg (58%); mp: 96 °C. IR (KBr): ν = 3203, 3108, 1701, 1642, 1597, 1579, 1539, 1506, 1348 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 3.96 (s, 2H, $–CH_2$), 7.23 (m, 1H, Ar), 7.37 (m, 1H, Ar), 7.41 (m, 1H, Ar), 7.52 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.66 (m, 3H, Ar), 8.38 (m, 1H, Ar), 8.42 (m, 1H, Ar), 8.89 (m, 1H, Ar), 11.01 (s, 1H, –NH). MS (EI): $m/z$ = 438 (0.5) [$M^+$], 359 (100), 269 (43), 169 (32), 171 (32), 105 (29), 191 (26).

***N*-(4-Amino-2-benzoylphenyl)-2-bromophenylacetic acid amide (3l).** From **2l** (1008 mg, 2.3 mmol) according to general procedure 2. Yield: 847 mg (87%); IR (KBr): ν = 3438, 3357, 3282, 1658, 1518s, 1325 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 3.83 (s, 4H, $–CH_2$ and $–NH_2$), 6.73 (m, 1H, Ar), 6.85 (m, 1H, Ar), 7.15 (m, 1H, Ar), 7.31 (m, 1H, Ar), 7.36 (m, 1H, Ar), 7.44 (m, 2H, Ar), 7.56 (m, 2H, Ar), 7.65 (m, 2H, Ar), 8.26 (d, $J$ = 9 Hz, 1H, Ar), 10.04 (s, 1H, –NH). MS (EI): $m/z$ = 408 (70) [$M^+$], 212 (100), 410 (70), 211 (50), 409 (17), 239 (16), 213 (15).

***N*-3-Benzoyl-4-(2-bromophenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4l).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and 3l (409 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 493 mg (76%); mp: 228 °C. IR (KBr): ν = 3356, 2925, 1666, 1628, 1598, 1553, 1508, 1332 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 3.57 (s, 2H, $–CH_2$), 6.77 (d, $J$ = 16 Hz, 1H, =CH), 7.04 (m, 1H, Ar), 7.17 (m, 2H, Ar), 7.28 (m, 2H, Ar), 7.41 (m, 2H, Ar), 7.52 (m, 3H, Ar), 7.62 (m, 2H, Ar), 7.68 (m, 2H, Ar), 7.79 (d, $J$ = 16 Hz, 1H, =CH), 7.90 (m, 1H, Ar), 7.99 (m, 2H, Ar), 8.32 (m, 2H, Ar), 10.14 (s, 1H, –NH), 10.43 (s, 1H, –NH). MS (EI): $m/z$ = 650 (3) [$M^+$], 212 (100), 410 (66), 408 (65), 211 (50), 242 (24), 238 (23), 105 (19). Anal. ($C_{34}H_{24}BrN_3O_6$): C, 62.78; H, 3.72; N, 6.46; found C, 62.58; H, 4.11; N, 6.47.

***N*-3-Benzoyl-4-(4-trifluoromethylphenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4m).** From (4-trifluoromethylphenyl)acetic acid chloride (166 mg, 0.75 mmol) and 8 (325 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 190 mg (30%); mp: 235 °C. IR (KBr): ν = 3426, 1684, 1626, 1598, 1511, 1331 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 3.52 (s, 2H, $–CH_2$), 6.77 (d, $J$ = 16 Hz, 1H, =CH), 7.03 (m, 1H, Ar), 7.32 (m, 2H, Ar), 7.41 (m, 2H, Ar), 7.44 (m, 2H, Ar), 7.58 (m, 4H, Ar), 7.66 (m, 2H, Ar), 7.78 (d, $J$ = 16 Hz, 1H, =CH), 7.90 (m, 1H, Ar), 7.99 (m, 2H, Ar), 8.32 (m, 2H, Ar), 10.12 (s, 1H, –NH), 10.39 (s, 1H, –NH). MS (EI): $m/z$ = 639 (1) [$M^+$], 43 (100), 55 (86), 41 (84), 57 (77), 59 (69), 69 (56), 44 (54). Anal. ($C_{35}H_{24}F_3N_3O_6$): C, 65.73; H, 3.78; N, 6.57; found C, 65.42; H, 4.09; N, 6.46.

***N*-3-Benzoyl-4-(2-trifluoromethylphenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4n).** From (2-trifluormethylphenyl)acetic acid chloride (133 mg, 0.6 mmol) and 8 (260 mg, 0.6 mmol) according to general procedure 1. Purification: recrystallisation from

toluene. Yield: 155 mg (40%); mp: 220 °C. IR (KBr): ν = 3437, 1654, 1627, 1598, 1554, 1511, 1332 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 3.60 (s, 2H, $–CH_2$), 6.75 (d, $J$ = 16 Hz, 1H, =CH), 7.00 (m, 1H, Ar), 7.23 (m, 1H, Ar), 7.37 (m, 1H, Ar), 7.39 (m, 1H, Ar), 7.47 (m, 3H, Ar), 7.53 (m, 1H, Ar), 7.59 (m, 3H, Ar), 7.65 (m, 2H, Ar), 7.77 (d, $J$ = 16 Hz, 1H, =CH), 7.86 (m, 1H, Ar), 7.96 (m, 2H, Ar), 8.28 (m, 2H, Ar), 10.05 (s, 1H, –NH), 10.36 (s, 1H, –NH). MS (EI): $m/z$ = 639 (20) [$M^+$], 242 (100), 212 (69), 139 (44), 398 (42), 43 (38), 159 (32), 196 (31). Anal. ($C_{35}H_{24}F_3N_3O_6$): C, 65.73; H, 3.78; N, 6.57; found C, 65.68; H, 3.43; N, 6.77.

***N*-3-Benzoyl-4-(4-nitrophenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4o).** From 4-nitrophenylacetic acid chloride (109 mg, 0.6 mmol) and *N*-(4-amino-3-benzoylphenyl)- 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (272 mg, 0.6 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 309 mg, (84%); mp: 240 °C. IR (KBr): ν = 3377, 1686, 1598, 1516, 1332 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 3.52 (s, 2H, $–CH_2$), 6.71 (d, $J$ = 16 Hz, 1H, =CH), 6.97–6.98 (m, 1H, Ar), 7.30–7.62 (m, 10H, Ar and d, $J$ = 16 Hz, 1H, =CH), 7.72–8.04 (m, 5H, Ar), 8.25–8.27 (m, 2H, Ar), 10.08 (s, 1H, –NH), 10.33 (s, 1H, –NH). MS (EI): $m/z$ = 616 (1) [$M^+$], 212 (100), 357 (79), 242 (64) 211 (53), 44 (53), 356 (43), 453 (34). Anal. ($C_{34}H_{23}N_4O_8$): C, 66.23; H, 3.92; N, 9.09; found C, 66.47; H, 3.87; N, 8.87.

***N*-3-Benzoyl-4-(2,4-dichlorophenyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4p).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and **3p** (399 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 515 mg (80%); mp: 245 °C. IR (KBr): ν = 3365, 3109, 1669, 1628, 1597, 1549, 1512, 1479, 1403, 1332 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 3.42 (s, 2H, $–CH_2$), 6.78 (d, $J$ = 16 Hz, 1H, =CH), 7.05 (m, 2H, Ar), 7.33 (m, 1H, Ar), 7.44 (m, 6H, Ar), 7.59 (m, 1H, Ar), 7.64 (m, 2H, Ar), 7.77 (d, $J$ = 16 Hz, 1H, =CH), 7.88 (m, 1H, Ar), 7.99 (m, 2H, Ar), 8.31 (m, 2H, Ar), 10.12 (s, 1H, –NH), 10.44 (s, 1H, –NH). MS (EI): $m/z$ = 212 (100), 380 (62), 242 (62), 453 (46), 382 (41), 211 (40), 379 (38), 381 (37), 398 (30), 640 (1) [$M^+$]. Anal. ($C_{34}H_{23}Cl_3N_3O_6$): C, 63.76; H, 3.62; N, 6.56; found C, 63.39; H, 3.94; N, 6.48.

***N*-3-Benzoyl-4-(1-naphthoylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4q).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and **3q** (366 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 120 mg (20%); mp: = 260 °C. IR (KBr): ν = 3442, 1676, 1653, 1623, 1598, 1514, 1332 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 6.83 (d, $J$ = 16 Hz, 1H, =CH), 7.04 (m, 1H, Ar), 7.31 (m, 1H, Ar), 7.42 (m, 2H, Ar), 7.48 (m, 2H, Ar), 7.53 (m, 3H, Ar), 7.65 (m, 2H, Ar), 7.79 (m, 2H, Ar), 7.89 (d, $J$ = 16 Hz, 1H, =CH), 7.94 (m, 1H, Ar), 8.00 (m, 5H, Ar), 8.31 (m, 2H, Ar), 10.49 (s, 1H, –NH), 10.58 (s, 1H, –NH). MS (EI): $m/z$ = 607 (5) [$M^+$], 366 (100), 155 (94), 367 (28), 392 (13), 156 (12), 242 (5), 127 (4). Anal. ($C_{37}H_{25}N_3O_6$): C, 73.14; H, 4.15; N, 6.92; found C, 73.13; H, 4.32; N, 6.98.

***N*-(2-Benzoyl-4-nitrophenyl)-2-naphthoic acid amide (2r).** From 2-naphthoic acid chloride (763 mg, 4.0 mmol) and 2-amino-5-nitrobenzophenone according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 941 mg, (59%); mp: 206 °C. IR (KBr): ν = 3135, 1634, 1617, 1547, 1510, 1411, 1335, 1233, 1197, 963, 773, 696 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 7.60–7.74 (m, 7H, Ar), 7.91–8.10 (m, 4H, Ar), 8.50–8.60 (m, 3H, Ar), 9.18–9.19 (m, 1H, Ar), 12.41 (s, 1H, –NH).

**N-(4-Amino-2-benzoylphenyl)-2-naphthoic acid amide (3r).** From **2q** (605 mg, 1.5 mmol) according to general procedure 2. Yield: 488 mg, (89%). $^1H$ NMR ($CDCl_3$): δ = 3.68 (s, 2H, $–NH_2$), 6.90–7.01 (m, 2H, Ar), 7.46–7.80 (m, 11H, Ar), 8.53–8.67 (m, 2H, Ar), 11.59 (s, 1H, –NH).

***N*-3-Benzoyl-4-(2-naphthoylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4r).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and **3r** (275 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 227 mg, (50%); mp: 279 °C. IR (KBr): ν = 3370, 1599, 1507, 1333, 966, 853, 753 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): 6.79 (d, $J$ = 16 Hz, 1H, =CH), 7.04–7.05 (m, 1H, Ar), 7.41–7.63 (m, 6H, Ar and d, $J$ = 16 Hz, 1H, =CH), 7.75–8.01 (m, 11H, Ar), 8.23–8.33 (m, 3H, Ar), 10.44 (s, 1H, –NH), 10.72 (s, 1H, –NH). MS (EI): $m/z$ = 607 (1) [$M^+$], 155 (100), 366 (57), 392 (29), 127 (19), 242 (17). Anal. ($C_{37}H_{25}N_3O_6$): C, 73.14; H, 4.15; N, 6.92; found C, 73.30; H, 4.30; N, 6.85.

***N*-3-Benzoyl-4-(1-naphthyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4s).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-1-naphthyl-acetic acid amide (286 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 322 mg, (69%); mp: 227 °C. IR (KBr): ν = 3360, 1598, 1510, 1402, 1333, 967, 863 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): 3.89 (s, 2H, $–CH_2$), 6.71 (d, $J$ = 16 Hz, 1H, =CH), 6.97– 6.98 (m, 1H, Ar), 7.27–7.57 (m, 7H, Ar and d, $J$ = 16 Hz, 1H, =CH), 7.59–7.95 (m, 11H, Ar), 8.26–8.28 (m, 2H, Ar), 10.13 (s, 1H, –NH), 10.32 (s, 1H, –NH). MS (EI): $m/z$ = 621 (1) [$M^+$], 212 (100), 380 (78), 239 (39), 211 (37), 141 (25), 238 (24), 381 (23), 156 (22). Anal. ($C_{38}H_{27}N_3O_6$): C, 73.42; H, 4.38; N, 6.76; found C, 73.53; H, 4.53; N, 6.85.

***N*-3-Benzoyl-4-(2-naphthyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4t).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-naphthyl-acetic acid amide (286 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 342 mg, (73%); mp: 209 °C. IR (KBr): ν = 3309, 2342, 1598, 1508, 1332, 969, 853, 752 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): 3.57 (s, 2H, $–CH_2$), 6.76 (d, $J$ = 16 Hz, 1H, =CH), 7.03–7.04 (m, 1H, Ar), 7.26–7.28 (m, 1H, Ar), 7.41–8.00 (m, 17H, Ar and d, $J$ = 16 Hz, 1H, =CH), 8.31–8.33 (m, 2H, Ar), 10.13 (s, 1H, –NH), 10.38 (s, 1H, –NH). MS (EI): $m/z$ = 621 (1)$M^+$, 212 (100), 380 (74), 156 (41), 211 (37), 155 (31), 238 (27), 141 (27). Anal. ($C_{38}H_{27}N_3O_6$): C, 73.42; H, 4.38; N, 6.76; found C, 73.39; H, 4.52; N, 6.84.

***N*-3-Benzoyl-4-(4-biphenylyl)acetylaminophenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4u).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (139 mg, 0.5 mmol) and *N*-(4-amino-2-benzoylphenyl)biphenylylacetic acid amide (203 mg, 0.5 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 215 mg (34%); mp: 250 °C. IR (KBr): ν = 3390, 1698, 1628, 1597, 1552, 1515, 1402, 1333 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 3.46 (s, 2H, $–CH_2$), 6.79 (d, $J$ = 16 Hz, 1H, =CH), 7.04 (m, 1H, Ar), 7.19 (m, 2H, Ar), 7.34 (m, 1H, Ar), 7.42 (m, 4H, Ar), 7.52 (m, 4H, Ar), 7.61 (m, 4H, Ar), 7.69 (m, 2H, Ar), 7.79 (d, $J$ = 16 Hz, 1H, =CH), 7.90 (m, 1H, Ar), 8.00 (m, 2H, Ar), 8.32 (m, 2H, Ar), 10.11 (s, 1H, –NH), 10.40 (s, 1H, –NH). MS (EI): $m/z$ = 647 (1) [$M^+$], 212 (100), 406 (61), 242 (39), 211 (33), 182 (31), 181 (28), 167 (28). Anal. ($C_{40}H_{29}N_3O_6$): C, 74.18; H, 4.51; N, 6.49; found C, 74.18; H, 4.51; N, 6.75.

***N*-3-Benzoyl-4-(2-phenylpropionylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4v).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-phenylpropionic acid amide (344 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 309 mg (53%); mp: 208 °C. IR (KBr): ν = 3374, 3304, 2932, 1686, 1629, 1597, 1548, 1508, 1330 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 1.14 (d, $J$ = 7 Hz, 3H, $–CH_3$), 3.58 (q, $J$ = 7 Hz, 2H, $–CH_2$), 6.77 (d, $J$ = 16 Hz, 1H, =CH), 7.04 (m, 1H, Ar), 7.19 (m, 3H, Ar), 7.24 (m, 2H, Ar), 7.41 (m, 2H, Ar), 7.48 (m, 2H, Ar), 7.55 (m, 1H, Ar), 7.62 (m, 1H, Ar), 7.66 (m, 2H, Ar), 7.79 (d, $J$ = 16 Hz, 1H, =CH), 7.89 (m, 1H, Ar), 7.99 (m, 2H, Ar), 8.32 (m, 2H, Ar), 10.09 (s, 1H, –NH), 10.42 (s, 1H, –NH). MS (EI): $m/z$ = 585 (18) [$M^+$], 242 (100), 344 (34), 212 (31), 105 (25), 40 (15), 196 (9), 586 (4). Anal. ($C_{35}H_{27}N_3O_6$): C, 71.79; H, 4.65; N, 7.18; found C, 71.75; H, 4.50; N, 7.37.

***N*-3-Benzoyl-4-(diphenylacetylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (4w).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (277 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)diphenylacetic acid amide (406 mg, 1.0 mmol) according to general procedure 1. Purification: recrystallisation from toluene. Yield: 433 mg (67%); mp: 248 °C. IR (KBr): ν = 3453, 1598, 1516, 1332 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 4.96.(s, 1H, –CH), 6.74 (d, $J$ = 16 Hz, 1H, =CH), 6.97 (m, 1H, Ar), 7.09 (m, 3H, Ar), 7.15 (m, 3H, Ar), 7.19 (m, 4H, Ar), 7.35 (m, 1H, Ar), 7.40 (m, 3H, Ar), 7.55 (m, 2H, Ar), 7.62 (m, 2H, Ar), 7.74 (d, $J$ = 16 Hz, 1H, =CH), 7.83 (m, 1H, Ar), 7.94 (m, 1H, Ar), 8.26 (m, 5H, Ar), 10.24 (s, 1H, –NH), 10.37 (s, 1H, –NH). MS (EI): $m/z$ = 647 (5) [$M^+$], 182 (100), 105 (96), 239 (80), 406 (77), 265 (53), 212 (48), 167 (24). Anal. ($C_{40}H_{29}N_3O_6$): C, 74.18; H, 4.51; N, 6.49; found C, 73.98; H, 4.90; N, 6.68.

***N*-3-Benzoyl-4-(4-chlorobenzoylamino)phenyl-3-[5-(4-nitrophenyl)-2-furyl]acrylic Acid Amide (4x).** From 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride (195 mg, 0.75 mmol) and *N*-(4-amino-2-benzoylphenyl)-4-chlorobenzoic acid amide (263 mg, 0.75 mmol) according to general procedure 1. Purification: recrystallisation from ethanol. Yield: 257 mg, (73%); mp: 257 °C. IR (KBr): ν = 3384, 1678, 1627, 1597, 1546, 1513, 1403, 1333, 1247, 853, 799 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ = 6.70 (d, $J$ = 16 Hz, 1H, =CH), 7.04–7.05 (m, 1H, Ar), 7.42–7.78 (m, 11H, Ar and d, $J$ = 16 Hz, 1H, =CH), 7.89–8.02 (m, 4H, Ar), 8.31–8.33 (m, 2H, Ar), 10.44 (s, 1H, –NH), 10,58 (s, 1H, –NH). MS (EI): $m/z$ = 592 (1) [$M^+$], 591 (3) [$M^+$], 139 (100), 350 (97), 352 (37), 351 (34), 211 (34). Anal. ($C_{33}H_{22}ClN_3O_6$): C, 66.95; H, 3.75; N, 7.10; found C, 66.78; H, 3.91; N, 6.89.

## 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.[22] The enzyme was purified by standard procedures with glutathione-agarose beads for selective binding of the target protein.

## Farnesyltransferase assay

The assay was conducted as described.[21] Farnesylpyrophosphate (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 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 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.

## Molecular modeling

All molecular modeling was carried out using SYBYL[25] version 6.7/6.8 running on a Silicon Graphics O2 (R5000). Flexible docking was performed using FlexX[23] version 1.10. The FlexX command MAPREF and the perturbate mode of the PLACEBAS command were used. Default parameters were employed except the MAX_ENERGY value which was set to 10 kJ $mol^{-1}$.

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## References and Notes

1. Zhang, F. L.; Casey, P. J. *Annu. Rev. Biochem.* **1996**, *65*, 241.
2. Fu, H.-W.; Casey, P. J. *Rec. Prog. Hormon. Res.* **1999**, *54*, 315.
3. Leonard, D. M. J. *Med. Chem.* **1997**, *40*, 2971.
4. Qian, Y. S.; Sebti, M.; Hamilton, A. D. *Biopolymers* **1997**, *43*, 25.
5. Williams, T. M. *Exp. Opin. Ther. Pat.* **1998**, *8*, 553.
6. Williams, T. M. *Exp. Opin. Ther. Pat.* **1999**, *9*, 1263.
7. Williams, T. M.; Dinsmore, C. J. *Adv. Med. Chem.* **1999**, *4*, 273.
8. Wittinghofer, A.; Waldmann, H. *Angew. Chem.* **2000**, *112*, 4360. Wittinghofer, A.; Waldmann, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 4192.
9. Rowinsky, E. K.; Patnaik, A. *Emerg. Drugs* **2000**, *5*, 161.
10. Sebti, S. M.; Hamilton, A. D. *Oncogene* **2000**, *19*, 6584.
11. Cox, A. D. *Drugs* **2001**, *61*, 723.
12. Bell, I. M. *Exp. Opin. Ther. Pat.* **2000**, *10*, 1813.
13. Reynolds, J. E. F., Ed. *Martindale The Extra Pharmacopeia*, 31st ed. Royal Pharmaceutical Society of Great Britain: London, 1996; p 821.
14. Hunt, J. T.; Lee, V. G.; Leftheris, K.; Seizinger, B.; Carboni, J.; Mabus, J.; Ricca, C.; Yan, N.; Manne, V. J. *Med. Chem.* **1996**, *39*, 353.
15. O'Connor, S. J.; Barr, K. J.; Wang, L.; Sorensen, B. K.; Tasker, A. S.; Sham, H.; Ng, A.-C.; Cohen, J.; Devine, E.; Cherian, S.; Saeed, B.; Zhang, H.; Lee, J. Y.; Warner, R.; Tahir, S.; Kovar, P.; Ewing, P.; Alder, J.; Mitten, M.; Leal, J.; Marsh, K.; Bauch, J.; Hoffman, D. J.; Sebti, S. M.; Rosenberg, S. H. J. *Med. Chem.* **1999**, *42*, 3701.
16. Augeri, D. J.; Janowick, D.; Kalvin, D.; Sullivan, G.; Larsen, J.; Dickman, D.; Ding, H.; Cohen, J.; Lee, J.; Warner, R.; Kovar, P.; Cherian, S.; Saeed, B.; Zhang, H.; Tahir, S.; Ng, S.-C.; Sham, H.; Rosenberg, S. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1069.
17. Breslin, M. J.; deSolms, J.; Giuliani, E. A.; Stokker, G. E.; Graham, S. L.; Pompliano, D. L.; Mosser, S. D.; Hamilton, K. A.; Hutchinson, J. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3311.
18. Ciccarone, T. M.; MacTough, S. C.; Williams, T. M.; Dinsmore, C. J.; O'Neill, T. J.; Shah, D.; Culberson, J. C.; Koblan, K. S.; Kohl, N. E.; Gibbs, J. B.; Oliff, A. I.; Graham, S. L.; Hartman, G. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1991.
19. Böhm, M.; Mitsch, A.; Wiβner, P.; Sattler, I.; Schlitzer, M. *J. Med. Chem.* **2001**, *44*, 3117.
20. Sakowski, J.; Böhm, M.; Sattler, I.; Dahse, H.-M.; Schlitzer, M. *J. Med. Chem.* **2001**, *44*, 2886.
21. Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7945.
22. Del Villar, K.; Mitsuzawa, H.; Yang, W.; Sattler, I.; Tamanoi, F. *J. Biol. Chem.* **1997**, *272*, 680.
23. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **1996**, *261*, 470.
24. Strickland, C. L.; Windsor, W. T.; Syto, R.; Wang, L.; Bond, R.; Wu, R.; Schwartz, J.; Le, H. V.; Beese, L. S.; Weber, P. C. *Biochemistry* **1998**, *37*, 16601.
25. SYBYL Molecular Modeling software; Tripos Inc.: 1699 South Hanley Rd, Suite 303, St. Louis, MO 63144.
26. Gerber, P. R. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 37.
27. Gohlke, H.; Hendlich, M.; Klebe, G. *J. Mol. Biol.* **2000**, *295*, 337.
28. Long, S. B.; Casey, P. J.; Beese, L. S. *Nature* **2002**, *419*, 645.