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Aromatic Carboxylic Acids as Farnesyl Surrogates in Farnesylpyrophosphate-Based Farnesyltransferase Inhibitors

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Abstract—With the help of easily obtainable *N*-acylaspartic acids, the 4-phenylcinnamoyl and the 4-benzyloxycinnamoyl moiety were identified as structurally simple, readily available farnesyl surrogates, yielding more potent inhibitors of farnesyltransferase than the literature known *N*-farnesoylaspartic acid.

Introduction

Ras proteins play an important role in the transduction of growth signals from membrane bound receptor tyrosine kinases to the cell nucleus. In approximately 30% of all human cancers mutated Ras proteins are found. These proteins are locked in their active state and are thus constantly transferring growth signals into the nucleus which results in a malignant transformation of the cells. Because a post-translational modification is obligatory for Ras activity, the enzyme farnesyltransferase has been identified as a promising target in the development of novel cancer therapeutics. Farnesyltransferase catalyzes the crucial step of the post-translational modification of Ras proteins, the transfer of a farnesyl residue from farnesylpyrophosphate to the thiol of a cysteine side chain. This cysteine is part of the C-terminal CAAX tetrapeptide (C: cysteine, A: aliphatic amino acid, X: methionine or serine) of the Ras protein which is recognized by the farnesyltransferase. (for reviews see refs 1–8).

Most work towards the design of inhibitors of the farnesyltransferase has been done in the area of peptidomimetic analogues of the CAAX recognition sequence. Analogues of the second substrate, farnesylpyrophosphate (FPP), are far less in number. Efforts in this field have mainly focused on the replacement of the hydrolyzable pyrophosphate moiety. Most of the FPP analogues have retained the farnesyl moiety in a more or less unmodified form.[1–8]

In the course of our studies towards the design of novel farnesyltransferase inhibitors we wanted to explore the potency of non-prenylic lipophilic structures as possible farnesyl surrogates. We chose *N*-farnesoylaspartic acid (**1**) which has been described[9] to inhibit farnesyltransferase with an IC_{50} of 25 μM as our lead structure and replaced the farnesoic acid moiety by various acyl groups (Fig. 1).

The focus of our study was not the preparation of inhibitors as active as possible but the identification of potential farnesyl surrogates using inexpensive and easily accessible test compounds. Therefore, we chose the commercially available aspartic acid as the pyrophosphate mimetic substructure instead of potentially more active moieties as for instance β-phosphonoalanine.[2] The lipophilic residues thus identified would later also be used as farnesyl surrogates in the design of other farnesyltransferase inhibitors. Lipophilic residues employed in this study were mainly chosen on the basis of similarity of their size compared to the farnesyl residue, their presumed stability and simplicity of preparation. Due to the rigidity of their backbone, special attention was paid to aromatic structures as possible farnesyl replacements.

Chemistry

All *N*-acylaspartic acid derivatives **4** employed in this study were prepared by alkaline hydrolysis of the corresponding *N*-acylaspartic acid dimethyl esters **3**. For

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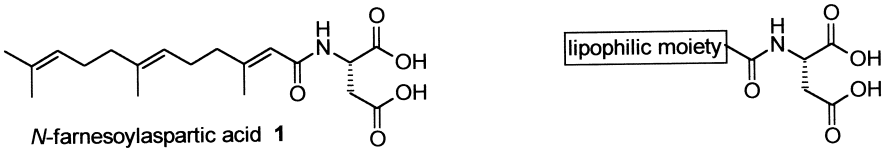


Figure 1.

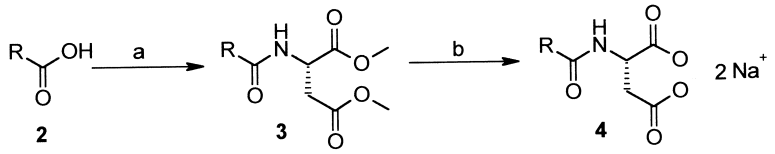
the preparation of the diester derivatives **3** appropriate carboxylic acids **2** were activated as mixed anhydrides using isobutyl chloroformate and coupled to aspartic acid dimethyl ester hydrochloride (Scheme 1). All 3-arylacrylic acids but α-methylcinnamic acid were prepared from the corresponding aldehydes and malonic acid.[10] Phenoxybenzyloxyacetic acid was prepared as described.[11]

Biological Evaluation

The inhibitory activity of the compounds on farnesyltransferase was determined using the fluorescence enhancement assay as described by Pompliano.[12] The assay employed yeast farnesyltransferase (FTase) fused to glutathione *S*-transferase at the N-terminus of the β-subunit.[13] 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. The results are shown in Table 1.

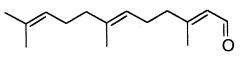
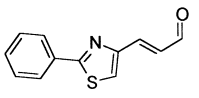
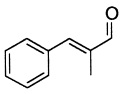
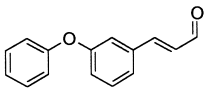
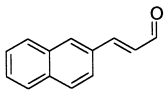
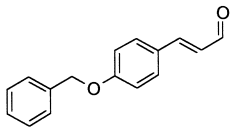
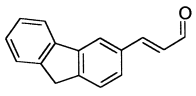
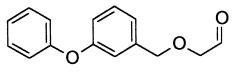
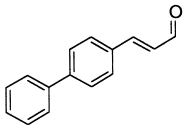
Results and Discussion

In the farnesyltransferase inhibiton assay some of the 3-arylacryloyl derivatives **4** showed inhibition of the farnesyltransferase in the low micromolar range. In the series of compounds ranging from **4a** to **4d** a tendency is visible that an increase in the size of the substituent results in better inhibitory activity. Best results are obtained with biphenylyl derivative **4d**, the 3-phenoxybenzyloxyacetic acid derivative **4h** and the 4-benzyloxycinnamoyl compound **4g** which is approximately 1 order of magnitude more active than the *N*-farnesoylaspartic acid (**1**)[9] (IC_{50}: 1.4 μM versus 25 μM). Compounds **4c, e** and **f** have approximately the same



Scheme 1. (a) *i*-BuOCOCl, *N*-methylmorpholine, DMF, −15°C, 5 min., (2) H-Asp(OMe)-OMe·HCl, *N*-methylmorpholine, DMF, −15°C→rt, overnight; (b) 1 N NaOH, THF/MeOH, rt.

Table 1. Farnesyltransferase inhibition of *N*-acylaspartic acids **4**

Compd	R-CO-	FTase inhibition	Compd	R-CO-	FTase inhibition
1		$IC_{50} = 25\ \mu M$[2]	**4e**		28% at 100 μM
4a		0% at 100 μM	**4f**		3% at 100 μM
4b		12% at 100 μM	**4g**		$IC_{50} = 1.4 \pm 0.2\ \mu M$
4c		$IC_{50} > 50\ \mu M$	**4h**		$IC_{50} = 5.4 \pm 0.9\ \mu M$
4d		$IC_{50} = 5.4 \pm 0.8\ \mu M$			

molecular size as **4d, g** and **h** but show a different spatial arrangement in the lipophilic part. It seems that the residues of **4d, g** and **h** produce the best resemblance of the enzyme bond conformation of the farnesyl moiety.

So it can be concluded that the farnesyl moiety can be replaced by certain aromatic structures. The same result was very recently reported by a group from Banyu Pharmaceuticals who also used much more complicated aromatic structures as farnesyl mimetics.[14]

With this study we discovered some structurally very simple farnesyl surrogates which are cheaper and much easier to prepare than the original farnesyl residue. Furthermore, incorporation of these novel farnesyl surrogates in farnesylpyrophosphate-based farnesyltransferase inhibitors yielded compounds of about one order of magnitude better activity than obtained with the original farnesoyl moiety. We used these novel farnesyl surrogates as partial structures in a novel class of bisubstrate analogue farnesyltransferase inhibitors.[15]

Experimental

^{1}H and ^{13}C 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 7070 H using a Vector 1 data aquisition system from Tecnivent 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. Column chromatagraphy was carried out using silica gel 60 (0.062–0.200 mm) from Merck.

General protocol for the coupling of carboxylic acids with aspartic acid dimethyl ester hydrochloride using mixed anhydride activation (Protocol 1)

The appropriate carboxylic acid was dissolved in a sufficient amount of dry DMF in a flame dried flask under an atmosphere of Ar. After addition of *N*-methylmorpholine [NMM] (0.25 mL per mmol acid) the solution was cooled to −15°C and isobutyl chloroformate (0.13 mL per mmol acid) was added. A solution of 1 equivalent of aspartic acid dimethyl ester hydrochloride in dry DMF which contained additional NMM (0.25 mL per mmol) was added after 5 min. The mixture was allowed to warm up to room temperature overnight and then poured into brine (400–800 mL). The aqueous mixture was extracted with EtOAc (3×100 mL) and the combined organic extracts were washed successively with 2 N citric acid, satd. $NaHCO_3$-solution and brine and dried over $MgSO_4$. The residue obtained after removal of the solvent was purified by recrystallisation or flash chromatography.

General protocol for the *O*-deprotection of *N*-acylaspartic acid dimethyl esters (Protocol 2)

The *N*-acylaspartic acid dimethyl esters **3** were dissolved in a 1:1 mixture of THF and methanol (50 mL per mmol) and stirred after addition of 2 equvalents of 1 N NaOH at rt until the reaction was complete (TLC). Then the solvents were evaporated.

***N*-(2-Methyl-3-phenylacryloyl)aspartic acid dimethyl ester 3a**. This compound was prepared according to protocol 1. Yield: 281 mg (46%); mp 200°C (ethanol/*n*-pentane). IR (KBr): ν = 3305, 2950, 1735 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.11 (s, 3H), 2.94 (dd, *J* = 17, 4.5 Hz, 1H), 3.09 (dd, *J* = 17, 4.5 Hz, 1H), 3.69 (s, 3H), 3.79 (s, 3H), 4.97 (m, 1H), 6.94 (m, 1H), 7.26–7.40 (m, 6H). ^{13}C NMR ($CDCl_3$): = 14.1, 36.0, 48.9, 52.1, 52.9, 128.0, 128.4, 129.6, 131.2, 135.0, 135.9. MS: *m*/*z* (%) 305 (18) [M$^+$], 145 (100). Anal. calcd for $C_{16}H_{19}NO_5$ (262.35): C, 62.94; H, 6.27; N, 4.29; found: C, 62.90; H, 6.29; N, 4.58.

***N*-(2-Methyl-3-phenylacryloyl)aspartic acid, disodium salt 4a**. This compound was prepared according to protocol 2. Yield: 100 mg (57%); mp >250°C. IR (KBr): ν = 3425, 1655, 1605 cm^{-1}. ^{1}H NMR (D_2O): δ = 2.38 (s, 3H), 2.77 (dd, *J* = 16, 9 Hz, 1H), 2.90 (dd, *J* = 16, 4 Hz, 1H), 4.67 (dd, *J* = 9, 4 Hz, 1H), 7.45 (s, 1H), 7.55 (m, 1H), 7.60–7.63 (m, 5H). ESI-MS: *m*/*z* (%) 278 (5) [M-2Na + 3H]$^+$, 300 (100) [M + 2H-Na]$^+$, 322 (69) [M + H]$^+$, 344 (15) [M + Na]$^+$. ESI-HRMS calcd for [M + Na]$^+$ $C_{14}H_{13}Na_3NO_5$: 344.048682; found: 344.046431.

***N*-[3-(2-Naphthyl)acryloyl]aspartic acid dimethyl ester 3b**. This compound was prepared according to protocol 1. Yield: 450 mg (66%); mp 129°C (toluene). IR (KBr): ν = 3335, 2950, 1735, 1655, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.97 (dd, *J* = 16, 4.5 Hz, 1H), 3.11 (dd, *J* = 16, 4.5 Hz, 1H), 3.71 (s, 3H), 3.80 (s, 3H), 5.03 (m, 1H), 6.57 (d, *J* = 16 Hz, 1H), 6.72 (d, *J* = 8 Hz, 1H), 7.47–7.51 (m, 2H), 7.63–7.66 (m, 1H), 7.78 (m, 4H), 7.91 (s, 1H). ^{13}C NMR ($CDCl_3$): = 36.2, 48.7, 52.1, 52.9, 120.2, 123.6, 126.7, 127.1, 127.6, 128.6, 128.7, 129.6, 132.2, 133.4, 134.1, 142.1, 165.5, 171.2, 171.7,. MS: *m*/*z* (%) 341 (30) [M$^+$], 181 (100), 152 (51). Anal. calcd for $C_{19}H_{19}NO_5$ (341.36): C, 66.85; H, 5.61; N, 4.10; found: C, 66.83; H, 5.61; N, 4.05.

***N*-[3-(2-Naphthyl)acryloyl]aspartic acid, disodium salt 4b**. This compound was prepared according to protocol 2. Yield: 125 mg (quant.); mp >250°C. IR (KBr): ν = 3385, 1655, 1595 cm^{-1}. ^{1}H NMR (D_2O): δ = 2.69 (dd, *J* = 16, 10 Hz, 1H), 2.83 (dd, *J* = 16, 5 Hz, 1H), 4.63 (dd, *J* = 10, 5 Hz, 1H), 6.73 (d, *J* = 16 Hz, 1H), 7.58–7.64 (m, 3H), 7.71–7.74 (m, 1H), 7.91–7.98 (m, 4H). ESI-MS: *m*/*z* (%) 336 (71) [M + 2H-Na]$^+$, 358 (100) [M + H]$^+$. ESI-HRMS calcd for [M + H]$^+$ $C_{17}H_{14}Na_2NO_5$: 358.066737; found: 358.071471.

***N*-[3-(2-Fluorenyl)acryloyl]aspartic acid dimethyl ester 3c**. This compound was prepared according to protocol 1. Yield: 640 mg (85%); mp 138°C (toluene). IR (KBr): ν = 3310, 2955, 1745, 1735, 1650, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.98 (dd, *J* = 17, 4 Hz, 1H), 3.12 (dd, *J* = 17, 4 Hz, 1H), 3.71 (s, 3H), 3.80 (s, 3H), 3.91 (s, 2H), 5.03 (m, 1H), 6.49 (d, *J* = 16 Hz, 1H), 6.67 (d, *J* = 8 Hz, 1H), 7.30–7.43 (m, 2H), 7.55 (m, 2H), 7.69–7.71 (m, 1H), 7.74–7.83 (m, 3H). ^{13}C NMR ($CDCl_3$): δ = 36.2,

36.8, 48.6, 52.0, 52.9, 115.8, 119.0, 120.3, 124.4, 125.1, 126.9, 127.2, 127.4, 128.2, 133.1, 141.0, 142.5, 143.8, 149.0, 165.6, 171.3, 171.7. MS: *m/z* (%) 379 (29) [M^+], 219 (100), 189 (43). HRMS calcd for $C_{22}H_{21}NO_5$ (379.41): 379.1420; found: 379.1402.

***N*-[3-(2-Fluorenyl)acryloyl]aspartic acid, disodium salt 4c**. This compound was prepared according to protocol 2. Yield: 276 mg (quant.); mp >250°C. IR (KBr): $\nu = 3385$, 1600 cm^{-1}. ^1H NMR (D_2O): $\delta = 2.73$ (dd, $J = 16$, 9 Hz, 1H), 2.86 (dd, $J = 16$, 4 Hz, 1H), 3.80 (s, 2H), 4.68 (dd, $J = 9$, 4Hz, 1H), 6.59 (d, $J = 16$ Hz, 1H), 7.41–7.49 (m, 4H), 7.58–7.63 (m, 2H), 7.67–7.69 (m, 1H), 7.80–7.82 (m, 1H). ESI-MS: *m/z* (%) 374 (27) $[M + 2H\text{-}Na]^+$, 396 (63) $[M + H]^+$, 418 (100) $[M + Na]^+$. ESI-HRMS calcd for $[M + Na]^+$ $C_{20}H_{15}Na_3NO_5$: 418.064332; found: 418.060414.

***N*-[3-(4-Biphenylyl)acryloyl]aspartic acid dimethyl ester 3d**. This compound was prepared according to protocol 1. Yield: 650 mg (88%); mp 150°C (toluene). IR (KBr): $\nu = 3315$, 3035, 2960, 1745, 1730, 1650, 1620 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.96$ (dd, $J = 17$, 4.5 Hz, 1H), 3.11 (dd, $J = 17$, 4.5 Hz, 1H), 3.71 (s, 3H), 3.80 (s, 3H), 5.01 (m, 1H), 6.49 (d, $J = 16$ Hz, 1H), 6.68 (d, $J = 8$ Hz, 1H), 7.34–7.38 (m, 1H), 7.44 (m, 2H), 7.56–7.62 (m, 6H), 7.68 (d, $J = 16$ Hz, 1H). ^{13}C NMR ($CDCl_3$): = 36.2, 48.6, 52.0, 52.9, 119.8, 127.0, 127.5, 127.7, 128.8, 133.6, 140.2, 141.5, 142.7, 165.4, 171.2, 171.7. MS: *m/z* (%) 367 (33) [M^+], 207 (100), 178 (57). Anal. calcd for $C_{21}H_{21}NO_5$ (367.40): C, 68.65; H, 5.76; N, 3.81; found: C, 68.30; H, 5.76; N, 3.85.

***N*-[3-(4-Biphenylyl)acryloyl]aspartic acid, disodium salt 4d**. This compound was prepared according to protocol 2. Yield: 275 mg (98%); mp >250°C. IR (KBr): $\nu = 3385$, 1655, 1600 cm^{-1}. ^1H NMR (D_2O): $\delta = 2.72$ (dd, $J = 16$, 9 Hz, 1H), 2.87 (dd, $J = 16$, 4 Hz, 1H), 4.66 (m, 1H), 6.82 (d, $J = 16$ Hz, 1H), 7.54–7.58 (m, 1H), 7.62–7.67 (m, 3H), 7.80–7.84 (m, 6H). ESI-MS: *m/z* (%) 362 (64) $[M + 2H\text{-}Na]^+$, 384 (100) $[M + H]^+$. ESI–HRMS calcd for $[M + H]^+$ $C_{19}H_{16}Na_2NO_5$: 384.082387; found: 384.076545.

***N*-[3-(2-Phenyl-1,3-thiazol-4-yl)acryloyl]aspartic acid dimethyl ester 3e**. This compound was prepared according to protocol 1 and obtained as an oil. Yield: 470 mg (63%); IR (neat): $\nu = 3420$, 3100, 1795, 1720, 1630 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.85$ (dd, $J = 17$, 4 Hz, 1H), 3.02 (dd, $J = 17$, 4 Hz, 1H), 3.60 (s, 3H), 3.70 (s, 3H), 4.92 (m, 1H), 6.65 (d, $J = 8$ Hz, 1H), 6.86 (d, $J = 15$ Hz, 1H), 7.16 (s, 1H), 7.35–7.38 (m, 3H), 7.50 (d, $J = 15$ Hz, 1H), 7.87–7.91 (m, 2H). ^{13}C NMR ($CDCl_3$): = 36.3, 48.7, 52.1, 52.9, 120.9, 122.8, 126.8, 129.1, 130.5, 133.3, 133.8, 152.8, 165.6, 168.8, 171.2, 171.6. MS: *m/z* (%) 374 (19) [M^+], 215 (40), 214 (100). HRMS calcd for $C_{18}H_{18}N_2O_5S$ (374.41): 374.093644; found: 374.090290.

***N*-[3-(2-Phenyl-1,3-thiazol-4-yl)acryloyl]aspartic acid, disodium salt 4e**. This compound was prepared according to protocol 2. Yield: 430 mg (96%); mp >250°C. IR (KBr): $\nu = 3415$, 1655 cm^{-1}. ^1H NMR (D_2O): $\delta = 2.73$ (dd, $J = 16$, 9 Hz, 1H), 2.89 (dd, $J = 16$, 4 Hz, 1H), 4.69 (m, 1H), 6.91 (d, $J = 16$ Hz, 1H), 7.53 (d, $J = 16$ hz, 1H), 7.61–7.67 (m, 3H), 7.85 (s, 1H), 8.00–8.03 (m, 2H). ESI-MS: *m/z* (%) 347 (7) $[M + 3H\text{-}2Na]^+$, 369 (18) $[M + 2H\text{-}Na]^+$, 413 (45) $[M + Na]^+$. ESI–HRMS calcd for $[M + Na]^+$ $C_{16}H_{12}Na_3NO_5S$: 413.016003; found: 413.016817.

***N*-[3-(3-Phenyloxyphenyl)acryloyl]aspartic acid dimethyl ester 3f**. This compound was prepared according to protocol 1. Yield: 400 mg (52%); mp 50°C. IR (KBr): $\nu = 3320$, 3030, 2965, 1765, 1725, 1655, 1620 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.85$ (dd, $J = 17$, 4.5 Hz, 1H), 3.03 (dd, $J = 17$, 4.5 Hz, 1H), 3.62 (s, 3H), 3.71 (s, 3H), 4.92 (m, 1H), 6.32 (d, $J = 16$ Hz, 1H), 6.60 (d, $J = 6$ Hz, 1H), 6.92–6.96 (m, 3H), 7.06–7.08 (m, 2H), 7.15 (m, 1H), 7.24–7.31 (m, 3H), 7.52 (d, $J = 16$ Hz, 1H). ^{13}C NMR ($CDCl_3$): $\delta = 36.2$, 48.7, 52.1, 52.9, 117.4, 119.2, 120.2, 120.7, 123.0, 123.8, 129.9, 130.2, 136.5, 141.4, 157.9, 165.3, 171.2, 171.7. MS: *m/z* (%) 383 (14) [M^+], 223 (100). Anal. calcd for $C_{21}H_{21}NO_5$ (383.40): C, 65.79; H, 5.52; found: C, 65.84; H, 5.64.

***N*-[3-(3-Phenyloxyphenyl)acryloyl]aspartic acid, disodium salt 4f**. This compound was prepared according to protocol 2. Yield: 282 mg (97%); mp >250°C. IR (KBr): $\nu = 3390$, 1575 cm^{-1}. ^1H NMR (D_2O): $\delta = 2.71$ (dd, $J = 15$, 9 Hz, 1H), 2.88 (dd, $J = 15$, 4 Hz, 1H), 4.66 (dd, $J = 9$, 4 Hz, 1H), 6.78 (d, $J = 16$ Hz, 1H), 7.21–7.24 (m, 3H), 7.34–7.41 (m, 2H), 7.54–7.62 (m, 5H). ESI-MS: *m/z* (%) 378 (65) $[M + 2H\text{-}Na]^+$, 400 (100) $[M + H]^+$, 422(68) $[M + Na]^+$. ESI-HRMS calcd for $[M + H]^+$ $C_{19}H_{16}Na_2NO_6$: 400.077302; found: 400.080451.

***N*-[3-(4-Benzyloxyphenyl)acryloyl]aspartic acid dimethyl ester 3g**. This compound was prepared according to protocol 1. Yield: 600 mg (76%); mp 124°C (toluene). IR (KBr): $\nu = 3310$, 1745, 1730, 1650 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.94$ (dd, $J = 17$, 5.5 Hz, 1H), 3.09 (dd, $J = 17$, 4.5 Hz, 1H), 3.69 (s, 3H), 3.78 (s, 3H), 4.99 (m, 1H), 5.08 (s, 2H), 6.31 (d, $J = 16$ Hz, 1H), 6.61 (d, $J = 8$ Hz, 1H), 6.94–6.98 (m, 2H, 7.30–7.47 (m, 7H), 7.59 (d, $J = 16$ Hz, 1H). ^{13}C NMR ($CDCl_3$): $\delta = 36.3$, 48.7, 52.1, 52.9, 70.2, 115.3, 117.7, 127.5, 127.6, 128.2, 128.7, 129.6, 136.6, 141.6, 160.3, 165.8, 171.4, 171.8. MS: *m/z* (%) 397 (41) [M^+], 237 (29), 91 (100). Anal. calcd for $C_{22}H_{23}NO_6$ (397.43): C, 66.49; H, 5.83; N, 3.52; found: C, 6.33; H, 5.81; N, 3.51.

***N*-[3-(4-Benzyloxyphenyl)acryloyl]aspartic acid, disodium salt 4g**. This compound was prepared according to protocol 2. Yield: 192 mg (95%); mp >250°C. IR (KBr): $\nu = 3405$, 1600 cm^{-1}. ^1H NMR (D_2O): $\delta = 2.73$ (dd, $J = 16$, 10 Hz, 1H), 2.88 (dd, $J = 16$, 4 Hz, 1H), 4.68 (dd, $J = 10$, 4 Hz, 1H), 5.35 (s, 2H), 6.72 (d, $J = 16$ Hz, 1H), 7.21–725 (m, 2H), 7.54–7.66 (m, 6H), 7.73–7.76 (m, 2H). MS: *m/z* (%) 392 (100) $[M + 2H\text{-}Na]^+$, 414 (70) $[M + H]^+$. ESI–HRMS calcd for $[M + 2H\text{-}Na]^+$ $C_{20}H_{19}NaNO_6$: 392.111007; found: 392.113286.

***N*-[2-(3-Phenyloxybenzyloxy)acetyl]aspartic acid dimethyl ester 3h**. This compound was prepared according to protocol 1 and purified by flash chromatography (EtOAc:*n*-hexane 3:2) and obtained as an oil. Yield: 470

mg (52%); IR (neat): ν = 3415, 3040, 2955, 2855, 1740, 1685 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.96 (dd, *J* = 17, 4 Hz, 1H), 3.00 (dd, *J* = 17, 4 Hz, 1H), 3.60 (s, 3H), 3.70 (s, 3H), 3.93 (s, 2H), 4.46–4.51 (m, 2H), 4.83 (m, 1H), 6.86–6.88 (m, 1H), 6.93–6.96 (m, 3H), 7.01–7.06 (m, 2H), 7.23–7.29 (m, 3H), 7.47 (d, *J* = 7 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ = 36.1, 47.8, 52.0, 52.8, 69.2, 73.1, 118.1, 118.4, 119.0, 122.5, 123.4, 129.8, 129.9, 138.8, 156.9, 157.5, 169.4, 170.8, 171.1. MS: *m*/*z* (%) 402 (3) $[M^+]$, 203 (100), 183 (74), 102 (84). HRMS calcd for $C_{21}H_{23}NO_7$ (262.35): 402.155277; found: 402.160248.

***N*-[2-(3-Phenyloxybenzyloxy)acetyl]aspartic acid, disodium salt 4h**. This compound was prepared according to protocol 1. Yield: 350 mg (quant.); mp 182°C. IR (KBr): = 3395, 1585 cm^{-1}. ^1H NMR (DMSO-d_6): δ = 2.60 (m, 2H), 3.84–3.92 (m, 2H), 4.22 (s, 1H), 4.48 (s, 2H), 6.83–6.85 (m, 1H), 6.95–6.97 (m, 3H), 7.07–7.10 (m, 2H), 7.27–7.36 (m, 3H). ESI–MS: *m*/*z* (%) 418 (10) $[M+H]^+$, 440 (100) $[M+Na]^+$. ESI-HRMS calcd for $[M+H]^+$ $C_{19}H_{18}Na_2NO_7$: 418.086867; found: 418.088020.

Farnesyltransferase assay

The assay was carried out as described.[5] The assay mixture (100 μL volume) contained 50 mM Tris/HCl pH 7.4, 5 mM $MgCl_2$, 10 μM, $ZnCl_2$, 5 mM DTT, 7 μM Ds-GCVLS, 20 μM FPP and 5 nmol GST-FTase[13] and 1% of various concentrations of the test compounds dissolved in DMSO. The progress of the enzyme reaction was followed by the enhancement of the fluorescence emission at 460 nm (excitation: 320 nm). The slight deviatation from the emission maximum (505 nm) was chosen for technical reasons. Fluorescence emission was recorded with BMG Polarstar microplate reader. IC_{50}s were calculated from initial velocity of three independent measurements of each inhibitor concentration and expressed as mean ± SD.

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References

1. Casey, P. J. *J. Biol. Chem.* **1996**, *271*, 5289.
2. Leonard, D. M. *J. Med. Chem.* **1997**, *40*, 2971.
3. Graham, S. L. *Exp. Opin. Ther. Patents* **1995**, *5*, 1269.
4. Ayral-Kaloustian, S.; Skotnicki, J. S. *Ann. Rep. Med. Chem.* **1996**, *31*, 171.
5. Clarke, S. *Ann. Rev. Biochem.* **1992**, *61*, 355.
6. Lerner, E. C.; Hamilton, A. D.; Sebti, S. M. *Anti-Cancer Drug Design* **1997**, *12*, 229.
7. Qian, Y.; Sebti, S. M.; Hamilton, A. D. *Biopolymers* **1997**, *43*, 25.
8. Sattler, I.; Tamanoi, F. In *Regulation of the RAS Signaling Network*; Maruta, H.; Burgess, A. W., Eds.; Springer: Heidelberg. 1996; pp 95–138.
9. Patel, D. V.; Schmidt, R. J.; Biller, S. A.; Gordon, E. M.; Robinson, S. S.; Manne, V. J. *Med. Chem.* **1995**, *38*, 2906.
10. Jones, G. The Knoevenagel Condensation. In *Organic Reactions*, Vol. 15; Cope, A. C., Ed.; John Wiley & Sons, Inc.: New York, 1967. pp 204–599.
11. Abraham, D. J.; Kennedy, P. E.; Mehanna, A. S.; Patwa, D. C.; Williams, F. L. *J. Med. Chem.* **1984**, *27*, 967.
12. Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7945.
13. Del Villar, K.; Mitsuzawa, H.; Yang, W.; Sattler, I.; Tamanoi, F. *J. Biol. Chem.* **1997**, *272*, 680.
14. Aoyama, T.; Satoh, T.; Vonemoto, M.; Shibata, J.; Nonoshita, K.; Arai, S; Kawakami, K.; Iwasawa, Y.; Sano, H.; Tanaka, K.; Monden, Y.; Kondera, T.; Arakawa, H.; Suzuki-Takahashi, I.; Kamei, T.; Tomimoto, K. *J. Med. Chem.* **1998**, *41*, 143.
15. Schlitzer, M.; Sattler, I. *Angew. Chem.* **1999**, *111*, 2150; *Angew. Chem., Int. Ed.* **1999**, *38*, 2032.