Synthesis and Biological Evaluation of Cycloalkylidene Carboxylic Acids as Novel Effectors of Ras/Raf Interaction

Anke Friese,[†,‡] Katja Hell-Momeni,[‡,§] Ilse Zündorf,[†] Thomas Winckler,*[,†] Theodor Dingermann,[†] and Gerd Dannhardt[§]

Institut für Pharmazeutische Biologie, Universität Frankfurt/M. (Biozentrum), D-60439 Frankfurt am Main, Germany, and Institut für Pharmazie, Johannes Gutenberg-Universität, D-55099 Mainz, Germany

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The protooncogenes Ras and Raf play important roles in signal transduction pathways regulated by mitogen-activated protein kinases. Mutations of Ras that arrest the protein in its active state are frequently implicated in tumor formation. We used Ras and Raf proteins in the yeast two-hybrid system to search for natural or synthesized substances capable of modulating Ras/Raf interaction by specifically binding to one of the interacting partners. We found that cycloalkylidene carboxylic acids enhanced Ras/Raf interaction by acting on the cysteine-rich domain of Raf. Several analogues of the active substance 2-cyclohexylidene propanoic acid were synthesized and the importance of the semicyclic double bond in the stabilization of Ras/Raf interaction was demonstrated. Variation of the size and the substituents of the cyclic system as well as the length of the carboxylic acid resulted in enhanced Ras/Raf interaction.

Introduction

The small guanosine 5′-triphosphate (GTP) binding protein p21 Ras and the serine/threonine protein kinase Raf-1 play a pivotal, regulatory role in oncogenic, mitogenic, and developmental signaling pathways. The *ras* genes were originally found as the transforming agents of the Harvey and Kirsten murine sarcoma viruses.[1] Identification of cellular *ras* homologues, the presence of mutated alleles in human tumors, and the strong conservation of *ras* genes in eukaryotes emphasize their importance in cell growth control. The Ras protein functions as a molecular switch, which cycles between a GDP-bound inactive state and a GTP-bound active state. Activation occurs transiently in response to an array of extracellular signals triggered by growth factors, cytokines, hormones, or neurotransmitters.[2] Single amino acid substitutions of Gly-12, Gly-13, or Gln-61 of the Ras protein create mutant proteins that are locked in the active, GTP-bound conformation, thus leading to constitutive, dysregulated activation of Ras function.[3] Mutated Ras proteins were detected in as many as 30% of human tumors with the highest incidence in ductal tumors of the exocrine pancreas,[4] suggesting that drugs interacting with Ras to disturb its interaction with downstream effectors may be a promising tumor therapy.

Activated Ras utilizes a multitude of functionally diverse effectors. One of the best characterized effectors of Ras is the serine/threonine kinases of the Raf family. The Raf family consists of at least three isoforms: c-Raf-1 (herein referred to as Raf), B-Raf, and A-Raf.[5] The proteins share homologous structural segments and designated conserved regions (CRs) CR1, CR2, and CR3. Ras binds to two sites in the regulatory region of Raf, which causes its translocation to the plasma membrane.[6] The structures of both Ras binding sites in Raf, spanning residues 51−131 and 139−184, have been solved.[7,8] Conformational changes in Raf upon binding of Ras induce the exposure of the kinase domain (CR3) and initiate a phosphorylation/activation cascade, which activates the mitogen-activated protein kinases MEK and ERK.[9] ERK phosphorylates numerous substrates including nuclear transcription factors, which serve to activate the expression of genes involved in cell proliferation and differentiation. They also activate p90RSK, which plays an important role in apoptosis.[10] The dual role of Ras in the regulation of proliferation and apoptosis is still controversially discussed.[11]

Presently, only a few substances, e.g., sulindac sulfide[12] or several peptides[13−15] are known to directly influence Ras/Raf interaction. In this paper, we describe the synthesis and analysis of structure−activity relationships of cycloalkylidene carboxylic acids in Ras/Raf interaction. 2-Cyclohexylidene propanoic acid (compound **5**) was originally developed as an analogue of valproic acid in order to verify its antiepileptic potency.[16,17] It represents the first compound of this structure class that showed a significant effect on the Ras/Raf interaction. Using compound **5** as a lead, we synthesized derivatives by varying the length of the carboxylic acid as well as the size of the cyclic system. Furthermore, substituents were introduced and varied by their size and place (compounds **6**−**12**) and the carboxylic group was once interchanged by an aldehyde function.

Chemistry

The cycloalkylidene alkanes evaluated in this study are shown in Figure 2. The preparation of the cycloheptylidene carboxylic acids was based on the Wittig−Horner−Emmons reaction[18] as shown in Scheme 1. The corresponding ketone was treated with carbethoxyalkylphosphonate obtained by means of the Michaelis−

* To whom correspondence should be addressed. Tel.: +49-69-798-29648. Fax: +49-69-798-29662. E-mail: winckler@em.uni-frankfurt.de.
† Universität Frankfurt/M. (Biozentrum).
‡ These authors contributed equally to this work.
§ Johannes Gutenberg-Universität.

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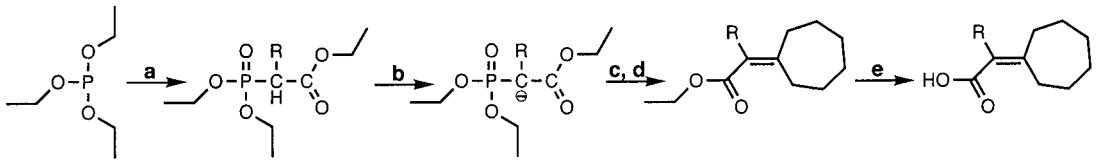
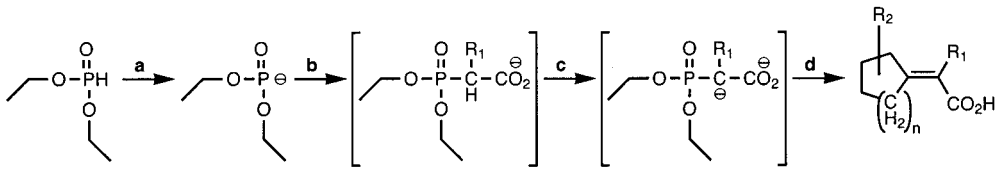
Scheme 1[a]



15 R = CH_3
16 R = H
17 R = CH_3
18 R = H
1 R = CH_3
2 R = H

[a] Reagent: (a) $RCHBrCO_2Et$; (b) NaH/DME; (c) cycloheptanone; (d) H_2O; (e) NaOH, EtOH.

Scheme 2[a]



n = 1
13 $R_1 = CH_3$ $R_2 = H$
14 $R_1 = H$ $R_2 = H$

n = 2
5 $R_1 = CH_3$ $R_2 = H$
6 $R_1 = CH_3$ $R_2 = 3'-CH_3$
7 $R_1 = CH_3$ $R_2 = 4'-CH_3$
8 $R_1 = CH_3$ $R_2 = 4'-C_2H_5$
9 $R_1 = CH_3$ $R_2 = 4'-C_3H_7$
10 $R_1 = CH_3$ $R_2 = 4'-C(CH_3)_3$
11 $R_1 = CH_3$ $R_2 = 4'-Ph$
12 $R_1 = CH_3$ $R_2 = 4'-Ph-O-CH_3$

[a] Reagent: (a) NaH; (b) NaH, $R_1CHBrCO_2H$; (c) NaH; (d) cycloalkanone, EtOH, HCl.

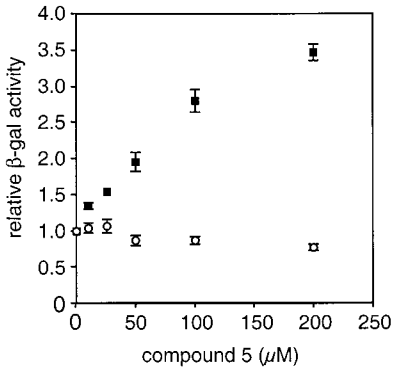


Figure 1. Compound **5** (2-cyclohexylidenpropanic acid) enhances Ras/Raf interaction. The basal β-galactosidase activity achieved with the tester strains BD-Ras(G12V)$^{1-166}$ and AD-Raf$^{1-275}$ (compare to Figure 4) in the presence of solvent (black squares) and the control strain (open circles) was set equal to 1. Relative enzyme activities were calculated as the ratio of the activity in the presence of compound divided by the effect of pure solvent. Experiments are presented ± standard deviation (SD) calculated from five individual experiments.

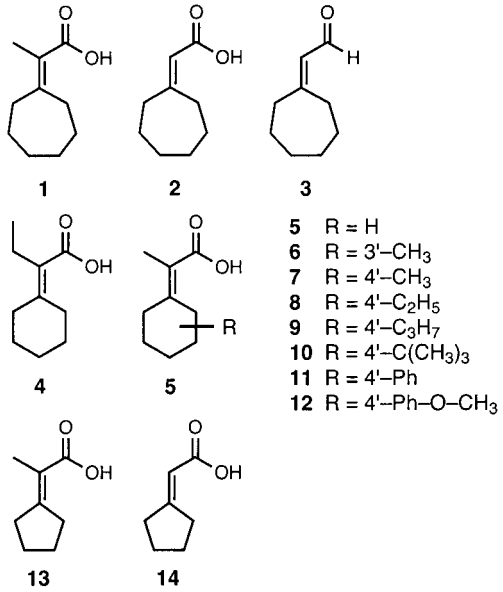


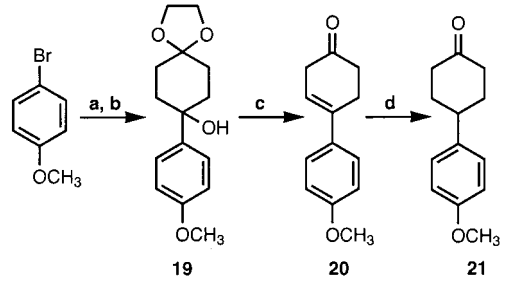
Figure 2. Synthesized compounds sorted according to the size of the cycloalkylidene ring. First row, cycloheptylidenes; second row, cyclohexylidenes; third row, cyclopentylidenes.

Arbuzov reaction[19] of triethyl phosphite and 2-bromocarboxylic acid. The release of the free acids occurred easily by hydrolysis of the resultant ester. To ensure the semicyclic position of the double bond, the products were characterized by ^{13}C nuclear magnetic resonance (NMR) as well as ^1H NMR.

Because of isomerization of both the cyclohexylidenic and the cyclopentylidenic derivatives, another way to obtain compounds **4**−**14** had to be pursued. The method of Britelli[20] allowed us to synthesize the desired acids in an one-vessel synthesis as shown in Scheme 2. Addition of diethyl phosphite to a suspension of 3 equiv of sodium hydride in 1,2-dimethoxyethane, followed by addition of first the bromo-carboxylic acid and then cycloalkanone, led to a good yield of the five- and six-membered cycloalkylidenic acids existing as pure semicyclic isomers.

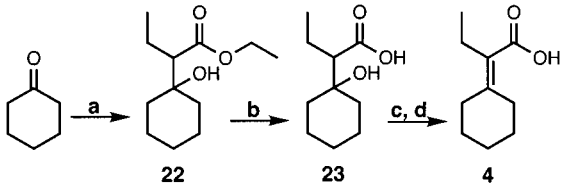
The applied cycloalkanones were commercially available except for the adduct of compound **12**, which was synthesized as outlined in Scheme 3. The Grignard reagent of 4-bromoanisole was treated with 1,4-cyclohexanedionemonoethylenketale to give the 4-hydroxycyclohexanone derivative. Treatment of the latter with trifluoroacetic acid[21] led to the corresponding cyclohex-

Scheme 3[a]



[a] Reagent: (a) Mg/THF; (b) 1,4-cyclohexanedionmonoethyleneketale; (c) CF_3COOH; (d) Pd/C, H_2.

Scheme 4[a]



[a] Reagent: (a) Zn/I_2, $CH_3CH_2CHBrCO_2Et$; (b) NaOH, EtOH; (c) acetic anhydride; (d) steam distillation.

enone, which could be hydrated by the means of hydrogen catalyzed by palladium on charcoal.

Efforts to achieve butyric acids by the Britelli reaction were unsuccessful. Compound **4** was therefore derived from the Reformatzky[22] reaction as shown in Scheme 4. In a first step, the ethyl 2-(1-hydroxycyclohexyl)-butanoate was obtained.[23] To prevent formation of the endocyclic derivative, hydrolysis of the ester had to occur before the dehydratization step. To separate the desired semicyclic derivative from its endocyclic isomer, steam distillation was carried out.

To examine the relevance of the functional group, the carboxylic group was exchanged for an aldehyde function as demonstrated by compound **3**. As discussed above, the direct conversion of the cycloalkanones into the α,β-unsaturated aldehydes could not be achieved due to unwanted condensation reactions.[24,25] For the formylolefination of cycloheptanone, we used a method developed by Nagata and Hyase,[26] which is based on the Wittig reaction (Scheme 5). Because the diethyl 2-(cyclohexylimino)ethylphosphonate (**24**) was considered to be less electrophilic than the parent carbonyl compound, considerable self-condensation was not expected using this reaction.

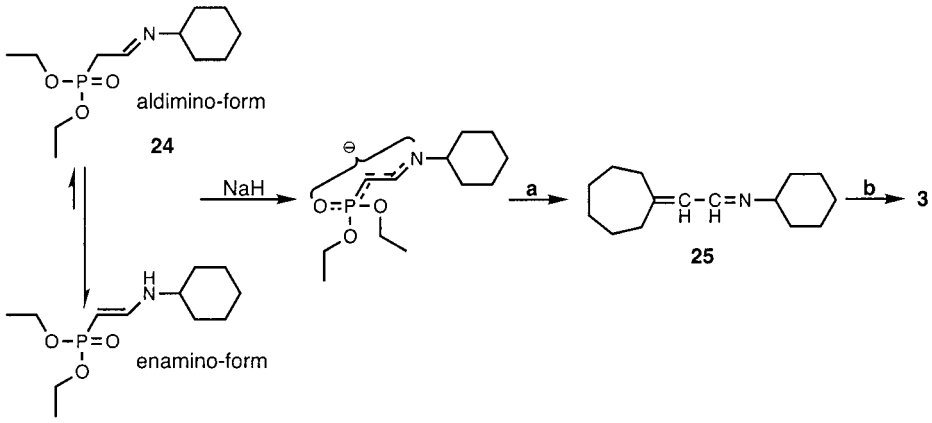
Compound **24** was prepared by the treatment of diethyl formylmethylphosphonate with an equimolar amount of cyclohexylamine. According to its acid sensibility, distillation should be carried out in the presence of anhydrous potassium carbonate. The IR spectra indicated that the compound existed predominantly in an enamino form. For completion of the formylolefination, the carbanion was generated in a convenient manner by treatment of a tetrahydrofuran (THF) solution of **24** with an equimolar amount of sodium hydride in the cold. After reaction of the carbanion with a slight minority of the cycloheptanone, the amine was leniently hydrolyzed with diluted oxalic acid in order to avoid isomerization.

Results and Discussion

The yeast two-hybrid system takes advantage of the modular construction of the yeast GAL4 transcriptional activator. DNA binding (BD) and transactivating (AD) domains of GAL4 can be dissociated and expressed separately from two different plasmids.[27,28] The plasmid-borne BD and AD domains of GAL4 can only induce transcription of reporter genes such as the bacterial β-galactosidase if proteins expressed as genetic fusions with BD and AD are capable of interacting to reconstitute GAL4. When Ras and Raf are fused to BD and AD, respectively, compounds that bind specifically to either Ras or Raf may influence Ras/Raf interaction and hence reconstitution of active GAL4, which in turn results in altered β-galactosidase reporter gene expression.

The assay applied to this study was performed with BD-Ras and AD-Raf proteins. We made use of microtiter plates in a high-throughput design in order to allow an extended and fast screening of natural and synthesized compounds. A tester strain was used, which expressed the catalytic domain of the constitutively active H-RasG12V (amino acids 1−166) fused to BD and the regulatory domain of Raf (amino acids 1−275) fused to AD. To demonstrate the specificity of effects of the tested compounds on β-galactosidase reporter activity, a control strain was used that expressed genetic fusions of AD and BD with the tumor suppressor protein p53 and the large T-antigen of the Simian virus 40, which are known to interact strongly.[29,30] Interference of test compounds with Ras and Raf was considered as specific only if dose-dependent changes of β-galactosidase reporter activity in the tester strain but not in the control strain were obtained.

Scheme 5[a]



[a] Reagent: (a) cycloheptanone; (b) H_3O^+.

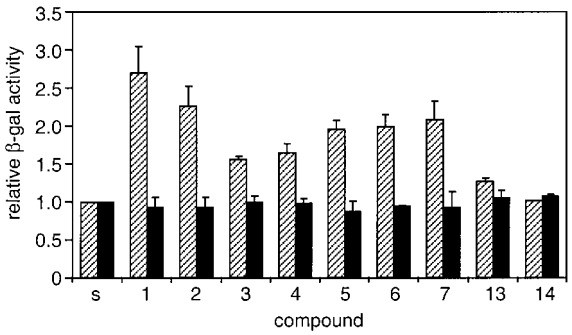


Figure 3. Cycloalkylidene carboxylic acids affect Ras/Raf interaction. The experiments were performed with 50 μM of the indicated compounds. Reporter gene activity (hatched columns) and control strain activity (black columns) obtained with pure solvent was set equal to 1 (column "s"). Experiments are presented ± SD calculated from three individual experiments.

In a random screening of natural and synthesized substances as well as plant and fungal extracts, we found that 2-cyclohexylidenpropanic acid (**5**) mediated a significant stabilization of Ras/Raf binding (Figure 1). An EC_{50} value for the specific enhancement of Ras/Raf interaction by compound **5** was calculated as 64 μM.

To obtain more potent effectors of the Ras/Raf interaction, various cycloalkylidene carboxylic acids were synthesized (Figure 2) and tested for effects on Ras/Raf interaction in yeast two-hybrid screens. Structure−activity relationships could be derived from these experiments, which are summarized in Figure 3. The compounds can be divided into three groups by their effects on Ras/Raf interaction as compared to compound **5**: (i) Modifications led to moderate changes of reporter gene activity; substitution in position 3′ in compound **6** had almost no effect on Ras/Raf interaction (expressed as changes in β-galactosidase activity). A methyl group in position 4′ (compound **7**) resulted in a slightly increased β-galactosidase activity. (ii) Modifications enhanced the reporter activity; enlargement of the ring to cycloheptylidene in compounds **1** and **2** led to increased β-galactosidase activities, indicating stabilization of Ras/Raf interaction. The best results were seen by treating the cells with compound **1**, which differed from **2** in the length of the acid chain. (iii) Modifications resulted in decreased reporter activity; replacement of the acid group in compound **2** by an aldehyde group in compound **3** reduced the observed effects. The acid group thus seemed to be of critical importance. Elongation of the propanoic acid in compound **5** to butanoic acid in compound **4** showed only slight changes of reporter activity. Decreasing the size of the ring system to cyclopentylidenic acids in compounds **13** and **14** resulted in a complete loss of the Ras/Raf stabilizing effect. To test the influence of semicyclic location of the double bond, compound **5** was also tested as a mixture of both the semicyclic and the endocyclic isomer in a ratio of 1:1 (compound **5***). A significant decrease of activity was observed (not shown).

Because of the fact that substituents in position 4′ as well as enlargement of the ring system to cycloheptylidenes led to more potent effectors, compounds **8**−**12** were synthesized and tested. These new cyclohexylidene carboxylic acids exhibited unexpected cytotoxic effects, such that no data on the potential of these drugs to stabilize Ras/Raf interactions could be obtained (data not shown). The cytotoxicity of compounds **1**−**7**, **13**, and **14** was calculated as EC_{50} values and was well above 300 μM, which is at least 6-fold above the concentration used in the experiments (50 μM). Elongation of the substituent in position 4′ from an ethyl group in compound **8** to a propyl group in compound **9** resulted in increased cytotoxicity (EC_{50} values of 125 and 74 μM, respectively). Incubation of the yeast cells with compound **10**, which had an isobutylic substituent, revealed a similar cytotoxicity as compound **9** (EC_{50} values of 81 μM). The correlation between larger substituents and growth inhibition was additionally found by comparing compounds **11** and **12**, which differ by a methoxy group (EC_{50} values of 143 and 130 μM).

To identify domains in Ras and Raf required for the stabilizing effects of the synthetic compounds, several truncated derivatives of Ras and Raf were expressed as AD and BD fusions, respectively (summarized in Figure 4A). In the experiments described above, amino acids 1−166 of H-Ras that fused to BD, named BD-Ras-$(G12V)^{1-166}$, were active in interacting with Raf. Unfortunately, it was impossible to achieve Ras/Raf interaction with any Ras derivative shorter than amino acids 1−166, probably because the loss of correct protein structure or amino acids is critical for Raf binding. Hence, the BD-Ras$(G12V)^{1-166}$ protein was used in this analysis. Yeast cells expressing wild-type Ras in fusion with BD (BD-Ras^{1-166}) had a significantly lower β-galactosidase activity than cells expressing the constitutively active Ras mutant Ras^{G12V} (Figure 4B). Cycloalkylidene carboxylic acids increased the interaction of both the mutant Ras$(G12V)^{1-166}$ and the wild-type Ras^{G12V} (Figure 4B). The minimal requirement of the Raf protein to interact with Ras is the Ras binding domain (RBD), which is located in CR1 at amino acid positions 51−131 of the Raf protein (Figure 4A). Interaction of AD-Raf^{51-131} with Ras was not enhanced by compound **5** (Figure 4B, column 2), suggesting that the compound did not stabilize Ras/Raf interaction by binding to the RBD of the Raf protein. We found that elimination of CR2 and CR3 from AD-Raf fusions still allowed effects of compound **5** on Ras/Raf interaction. On the other hand, the cysteine-rich domain of Raf (amino acids 131−194) seemed to be required to exert effects of compound **5** (Figure 4B). These data argue that the cycloalkylidene carboxylic acids bind to the zinc fingerlike motif, which is located between amino acids 131 and 194 of Raf.

Preliminary data support that the cycloalkylidene carboxylic acids described in this paper may be able to activate Ras/Raf-dependent pathways in mammalian cell culture cells (data not presented). Hence, cycloalkylidene carboxylic acids may represent an interesting new class of Ras/Raf effectors, which may be useful tools in basic research of Ras/Raf-regulated pathways and may also serve as lead structures in the development of novel drugs.

Experimental Section

Plasmids. The yeast two-hybrid $Ras^{G12V}(1-166)$ construct was generated by polymerase chain reaction (PCR) using the DNA template pPC97Ras^{G12V}verk. [31] The Ras carboxy terminus containing the CaaX motif was deleted to prevent association with the plasma membrane. Wild-type Ras was generated by site specific mutation[32,33] of Ras^{G12V}. All Ras constructs were

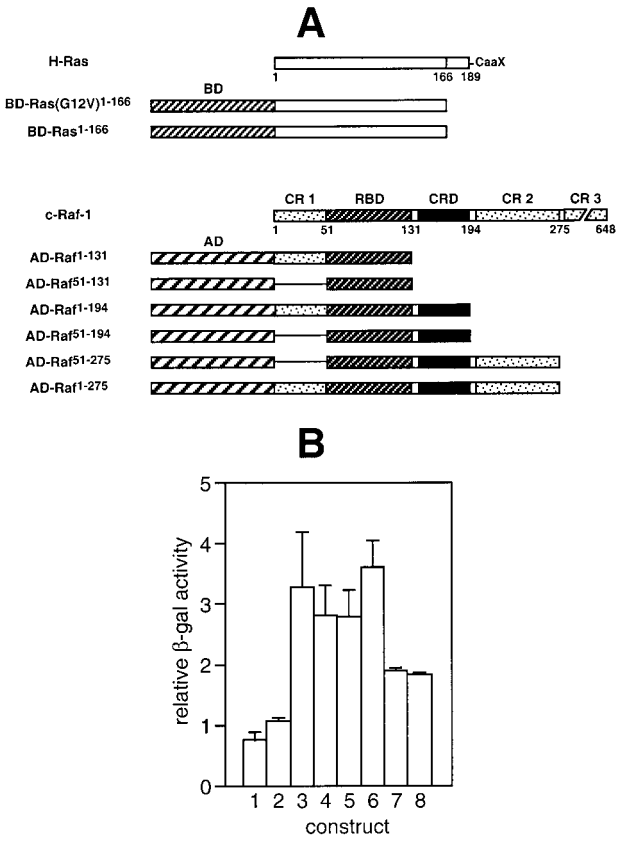


Figure 4. Evaluation of Raf domains required for effects by cycloalkylidene carboxylic acids on Ras/Raf interaction. (A) Schematic presentation of Ras and Raf proteins used in this study. Ras is a 189 amino acid full-length protein, which carries a carboxy-terminal farnesylation motif (CaaX motif). We expressed genetic fusions of GAL4 BD with amino acids 1−166 of mutant H-Ras(G12V) or wild-type Ras. Full-length Raf is 648 amino acids long. We expressed several truncated versions of Raf as indicated by the numbers. The Ras-interacting domain RBD spans amino acids 51−131. The cysteine-rich, zinc fingerlike domain CRD is located between positions 131 and 194. (B) Effects of 2-cyclohexylidenpropanic acid **5** (100 μM) on reporter gene activity in the presence of various truncated Raf mutants. Columns 1−6 represent experiments performed with BD-Ras(G12V)$^{1-166}$ as the Raf binding partner, whereas wild-type Ras (BD-Ras$^{1-166}$) was used in experiments presented in columns 7 and 8. The following Raf derivatives were used as Ras binding partners: column 1, AD-Raf$^{1-131}$; column 2, AD-Raf$^{51-131}$; column 3, AD-Raf$^{1-194}$; column 4, AD-Raf$^{51-194}$; column 5, AD-Raf$^{1-275}$; column 6, AD-Raf$^{51-275}$; column 7, AD-Raf$^{1-194}$; column 8, AD-Raf$^{1-275}$. Reporter gene activity obtained with pure solvent was set equal to 1 (not shown in the graph). Experiments are presented ± SD calculated from three individual experiments.

cloned as *Eco*R I/*Sal* I DNA fragments into the pBD-GAL4Cam vector (Stratagene). The Raf regulatory domain (amino acids 1−275) and truncated Raf mutants were PCR amplified from the template pPC86HCRC2Raf [30] and cloned either with *Eco*R I/*Sal* I (for DNA fragments coding for amino acids 1−131 and 51−131 of Raf) or *Eco*R I/*Xba* I (for DNA fragments coding for amino acids 1−194, 51−194, 1−275, and 51−275 of Raf) into the pAD-GAL4 vector (Stratagene).

Yeast Two-Hybrid System. Competent Y190 yeast cells[34,35] were cotransformed with 1 μg of each of the two-hybrid vectors according to Klebe[36] and grown on synthetic medium lacking leucine, tryptophane, and histidine. Single colonies were picked and inoculated overnight at 30 °C. In microtiter plates, 140 μL per well liquid culture diluted to A_{600} 0.2 were shaken with 10 μL of probe dissolved in 50% dimethyl sulfoxide (DMSO) for 12 h at 30 °C. Cells were washed twice with 150 μL of buffer Z (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, and 1 mM $MgSO_4$) and frozen as pellets for 15 min at −80 °C. After resuspension in 90 μL of buffer Z, the cell densities were measured at 630 nm. Cells were lysed by addition of 4 μL of 0.1% sodium dodecyl sulfate and 6 μL of $CHCl_3$ and incubated for 10 min at 30 °C. β-Galactosidase activity was tested by adding 100 μL of 0.2 mg/mL chlorophenolred-β-D-galactopyranoside (CPRG), and the amount of reaction product was measured time dependently in an enzyme-linked immunosorbent assay reader at $A_{550/630}$. Enzyme activity was calculated as Miller units[37] and presented as the ratio of β-galactosidase activity obtained in the presence of compound vs pure solvent. One Miller unit defines the hydrolization of 1 μmol of CPRG to chlorophenol red and D-galactose per minute and cell.

General Procedures. NMR spectra were recorded on Bruker AC 300 or AM 400 MHz spectrometers with chemical shifts reported as δ values in parts per million downfield from tetramethylsilane as the internal standard. Splitting patterns are designated as follows: s, singlet; d, double; t, triplet; q, quartet; m, multiplet. IR spectra were recorded on a Beckmann IR 42200, Perkin-Elmer 1310 Infrared Spectrophotometer or Shimadzu Infrared Spectrophotometer IR-470. Mass spectra were obtained on a Varian MAT 311A (70 eV) and a Varian CH 7a (90 eV). Elemental analyses were performed at the department of Organic Chemistry at the Johannes Gutenberg-University, Mainz. Melting points were determined with a Dr. Tottoli melting point apparatus and are uncorrected. Flash column chromatography was carried out with silica gel 60 (Merck).

Reagents. All reagents were purchased from Aldrich Chemical Co. and used without further purification except anhydrous THF, which was distilled over potassium metal under nitrogen or glyme, distilled over calcium hydride.

Compounds of First Stage Triethyl Phosphonopropionate (15).[19] A total of 40 g (221 mmol) of ethyl 2-bromopropionate and 36.7 g (221 mmol) of triethyl phosphite were heated at 155−160 °C for about 10 h during which bromoethane formed. Bromoethane was removed continuously by the help of a distillation bridge. The mixture was distilled under reduced pressure to give 29.2 g (55.6%) of a colorless liquid; bp 90 °C (1.6×10^{-1} mbar).

Triethyl Phosphonoacetate (16).[19] Ethyl 2-bromoacetate was used in the manner described for **15**; 4.8%; 156 °C (18 mbar).

Ethyl 2-Cycloheptylidenpropanoat (17).[18] A 13.09 g (55 mmol) amount of **15** was added dropwise at 20 °C to a slurry of NaH (1.2 g, 50 mmol) in 100 mL of dry glyme. The reaction mixture was stirred for 1 h at room temperature until gas evolution had ceased. Cycloheptanone was added dropwise at such a rate that the temperature was maintained below 30 °C. After an additional stirring of 15 min, during which time a viscous semisolid appeared, the mixture was taken up in a large excess of water. The aqueous solution was extracted with ether, and the organic layer was dried over magnesium sulfate and evaporated. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate; 9.5:0.5; R_f 0.58) to give 2.1 g (18%) of a colorless liquid. IR 2900, 1700, 1610 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.14 (dq, J = 7.39 Hz, 2H, O−CH_2), 2.48 (t, J = 7.75 Hz, 2H, CH_2−C=C), 2.47 (t, J = 7.75 Hz, 2H, CH_2−C=C), 1.73−1.55 (m, 4H, CH_2−C−C−CH_2), 1.68 (s, 3H, CH_3−C−CO), 1.50−1.45 (m, 2H, CH_2), 1.34−1.29 (m, 2H, CH_2), 1.26 (dt, J = 7.15 Hz, 3H, CH_3−CO). ^{13}C NMR (DMSO): δ 169.37 (C-1), 149.76 (C-1′), 122.69 (C-2); 59.84 (C-1″), 30.19, 28.77 (C-2′; C-7′), 28.42; 27.67 (C-3′; C-6′), 25.94, 24.00 (C-4′; C-5′), 15.33 (C-3), 14.42 (C-2″). MS *m*/*z* 196, 181, 168, 151, 123, 108, 102. Anal. ($C_{12}H_{20}O_2$) C, H.

Ethyl 2-Cycloheptylidenacetate (18).[18] Preparation was as described for **15** by use of triethyl phosphonoacetate (12.32 g, 55 mmol). The crude product was distilled giving 6.53 g (71.7%) of a colorless liquid; bp 80 °C (4×10^{-2} mbar). IR 3020, 2900, 1700, 1610, 830 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.65 (s, 1H, H−C=C), 4.12 (q, J = 7.15 Hz, 2H, O−CH_2), 2.86 (t, J = 6.08 Hz, 2H, CH_2−C=C); 2.35 (t, J = 5.72 Hz, 2H, CH_2−C=C),

1.74−1.44 (m, 8H, $(CH_2)_4$), 1.24 (t, J = 7.15 Hz, 3H, CH_3−C−O). ^{13}C NMR (DMSO): δ 172.27 (C-1), 166.67 (C-1′), 115.64 (C-2), 59.35 (C-1″), 39.02, 32.09, 29.86, 29.02, 28.06, 26.88 (C-2′; C-3′; C-4′; C-5′; C-6′; C-7′), 14.28 (C-2″). MS *m*/*z* 182, 153, 137, 136, 109, 96, 82, 68, 56. Anal. ($C_{11}H_{18}O_2$) C, H.

4-Hydroxy-4-(4-methoxyphenyl)cyclohexanonethylenketale (19). A 3.45 g (144 mmol) amount of magnesium splinters was added to a solution of 25.5 g (136 mmol) *p*-bromoanisole in dry THF (180 mL). The mixture was stirred heavily and heated carefully until the reaction started. The reaction was maintained until all magnesium had disappeared. After the mixture was cooled to room temperature, 1,4-cyclohexandionethylenketale (20.3 g, 123 mmol) in 180 mL of dry THF was added dropwise and the resulting solution was refluxed for 24 h. The reaction was stopped by adding 200 mL of saturated NH_4Cl and extracted three times with 100 mL portions of ether. The organic layers were washed with NaOH (10%, 40 mL) and water (100 mL) and dried over magnesium sulfate. The solvent was evaporated, and the resulting product was recrystallized from ether; 26.5 g (77.2%); mp 122 °C. ^{1}H NMR ($CDCl_3$): δ 7.43 (d, J = 8.82 Hz, 2H, AA′), 6.86 (d, J = 8.82 Hz, 2H, BB′), 3.96 (dd, J = 2.63 Hz, 4H, O−$(CH_2)_2$−O), 3.79 (s, 3H, O−CH_3), 2.19−2.01 (m, 4H, CH_2−C−CH_2), 1.86−1.62 (m, 4H, OH, CH_2−C−CH_2). MS *m*/*z* 265, 204, 189, 165, 160, 150, 135, 101.

4-(4-Methoxyphenyl)cyclohexene-1-one (20). A 2.6 g (100 mmol) amount of **19** was treated with 100 mL of trifluoracetic acid and stirred at room temperature for 30 min. The mixture was then given into a solution of saturated $NaHCO_3$ (50 mL) and extracted with chloroform. The organic layer was washed with brine and dried over magnesium sulfate, and the solvent was evaporated. Purification of the crude product by flash chromatography (*n*-hexane/ethyl acetate 3:1, R_f 0.32) led to 8.3 g (41%) of orange crystals; mp 52 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (d, J = 8.83 Hz, 2H, AA′), 6.88 (d, J = 8.82 Hz, 2H, BB′), 5.99 (t, J = 3.94 Hz, 1H, C=CH), 3.04 (m, 2H, CH−CH_2), 2.86 (t, J = 6.91 Hz, 2H, CH_2), 2.63 (t, J = 6.91 Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 210.35 (C-1), 159.08 (C-4′), 137.12 (C-4), 133.32 (C-1′), 126.37 (C-2′; C-6′), 119.30 (C-3), 113.85 (C-3′; C-5′), 55.37 (C-1″), 39.97, 38.76 (C-5; C-6), 28.01 (C-2). MS *m*/*z* 202, 185, 173, 160, 147, 145, 129, 115, 77.

4-(4-Methoxyphenyl)cyclohexanone (21). A 800 mg amount of Pd/C was given to a solution of **20** (8.3 g, 41 mmol) in ethyl acetate (180 mL). The suspension was put under hydrogen atmosphere and stirred at room temperature for about 3 h (thin-layer chromatography (TLC) control). After the catalyst was filtered and the solvent was evaporated, the crude product (white crystals) was purified by flash chromatography (*n*-hexane/ethyl acetate 3:1, R_f 0.32); 8.3 g (41%); mp 74 °C. ^{1}H NMR ($CDCl_3$): δ 7.16 (d, J = 8.58 Hz, 2H, AA′), 6.87 (d, J = 8.82 Hz, 2H, BB′), 3.79 (s, 3H, CH_3), 3.04−2.92 (m, 1H, CH−Ar), 2.53−2.46 (m, 4H, CH_2−C−CH_2), 2.26−2.15 (m, 2H, CH_2), 1.99−1.83 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 211.42 (C-1), 158.30 (C-4′), 136.97 (C-1′), 127.61 (C-2′; C-6′), 113.83 (C-3′; C-5′), 55.31 (C-1″), 41.85 (C-4), 41.46 (C-2; C-6), 34.26 (C-3; C-5). MS *m*/*z* 204, 147, 134, 121, 119, 91, 77.

Ethyl 2-(1-Hydroxycyclohexyl)butanoate (22).[23] In a dry three neck flask, supplied with a drying tube filled with calcium chloride, 12 g of zinc powder and a few crystals of iodine were treated with 20 mL of a mixture of cyclohexanone (16.6 g, 170 mmol), ethyl 2-bromobutanoic acid (31.5 g, 160 mmol), and glyme (25 mL). The suspension was stirred heavily and heated on an oil bath (100−105 °C). As soon as the reaction started, the rest of the solution was added dropwise and the reaction was maintained for an additional hour. After the mixture was cooled to room temperature, a mixture of ice and 15 mL of concentrated sulfuric acid was added and the solution was extracted several times with toluene. The organic layer was washed with ice-cold solution of soda (10%) and then with ice water. After the mixture was dried over sodium sulfate, the toluene was evaporated and the resulting ester was distillated under reduced pressure; 15.4 g (44.8%); bp 74 °C (10^{-2} mbar).

2-(1-Hydroxycyclohexyl)butanoic Acid (23).[23] A 15.4 g (72 mmol) amount of **22** was treated with a solution of 3 g of NaOH in 60 mL of ethanol (50%). After the solution was refluxed for 4 h, the resulting solution was reduced to its half and diluted with the same amount of water. The solution was extracted with ether, and the aqueous layer was acidified with HCl. After it was extracted with ether, the organic layer was washed with water and dried over sodium sulfate, and the solvent was evaporated. The resulting crystals were recrystallized with a mixture of ether and petroleum ether; 5.2 g (38.5%); mp 77 °C. IR 3400, 2900, 2550, 1680, 1430, 1310, 1265, 1180, 1120, 940 cm^{-1}. ^{1}H NMR (DMSO): δ 11.99 (s, 1H, CO_2H), 2.37 (dt, J = 6.78 Hz, 1H, CH−C−O), 2.17 (s, 1H, OH), 1.62−1.30 (m, 10H, C_6H_{10}), 1.34 (m, 1H, CH_2−C−C−O), 1.24 (m, 1H, CH_2−C−C−O), 0.96 (t, J = 7.54 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 176.04 (C-1), 70.79 (C-1′), 59.41 (C-2), 35.40, 33.70 (C-2′; C-6′), 25.85 (C-4′), 21.54 (C-3′; C-5′), 19.59 (C-3), 12.98 (C-4). MS *m*/*z* 186, 169, 143, 125, 112, 102, 99, 81. Anal. ($C_{10}H_{18}O_3$) C, H.

Diethyl Formylmethylphosphonate.[26] A total of 71 g (474 mmol) of triethyl phosphite and 94 g (474 mmol) of 2-bromo-1,1-diethoxyethane were refluxed for 3.5 h during which bromoethane formed. Bromoethane was continuously removed by the help of a distillation apparatus. After distillation of the formed diethyl (2,2-diethoxyethyl)phosphonate (bp 128−130 °C, 2 mbar), the latter was given into its 3.5-fold amount of refluxing HCl (2%) and extracted with ether for about 3 days. Evaporation of the ether led to 33.91 g (75%) of a colorless liquid.

Diethyl 2-(Cyclohexylimino)ethylphosphonate (24).[26] A solution of diethyl formylmethylphosphonate (30 g, 168 mmol) in methanol was cooled to 0 °C and treated under an argon atmosphere with freshly distillated cyclohexylamine. After the mixture was stirred for 10 min at room temperature, the solvent was evaporated and the crude product was distillated twice over K_2CO_3 to give 21.3 g (48.52%) of a colorless liquid; bp 151 °C (0.04 mbar). IR 3300, 1430, 1290, 1210 cm^{-1}.

***N*-(2-Cycloheptylidenethylidene)-*N*-cyclohexylamine (25).**[26] Under an argon atmosphere and cooling in an ice bath, a solution of **24** (10 g, 38 mmol) in 150 mL of dry THF was given dropwise to a suspension of NaH (0.9 g, 38 mmol) in dry THF (10 mL). A solution of 3.6 g of cycloheptanone (32 mmol) in dry THF (10%) was added slowly, and the mixture was stirred for 2 h during which TLC was conducted. The reaction mixture was given onto ice water, extracted with ether, washed with saturated brine, and dried over magnesium sulfate. Evaporation of the ether led to 3.1 g (47.6%) of a colorless liquid.

Test Compounds. General Procedure for Compounds 1 and 2. A 44 mmol amount of the ester was added to 4.1 g of NaOH dissolved in a mixture of 40 mL of ethanol and 30 mL of water. The solution was refluxed for 3 h. After the solution was cooled to room temperature, the ethanol was evaporated and the residual was acidified with 8% HCl. The resulting cloudy liquid was cooled in ice, which resulted in a white precipitate. The acid was filtered and recrystallized in 20% ethanol.

2-Cycloheptylidenpropanoic Acid (1). 20.73%; mp 66 °C. ^{1}H NMR (DMSO): δ 12.07 (s, 1H, CO_2H), 2.48 (t, J = 6.40 Hz, 2H, CH_2−C=C), 2.26 (t, J = 5.65 Hz, 2H, CH_2−C=C), 1.74 (s, 3H, CH_3), 1.49 (m, 8H, $(CH_2)_4$). MS *m*/*z* 168, 159, 123, 110, 66. Anal. ($C_{10}H_{16}O_2$) C, H.

2-Cycloheptylidenacetic Acid (2).[16] 43.65%; mp 56 °C. IR 3020, 2900, 1700, 1610, 830 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.65 (s, 1H, H−C=C), 4.12 (q, J = 7.15 Hz, 2H, O−CH_2), 2.86 (t, J = 6.08 Hz, 2H, CH_2−C=C), 2.35 (t, J = 5.72 Hz, 2H, CH_2−C=C), 1.74−1.44 (m, 8H, $(CH_2)_4$), 1.24 (t, J = 7.15 Hz, 3H, CH_3−C−O). ^{13}C NMR (DMSO): δ 167.51 (C-1), 165.57 (C-1′), 116.53 (C-2), 38.44, 31.51 (C-2′; C-7′), 29.59; 28.63 (C-5′; C-4′), 27.78, 26.49 (C-6′; C-3′). MS *m*/*z* 155, 137, 127, 112, 110, 101, 95, 80, 68. Anal. ($C_9H_{14}O_2$) C, H.

2-Cycloheptylidenacetaldehyde (3). Compound **25** was dissolved in the ratio of 1:40 in a mixture of CH_2Cl_2/THF (4:1). An amount totaling 150 parts of 1% oxalic acid was

added, and the mixture was refluxed for several hours. After the mixture was cooled to room temperature, the solvent was evaporated and the crude product was distillated by means of bulb to bulb distillation; 888 mg (42.28%); bp 103 °C, 19 mbar. IR 2820, 1725, 1425, 1385, 1270 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.80 (d, J = 8.08 Hz, 1H, CHO), 5.57 (d, J = 7.84 Hz, 1H, C=CH), 2.65 (t, J = 5.89 Hz, 2H, CH_2−C=C), 2.42 (t, J = 5.52 Hz, 2H, CH_2−C=C), 1.76−1.59 (m, 8H, $(CH_2)_4$). Anal. ($C_9H_{14}O$) C, H.

2-Cyclohexylidenbutanoic Acid (4).[23] A 5.2 g (29 mmol) amount of **23** in 6.0 mL of acetic anhydride were heated at 150 °C for 3 h. After the mixture was cooled to room temperature, the solution was given into a big amount of water and subjected to steam distillation. The distillate was cooled to 0 °C during which the acid crystallized. The crystals were washed and dried under vacuum; 404 mg (8.1%); mp 49 °C. IR 2900, 2600, 1650, 1600, 1430, 1280, 1230, 1200, 930 cm^{-1}. ^1H NMR (DMSO): δ 12.14 (s, 1H, CO_2H), 2.33 (t, J = 5.46 Hz, 2H, CH_2−C=C), 2.21 (t, J = 7.54 Hz, 2H, CH_2−C−C−O), 2.15 (t, J = 6.03 Hz, 2H, CH_2−C=C), 1.51 (m, 6H, CH_2−CH_2−CH_2), 0.91 (t, J = 7.54 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 176.10 (C-1), 151.87 (C-1′), 125.73 (C-2), 32.74; 31.72, 28.50, 28.50, 26.57, 22.75 (C-2′; C-3′; C-4′; C-5′; C-6′; C-3), 11.37 (C-4). MS *m*/*z* 169, 155, 151, 124, 108, 95, 81, 68. Anal. ($C_{10}H_{16}O_2$) C, H.

General Procedure for Compounds 5−14.[20] To a suspension of 31 mmol of NaH in 100 mL of dry glyme (argon atmosphere), 4.0 mL (31 mmol) of diethyl phosphite was slowly added. After the gas development had ceased, 31 mmol of the 2-bromocarboxylic acid, dissolved in 30 mL of dry glyme, was added carefully and the solution was stirred until no more gas development occurred. Afterward, 31 mmol of the cycloalkanone was added dropwise and the mixture was stirred for an additional hour. The reaction was stopped by adding 5 mL of ethanol, and the mixture was tilted into a big excess of ice-cold water. After it was extracted with ether, the aqueous layer was brought to pH 4 with concentrated HCl and extracted once again with ether. The organic layer was dried over magnesium sulfate, and the solvent was evaporated. The resulting acid was purified by recrystallization or flash chromatography.

2-Cyclohexylidenpropanoic Acid (5).[20] 41.72%; mp 78 °C. IR 3400, 2900, 1670, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.70 (s, 1H, CO_2H), 2.59 (t, J = 5.86 Hz, 2H, CH_2−C=C), 2.25 (t, J = 5.86 Hz, 2H, CH_2−C=C), 1.88 (s, 3H, CH_3), 1.68−1.50 (m, 6H, CH_2−CH_2−CH_2). ^{13}C NMR (DMSO): δ 175.99 (C-1), 152.89 (C-1′), 118.60 (C-2), 32.38, 32.06 (C-2′; C-6′), 27.33, 27.83 (C-3′; C-5′), 26.39 (C-4′), 15.06 (C-3). MS *m*/*z* 154, 136, 111, 109, 81. Anal. ($C_9H_{14}O_2$) C, H.

2-(3-Methylcyclohexyliden)propanoic Acid (6). 10.56%; mp 76 °C. IR 2840, 2600, 1650, 1430, 1380, 1270, 1210, 1100 cm^{-1}. ^1H NMR (DMSO): δ 12.15 (s, 1H, CO_2H), 2.48 (m, 4H, 2 × CH_2−C=C), 1.76 (s, 3H, CH_3−C−C−O), 1.86−0.99 (m, 5H, CH_2−CH_2−CH), 0.99 (dd, J = 6.40 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 171.50 (C-1), 145.17 (C-1′), 120.80, 34.58, 31.45 (C-2′; C-6′), 33.72 (C-3′), 30.25 (C-4′), 26.88 (C-5′), 22.37 (C-7′), 15.35 (C-3). MS *m*/*z* 168, 150, 135, 123, 112, 111, 100, 95. Anal. ($C_{10}H_{16}O_2$) C, H.

2-(4-Methylcyclohexyliden)propanoic Acid (7). 8.5%; mp 54 °C. IR 2840, 2550, 1650, 1610, 1430, 1380, 1270, 920 cm^{-1}. ^1H NMR (DMSO): δ 12.17 (s, 1H, CO_2H), 2.98 (m, 1H, CH_2−C=C), 2.52 (m, 1H, CH_2−C=C), 1.86−1.69 (m, 4H, CH_2−C−CH_2), 1.75 (s, 3H, CH_3−C−C−O), 1.56 (m, 1H, CH), 0.96 (m, 2H, CH_2−C=C), 0.85 (d, J = 6.40 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 171.45 (C-1), 145.48 (C-1′), 120.64 (C-2), 36.20, 35.73 (C-2′; C-6′), 32.12 (C-4′), 31.15, 30.09 (C-3′; C-5′), 21.96 (C-7′), 15.40 (C-3). MS *m*/*z* 168, 150, 139, 135, 125, 123, 111, 108, 100, 94. Anal. ($C_{10}H_{16}O_2$) C, H.

2-(4-Ethylcyclohexyliden)propanoic Acid (8). 14.39%; mp 41 °C. IR 2900, 2500, 1650, 1600, 1270, 1220 cm^{-1}. ^1H NMR (DMSO): δ 12.16 (s, 1H, CO_2H), 3.03 (m, 2H, CH_2−C=C), 2.53 (m, 2H, CH_2−C=C), 1.82 (m, 4H, CH_2−C−CH_2), 1.75 (s, 3H, CH_3−C−CO), 1.32 (m, 1H, CH), 1.18 (m, 2H, CH_2−Cyclohexyl), 0.84 (t, J = 7.16 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 171.42 (C-1), 145.89 (C-1′), 120.50 (C-2), 38.74 (C-4′), 33.80, 33.35 (C-2′; C-6′), 31.11, 30.07 (C-3′, C-5′), 28.92 (C-7′), 15.31 (C-3), 11.70 (C-8′). MS *m*/*z* 182, 108, 79, 81, 67, 56. Anal. ($C_{11}H_{18}O_2$) C, H.

2-(4-Propylcyclohexyliden)propanoic Acid (9). 59.55%; mp 46 °C. IR 2900, 2600, 1670, 1620 cm^{-1}. ^1H NMR (DMSO): δ 12.18 (s, 1H, CO_2H), 2.77 (m, 2H, CH_2−C=C), 2.53 (m, 2H, CH_2−C=C), 1.88−1.70 (m, 4H, CH_2−C−CH_2), 1.75 (s, 3H, CH_3−C−CO), 1.43 (m, 1H, CH), 1.29 (m, 2H, CH_2−C−Cyclohexyl), 1.14 (m, 2H, CH_2−Cyclohexyl), 0.84 (t, J = 7.16 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 171.42 (C-1), 145.96 (C-1′), 120.47 (C-2), 38.62 (C-7′), 36.74 (C-4′), 34.20, 33.75 (C-2′; C-6′), 31.12, 30.13 (C-3′; C-5′), 19.92 (C-8′), 15.36 (C-9′), 14.49 (C-3). MS *m*/*z* 196, 162, 153, 122, 111, 100, 81, 79, 56, 44. Anal. ($C_{12}H_{20}O_2$) C, H.

2-[4-(*tert*-Butyl)cyclohexyliden]propanoic Acid (10). 48.32%; mp 114 °C. IR 2910, 2600, 1680, 1630 cm^{-1}. ^1H NMR (DMSO): δ 12.14 (s, 1H, CO_2H), 2.87 (m, 2H, CH_2−C=C), 1.88−1.65 (m, 4H, CH_2−C−CH_2), 1.75 (s, 3H, CH_3−C−CO), 1.20 (m, 1H, CH), 0.99 (m, 2H, CH_2−C=C), 0.80 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (DMSO): δ 171.08 (C-1), 146.04 (C-1′), 120.20 (C-2), 47.55 (C-4′), 32.41, 31.57, 30.67, 28.66, 28.26 (C-2′; C-3′; C-5′; C-6′; C-1″), 27.66 (3 × CH_3), 15.35 (C-3). MS *m*/*z* 211, 210, 166, 195, 154, 139, 136, 125, 57. Anal. ($C_{13}H_{22}O_2$) C, H.

2-(4-Phenylcyclohexyliden)propanoic Acid (11). 69.35%; mp 146 °C. IR 3000, 2900, 2530, 1670, 1640, 1600, 790 cm^{-1}. ^1H NMR (DMSO): δ 12.25 (s, 1H, CO_2H), 7.28−7.01 (m, 5H, Ar), 3.19 (m, 2H, CH_2−C=C), 2.76 (m, 2H, CH_2−C=C), 1.93 (m, 4H, CH_2−C(Ar)-CH_2), 1.81 (s, 3H, CH_3), 1.45 (m, 1H, CH). ^{13}C NMR (DMSO): δ 171.45 (C-1), 146.58 (C-1′), 144.73 (C-1″), 128.62 (C-3″; C-5″), 126.99 (C-2″; C-6″), 126.27 (C-4″), 121.15 (C-2), 43.79 (C-4′), 35.24, 34.84 (C-2′; C-6′), 31.62, 30.57 (C-3′; C-5′), 15.49 (C-3). MS *m*/*z* 230, 161, 122, 111, 100, 91, 79, 77, 44. Anal. ($C_{15}H_{18}O_2$) C, H.

2-[4-(4-Methoxyphenyl)cyclohexyliden]propanoic Acid (12). Adduct **21** was used to give **12**; 21.43%; mp 138 °C. IR 3000, 2900, 2560, 1680, 1660, 1610, 1510, 1280, 1240, 1180, 1030, 810 cm^{-1}. ^1H NMR ($CDCl_3$): δ 12.27 (s, 1H, CO_2H), 7.12 (d, J = 8.58 Hz, 2H, AA′), 6.83 (d, J = 8.82 Hz, 2H, BB′), 3.78 (s, 3H, CH_3), 3.50 (m, 1H, CH−Ar), 2.76 (m, 2H, CH_2−C=C), 2.10−1.95 (m, 4H, CH_2−C−CH_2), 1.95 (s, 3H, CH_3), 1.63−1.48 (m, 2H, CH_2−C=CH). ^{13}C NMR (DMSO): δ 171.48 (C-1), 157.81 (C-4″), 144.76 (C-1′), 138.58 (C-1″), 127.84 (C-2″; C-6″), 121.07 (C-2), 114.02 (C-3″; C-5″), 55.26 (C-1‴), 42.92 (C-4′), 35.49, 35.09 (C-2′; C-6′), 31.66, 30.58 (C-3′; C-5′), 15.49 (C-3). MS *m*/*z* 260, 215, 186, 147, 134, 126, 107. Anal. ($C_{16}H_{20}O_2$) C, H.

2-Cyclopentylidenpropanoic Acid (13).[23] 21.53%; mp 100 °C. IR 2960, 2600, 1720, 1660, 1620, 1430, 1290, 730, 600, 440 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.43 (s, 1H, CO_2H), 2.76 (t, J = 6.40 Hz, 2H, CH_2−C=C), 2.39 (t, J = 6.40 Hz, 2H, CH_2−C=C), 1.86 (s, 1H, CH_3), 1.70 (m, 4H, CH_2−CH_2). ^{13}C NMR (DMSO): δ 173.93 (C-1), 164.33 (C-1′), 118.01 (C-2), 34.66 (C-2′; C-5′), 27.13, 25.52 (C-3′; C-4′); 15.83 (C-3). MS *m*/*z* 140, 95, 79, 67. Anal. ($C_8H_{12}O_2$) C, H.

2-Cyclopentylidenacetic Acid (14).[16] 3.5%; mp 51 °C. IR 3180, 3080, 2960, 1680, 1630, 860 cm^{-1}. ^{13}C NMR ($CDCl_3$): δ 168.08 (C-1), 167.65 (C-1′), 112.44 (C-2), 35.54, 32.44 (C-2′; C-5′), 26.19, 25.29 (C-3′; C-4′). MS *m*/*z* 126, 125, 108, 97, 81, 80, 79, 71, 68, 67, 52. Anal. ($C_7H_{10}O_2$) C, H.

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References

(1) Ellis, R. W.; DeFeo, D.; Shih, T. Y.; Gonda, M. A.; Young, H. A. Tsuchida, N.; Lowy, D. R.; Scolnick, E. M. The p21 src genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. *Nature* **1981**, *292*, 506−511.

(2) Campbell, S. L.; Khosravi-Far, R.; Rossman, K. L.; Clark, G. J.; Der, C. J. Increasing complexity of Ras signaling. *Oncogene* **1998**, *17*, 1395−1413.

(3) Bos, J. L. p21Ras: an oncoprotein functioning in growth factor-induced signal transduction. *Eur. J. Cancer* **1995**, *31A*, 1051−1054.
(4) Bos, J. L. Ras oncogenes in human cancer: a review. *Cancer Res.* **1989**, *49*, 9 (17), 4682−4689.
(5) Yurvey, A.; Wennogle, L. P. The RAF family: an expanding network of posttranslational controls and protein−protein interactions. *Cell Res.* **1998**, *8*, 81−98.
(6) Morrison, D. K.; Cutler, R. E. The complexity of Raf-1 regulation. *Curr. Opin. Cell Biol.* **1997**, *9* (2), 174−179.
(7) Nassar, N.; Horn, G.; Herrmann, C.; Scherer, A.; McCormick, F.; Wittinghofer, A. The 2.2A crystal structure of the Ras-binding domain of the serine/threonine kinase c-Raf-1 in complex with Rap1A and a GTP analogue. *Nature* **1995**, *375*, 554−560.
(8) Mott, H. R.; Carpenter, J. W.; Zhong, S.; Ghosh, S.; Bell, R. M.; Campbell, S. L. The solution structure of the Raf-1 cysteine-rich domain: a novel Ras and phospholipid binding site. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (16), 8312−8317.
(9) Marais, R.; Marshall, C. J. Control of the ERK MAP kinase cascade by Ras and Raf. *Cancer Surv.* **1996**, *27*, 101−125.
(10) Frodin, M.; Gammeltoft, S. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. *Mol. Cell. Endocrinol.* **1999**, *151* (1−2), 65−77.
(11) Downward, J. Ras signaling and apoptosis. *Curr. Opin. Genet. Dev.* **1998**, *8*, 49−54.
(12) Herrmann, C.; Block, C.; Geisen, C.; Haas, K.; Weber, C.; Winde, G.; Moroy, T.; Muller, O. Sulindac sulfide inhibits Ras signaling. *Oncogene* **1998**, *17* (14), 1769−1776.
(13) Clark, G. J.; Drugan, J. K.; Terrel, R. S.; Bradham, C.; Der, C. J.; Ball, R. M.; Campbel, S. Peptides containing a consensus Ras binding sequence from Raf-1 and the GTPase activating protein NF1 inhibit Ras function. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1577−1581.
(14) Barnard, D.; Diaz, B.; Hettich, L.; Chuang, E.; Zhang, X. F.; Avruch, J.; Marshall, M. Identification of the sites of interaction between c-Raf-1 and Ras-GTP. *Oncogene* **1995**, *10* (7), 1283−1290.
(15) Barnard, D.; Sun, H.; Baker, L.; Marshall, M. S. In vitro inhibition of Ras-Raf association by short peptides. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 176−180.
(16) Palaty, J.; Abbott, F. S. Structure−activity relationships of unsaturated analogues of valproic acid. *J. Med. Chem.* **1995**, *38*, 3398−3408.
(17) Löscher, W. Pharmacological, toxicological and neurochemical effects of Δ^2(E)-valproate in animals. *Pharm. Weekbl. Sci. Ed.* **1992**, *14*, 139−143.
(18) Wadsworth, W. S., Jr.; Emmons, W. D. The utility of phosphonates carbanions in olefin synthesis. *J. Am. Chem. Soc.* **1961**, *83*, 1733−1738.
(19) Arbusov, B. A. Michaelis-Arbusow- und Perkow-Reaktion. *Pure Appl. Chem.* **1964**, *9*, 307−335.
(20) Britelli, D. R. Phosphite-mediated in situ carboxyvinylation: A new general acrylic acid synthesis. *J. Org. Chem.* **1981**, *46*, 2514−2520.
(21) Kaiho, T.; San-nohe, K.; Kajiya, S.; Suzuki, T.; Otsuka, K.; Ito, T.; Kamiya, J.; Maruyama, M. Cardiotonic agents. 1-Methyl-7-(4-pyridyl)-5,6,7,8-tetrahydro-3(2*H*)-isoquinolinones and related compounds. Synthesis and Activity. *J. Med. Chem.* **1989**, *32*, 351−357.
(22) Reformatsky, S. Neue Synthese zweiatomiger einbasischer Säuren aus den Ketonen. *Ber. Dtsch. Chem. Ges.* **1887**, *20*, 1210−1211.
(23) Maillard, J.; Benard, M.; Morin, R. Acides cycloalphatiques doués d'activité cholérétique. *Bull. Soc. Chim. Fr.* **1958**, 244−248.
(24) Carnduff, J. Recent Advances in Aldehyd Synthesis. *Quart. Rev.* **1966**, *20*, 169−189.
(25) Buddrus, J. Wittig Reaktion mit Äthylenoxid. *Angew. Chem.* **1968**, *80*, 535−536.
(26) Nagata, W.; Hayase, Y. Formylolefination of carbonyl compounds. *J. Chem. Soc.* **1969**, 460−466.
(27) Fields, S.; Song, O. A novel genetic system to detect protein−protein interaction. *Nature* **1989**, *340*, 245−246.
(28) Chien, C.; Bartel, P.; Sternglanz, R.; Fields, S. The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9578−9582.
(29) Iwabuchi, K.; Li, B.; Bartel, P.; Fields, S. Use of the two-hybrid system to identify the domain of p53 involved in oligomerization. *Oncogene* **1993**, *8*, 1693−1696.
(30) Li, B.; Fields, S. Identification of mutations in p53 that affect its binding to SV40 T antigen by using the yeast two-hybrid system. *FASEB J.* **1993**, *7*, 957−963.
(31) Jaitner, B. K.; Becker, J.; Linnemann, T.; Herrmann, C.; Wittinghofer, A.; Block, C. Discrimination of amino acids mediating Ras binding from noninteracting residues affecting Raf activation by double mutant analysis. *J. Biol. Chem.* **1997**, *272* (47), 29927−29933.
(32) Kunkel, T. A. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 488−492.
(33) Kunkel, T. A.; Roberts, J. D.; Zakour, R. A. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* **1987**, *14*, 367−382.
(34) Harper, J. W.; Adami, G. R.; Wei, N.; Keyomarsi, K.; Elledge, S. J. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **1993**, *75* (4), 805−816.
(35) Flick, J. Two systems of glucose repression of the GAL1 promotor in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **1990**, *10* (9), 4757−4769.
(36) Klebe, R. J.; Harriss, J. V.; Sharp, Z. D.; Douglas, M. G. A general method for polyethylene-glycol-induced genetic transformation of bacteria and yeast. *Gene* **1983**, *25*, 333−341.
(37) Miller, J. H. *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1972.

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