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New Indene-Derivatives with Anti-Proliferative Properties

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Abstract—Metabolites of the non-steroidal anti-inflammatory drug Sulindac inhibit cell proliferation by affecting several intracellular signaling pathways including the tumorigenic Ras/Raf/MAPK pathway. Here, we report the synthesis of eight new indene derivatives derived from the Sulindac structure, and present data on their anti-proliferative properties and their effects on the p21ras protein.

Sulindac belongs to the chemically heterogeneous group of non-steroidal anti-inflammatory drugs (NSAIDs).[1] Many NSAIDs delay or inhibit the growth of malignant tumors.[2,3] However, unspecific side effects of Sulindac still prevent its broad use in clinical cancer prevention and therapy. The Sulindac molecule itself has no biological activity.[4] Sulindac is metabolized into the metabolites Sulindac sulfide and Sulindac sulfone, which affect several physiological functions including blood coagulation (sulfide), inflammation (sulfide) or cell proliferation (sulfide, sulfone). Originally, the effects of these metabolites were only ascribed to their inhibitory influence on cyclooxygenase (COX) 1, one of the two key enzymes of the eicosanoid metabolism.[5,6] Recent studies revealed, however, that the effects of Sulindac metabolites do not solely rely on COX inhibition. Sulindac sulfone causes apoptosis and inhibits tumor growth independently of COX.[7–11] Other reports demonstrated that Sulindac sulfone can inhibit the growth of tumors harboring mutations in the Ras proto-oncogene more effectively than the growth of tumors bearing the wild-type Ras gene. In addition, the apoptosis inducing effect of Sulindac was shown to be overcome by transfection of the oncogenic Ras gene.[12,13] Hence, it was suggested that Sulindac metabolites negatively interfere with the oncogenically activated p21ras protein and that they are able to block the activation of p21ras downstream effectors and p21ras dependent gene expression.[14–16] These results demonstrated that Sulindac and its main metabolites differ drastically in their biological activities although they are structurally very similar. Therefore, variations of the Sulindac structure could be a reasonable strategy to search for new compounds with effects on different important intracellular signaling pathways.

We synthesized a library of novel indene derivatives using 5-fluoro-2-methylindene-3-acetic acid (FMIA) as the starting component (Fig. 1).[17] A one-step con-

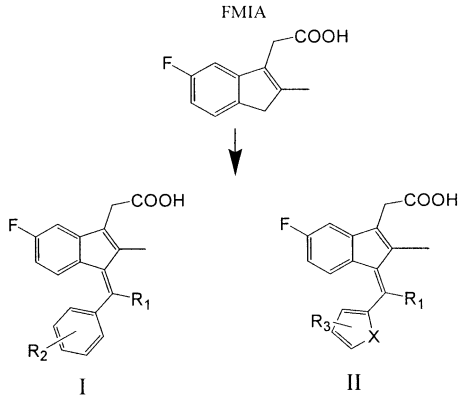

Figure 1. A schematic overview of the synthetic strategy. The library of indene derivatives can be classified into two groups, I and II: the phenyl substituents (I) and the five-membered heterocycle substituents (II).

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densation with the substituted aldehydes via the methylene moiety at position 1 of FMIA afforded in high yield the new compounds. The first group of substances is very similar to Sulindac ($R_1 = H$, $R_2 = SOCH_3$). We synthesized several isomers of (I), for example, $R_1 = H$, $R_2 = O–CH_3$, where R_2 is localized either in the *ortho*-, *meta*- or *para*-position of the aromatic system. The second group of substances contains a five-membered heterocyclic ring including the substituted thiophene (X = S) or furane moieties (X = O). The substituents (R_3) in the heterocyclic rings include $–CH_2–OH$, $–SO_3H$ or $–CH_3$ functionalities (Fig. 2).

First, we wished to test the influence of the newly synthesized compounds on cell proliferation and compare the effect of Sulindac and Sulindac sulfide and eight new substances (**1**–**8**) on the growth of five different cell types in a cell toxicity assay in a concentration dependent manner.[20] The IC_{50} values were determined for each compound for five different cell lines (Table 1). Whereas **3** showed no measurable effect up to 1 mM, all other compounds showed a growth inhibitory effect at concentrations similar to Sulindac and Sulindac sulfide.[15] All compounds with the exception of **2** inhibited the proliferation of REF cells at lower concentrations than the proliferation of NIH3T3 and SW480 cells. This can be explained by the fact that NIH3T3 and SW480 cells are immortalized by mutations in several different proliferation activating pathways rendering these cells less sensitive to the inhibitory influence of anti-proliferative drugs compared to the primary REF cells that bear no mutations. Most remarkably, the drugs **2**, **6**, **7a** and **8** inhibited the proliferation of MDCK-f3 cells, which had been transformed with an oncogenically activated H-Ras gene, at lower concentrations than the corresponding untransformed MDCK cells. These results provided a first indication for an inhibitory effect of these drugs on the Ras/Raf/MAPK pathway. Therefore, these four drugs were selected for further biochemical analysis.

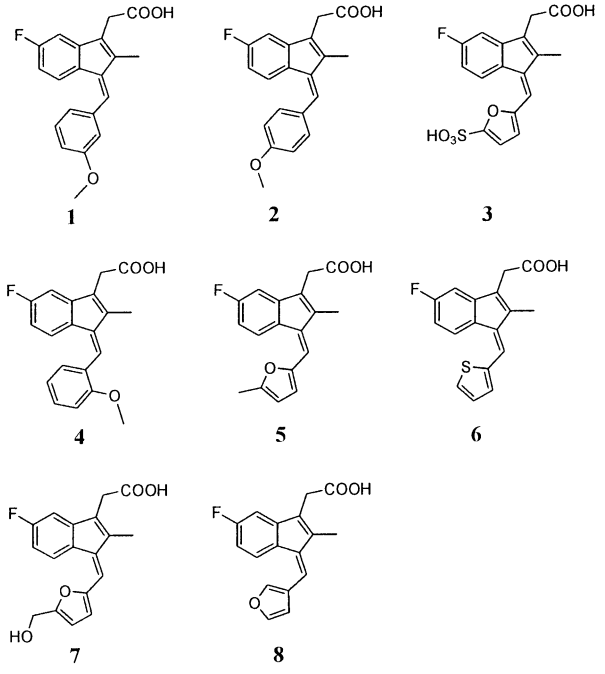

Figure 2. Examples of substances of the library of synthesized molecules.[19] In addition to the depicted compounds, the sodium salts of **5** (**5a**) and **7** (**7a**) were included in the study.

The four compounds **2**, **6**, **7a** and **8** were tested for their ability to inhibit COX activity.[24] The micromolar concentrations, which led to half-maximal inhibition of COX activity, were determined and compared to the value for Sulindac sulfide. We found that **6** and **8** inhibited COX activity at significantly lower concentrations than Sulindac sulfide (Table 2). Since the COX activity was measured in sheep seminal vesicles, where the constitutively expressed isoform COX 1 predominates, we can not rule out the possibility that the compounds tested here affect also the inducible isoform COX 2.[26] Assuming analogous biochemical mechanisms for the new compounds and for Sulindac sulfide that is ineffective on COX 2, an inhibitory effect of the new agents on COX 2 appears unlikely. **6** and **8** were found to inhibit COX activity at significantly lower concentrations than **2** and **7a**. Nevertheless, all four compounds inhibited cell proliferation at similar concentration ranges (Table 1).

Table 1. The micromolar IC_{50} values of the compounds, which lead to 50% decrease of the cell number of the corresponding cell line compared to the untreated control

Compd	NIH3T3	REF	SW480	MDCK	MDCK-f3
Sulindac	200 (±60)	100 (±30)	800 (±150)	300 (±50)	320 (±30)
Sulfide	350 (±50)	200 (±30)	200 (±110)	180 (±40)	200 (±60)
1	520 (±50)	200 (±100)	360 (±100)	275 (±60)	300 (±40)
2	480 (±50)	500 (±150)	na	460 (±50)	310 (±100)
3	na	na	na	na	na
4	500 (±200)	200 (±70)	850 (±200)	nd	360 (±60)
5	100 (±20)	20 (±10)	380 (±150)	250 (±60)	310 (±110)
5a	60 (±20)	10 (±3)	80 (±10)	60 (±10)	60 (±20)
6	400 (±100)	100 (±25)	320 (±120)	470 (±50)	80 (±20)
7	490 (±50)	80 (±30)	70 (±50)	50 (±20)	120 (±90)
7a	450 (±100)	90 (±10)	310 (±140)	250 (±10)	130 (±30)
8	170 (±70)	30 (±5)	400 (±200)	300 (±10)	50 (±10)

Values are means of three experiments, standard deviations are given in parentheses. Na, not active; nd, not determined. The compounds **2**, **6**, **7a** and **8** inhibited the proliferation of Ras transformed MDCK-f3 cells at lower concentrations than the proliferation of untransformed MDCK cells, what indicates an inhibitory effect on the Ras pathway. Data for Sulindac and Sulindac sulfide were taken from a recent publication.[14]

Table 2. The micromolar concentrations of Sulindac, Sulindac sulfide and the four compounds **2**, **6**, **7a** and **8** at which a half-maximal inhibition of the COX activity or of the p21ras/Raf interaction was achieved

Compd	COX activity	p21ras/Raf interaction
Sulindac	na	na
Sulfide	2.6	210
2	1.6	10
6	0.03	na
7a	570	45
8	0.24	30

Data for Sulindac and Sulindac sulfide were taken from recent publications.[14,27] na, not active.

The p21ras protein is activated by exchange of the bound GDP with GTP. In the activated GTP-bound state, p21ras interacts with the Raf kinase and activates the kinase activity by translocating Raf to the cytoplasmic membrane. The association of p21ras with a GTPase activating protein (GAP) leads to the activation of the intrinsic GTPase activity and to the hydrolysis of GTP to GDP. The inactivated GDP-bound p21ras dissociates from Raf and the signal transduction process is terminated. Sulindac sulfide inhibits the interaction between the p21ras protein and its main effector Raf kinase and thus the Ras/Raf/MAPK pathway.[14] In control experiments, none of the four new substances showed any effect on the intrinsic GTPase activity of the p21ras protein that is independent of the interaction with GAP. In addition, none of the compounds affected a spontaneous GDP/GTP exchange of p21ras up to a concentration of 500 μM (not shown) excluding unspecific effects of **2**, **6**, **7a** or **8** on the p21ras protein. We found that **2**, **7a** and **8** were able to inhibit the interaction between p21ras and Raf, **7a** and **8** even at lower half-maximal concentrations than Sulindac sulfide. Remarkably, **6** had no effect on the interaction between p21ras and Raf, despite it was highly effective on MDCK-f3 cells compared to MDCK cells. This discrepancy between the effect on living cells and the biochemical inhibitory property indicate that **6** might affect the Ras pathway at a level that is different from the interaction between p21ras and Raf.

Oncogenic mutations in the H-Ras gene lead to the production of p21ras isoforms that possess a prolonged half-life of the active GTP bound state. As a consequence the activation of Raf and the downstream MAP kinases is sustained and can not be switched off.[24] Thus, a compound, which inhibits the p21ras–Raf interaction represents a promising drug candidate with inhibiting potential against tumor cells that bear mutations in the H-Ras gene.[28] We have analyzed different properties of eight new derivatives of indenylacetic acid structurally related to the anti-inflammatory and anticancer drug Sulindac. Although Sulindac related molecules have been described in other recent reports,[27,29–31] this is the first study showing inhibitory effects on both the COX activity and on the signaling activity of the p21ras oncoprotein at lower concentrations than Sulindac sulfide itself. Among the novel substances, **8** appeared to be the most promising since this compound showed the lowest IC_{50} values in proliferation assays with H-Ras transformed cells in comparison to control cells that bear no oncogenic p21ras protein. In addition, **8** inhibited the interaction between p21ras and its main effector kinase Raf at the lowest concentration compared to the other drugs that were analyzed here and in particular in comparison to Sulindac sulfide. Since the interaction between p21ras and Raf is a key element in the Ras/Raf/MAPK signal transduction cascade, **8** may be most effective in blocking this pathway. Of the new compounds, **8** is the only indenylacetic acid with an unsubstituted furane substituent (Fig. 2). The unexpected biochemical effects of **6** showed that the substances might have yet unidentified effects on the Ras pathway or on other proliferation controlling pathways. Taken together, our results confirm that structurally very similar compounds of the family of Sulindac-like molecules can differ strongly in their biochemical and biological activities. Future studies will show whether these compounds might be suitable for application in cancer therapy.

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References and Notes

1. Vane, J. R.; Botting, R. M. *Scand. J. Rheumatol. Suppl.* **1996**, *102*, 9.
2. Giardiello, F. M. *Cancer Metast. Rev.* **1994**, *13*, 279.
3. Luk, G. D. *J. Suisse Med.* **1996**, *126*, 801.
4. Duggan, D. E.; Hooke, K. F.; Risley, E. A.; Shen, T. Y.; Arman, C. G. *J. Pharmacol. Exp. Ther.* **1977**, *201*, 8.
5. Rao, C. V.; Rivenson, A.; Simi, B.; Zang, E.; Kelloff, G.; Steele, V.; Reddy, B. S. *Cancer Res.* **1995**, *55*, 1464.
6. Qiao, L.; Kozoni, V.; Tsioulias, G. J.; Koutsos, M. I.; Hanif, R.; Shiff, S. J.; Rigas, B. *Biochim. Biophys. Acta* **1995**, *1258*, 215.
7. Pasricha, P. J.; Bedi, A.; O'Connor, K.; Rashid, A.; Akhtar, A. J.; Zahurak, M. L.; Piantadosi, S.; Hamilton, S. R.; Giardiello, F. M. *Gastroenterology* **1995**, *109*, 994.
8. Shiff, S. J.; Qiao, L.; Tsai, L. L.; Rigas, B. *J. Clin. Invest.* **1995**, *96*, 491.
9. Piazza, G. A.; Alberts, D. S.; Hixson, L. J.; Paranka, N. S.; Li, H.; Finn, T.; Bogert, C.; Guillen, J. M.; Brendel, K.; Gross, P. H.; Sperl, G.; Ritchie, J.; Burt, R. W.; Ellsworth, L.; Ahnen, D. J.; Pamukcu, R. *Cancer Res.* **1997**, *57*, 2909.
10. Piazza, G. A.; Rahm, A. K.; Finn, T. S.; Fryer, B. H.; Li, H.; Stoumen, A. L.; Pamukcu, R.; Ahnen, D. J. *Cancer Res.* **1997**, *57*, 2452.
11. Chiu, C. H.; McEntee, M. F.; Whelan, J. *Cancer Res.* **1997**, *57*, 4267.
12. Thompson, H. J.; Jiang, C.; Lu, J.; Mehta, R. G.; Piazza, G. A.; Paranka, N. S.; Pamukcu, R.; Ahnen, D. J. *Cancer Res.* **1997**, *57*, 267.
13. Arber, N.; Han, E. K.; Sgambato, A.; Piazza, G. A.; Delohery, T. M.; Begemann, M.; Weghorst, C. M.; Kim, N. H.; Pamukcu, R.; Ahnen, D. J.; Reed, J. C.; Weinstein, I. B.; Holt, P. R. *Gastroenterology* **1997**, *113*, 1892.
14. Herrmann, C.; Block, C.; Geisen, C.; Haas, K.; Weber, C.; Winde, G.; Möröy, T.; Müller, O. *Oncogene* **1998**, *17*, 1769.
15. Rice, P. L.; Goldberg, R. J.; Ray, E. C.; Driggers, L. J.; Ahnen, D. J. *Cancer Res.* **2001**, *61*, 1541.
16. Taylor, M. T.; Lawson, K. R.; Ignatenko, N. A.; Marek, S. E.; Stringer, D. E.; Skovan, B. A.; Gerner, E. W. *Cancer Res.* **2000**, *60*, 6607.
17. One mmol (206 mg) FMIA was solubilized in 10 mL dry methanol and 2.5 equivalents sodium-methoxide and 1.05 mmol of the corresponding aldehyde were added. The resulting colorless solution was heated under reflux conditions in an argon atmosphere while controlling the reaction using analytical HPLC [RP-18, methanol/0.05% ammoniumformiate/water (pH 5)]. After 10 min, the solution was turning light yellow and after 30 min a yellow precipitate formed. After 4 h, the reaction was complete. The solution was cooled, neutralized

with HCl and precipitated in ether. The filtrated crystals were re-crystallized with ether/hexane. A typical reaction yielded in fine yellow crystals (relative yield 70–95%). HPLC analysis showed for some compounds two isomeric forms of condensation products. In analogy to Sulindac synthesis, the main product was in all cases the *Z*-isomer.[18] All compounds were revealed at purities higher than 97%.

18. Shen, T. Y.; Winter, C. A. *Adv. Drug Res.* **1977**, *12*, 90.

19. All compounds are soluble in organic solvents (DMSO, $CHCl_3$, ethanol) up to molar concentrations and in aqueous buffers up to millimolar concentrations. The linear correlation between drug concentration and UV-absorption at the characteristic maximal wavelength revealed no hints of insolubility or micelle formation in aqueous buffered solutions at the applied concentrations. All compounds are stable at room temperature as solid freeze-dried powder for at least 6 months and solubilized in PBS at pH 7.5 for at least 24 h.

(*Z*)-5-Fluoro-2-methyl-1-(3′-benzyliden-1′-methoxy)-3-indenyl-acetic acid (1). 245 mg yellow crystals (76%), single isomer, mp 118 °C; UV (methanol) λ_{max} (log ε) = 224 (4.45), 263 (4.19), 279 (4.19), 325 (4.10); ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 3.56 (s, 2H, CH_2), 3.76 (s, 3H, CH_3), 6.71 (s, 1H, olefin–H), 6.95 (m, 2H, ArH), 7.17 (m, 2H, ArH), 7.25 (m, 1H, ArH), 7.35 (s, 1H, ArH), 7.40 (s, m, 1H, ArH), 12.35 (s, 1H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.21 (CH_3), 31.06 (CH_2), 55.13 (CH_3), 105.75 (Ar–C), 110.32 (Ar–C), 114.48 (Ar–C), 121.29 (olefin–C), 123.22 (Ar–C), 129.55 (Ar–C), 129.71 (Ar–C), 130.76 (Ar–C), 131.98 (Ar–C), 137.87 (Ar–C), 139.54 (Ar–C), 146.84 (Ar–C), 146.92 (Ar–C), 159.22 (Ar–C), 161.46 (olefin–C), 163.33 (olefin–C), 171.57 (carbonyl–C); LRMS (CI, rel intensity) (324 (M+, 100), 264 (50), 263 (15)); HRMS (CI, *m*/*z* calcd for $C_{20}H_{17}FO_3$) (M+, 324.116323, found 324.116173); −0.5 ppm.

(*Z*)-5-Fluoro-2-methyl-1-(4′-benzyliden-1′-methoxy)-3-indenyl-acetic acid (2). 280 mg yellow crystals (86%), single isomer, mp 168 °C; UV (methanol) λ_{max} (log ε) = 224 (4.45), 256 (4.19), 294 (4.07), 339 (4.22); ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.79 (s, 3H, CH_3), 6.71 (s, 1H, olefin–H), 6.95 (m, 1H, ArH), 7.18 (m, 2H, ArH), 7.29 (s, 1H, ArH), 7.35 (m, 1H, ArH), 7.50 (m, 2H, ArH), 12.32 (s, 1H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.28 (CH_3), 31.08 (CH_2), 55.15 (CH_3), 105.75 (Ar–C), 110.02 (Ar–C), 114.03 (Ar–C), 122.82 (olefin–C), 123.89 (Ar–C), 129.64 (Ar–C), 129.99 (Ar–C), 130.95 (Ar–C), 131.19 (Ar–C), 138.07 (Ar–C), 138.24 (Ar–C), 146.70 (Ar–C), 146,77 (Ar–C), 159.46 (Ar–C), 161.24 (olefin–C), 163.17 (olefin–C), 171.65 (carbonyl–C); LRMS (CI, rel intensity) (324 (M+, 100), 279 (45), 264 (25)); HRMS (CI, *m*/*z* calcd for $C_{20}H_{17}FO_3$) (M+, 324.116547, found 324.116173); −1.2 ppm.

(*Z*)-5-Fluoro-2-methyl-1-(2′-furfuryliden-5′-sulfonyl)-3-indenyl-acetic acid (3). 255 mg yellow crystals (70%), mixture of isomers (*Z*/*E* = 23:1, mp 190 °C (decomp); UV (methanol) λ_{max} (log ε) = 224 (4.43), 308 (3.84), 352 (4.27), 364 (4.27); ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.11 (s, 3H, CH_3), 3.50 (s, 2H, CH_2), 6.62 (d, *J* = 3.5 Hz, 1H, olefin–H), 6.79 (m, 1H, ArH), 6.91 (m, 1H, ArH), 6.94 (s, 1H, ArH), 7.02 (m, 1H, ArH), 8.60 (s, 1H, ArH), 12.35 (s, 2H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.50 (CH_3), 31.92 (CH_2), 105.53 (Ar–C), 110.30 (Ar–C), 115.38 (Ar–C), 117.62 (olefin–C), 125.69 (Ar–C), 129.01 (Ar–C), 131.98 (Ar–C), 135.78 (Ar–C), 138.10 (Ar–C), 146.95 (Ar–C), 150.14 (Ar–C, furane), 159.05 (Ar–C, furane), 161.41 (olefin–C), 163.34 (olefin–C), 171.93 (carbonyl–C); LRMS (CI, rel intensity) (364 (M+, 15), 324 (41), 159 (100)).

(*Z*)-5-Fluoro-2-methyl-1-(2′-benzyliden-1′-methoxy)-3-indenyl-acetic acid (4). 278 mg yellow crystals (85%), mixture of isomers (*Z*/*E* = 11:1 mp 122–128 °C; UV (methanol) λ_{max} (log ε) = 223 (4.36), 259 (3.92), 328 (3.68). ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.91 (s, 3H, CH_3), 6.71 (s, 1H, olefin–H), 6.99 (m, 2H, ArH), 7.08 (m, 2H, ArH), 7.37 (m, 1H, ArH), 7.38 (s, 1H, ArH), 7.44 (s, m, 1H, ArH), 12.30 (s, 1H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.25 (CH_3), 31.05 (CH_2), 55.45 (CH_3), 105.63 (Ar–C), 110.24 (Ar–C), 111.35 (Ar–C), 120.19 (olefin–C), 122.97 (Ar–C), 124.32 (Ar–C), 127.72 (Ar–C), 129.82 (Ar–C), 130.38 (Ar–C), 137.62 (Ar–C), 139.33 (Ar–C), 142.13 (Ar–C), 147.97 (Ar–C), 157.14 (Ar–C), 161.54 (olefin–C), 164.37 (olefin–C), 171.62 (carbonyl–C); LRMS (CI, rel intensity) (324 (M+, 10), 296 (50), 263 (15)).

(*Z*)-5-Fluoro-2-methyl-1-(2′-furfuryliden-5′-methyl)-3-indenyl-acetic acid (5). 220 mg yellow crystals (73%), mixture of isomers (*Z*/*E* = 27:1, mp 181–183 °C; UV (methanol) λ_{max} (log ε) = 224 (4.41), 370 (4.35) ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.11 (s, 3H, CH_3), 2.47 (s, 3H, CH_3) 3.54 (s, 2H, CH_2), 6.36 (d, *J* = 1.5 Hz, 1H, olefin–H), 6.90–7.01 (m, 3H, ArH), 6.91 (m, 1H, ArH), 8.45 (s, 1H, ArH), 12.38 (s, 2H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.47 (CH_3), 13.82 (CH_3), 31.19 (CH_2), 105.54 (Ar–C), 110.11 (Ar–C), 115.88 (Ar–C), 119.62 (olefin–C), 124.74 (Ar–C), 129.29 (Ar–C), 130.33 (Ar–C), 133.58 (Ar–C), 138.62 (Ar–C), 146.33 (Ar–C), 149.93 (Ar–C, furane), 155.39 (Ar–C, furane), 161.19 (olefin–C), 163.12 (olefin–C), 171.75 (carbonyl–C); LRMS (CI, rel intensity) (298 (M+, 100), 253 (90), 209 (35)), HRMS (CI, *m*/*z* calcd for $C_{18}H_{15}FO_3$) (M+, 298.100693, found 298.100523); −0.6 ppm.

(*Z*)-5-Fluoro-2-methyl-1-(2′-thiophenyliden)-3-indenyl-acetic acid (6). 190 mg yellow crystals (63%) mp 171 °C; UV (methanol) λ_{max} (log ε) = 223 (4.41), 352 (4.24); ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.12 (s, 3H, CH_3), 3.33 (s, 2H, CH_2), 6.85 (m, 1H, olefin–H), 7.04 (m, 1H, ArH), 7.21 (m, 1H, ArH), 7.35 (s, 1H, ArH), 7.49 (m, 1H, ArH), 7.79 (s, 1H, ArH), 7.90 (s, 1H, ArH), 12.50 (s, 1H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.31 (CH_3), 31.07 (CH_2), 105.69 (Ar–C), 110.41 (Ar–C), 122.79 (Ar–C), 123.34 (Ar–C), 128.24 (Ar–C), 129.15 (Ar–C), 131.09 (Ar–C), 131.70 (Ar–C), 137.91 (Ar–C), 138.32 (Ar–C), 138.68 (Ar–C), 146.72 (olefin–C), 161.41 (olefin–C), 163.35 (olefin–C), 171.56 (carbonyl–C); LRMS (CI, rel intensity) (300 (M+, 100), 255 (80), 239 (45), 97 (40)); HRMS (CI, *m*/*z* calcd for $C_{17}H_{13}FO_2S$) (M+, 300.061734, found 300.062030); −1.0 ppm.

(*Z*)-5-Fluoro-2-methyl-1-(2′-furfuryliden-5′-hydroxy-methyl)-3-indenyl-acetic acid (7). 140 mg yellow crystals (44.5%), mixture of isomers (*Z*/*E* = 9:1, mp 82 °C (decomp); UV (methanol) λ_{max} (log ε) = 224 (4.44), 361 (4.35) ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.12 (s, 3H, CH_3), 2.21 (s, 1H, OH), 3.54 (m, 4H, CH_2), 6.36 (m, 1H, olefin–H), 6.90–7.20 (m, 4H, ArH), 8.48 (m, 1H, ArH), 12.43 (s, 2H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.38 (CH_3), 31.82 (CH_2), 31.19 (CH_2), 103.14 (Ar–C), 111.01 (Ar–C), 117.98 (Ar–C), 120.32 (olefin–C), 125.81 (Ar–C), 129.41 (Ar–C), 132.13 (Ar–C), 133.19 (Ar–C), 138.14 (Ar–C), 147.13 (Ar–C), 149.51 (Ar–C, furane), 157.28 (Ar–C, furane), 162.78 (olefin–C), 164.59 (olefin–C), 172.75 (carbonyl–C); LRMS (CI, rel intensity) (314 (M+, 38)), HRMS (CI, *m*/*z* calcd for $C_{18}H_{15}FO_4$) (M+, 314.095945, found 314.095437); −1.6 ppm.

(*Z*)-5-Fluoro-2-methyl-1-(3′-furfuryliden)-3-indenyl-acetic acid (8). 210 mg yellow crystals (74%), mixture of isomers (*Z*/*E* = 30:1, mp 150–153 °C; UV (methanol) λ_{max} (log ε) = 223 (4.31), 264 (4.11), 327 (4.00); ^{1}H NMR (500 MHZ, DMSO-d_6): δ 2.08 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 6.74 (s, 1H, olefin–H), 6.78 (m, 1H, ArH), 6.95 (m, 1H, ArH), 7.02 (s, 1H, ArH), 7.65 (m, 1H, ArH), 7.80 (s, 1H, ArH), 8.00 (s, 1H, ArH), 12.35 (s, 1H, OH); ^{13}C NMR (500 MHZ, DMSO-d_6): δ 10.19 (CH_3), 31.13 (CH_2), 105.67 (Ar–C), 110.35 (Ar–C), 111.48 (Ar–C), 120.82 (olefin–C), 123.3 (Ar–C), 129.64 (Ar–C), 131.27 (Ar–C), 138.12 (Ar–C), 139.28 (Ar–C), 143.81 (Ar–C, furane), 144.16 (Ar–C, furane), 146.72 (olefin–C), 161.29 (olefin–C), 163.22 (olefin–C), 171.64 (carbonyl–C); LRMS (CI, rel intensity) (284 (M+, 100), 239 (55), 225 (45), 196 (65)); HRMS (CI,

m/z calcd for $C_{17}H_{13}FO_3$) (M+, 284.085526, found 284.084873); −2.3 ppm.
20. The anti-proliferative activities of Sulindac sulfide and eight derivatives were quantified in an assay, which relies on the metabolization of the yellow dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into the blue thiazole form by cellular mitochondrial dehydrogenases by intact and living cells.[21] We chose murine NIH3T3 fibroblasts, primary Rat Embryonic Fibroblasts (REFs) and the SW480 cell line, the latter being an example of a human cancer cell line harboring mutational defects in several signaling pathways (American Type Culture Collection). The MDCK-f3 (Madin-Darby canine kidney f3) cell line is a subclone of the MDCK-ras-f cells,[22] which had been derived from MDCK cells after stable transfection with a construct directing the expression of the oncogenically activated p21ras protein (kindly provided by John G. Collard, Amsterdam). The compounds were dissolved as 1000× stock solutions in DMSO and were added to final concentrations in the range between 10 nM and 1 mM. The assay was done as described.[21,23] The growth inhibition was calculated as percentage of the respective control cells without drug. The percentages were plotted against the compound concentration, and the IC_{50} values, were determined from the graphic plots. Vehicle control showed no detectable inhibition of cell proliferation.
21. Denizot, F.; Lang, R. *J. Immunol. Meth.* **1986**, *89*, 271.
22. Vleminckx, K.; Vakaet, L.; Mareel, M.; Fiers, W.; van Roy, F. *Cell* **1991**, *66*, 107.
23. Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55.
24. The COX inhibitory activities of the compounds were measured by the direct analysis of the consumed O_2 using isolated sheep seminal vesicles.[25] The effects of selected compounds on the interaction of the p21ras protein with its main effector Raf kinase was measured in vitro using recombinant proteins.[14]
25. Gierse, J. K.; Hauser, S. D.; Creely, D. P.; Koboldt, C.; Rangwala, S. H.; Isakson, P. C.; Seibert, K. *Biochem. J.* **1995**, *305*, 479.
26. Tsuji, S.; Tsujii, M.; Kawano, S.; Hori, M. *J. Exp. Clin. Cancer Res.* **2001**, *20*, 117.
27. Jung, M.; Wahl, A. F.; Neupert, W.; Geisslinger, G.; Senter, P. *Pharm. Pharmacol. Commun.* **2000**, *6*, 217.
28. Sigal, I. S.; Smith, G. M.; Jurnak, F.; Marsico-Arhern, J. D.; D'Alonzo, J. S.; Scolnick, E. M.; Gibbs, J. B. *Anti-cancer Drug Des.* **1987**, *2*, 107.
29. Thompson, W. J.; Piazza, G. A.; Li, H.; Liu, L.; Fetter, J.; Zhu, B.; Sperl, G.; Ahnen, D.; Pamukcu, R. *Cancer Res.* **2000**, *60*, 3338.
30. Pamukcu, R.; Piazza, G. US Patent 6,046,199, 2000.
31. Sperl, G. US Patent 6,020,379, 2000.