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Synthesis and biological study of a flavone acetic acid analogue containing an azido reporting group designed as a multifunctional binding site probe

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Abstract—Flavone-8-acetic acid (FAA) is a potent immunomodulatory small molecule that is uniquely characterized as being active on mouse but not human cells. Although FAA is a potent inducer of murine cytokine, chemokine and interferon gene expression, its mode of action remains unknown. In this report, we describe the synthesis of a new flavone acetic acid (FAA) analogue, (2-[2-(4-azidophenyl)-4-oxochromen-8-yl-]acetic acid (compound **2**). We demonstrate that compound **2** is equally active as the parent FAA in inducing chemokine gene expression and that the azide functional group is capable of reacting with a reporter molecule, such as the FLAG peptide–phosphine, under mild conditions. This reaction will be useful for detecting the drug-bound protein active complex utilizing an anti-FLAG antibody.
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1. Introduction

Almost two decades ago, a synthetic flavonoid, flavone-8-acetic acid (**1**, FAA),[1] was widely investigated when its ability to reduce tumor growth in a number of murine solid tumors was discovered.[2–6] Unfortunately, the antitumor activity observed in murine models did not translate to humans and FAA failed in early clinical trials.[7,8] Despite extensive studies, the mechanism of FAA remains unknown and to date no specific biological target has been identified. Its antitumor activity appears to be the result of indirect effects rather than direct cytotoxicity, and it differs from most conventional chemotherapeutic agents in that it does not induce myelosuppression.[9] The compound is able to activate macrophages[10] and natural killer (NK) cells;[11–13] it increases production of interferon,[14] tumor necrosis factor-α (TNF-α),[14] and nitric oxide and other cytokines from activated mouse but not human macrophages.[15] One of the main effects of FAA is TNF-α-mediated tumor vascular collapse[16–18] followed by tumor necrosis.[19] More recently, the reported effects of FAA on endothelial cell proliferation demonstrates that this flavone possesses antiangiogenic properties.[20] Therefore, FAA is capable of engaging the immune system by acting as a biological response modifier.

The immunomodulatory activity of FAA in murine systems has been linked to its ability to rapidly induce cytokine gene expression. Because the drug fails to do the same in humans,[21] knowledge of its mechanism of action in mice would be critically important to provide new leads for drug development to treat human cancers. Despite the reported syntheses of a number of analogues, none of these compounds have been designed to identify any of the possible molecular targets.[22–25] To achieve such a goal, we decided to synthesize a compound with a close structural resemblance to the parent FAA, but modified with a functional group capable of

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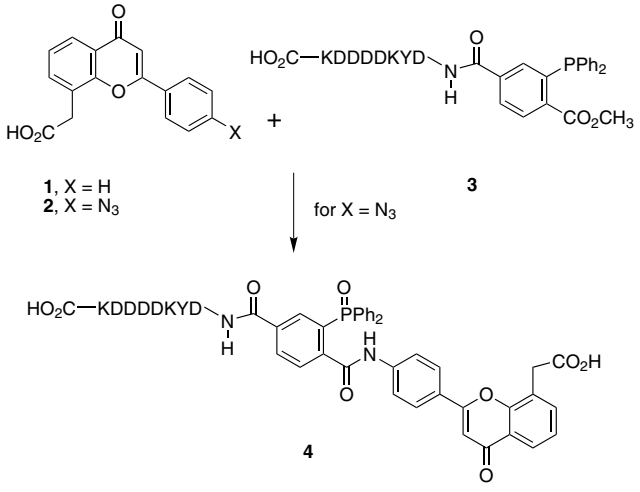
reacting in a biological milieu with a specific peptide tag. With the use of a recently developed rapid tissue culture screen, we have been able to dissect the molecular structure of FAA and determine, which chemical variations enhance, maintain or abrogate its functional activity. Using a mouse macrophage cell line we were able to measure the direct induction of the CCL5 chemokine (Rantes) within 6 h by FAA and its derivatives utilizing 100,000 target cells in a 96 well format (data not shown). This assay led to the identification of a substitution ($X = N_3$) of the molecule (2-[2-(4-X-phenyl)-4-oxochromen-8-yl-]acetic acid, **2**) that preserved the full biological activity of FAA.

A major objective of this project is to ultimately be able to identify a target macromolecule bound by FAA by using the N_3 group as an affinity label, which can be activated either photochemically[26] or through conventional chemistry.[27] In view of the technical difficulties anticipated with photochemical activation, our attention was directed to the use of the Staudinger reaction to locate the binding site of our azide-containing FAA by reacting it with a FLAG peptide–phosphine (Scheme 1).[27,28] Detection of the drug-bound complex could then possibly be achieved by Western blot or immunoprecipitation with a mouse anti-FLAG antibody in a manner similar to the elegant work of Bertozzi and co-workers.[27,28] The conditions for the Staudinger reaction between the phosphine and the azide are very mild and ligations could be performed in a cell supernatant maintained between pH 7.0 and 7.4 after FAA treatment.[28]

In order to reach our goal of developing a new reagent that will permit us to identify the molecular target of FAA, we needed to (1) synthesize (2-[2-(4-azidophenyl)-4-oxochromen-8-yl-]acetic acid, **2**), (2) confirm that it was biologically equivalent to FAA, and (3) demonstrate that the Staudinger reaction occurred readily between the FLAG peptide–phosphine probe and **2**. The realization of these three objectives is described below.



Scheme 1.

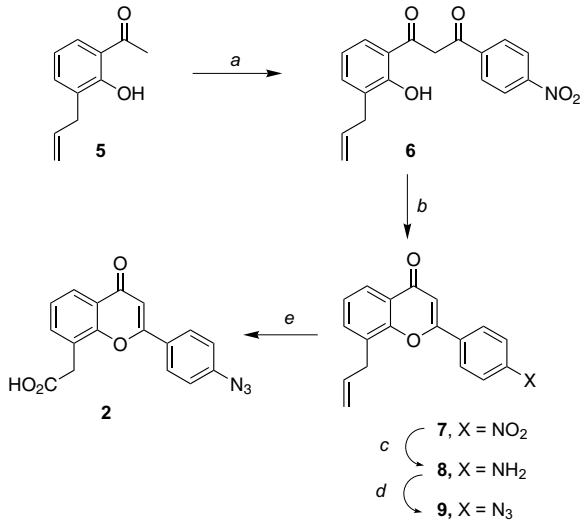
2. Results

2.1. Synthesis of 2-[2-(4-azidophenyl)-4-oxochromen-8-yl-]-acetic acid (2)

Diketone **6** was readily formed by *O*-acylation of the known 1-(2-hydroxy-3-prop-2-enylphenyl)ethan-1-one (**5**) in the presence of K_2CO_3 (Scheme 2). The ensuing intramolecular rearrangement resulted in the migration of the *p*-nitrobenzoyl moiety from oxygen to carbon according to the well-known Baker–Venkataraman rearrangement.[29] Cyclization to chromone **7** occurred readily in acid, and conversion of the nitro group to the azide was performed in two simple steps via the amine intermediate to give **9**. The allyl group was finally oxidized with potassium permanganate[22] in a mixture of acetic acid, water, and acetone to give the desired target **2**. Oxidation with $NaIO_4/RuO_4$ provided no decisive advantage despite the excellent yields reported with this methodology by Sharpless and co-workers.[30]

2.2. Staudinger reaction between 2-[2-(4-azidophenyl)-4-oxochromen-8-yl-]acetic acid (2) and the FLAG peptide–phosphine probe (3)

A sample of the FLAG peptide–phosphine probe, synthesized according to Ref. 27 was kindly provided by Dr. Carolyn Bertozzi. The Staudinger ligation depicted in Scheme 1 was carried out on a microscale in aqueous media at pH 7.2 to mimic as closely as possible the conditions of a potential biological experiment. Since mass spectrometric analysis was used to follow and confirm formation of the expected FAA–FLAG peptide conjugate (**4**), a volatile ammonium carbonate buffer was employed to maintain pH and yet allow lyophilization prior to analysis in order to minimize inorganic salt contamination of the sample. MALDI MS indicated com-



Scheme 2. Reagents and conditions: (a) *p*-nitrobenzoyl chloride, K_2CO_3, butanone, Δ (62%); (b) H_2SO_4, MeOH, Δ (90%); (c) $Na_2S_2O_4$, AcOH/acetone/H_2O; (d) $NaNO_2$, HCl, NaN_3, CH_2Cl_2/H_2O (biphasic) (57%, two steps); (e) $KMnO_4$, AcOH/acetone/H_2O (18%).

plete reaction of the FLAG peptide–phosphine **3** with **2** to form expected FAA–FLAG peptide conjugate **4** after 1 h at room temperature (25 °C). The product mass spectrum exhibited the correct mass (*m*/*z* 1638.52) and appropriate isotopic distribution for the expected protonated molecule (MH^+) with minimal cationization by Na^+ and K^+. No indication of the presence of FLAG peptide–phosphine **3** starting material (*m*/*z* 1359.48, MH^+) was observed in the product spectrum. Similar MALDI MS analysis of a control sample containing only the FLAG peptide–phosphine **3** indicated the appropriate mass and isotopic distribution for this compound and showed only the minor presence of the oxidized phosphine (*m*/*z* 1375, MH^+).

2.3. Biological activity

The biological activity of azido-FAA (**2**) relative to FAA was determined by treating a mouse C57BL/6 macrophage cell line with the two compounds (at varying concentrations) for 6 h, isolating total cellular RNA from the treated cells, and analyzing the extracted RNA with the chemokine Multiprobe RNAse Protection assay. As shown in Figure 1, the azido-FAA (**2**) derivative was equally as active as FAA in inducing chemokine gene expression in the mouse macrophage cell line.

3. Discussion and conclusion

The synthesis of the target azido compound **2** proceeded uneventfully according to Scheme 2. As demonstrated in Figure 1, the azido group did not interfere with the desired biological activity and the compound essentially behaved as the parent FAA (**1**). The Staudinger reaction between **2** and the FLAG peptide–phosphine probe occurred under mild conditions that were compatible with those of potential biological experiments (Scheme 1), and the formation of the product was unequivocally confirmed by MALDI MS analysis. One possible limitation of this reaction in a biological system is the accessibility of the azido group to react with the FLAG peptide–phosphine probe after compound **2** binds to its target protein. If the azido group finds itself in a deep pocket at the active site, the Staudinger reaction will probably fail. In this case, the reactive azido group could be photoactivated and the resulting nitrene would react with a proximal functional group on the target macromolecule forming a covalent bond.[25] The tagged protein could then be identified and degraded to identify the binding site. Whatever approach is to be followed, we propose that compound **2** should be a useful probe to define the active binding site of FAA. As FAA was a potent immunomodulatory that demonstrated significant activity in murine tumor models, the identification of its molecular target will permit us to identify the human counterpart and develop FAA analogues that may show equivalent biological responses when tested on human cells in vitro and in vivo. The approach described here represents an important model for identifying the molecular targets of novel small molecules whose mechanism of biological activity is unknown.

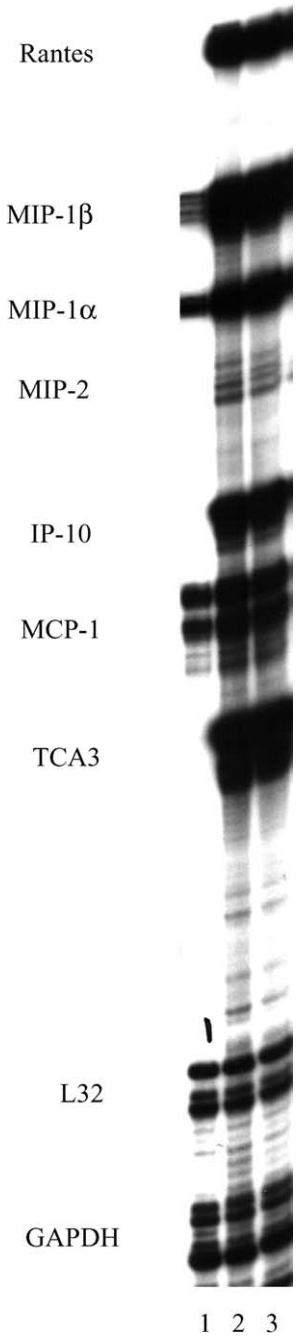


Figure 1. Ribonuclease protection assay (1 = no treatment; 2 = FAA (100 μg/mL); 3 = azido-FAA, 10 μg/mL).

4. Experimental

4.1. General

All chemical reagents were commercially available. Column chromatography was performed on silica gel 60, 230–240 mesh (E. Merck), and analytical TLC was performed on Analtech Uniplates silica gel GF. Routine IR, ^1H NMR and ^{13}C NMR spectra were recorded using standard methods. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

4.2. (2*Z*)-3-Hydroxy-1-(2-hydroxy-3-prop-2-enylphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (6)

A mixture of 1-(2-hydroxy-3-prop-2-enylphenyl)ethan-1-one (**5**, 6 g, 34 mmol), 4-nitrobenzoyl chloride (6 g, 32.4 mmol), and anhydrous K_2CO_3 (10 g) in anhydrous butanone (100 mL) was refluxed for 36 h. The reaction mixture was decanted to remove inorganic salts and evaporated to half its volume. Hexane (100 mL) was added and the precipitate formed was filtered off. The solid was re-dissolved in EtOAc (100 mL) and acidified with 6 N HCl to pH 1. The organic layer was separated and the aqueous layer was further washed with EtOAc (2×50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to afford pure **6** as an orange solid product (8 g, 62%), which was crystallized from EtOAc/hexanes; mp 177–179 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.26 (s, 1H), 8.34 (d, $J = 8.8$ Hz, 2H), 8.09 (d, $J = 8.8$ Hz, 2H), 7.69 (d, $J = 7.2$ Hz, 1H),7.40 (dd, $J = 7.2$ Hz, 1H), 6.88–6.93 (m, 2H), 6.00–6.07 (m, 1H), 5.10–5.14 (m, 2H), 3.47 (d, $J = 6.4$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.6, 173.27, 160.8, 149.7, 139.5, 136.6, 135.9, 130.0, 127.6, 126.7, 123.9, 118.8, 118.2, 116.1, 94.3, 33.5; IR (neat) 3077, 1636, 1438, 1244 cm^{-1}; FAB MS (negative ion) *m*/*z* 324 $(M-H)^-$. Anal. Calcd for ($C_{18}H_{15}NO_5 \cdot 0.33H_2O$): C, 65.25; H, 4.77; N, 4.23. Found: C, 65.27; H, 4.57; N, 4.21.

4.3. 2-(4-Nitrophenyl)-8-prop-2-enylchromen-4-one (7)

Five drops of concd H_2SO_4 were added to solution of **6** (2.1 g, 6.3 mmol) in MeOH (50 mL) and heated to reflux for 2 h. The solution was concentrated under reduced pressure, and the brown solid obtained dissolved in CH_2Cl_2 (100 mL) and washed with water (2×25 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The product was purified by crystallization from EtOAc/hexanes to afford **7** (1.4 g, 70%); mp 210–212 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.40 (d, $J = 9.2$ Hz, 2H), 8.09 (dd, $J = 8.0$, 1.7 Hz, 1H), 8.06 (d, $J = 9.1$ Hz, 2H), 7.57 (br d, $J = 7.4$ Hz, 1H), 7.37 (t, $J = 7.6$ Hz, 1H), 6.88 (s, 1H), 6.02–6.09 (m, 1H), 5.10–5.18 (m, 2H), 3.74 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.3, 160.3, 154.2, 149.4, 137.9, 135.10, 134.80, 129.50, 127.20, 125.50, 124.30, 124.10, 123.97, 117.20, 109.40, 34.00; IR (neat) 1747, 1525, 1346 cm^{-1}; FAB MS *m*/*z* (relative intensity) 308 (MH^+, 100). Anal. Calcd for ($C_{18}H_{13}NO_4 \cdot 0.2H_2O$): C, 69.54; H, 4.34; N, 4.51. Found: C, 69.49; H, 4.53; N, 4.23.

4.4. 2-(4-Azidophenyl)-8-prop-2-enylchromen-4-one (9)

A slurry of compound **7** (1.4 g, 4.5 mmol) and $Na_2S_2O_4$ (8.3 g, 48.5 mmol) in a mixture of acetone (40 mL) and water (20 mL) was at 50 °C for 1 h. The reaction mixture was concentrated to a yellow solid. The solid was washed with EtOAc (100 mL) and the washing was concentrated under reduced pressure to afford intermediate amine **8**. This product was considered pure enough for the subsequent reaction: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06 (dd, $J = 9.5$, 1.6 Hz, 1H), 7.71 (dm, $J = 8.8$ Hz, 2H), 7.48 (dd, $J = 8.9$, 1.6 Hz, 1H), 7.33 (t, $J = 7.6$ Hz, 1H), 6.76 (dm, $J = 8.8$, 2H), 6.66 (s, 1H), 6.03–6.10 (m, 1H), 5.11–5.16 (m, 2H), 3.72 (d, $J = 6.4$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.70, 163.60, 154.00. 149.90, 135.50, 133.70, 129.30, 127.95, 124.63, 124.00, 123.83, 116.85, 114.75, 104.83, 34.00; FAB MS *m*/*z* (relative intensity) 278 (MH^+, 100). Crude **8** was then dissolved in cold (0 °C) CH_2Cl_2 (5 mL), treated with cold (0 °C) 6 N HCl (10 mL), and stirred. Aqueous sodium nitrite (0 °C) was added slowly and stirred for 20 min. Sodium azide was then added slowly and stirred for an additional 25 min. The reaction mixture was diluted with CH_2Cl_2 (25 mL) and the layers separated. The aqueous layer was further washed with CH_2Cl_2 (2×25 mL) and the collected organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. Purification by column chromatography (SiO_2, 3:1 hexanes/EtOAc) afforded **9** (800 mg, 57% for two steps); mp 153–155 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (dd, $J = 8.0$, 1.6 Hz, 1H), 7.81 (d, $J = 8.9$ Hz, 2H), 7.47 (br d, $J = 8.0$ Hz, 1H), 7.27 (t, $J = 7.8$ Hz, 1H), 7.07 (d, $J = 8.9$ Hz, 2H), 6.68 (s, 1H), 5.98–6.05 (m, 1H), 5.08–5.14 (m, 2H), 3.67 (d, $J = 6.4$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.38, 161.88, 154.03, 143.42, 135.21, 134.10, 129.34, 128.36, 127.71, 124.97, 123.92, 123.88, 119.61, 117.03, 106.83, 33.97; IR (neat) 2093, 1635 cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{14}N_3O_2$ (MH^+): 304.1086. Found: 304.1084.

4.5. 2-[2-(4-Azidophenyl)-4-oxochromen-8-yl]acetic acid (2)

$KMnO_4$ (938 mg, 5.94 mmol) was added in aliquots to a stirred solution of **9** (360 mg, 1.19 mmol) in a mixture of AcOH (2.5 mL), acetone (5 mL), and water (1 mL) that was maintained at 0 °C for a period of 5 h. The reaction mixture was further stirred for 1 h at room temperature. The reaction was quenched with H_2O_2 and concentrated under vacuum to a residue. The solid obtained was washed with warm EtOH/EtOAc (1:1) and filtered. The solid was then dissolved in EtOAc and washed with water. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Pure product **2** (80 mg, 18%) was obtained as an amorphous yellow solid by precipitation from a solution in EtOH/EtOAc with hexanes; ^{1}H NMR ($CD_3OD/CDCl_3$, 400 MHz) δ 7.93 (dd, $J = 8.0$, 1.6 Hz, 1H), 7.80 (br d, $J = 8.9$ Hz, 2H), 7.50 (br d, $J = 7.2$ Hz, 1H), 7.24 (t, $J = 8.0$ Hz, 1H), 7.01 (dd, $J = 8.9$, 1.6 Hz, 2H), 6.64 (s, 1H), 3.80 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.20, 172.58, 162.95, 154.34, 143.73, 135.71, 127.91, 127.68, 125.10, 124.53, 124.43, 123.38, 119.45, 106.24, 35.64; IR (neat) 3500–3073, 2094, 1698, 1632 cm^{-1}; HRMS (FAB) calcd for $C_{17}H_{12}N_3O_2$ (MH^+): 322.0828. Found: 322.0841.

5. Experimental (mass spectrometry)

All fast-atom bombardment mass spectra (FABMS) were obtained on a VG 7070E-HF double-focusing mass spectrometer in positive ion mode, except where noted

otherwise. A sample matrix of 3-nitrobenzyl alcohol (NBA) was employed, and ionization was effected by a beam of xenon atoms generated in a saddle-field ion gun at 1.2 mA 8.2 kV. Nominal mass MS were obtained at a resolution of 1200, while accurate mass analysis (HRMS) was carried out at a resolution of 4000. For the latter, a limited-range V/E scan was employed under control of a MASPEC-II[32] data system for Windows (MasCom GmbH). Matrix-derived ions were utilized as the internal mass references for accurate mass determinations. ^{1}H and ^{13}C NMR data were used to set constraints for the calculation of all possible elemental compositions within 20 ppm of the measured accurate mass. In all cases, a unique molecular formula could be determined. MALDI mass spectra were used to follow the Staudinger reaction and were obtained on a Kratos-Axima-CRF mass spectrometer using α-cyano-4-hydroxycinnamic acid as the sample matrix. Both external and internal mass calibration was employed; for internal calibration, the peptide mass reference standards (*m/z* 757–2465) were directly co-mixed with the sample in the matrix.

5.1. Formation of FAA–FLAG peptide conjugate 4

To a screw-cap polypropylene Eppendorf tube of 1.5 mL capacity were added 125 μL of 0.24 mM FLAG peptide–phosphine **3** (29 nmol) in pH 7.2, 10 mM NH_4CO_3 buffer and 40 μL 1.0 mM FAA-azide **2** (40 nmol) in pH 7.2, 10 mM NH_4CO_3 buffer containing 2.5% DMSO. The resulting solution was mixed by vortexing and allowed to react for 1 h in an Eppendorf ThermoStat plus constant temperature block maintained at 25 ± 0.5 °C. A second 125 μL aliquot of 0.24 mM FLAG peptide–phosphine **3** (29 nmol) in pH 7.2 buffer was placed in an Eppendorf tube without FAA-azide **2** to serve as a control. At the end of the reaction period, each solution was diluted with an equal volume (165 and 125 μL, respectively) of distilled H_2O and frozen in dry ice prior to an initial lyophilization. The resulting residues were redissolved in 250 μL distilled H_2O and lyophilized two more times. After the third lyophilization, each residue was dissolved in 200 μL 4:1 CH_3CN/H_2O, and a 25 μL aliquot of each solution was further diluted with an equal volume of 0.05% trifluoroacetic acid in 50% CH_3CN/H_2O. Multiple 0.5 and 1.0 μL samples of these diluted solutions were taken for analysis by MALDI MS.

MALDI MS calcd for $C_{78}H_{85}N_{11}O_{27}P$ (MH^+): 1638.535. Found: 1638.522.

MALDI MS (control, **3**) calcd for $C_{62}H_{76}N_{10}O_{23}P$ (MH^+): 1359.482. Found: 1359.473.

5.2. Cell culture

A mouse C57BL/6 macrophage cell line, immortalized with the raf and myc oncogenes, was treated with azido-FAA (10 μg/mL) or FAA (100 g/mL) for 6 h at 37 °C. A that time the media was removed and total cellular RNA extracted with Trisol (Invitrogen, Carlsbad, CA). Total cellular RNA (5 μg) from each sample was utilized for the Multiprobe RNAse Protection Assay.

5.3. Multiprobe RNAse protection assay

This assay was performed according to the manufacturer's directions (Pharmingen, San Diego, CA) with the following modifications.

5.4. Hybridization

Probes were synthesized with ^{33}P UTP (70–80 μC/full reaction) utilizing the Pharmingen In Vitro Transcription kit and the mck-5b probe set. Following incubation, yeast tRNA and EDTA were added as described by the manufacturer (Pharmingen), the reaction was placed on Amersham-Pharmacia G25 Microspin columns and the probe purified by centrifugation for 2 min at 3000 rpm. $0.5–1.0 \times 10^6$ cpm was added to each RNA in a final hybridization volume of 10–20 μL (at least 50% Pharmingen hybridization buffer).

5.5. RNAse inactivation

A master cocktail, containing 200 μL Ambion RNAse inactivation/precipitation reagent III (Ambion, Inc., Austin, TX), 50 μL ethanol, 5 μg yeast tRNA, and 1 μL Ambion GycoBlue co-precipitate per RNA sample was utilized to precipitate the protected RNA. After adding the individual RNAse treated samples to 250 μL of the inactivation/precipitation cocktail, the samples were mixed well, placed at −70 °C for 15 min, and subjected to centrifugation at 14,000 rpm for 15 min in a room temperature microcentrifuge. The supernatants were decanted, a sterile cotton swab was used to remove excess liquid and the pellet was resuspended in 3 μL of Pharmingen sample buffer prior to gel electrophoresis and autoradiography.

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References and notes

1. Atassi, G.; Briet, P.; Berthelon, J.-J.; Collonges, F. Synthesis and antitumor activity of some 8-substituted-4-oxo-4H-1-benzopyrans. *Eur. J. Med. Chem. Chim. Ther.* **1985**, *20*, 393–402.
2. Plowman, J.; Narayanan, V. L.; Dykes, D.; Szarvasi, E.; Briet, P.; Yader, O. C.; Paull, K. D. Flavone acetic acid: a novel agent with preclinical anti-tumor activity against colon adenocarcinoma 38 in mice. *Cancer Treat. Rep.* **1986**, *70*, 631–635.
3. Corbett, T. H.; Bissery, M.-C.; Wozniak, A.; Plowman, J.; Polin, L.; Tapazoglou, E.; Dieckman, J.; Valeriote, F.

Activity of flavone acetic acid (NSC 347512) against solid tumors of mice. *Invest. New Drugs* **1986**, *4*, 207–220.
4. Finlay, G. J.; Smith, G. P.; Fray, L. M.; Baguley, B. C. Effect of flavone acetic acid (NSC 347512) on Lewis lung carcinoma: evidence for an indirect effect. *J. Natl. Cancer Inst.* **1988**, *80*, 241–245.
5. Hill, S. A.; Williams, K. B.; Denekamp, J. Studies with a panel of tumors having a variable sensitivity to FAA, to investigate its mechanism of action. *Int. J. Radiat. Biol.* **1991**, *60*, 379–384.
6. Bowler, K.; Pearson, J. A. Long-term effects of flavone acetic acid on the growth of a rat tumor. *Anticancer Res.* **1992**, *12*, 1275–1280.
7. Kerr, D. J.; Kaye, S. B. Flavone acetic-acid. Preclinical and clinical activity. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 1271–1272.
8. Bibby, M. C.; Double, J. A. Flavone acetic acid. From laboratory to clinic and back. *Anti-Cancer Drugs* **1993**, *4*, 3–17.
9. Capolongo, L. S.; Balconi, G.; Ubezio, P.; Giavazzi, R.; Taraboletti, G.; Regonesi, A.; Yoder, O. C.; D'Incalci, M. Antiproliferative properties of flavone acetic acid (NSC-347512) a new anticancer agent. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1529–1535.
10. Ching, L. M.; Baguley, B. C. Enhancement of in vitro cytotoxicity of mouse peritoneal-exudate cells by flavone acetic acid. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 1521–1525.
11. Ching, L. M.; Baguley, B. C. Induction of natural killer cell activity by the antitumor compound flavone acetic acid (NSC-347512). *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1047–1050.
12. Wiltrout, R. H.; Boyd, M. R.; Back, T. C.; Salup, R. R.; Arthur, J. A.; Hornung, R. L. Flavone-8-acetic acid augments systemic natural killer cell activity and synergizes with IL-2 for treatment of murine renal cancer. *J. Immunol.* **1988**, *9*, 3261–3265.
13. Thomsen, L. L.; Ching, L. M.; Baguley, B. C. Evidence for the production of nitric oxide by activated macrophages treated with antitumor agents flavone-8-acetic acid and xanthenone-4-acetic acid. *Cancer Res.* **1990**, *50*, 6966–6970.
14. Mace, K. F.; Hornung, R. L.; Wiltrout, R. H.; Young, H. A. Correlation between in vivo induction of cytokine gene-expression by flavone acetic-acid and strict dose dependency and therapeutic efficacy against murine renal cancer. *Cancer Res.* **1990**, *59*, 1742–1747.
15. Thomsen, L. L.; Ching, L. M.; Zhuang, L.; Gavin, J. B.; Baguley, B. C. Tumor-dependent increased plasma nitrate concentrations as an indication of the antitumor effect of flavone-8-acetic acid and analogs in mice. *Cancer Res.* **1991**, *51*, 77–81.
16. Bibby, M. C.; Double, J. A.; Loadman, P. M.; Duke, C. V. Reduction of tumor blood flow by flavone acetic acid. A possible component of therapy. *J. Natl. Cancer Inst.* **1989**, *81*, 216–222.
17. Zwi, L. J.; Baguley, B. C.; Gavin, J. B.; Wilson, W. R. Blood flow failure as a major determinant in the antitumor action of flavone acetic acid. *J. Natl. Cancer Inst.* **1989**, *81*, 1013–1015.
18. Hill, S.; Williams, K. B.; Denekamp, J. Vascular collapse after flavone acetic-acid. A possible mechanism of its antitumor action. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 1419–1424.
19. Smith, G. P.; Calveley, S. B.; Smith, M. J.; Baguley, B. C. Flavone acetic-acid (NSC-347512) induces hemorrhagic necrosis of mouse colon-26 and colon-38 tumors. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1209–1211.
20. Lindsay, C. K.; Gomez, D. E.; Thorgeirsson, U. P. Effect of flavone acetic acid on endothelial cell proliferation: evidence for antiangiogenic properties. *Anticancer Res.* **1996**, *16*, 425–431.
21. Futami, H.; Eader, L. A.; Komschlies, K. L.; Bull, R.; Gruys, M. E.; Ortaldo, J. R.; Young, H. A.; Wiltrout, R. H. Flavone acetic-acid directly induces expression of cytokine genes in mouse splenic leukocytes but not in human peripheral blood leukocytes. *Cancer Res.* **1991**, *51*, 6596–6602.
22. Aiken, R. A.; Bibby, M. C.; Double, J. A.; Laws, A. L.; Ritchie, R. B.; Wilson, D. W. J. Synthesis and antitumor activity of new derivatives of flavone-8-acetic acid (FAA). Part 2. Ring-substituted derivatives. *Arch. Pharm. Pharm. Med. Chem.* **1997**, *330*, 215–224.
23. Valenti, P.; Bisi, A.; Rampa, A.; Belluti, F.; Gobbi, S.; Zampiron, A.; Carrara, M. Synthesis and biological activity of some rigid analogues of flavone-8-acetic acid. *Bioorg. Med. Chem.* **2000**, *8*, 239–246.
24. Gobbi, S.; Rampa, A.; Bisi, A.; Belluti, F.; Piazzi, L.; Valenti, P.; Caputo, A.; Zampiron, A.; Carrara, M. Synthesis and biological evaluation of 3-alkoxy analogues of flavone-8-acetic acid. *J. Med. Chem.* **2003**, *46*, 3662–3669.
25. Bauvois, B.; Puiffe, M. L.; Bongui, J. B.; Paillat, S.; Monneret, C.; Dauzonne, D. Synthesis and biological evaluation of novel flavone-8-acetic acid derivatives as reversible inhibitors of aminopeptidase N/CD13. *J. Med. Chem.* **2003**, *46*, 3900–3913.
26. Schuster, G. B. Photochemistry of aryl and aroyl azides: application to photolabeling of biological systems. In *Photochemical Probes in Biochemistry*; Nielsen, P., Ed.; Kluwer Academic, 1989; pp 31–41.
27. Vocadlo, D. J.; Hang, H. C.; Kim, E.-J.; Hanover, J. A.; Bertozzi, C. R. A chemical approach for identifying O-GlcNAc-modified proteins in cells. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9116–9121.
28. Luchansky, S. J.; Hang, H. C.; Saxon, E.; Grunwell, J. R.; Yu, C.; Dube, D. H.; Bertozzi, C. R. Constructing azide-labeled cell surfaces using polysaccharide biosynthetic pathways. *Methods Enzymol.* **2003**, *362*, 249–272.
29. Wheeler, T. S. Flavone (method 1). *Org. Synth. Coll.* **1963**, *4*, 478–481.
30. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. A greatly improved procedure for ruthenium tetraoxide catalyzed oxidations of organic compounds. *J. Org. Chem.* **1981**, *46*, 3936–3938.