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# Synthesis of flavone-8-carboxylic acid analogues as potential antitumor agents*

SJ Cutler[1], FM El-Kabbani[1], C Keane[1], SL Fisher-Shore[1], FL McCabe[2], RK Johnson[2], C DeWitt Blanton Jr[1]**

[1]*Department of Medicinal Chemistry, College of Pharmacy, The University of Georgia, Athens, GA 30602-2352;*
[2]*Department of Biomolecular Discovery, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406-0939, USA*

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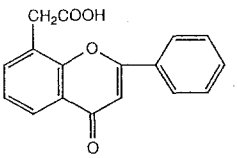
**Summary** — Furan *o*-aminonitriles may be utilized as precursors in the synthesis of flavone-8-carboxylic acids. Some results from *in vivo* evaluation against P388 leukemia, colon carcinoma 38, and B16 melanoma models suggest that selected examples of the acids are potentially as effective as the antitumor compound, flavone acetic acid. The flavone-8-carboxylic acids did not exhibit significant activity against an *in vitro* HIV screen or an *in vitro* antitumor screen consisting of a cell panel of 60 lines.

**flavone-8-acetic acid / flavone-8-carboxylic acids / furan *o*-aminonitriles / antitumor / melanoma / anti-HIV**

## Introduction

Flavone acetic acid (**1**, FAA, LM-975, NSC-347512) has been reported to have solid-tumor selective activity in experimental animal models [1–3]. FAA has a broad spectrum of activity against murine transplantable tumors including a pancreatic adenocarcinoma (No 2) which is unresponsive to all currently used chemotherapeutic agents [4, 5]. FAA is more effective on slow-growing solid tumors (*eg*, mouse colon 38 adenocarcinoma) than on rapidly proliferating leukemias (*eg*, L1210, P388 [1, 4, 6]). Furthermore, FAA possesses none of the usual properties of cytotoxic agents such as nausea, vomiting and myelosuppression [7]. FAA has been reported to have effects on shutting down blood flow in solid tumors [8–10] and induction of hemorrhagic necrosis [11] in experimental murine tumors. Unfortunately, results [8, 12–15] from clinical trials have not shown activity in human cancer. The unusual spectrum of antitumor activity, and reports on the ability of FAA to stimulate macrophages [16, 17] and to systemically augment natural killer (NK) cell activity in normal and tumor-bearing mice and human patients [18, 19], suggest a lead for further chemical studies to develop potential chemotherapeutic drugs with improved antitumor activity which may also function as biological response modifiers (BRM). Topologically related analogues of FAA, xanthenone-4-acetic acids, have recently been reported [20, 21] to have a similar biological profile.

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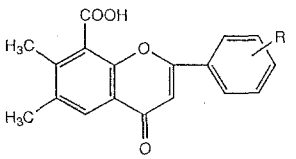


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With this background, we describe the synthesis and evaluation of a series of substituted flavone-8-carboxylic acid analogues **2** of FAA. The availability of uniquely substituted *o*-hydroxyacetophenones (*eg* **6**; scheme 1) should facilitate preparation of previously inaccessible flavone analogues which may potentially exert a more potent and selective activity against solid tumors.

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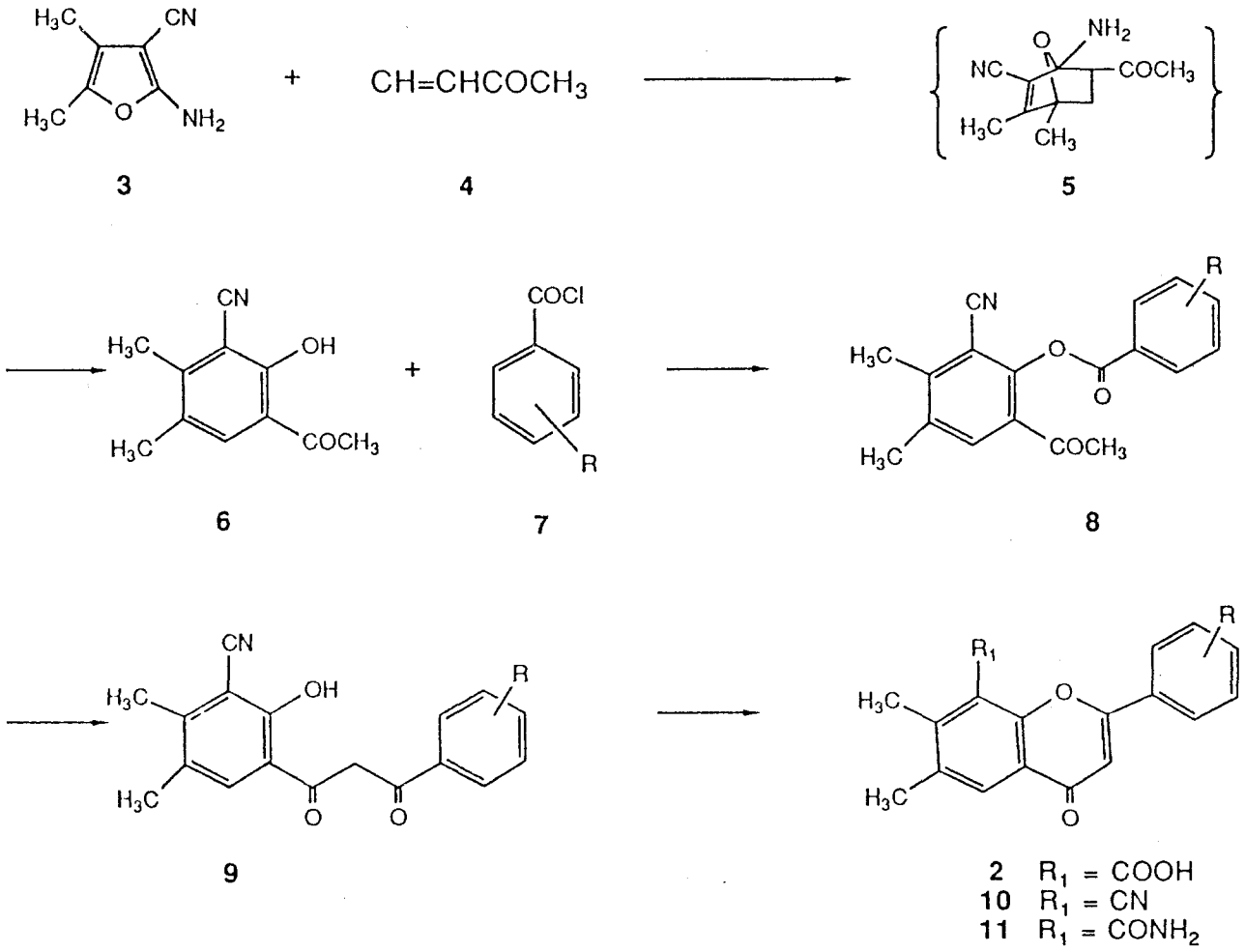


**Scheme 1.**

## Chemistry

In the course of our studies [22–26] utilizing various substituted furan *o*-aminonitriles [27] as dienes in Diels–Alder cycloaddition reactions to prepare various anthranilates, we noted [26] that alterations in experimental conditions led to *o*-hydroxyacetophenones (**6**; scheme 1). This provided a useful precursor for the synthesis of highly substituted flavones (**10**) by established procedures [28] as depicted in scheme 1. The present study concentrated on employing 2-amino-3-cyano-4,5-dimethylfuran **3** [27], but other substitution patterns are conceivable. Acid hydrolysis of the cyano-substituted flavones (**10**) gave the amides (**11**) which are subject to marked steric inhibition by *ortho* substituents. Such sterically hindered amides (**11**) could not be directly hydrolyzed to the acids, but they could be converted to the carboxylic acids (**2**) by treatment with nitrous acid [28].

With the 4'-methoxy and 3',4',5'-trimethoxy analogues (tables I, II), the strong 80% sulfuric acid conditions required for hydrolysis of the cyano group apparently led to cleavage of the 4'-methoxy moiety. Hence, conditions designed to convert the 8-cyano group to an 8-amido group without alkoxy cleavage were found by the use of 85% phosphoric acid (syrupy) (*eg* **11b**). In some cases, a mixture of syrupy phosphoric acid and acetic acid was found to be preferred (*eg* **2k**, **11n**). In addition, this procedural modification was employed in the preparation of the 4'-trifluoromethyl analogue (**2n**), since the strong sulfuric acid media also hydrolyzed the trifluoromethyl group to a carboxylic acid moiety (**2Φ**). In selected cases, the use of sulfuric acid and excess sodium nitrite led to nitration of the 2-aryl moiety (eg **2i**). This side-reaction was circumvented by the use of nitrosylsulfuric acid (*eg* **2d**).

**Table I.** Benzoyloxacetophenones **8**, α-benzoyl-2-hydroxyacetophenones **9**, 8-cyanoflavones **10**, and flavone-8-carboxamides **11**.

| *Compd* | *R* | *mp (°C)* | *Yield (%)* | *Formula* | *Anal*[a] |
|---|---|---|---|---|---|
| **8a** | H | 122–124 | 79[b] | $C_{18}H_{15}NO_3$ | C, H, N |
| **8b** | 4'-$CH_3O$ | 131–133 | 83[b] | $C_{19}H_{17}NO_4$ | |
| **8c** | 3'-$CH_3O$ | 155–156 | 77[b] | $C_{19}H_{17}NO_4$ | |
| **8d** | 4'-$CH_3$ | 127–129 | 93[b] | $C_{19}H_{17}NO_3$ | C, H, N |
| **8e** | 4'-F | 107–109 | 76[b] | $C_{18}H_{14}FNO_3$ | |
| **8f** | 2'-Cl | 118–121 | 85[b] | $C_{18}H_{14}ClNO_3$ | C, H, N |
| **8g** | 4'-Cl | 117–119 | 78[b] | $C_{18}H_{14}ClNO_3$ | C, H, N |
| **8h** | 2',4'-diCl | 145–147 | 81[b] | $C_{18}H_{13}Cl_2NO_3$ | |
| **8k** | 3'4',5'-tri$CH_3O$ | 169–170 | 90[b] | $C_{21}H_{21}NO_6$ | |
| **8m** | 3',4'-diCl | 110–112 | 81[b] | $C_{18}H_{13}Cl_2NO_3$ | |
| **8n** | 4'-$CF_3$ | 138–140 | 97[b] | $C_{19}H_{14}F_3NO_3$ | |
| **8p** | 2'-$CH_3$ | 123–124 | 85[b] | $C_{19}H_{17}NO_3$ | |
| **8q** | 3'-$CH_3$ | 116–117 | 94 | $C_{19}H_{17}NO_3$ | |
| **8r** | 3',4'-di$CH_3$ | 141–143 | 63[b] | $C_{20}H_{19}NO_3$ | |
| **8s** | 4'-$NO_2$ | 145–147 | 63[b] | $C_{18}H_{14}N_2O_5$ | C, H, N |
| **8t** | 2'-$CH_3O$ | 135–137 | 84[b] | $C_{19}H_{17}NO_4$ | |
| **9a** | H | 216–218 | 76[c] | $C_{18}H_{15}NO_3$ | C, H, N |
| **9b** | 4'-$CH_3O$ | 185–187 | 65[b] | $C_{19}H_{17}NO_4$ | |
| **9c** | 3'-$CH_3O$ | 192–193 | 74[c] | $C_{19}H_{17}NO_4$ | C, H, N |
| **9d** | 4'-$CH_3$ | 203–205 | 73[b] | $C_{19}H_{17}NO_3$ | |
| **9e** | 4'-F | 140–142 | 80[c] | $C_{18}H_{14}FNO_3$ | |
| **9f** | 2'-Cl | 171–173 | 87[d] | $C_{18}H_{14}ClNO_3$ | |
| **9g** | 4'-Cl | 194–196 | 74[c] | $C_{18}H_{14}ClNO_3$ | |
| **9h** | 2',4'-diCl | 202–204 | 65[c] | $C_{18}H_{13}Cl_2NO_3$ | |
| **9k** | 3',4',5'-tri$CH_3O$ | 184–185 | 77[d] | $C_{21}H_{21}NO_6$ | C, H, N |
| **9m** | 3',4'-diCl | 265–267 | 78[c] | $C_{18}H_{13}Cl_2NO_3$ | |
| **9n** | 4'-$CF_3$ | 167–170 | 61[c] | $C_{19}H_{14}F_3NO_3$ | |
| **9p** | 2'-$CH_3$ | 177–178 | 68[d] | $C_{19}H_{17}NO_3$ | |
| **9q** | 3'-$CH_3$ | 195–197 | 73[d] | $C_{19}H_{17}NO_3$ | |
| **9r** | 3',4'-di$CH_3$ | 222–224 | 82[d] | $C_{20}H_{19}NO_3$ | |
| **9s** | 4'-$NO_2$ | 224–226 | 23[d] | $C_{18}H_{14}N_2O_5$ | |
| **9t** | 2'-$CH_3O$ | 155–157 | 65[c] | $C_{19}H_{17}NO_4$ | |
| **10a** | H | 245–247 | 91[e] | $C_{18}H_{13}NO_2$ | C, H, N |
| **10b** | 4'-$CH_3O$ | 248–250 | 79[f] | $C_{19}H_{15}NO_3$ | C, H, N |
| **10c** | 3'-$CH_3O$ | 222–223 | 88[e] | $C_{19}H_{15}NO_3$ | |
| **10d** | 4'-$CH_3$ | 189–190 | 90[c] | $C_{19}H_{15}NO_2$ | C, H, N |
| **10e** | 4'-F | 206–208 | 98[e] | $C_{18}H_{12}FNO_2$ | C, H, N |
| **10f** | 2'-Cl | 171–173 | 79[f] | $C_{18}H_{12}ClNO_2$ | C, H, N, Cl |
| **10g** | 4'-Cl | 234–236 | 83[e] | $C_{18}H_{12}ClNO_2$ | C, H, N, Cl |
| **10h** | 2',4'-diCl | 228–230 | 78[e] | $C_{16}H_{11}Cl_2NO_2$ | C, H, N, Cl |
| **10k** | 3',4',5'-tri$CH_3O$ | 244–245 | 90[g] | $C_{21}H_{19}NO_5$ | C, H, N |
| **10m** | 3',4'-diCl | 275–277 | 84[e] | $C_{18}H_{11}Cl_2NO_2$ | C, H, N, Cl |
| **10n** | 4'-$CF_3$ | 231–233 | 75[e] | $C_{19}H_{12}F_3NO_2$ | C, H, N |
| **10p** | 2'-$CH_3$ | 186–187 | 56[e] | $C_{19}H_{15}NO_2$ | |
| **10q** | 3'-$CH_3$ | 202–203 | 83[e] | $C_{19}H_{15}NO_2$ | |
| **10r** | 3',4'-di$CH_3$ | 225–226 | 88[e] | $C_{20}H_{17}NO_2$ | C, H, N |
| **10s** | 4'-$NO_2$ | 263–265 | 77[f] | $C_{18}H_{12}N_2O_4$ | C, H, N |
| **10t** | 2'-$CH_3O$ | 209–211 | 86[e] | $C_{19}H_{15}NO_3$ | C, H, N |
| **11a** | H | 346–347 | 55[h] | $C_{18}H_{15}NO_3$ | C, H, N |
| **11b** | 4'-$CH_3O$ | 354–355 | 95[i] | $C_{19}H_{17}NO_4$ | C, H, N |
| **11d** | 4'-$CH_3$ | 344–345 | 75[e] | $C_{19}H_{17}NO_3$ | C, H, N |
| **11n** | 4'-$CF_3$ | 276–278 | 85[i] | $C_{19}H_{14}F_3NO_3$ | C, H, N |
| **11Φ** | 4'-COOH | 336–338 | 34[j] | $C_{19}H_{15}NO_5 \cdot 0.25\ H_2O$ | C, H, N[k] |
| **12a**[l] | – | 148–151 | 81[b] | $C_{20}H_{17}NO_3$ | |
| **12b**[l] | – | 179–181 | 80[c] | $C_{20}H_{17}NO_3$ | |
| **12c**[m] | – | 230–232 | 89[e] | $C_{20}H_{15}NO_2$ | C, H, N |
| **13a**[n] | – | 95–97 | 69[b] | $C_{16}H_{13}NO_3S$ | |
| **13b**[n] | – | 212–214 | 68[c] | $C_{16}H_{13}NO_3S$ | |
| **13c**[n] | – | 228–230 | 72[e] | $C_{16}H_{11}NO_2S$ | C, H, N |
| **14a**[o] | – | 129–130 | 90[b] | $C_{17}H_{14}N_2O_3$ | C, H, N |
| **14b**[o] | – | 244–145 | 78[e] | $C_{17}H_{14}N_2O_3$ | C, H, N |
| **14c**[o] | – | 266–268 | 77[e] | $C_{17}H_{12}N_2O_2$ | C, H, N |
| **14d**[o] | – | 344–345 | 93[e] | $C_{17}H_{14}N_2O_3$ | C, H, N |

[a]The results of elemental analyses are within ± 0.4% of the theoretical values unless otherwise noted; [b]EtOAc–petroleum ether (30–60°C); [c]EtOH; [d]benzene–hexane; [e]MeOH–DMF; [f]MeOH; [g]MeOH–chloroform; [h]dioxane; [i]dioxane–DMF; [j]EtOH–benzene; [k]MS, *m/z* 337; [l]the 4,5-tetramethylene analogue of **8a** and **9a**, respectively; [m]the 6,7-tetramethylene analogue of **10a**; [n]the 2-thienyl isosteres of **8a**, **9a**, and **10a** respectively; [o]the 4-pyridyl isosteres of **8a**, **9a**, **10a**, and **11a**, respectively.

**Table II.** Flavone-8-carboxylic acids **2**, **12**, **13**, **14**.

| *Compd* | *R* | *mp (°C)* | *Yield (%)* | *Formula* | *Anal*[a] |
|---|---|---|---|---|---|
| **2a** | H | 244–246 | 81[b] | $C_{18}H_{14}O_4$ | C, H |
| **2b** | 4'-$CH_3O$ | 290–292 | 71[c] | $C_{19}H_{16}O_5 \cdot 0.25\ H_2O$ | C, H[d] |
| **2c** | 3'-$CH_3O$ | 304–305 | 67[c] | $C_{19}H_{16}O_5$ | C, H |
| **2d** | 4'-$CH_3$ | 308–310 | 64[c] | $C_{19}H_{16}O_4 \cdot 0.25\ H_2O$ | C, H[e] |
| **2e** | 4'-F | 289–300 | 73[b] | $C_{18}H_{13}FO_4$ | C, H |
| **2f** | 2'-Cl | 248–249 | 75[b] | $C_{18}H_{13}ClO_4$ | C, H, Cl |
| **2g** | 4'-Cl | 302–304 | 72[f] | $C_{18}H_{13}ClO_4$ | C, H, Cl |
| **2h** | 2',4'-diCl | 289–290 | 72[f] | $C_{18}H_{12}Cl_2O_4$ | C, H, Cl |
| **2i** | 3'-$NO_2$,4'-$CH_3$ | 335–337 | 37[c] | $C_{19}H_{15}NO_6$ | C, H, N |
| **2j** | 3'-$NH_2$,4'-$CH_3$ | 320–321 | 58[c] | $C_{19}H_{17}NO_4$ | C, H, N |
| **2k** | 3',4',5'tri$CH_3O$ | 293–295 | 47[b] | $C_{21}H_{20}O_7$ | C, H |
| **2l** | 4'-OH | 333–334 | 59[f] | $C_{18}H_{14}O_5 \cdot 0.25\ H_2O$ | C, H[g] |
| **2m** | 3',4'-diCl | 331–332 | 79[b] | $C_{18}H_{12}Cl_2O_4$ | C, H, Cl |
| **2n** | 4'-$CF_3$ | 343–344 | 86[b] | $C_{19}H_{13}F_3O_4$ | C, H |
| **2Φ** | 4'-COOH | 381–383 | 91[b] | $C_{19}H_{14}O_6 \cdot 1/6H_2O$ | C, H[h] |
| **2p** | 2'-$CH_3$ | 218–219 | 85[b] | $C_{19}H_{16}O_4$ | C, H |
| **2q** | 3'-$CH_3$ | 286–287 | 56[i] | $C_{19}H_{16}O_4$ | C, H |
| **2r** | 3',4'-di$CH_3$ | 315–317 | 65[b] | $C_{20}H_{18}O_4 \cdot 1/3\ H_2O$ | C, H[j] |
| **2t** | 2'-$CH_3O$ | 305–307 | 60[l] | $C_{19}H_{16}O_5$ | C, H |
| **12**[k] | – | 258–260 | 98[l] | $C_{20}H_{16}O_4$ | C, H[m] |
| **13**[n] | – | 285–287 | 42[c] | $C_{16}H_{12}O_4S \cdot 0.25\ H_2O$ | C, H, N, S[o] |
| **14**[p] | – | 325–326 | 80[c] | $C_{17}H_{13}NO_4$ | C, H, N |

[a]The results of elemental analyses are within ± 0.4% of the theoretical values unless otherwise noted; [b]EtOH–benzene; [c]EtOH–DMF; [d]MS, *m/z* 324; [e]MS, *m/z* 308; [f]HOAc; [g]MS, *m/z* 310; [h]MS, *m/z* 338; [i]EtOH–EtOAc; [j]Ms, *m/z* 322; [k]the 6,7-tetramethylene analogue of **2a**; [l]EtOH; [m]MS, *m/z* 320; [n]the 2-thienyl isostere of **2a**; [o]MS, *m/z* 300; [p]the 4-pyridyl isostere of **2a**.

In an analogous fashion, starting with 3-cyano-4,5-tetramethylenefuran [27], it was possible to prepare 3-cyano-2-hydroxy-4,5-tetramethyleneacetophenone (*cf* **6**) [28]. With this *o*-hydroxyacetophenone, 6,7-tetramethyleneflavone-8-carboxylic acid **12** was prepared according to scheme 1. Similarly, by substituting 2-thiophenecarbonyl chloride for benzoyl chloride **7**, it was possible to synthesize according to scheme 1 the thiophene isostere of **2a**, 2-(2-thienyl)-6,7-dimethylchromone-8-carboxylic acid **13**. Substituting isonicotinoyl chloride for benzoyl chloride **7** led to a nitrogen isostere of **2a**, 2-(4-pyridyl)-6,7-dimethylchromone-8-carboxylic acid **14**.

## Biological results and discussion

Initial evaluation of selected 8-cyano substituted flavone analogues **10a**, **10b**, **10d**, **10f**, **10g**, **10s** and one 8-carboxamido analogue (**11a**) in the National Cancer Institute's prescreen system 3PS31 (P388) [29] (data provided by the National Cancer Institute Developmental Therapeutic Program) failed to reveal any significant activity. However, the acid **2a** exhibited confirmed activity with T/C = 128–161% at 120–140 mg/kg.

Since FAA was more effective on solid tumors than rapid proliferating leukemias, our emphasis was directed toward evaluating the flavone-8-carboxylic acids **2** in mice bearing B16 melanomas in an attempt to identify compounds possessing improved activity relative to FAA. The ideal tumor model for such an objective is one which demonstrates marginal but reproducible sensitivity to the parent compound. The results are presented in table III. Some flavone-8-carboxylic acids are potentially as effective as FAA in this model, and substituents on the 2-aryl ring can markedly influence activity and dose-potency. None of the flavone-8-carboxylic acids tested proved to be convincingly more effective than FAA in this tumor model.

One of the active compounds (**2b**) was evaluated in mice bearing colon carcinoma 38. The results (table IV) demonstrate that FAA is only active at a

**Table III.** Comparative antitumor activity in mice bearing ip B16 melanoma[a].

| *Compd* | *R* | *MTD*[b] | $ILS_{max}$[c] | $ILS_{max}$ *for FAA(1)*[d] |
|---|---|---|---|---|
| **2a** | H | 80 | 46 | 71 |
| **2b** | 4'-$OCH_3$ | 80 | 73, 68[e] | 71, 59[e] |
| **2c** | 3'-$OCH_3$ | 160 | 55 | 59 |
| **2d** | 4'-$CH_3$ | 80 | 125 | 125 |
| **2e** | 4'-F | 160 | 92 | 125 |
| **2f** | 2'-Cl | ≥ 160 | Inactive[f] | 71 |
| **2g** | 4'-Cl | 160 | 50 | 59 |
| **2h** | 2',4'-diCl | 80 | 41 | 59 |
| **2i** | 3'-$NO_2$,4'-$CH_3$ | 160 | 64 | 59 |
| **2j** | 3'-$NH_2$,4'-$CH_3$ | 160 | 120, 50[e] | 40, 95[e] |
| **2k** | 3',4',5'-tri$OCH_3$ | ≥ 160 | Inactive | 40 |
| **2l** | 4'-OH | 160 | 50 | 95 |
| **2m** | 3',4'-diCl | 40 | 45 | 95 |
| **2n** | 4'-$CF_3$ | 80 | 64 | 64 |
| **2Φ** | 4'-COOH | ≥ 160 | Inactive | 64 |
| **2p** | 2'-$CH_3$ | ≥ 160 | Inactive | 64 |
| **2q** | 3'-$CH_3$ | 80 | 88 | 64 |
| **2r** | 3',4'-di$CH_3$ | 80 | 62 | 100 |

[a]Female B6D2$F_1$ mice (17–21 g) were inoculated ip with B16 melanoma, randomized to groups of 7 mice each, and were treated ip on d 1–8. Drugs were formulated as aqueous solutions by preparation of sodium salts achieved by addition of a slight molar excess of sodium hydroxide. Mice were monitored for survival daily for 60 d; [b]maximally-tolerated dose. Compounds were tested at 160, 80, 40, 20, and 10 mg/kg/d x 8. If more than 1 mouse in a treatment group died prior to the first untreated control animal, a dose level was considered toxic; [c]maximum percent increase in lifespan relative to untreated controls. Determined by the formula: 100 (*T*/*C*-1) where *T* and *C* are the median survival time of treated and control groups, respectively. This drug effect had to occur at or below the MTD; [d]activity of FAA (**1**) which was evaluated in direct comparison with each analogue. FAA was administered ip on d 1 to 8 at 160, 80, 40, 20, and 10 mg/kg. The MTD of FAA was generally 80 mg/kg/d. [e]Results of 2 independent dose–response studies; [f]an increase in lifespan of > 40% is considered to reflect significant antitumor activity. Compounds listed as inactive did not prolong lifespan by 40% at any dose level tested. However, these compounds might prove to be active at much higher dose levels.

dose that produces some lethality consistent with previous studies of this drug in colon carcinoma 38. Compound **2b** produced marginal tumor growth inhibition (TGI) at a maximally-tolerated dose but did produce 2/7 long-term tumor-free survivors.

All of the flavone-8-carboxylic acids except **2a** and **2f** (table II) were evaluated in the National Cancer Institute's *in vitro* Anti-HIV Drug Testing System [30] (data provided by the National Cancer Institute Developmental Therapeutic Program). No activity was observed. Similarly, all of the flavone-8-carboxylic acids, except **2a** and **2f**, were evaluated in the NCI's new investigational *in vitro* disease-oriented primary antitumor screen [31] (data provided by the National Cancer Institute Developmental Therapeutic Program). The cell panel currently consists of 60 lines. No significant *in vitro* activity was observed. FAA is also non-toxic and non-selective in the current NCI *in vitro* cell line screen.

The available data suggest that a carboxylic acid moiety is essential for antitumor activity among flavone analogues since the cyano or carboxamido analogues were inactive. Furthermore, the acid moiety does not require separation from the flavone molecule by a methylene moiety for activity as seen in FAA. Since FAA and these flavone analogues **2** are inactive in the NCI *in vitro* system, processes involving biotransformation or BRM mechanism may be indicated for the observed *in vivo* activity. Although the data suggest substituents on the 2-aryl ring can markedly influence activity and dose-potency, none of the compounds screened exhibited activity patterns adequate to justify expanded testing or extension of the present group. However, as in other studies [20, 32– 34] va-riation of substituents on the flavone-8-carboxylic acid nucleus or its topologically related analogue, xanthenone-4-carboxylic acid, may offer a course to further delineate the SAR for this novel type of antitumor agents.

**Table IV.** Comparative activity of compound **2b** and flavone-8-acetic acid in mice bearing sc colon carcinoma 38.

| *Drug* | *Dose (mg/kg, ip, days 3, 10, 17 and 24* | *Toxic deaths* | *Tumor volume ($mm^3 \pm SE$ on day 36)* | *Proportion without tumors Day 36* | *TGI (%) Day 93* | *Tumor-free survivors* |
|---|---|---|---|---|---|---|
| Control | | 0/21 | 520 ± 70 | 0/21 | – | 0/21 |
| 5-Fluorouracil | 100 | 0/7 | 29 ± 12 | 4/7 | 94 | 1/7 |
| (Pos control) | 60 | 0/7 | 78 ± 42 | 4/7 | 85 | 1/7 |
| FAA | 150 | 2/7 | Toxic | | | 4/5 |
| | 90 | 0/7 | 380 ± 150 | 1/7 | 26 | 1/7 |
| | 54 | 0/7 | 410 ± 130 | 0/7 | 21 | 0/7 |
| Compound **2b** | 288 | 5/7 | Toxic | | | 1/2 |
| | 173 | 0/7 | 320 ± 130 | 2/7 | 37 | 2/7 |
| | 104 | 0/7 | 290 ± 120 | 3/7 | 44 | 2/7 |

## Experimental protocols

*Chemistry*

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Melting points in excess of 300°C were determined on a Mel-Temp capillary melting point apparatus. Elemental analyses were performed by Atlantic Microlab Inc (Atlanta, GA) and results were within ± 0.4 of the calculated values unless otherwise noted. Satisfactory IR (Perkin–Elmer 684 grating spectrophotometer, KBr) and $^1$H-NMR (90 MHz JEOL FX90Q spectrometer, $Me_4Si$ as internal reference) spectra were obtained for all new compounds. Low-resolution mass spectra were determined on a Finnigan 4023 chromatograph-mass spectrometer by a direct probe and are expressed in *m/z* units. TLC was performed on Analtech Chromatogram plates, type 02521, or Eastman chromatogram sheets, type 13181, coated with silica gel. General procedures for the synthesis of compounds **8a–8h**, **8p**, **8q**, **9a–9h**, **9p**, **9q**, **10a–10h**, **10p**, **10q**, **11a**, **11b**, **12a–12c**, **2a–2h**, **2p**, **2q**, and **12** have already been described [28]. The remaining compounds reported in tables I–III were prepared in an analogous manner or as described below.

*4-Oxo-4H-1-benzopyran-8-carboxylic acids*

*Method A. 6,7-Dimethyl-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxylic acid **2a** [28]*

Compound **10a** (5 g, 0.02 mol) was suspended in 50 ml of 80% sulfuric acid. This mixture was heated with stirring at 170 ± 5°C in an oil bath for 3 h. In general, the resulting amides (**11**) were typically not isolated. Thus, the dark reddish clear solution was cooled to room temperature and transferred to a 1-l beaker. The mixture was cooled to 5°C with an ice-salt bath and treated with 3 g sodium nitrite (0.4 mol) dissolved in a minimal amount of $H_2O$. For best results, the aqueous sodium nitrite must be added below the acidic layer. The addition of sodium nitrite solution generally required ≈ 30 min with much foaming occurring. The mixture was stirred for 1 h at 5°C once the addition of sodium nitrite was complete. After 1 h, urea (3 g) was added to the mixture. The temperature was slowly raised to 80°C and kept there for 2 h. The mixture was cooled and diluted with 100 ml $H_2O$. The resulting precipitate was collected, air dried, and recrystallized from EtOH and benzene to yield 4.8 g (81%) of **2a**, mp: 244–246°C. IR(KBr): 3340 (br), 3060, 1705, 1610 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.38 (s, 6H, 6,7–$CH_3$), 7.03 (s, 1H, 3–CH), 7.98–8.06 (m, 6H, aromatic protons). MS: *m/z* 294. Anal $C_{18}H_{14}O_4$ (C, H).

*Method B. 6,7-Dimethyl-2-(4'-methylphenyl)-4-oxo-4H-1-benzopyran-8-carboxylic acid **2d***

In a 1-l beaker equipped with an overhead stirrer was placed 200 ml of nitrosylsulfuric acid (40% in 87% sulfuric acid) and 3.0 g (0.0097 mol) of **11d**. This mixture was cooled < 20°C before ≈ 80 ml of $H_2O$ was slowly and carefully added over a 10-min period. The solution turned a dark color, then much foaming occurred. The mixture was allowed to warm to room temperature and stirred overnight. The white precipitate was collected, washed with $H_2O$, and recrystallized from absolute EtOH, with sufficient DMF for dissolution, to give 1.9 g (64%) of **2d**, mp: 308–310°C. IR (KBr): 2900 (br), 1720, 1610, 1590 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.37 (s, 6H, 4', 6-$CH_3$), 2.57 (s, 3H, 7-$CH_3$), 7.14 (s, 1H, 3-CH), 7.63–8.49 (m, 5H, aromatic protons). MS: *m/z* 308. Anal $C_{19}H_{16}O_4 \cdot 0.25\ H_2O$ (C, H).

*6,7-Dimethyl-2-(4'-methyl-3'-nitrophenyl)-4-oxo-4H-1-benzopyran-8-carboxylic acid **2i***

Compound **10d** (10 g, 0.035 mol) was suspended in 100 ml 80% sulfuric acid and heated with an oil bath at 170 ± 5°C for 3 h. The dark clear solution was poured into a 2-l beaker and cooled to 10°C. To this mixture was added below the acid layer 20 g (0.29 mol) of sodium nitrite dissolved in a minimal amount of $H_2O$. This addition required ≈ 1.5 h with much foaming occurring. The mixture was allowed to warm to room temperature. Then the temperature was slowly raised to 80°C

and kept there for 2 h. A white precipitate was collected, washed with $H_2O$, and air dried. The compound was recrystallized from boiling EtOH to which was added sufficient DMF to achieve solution. Pure white acid (**2i**) was obtained (3.9 g, 37%), mp: 335–337°C. IR (KBr): 2900 (br), 2500 (br), 1720, 1620, 1600, 1530 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.37 (s, 6H, 4', 6-$CH_3$), 2.57 (s, 3H, 7-$CH_3$), 7.05 (s, 1H, 3-CH), 7.56–8.44 (m, 4H aromatic protons). Anal $C_{19}H_{15}NO_6$ (C, H, N).

*2-(3'-Amino-4'-methylphenyl)-6,7-dimethyl-4-oxo-4H-1-benzopyran-8-carboxylic acid **2j***

Compound **2i** (3 g, 0.008 mol) was placed in 100 ml absolute EtOH. To this suspension was added ≈ 0.5 g of 5% palladium on powdered charcoal. The mixture was hydrogenated under 50 psi hydrogen using a Parr apparatus. This was shaken for 1 h after the theoretical amount of hydrogen had been consumed. As the zwitterionic product had precipitated, 40 ml DMF was added and the mixture was heated in the pressure bottle to achieve solution. The hot mixture was filtered to remove the palladium and charcoal. The solution was then allowed to stand at room temperature to complete precipitation of 1.5 g (58%) **2j**, mp: 320–321°C. IR (KBr): 3100 (br), 1630, 1560, 1510 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.14 (s, 3H, 4'-$CH_3$), 2.36 (s, 6H, 6,7-$CH_3$), 6.70 (s, 1H, 3-CH), 7.12–7.86 (m, 4H, aromatic protons). Anal $C_{19}H_{17}NO_4$ (C, H, N).

*6,7-Dimethyl-4-oxo-2-(3',4',5'-trimethoxyphenyl)-4H-1-benzopyran-8-carboxylic acid **2k***

Compound **10k** (5 g, 0.0137 mol) was heated in an oil bath for 10 h at 175 ± 5°C in a stirred mixture of syrupy phosphoric acid (75 ml) and glacial acetic acid (50 ml). The mixture was cooled to room temperature and poured into ice $H_2O$. The precipitate was collected, washed with $H_2O$, and air dried. The IR spectrum indicated some unreacted starting material was still present; there was a small cyano band at 2220 $cm^{-1}$. The precipitate was stirred with chloroform and filtered to remove insoluble product (4.5 g, 85%) which had an expected IR and $^1$H-NMR spectra for the 8-carboxamido analogue (evaporation of the chloroform filtrate gave unreacted **10k**). The amide (4.5 g, 0.0117 mol) was suspended in 100 ml sulfuric acid and chilled in an ice bath. About 50 g of crushed ice was slowly added (temp < 40°C) to the acidic solution. The mixture became turbid. An additional 50 ml sulfuric acid was added and the solution chilled to 0–5°C. Over ≈ 20 min, solid sodium nitrite was added to the stirred mixture maintaining the temperature < 10°C. The mixture was stirred for an additional 3 h at 0–10°C and then allowed to warm to room temperature. After standing for 24 h, the mixture was poured into 1 l ice $H_2O$. When this mixture had returned to room temperature, it was warmed at 75 ± 5°C for 2 h. The yellow precipitate was collected, washed with $H_2O$, and air dried. The product was dissolved in dilute aqueous NaOH and filtered to remove ≈ 0.9 g of insoluble starting amide. Acidification of the filtrate gave a tan product which was recrystallized from EtOH and benzene to yield 1.7 g (47%, based on amide actually converted to acid) of **2k**, mp: 293–295°C. IR (KBr): 3400 (br), 2500 (br), 1705, 1620, 1590 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.37 (s, 6H, 6,7-$CH_3$), 3.75 (s, 3H, 4'-$CH_3O$), 3.88 (s, 6H, 3',5'-$CH_3O$), 7.15 (s, 1H, 3-CH), 7.32 (s, 2H, 2',6'CH), 7.85 (s, 1H, 5-CH). Anal $C_{21}H_{20}O_7$ (C, H).

*6,7-Dimethyl-2-(4'-hydroxyphenyl)-4-oxo-4H-1-benzopyran-8-carboxylic acid **2l***

Compound **2b** (3 g, 0.0092 mol) was suspended in 150 ml glacial acetic acid and 50 ml 47% HI. The mixture was refluxed for 24 h, cooled, and poured into 500 ml ice $H_2O$. The yellow product was collected, washed with $H_2O$, and air dried. Recrystallization from acetic acid gave 1.7 g (59%) **2l**, mp: 333–334°C. IR (KBr): 3400 (br), 1710, 1617, 1620, 1610 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.37 (s, 6H, 6,7-$CH_3$), 6.86 (s, 1H, 3-CH), 7.85 (s, 1H, 5-CH), 6.91–7.93 (m, 4H, aromatic protons). MS: *m/z* 310. Anal $C_{18}H_{14}O_5 \cdot 0.25\ H_2O$ (C, H)

*6,7-Dimethyl-4-oxo-2-(4'-trifluoromethylplenyl)-4H-1-benzopyran-8-carboxylic acid **2n***

Compound **10n** (8 g, 0.023 mol) was suspended in a 100 ml mixture of syrupy phosphoric acid/acetic acid (3:1). This suspension was heated with stirring at 170 ± 5°C in an oil bath for 3 h. The dark clear solution was cooled and poured into 200 ml cold $H_2O$. The resulting white precipitate was collected, washed with $H_2O$, and recrystallized from EtOH and benzene to give 7.1 g (85%) of **11n**, mp: 276–278°C. IR (KBr): 3300, 1690, 1630, 1600 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.35 (s, 6H, 6,7-$CH_3$), 7.03 (s, 1H, 3-CH), 7.80–8.25 (m, 5H, aromatic protons). Anal $C_{19}H_{14}F_3NO_3$ (C, H, N).

In a 600-ml beaker was placed **11n** (2.5 g, 0.007 mol) and 80% sulfuric acid (70 ml). This mixture was cooled to 10°C with an ice bath and slowly treated with excess sodium nitrite dissolved in a minimal amount of $H_2O$. Once the addition was complete, the mixture was allowed to stir at 10°C for 2 h. The reaction mixture was then warmed to 80°C and held at that temperature for 2 h. After cooling and diluting the mixture with 100 ml $H_2O$, the precipitate was collected and recrystallized from EtOH and benzene to give 2.2 g (86%) **2n**, mp: 343–344°C. IR (KBr): 2900 (br), 1720, 1630, 1600 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.38 (s, 6H, 6,7-$CH_3$), 7.16 (s, 1H, 3-CH), 7.88–8.25 (m, 5H, aromatic protons). Anal $C_{19}H_{13}F_3O_4$ (C, H).

*2-(4'-Carboxyphenyl)-6,7-dimethyl-4-oxo-4H-1-benzopyran-8-carboxylic acid **2Φ***

In a 150-ml round-bottomed flask was placed 3 g (0.009 mol) of **10n** and 70 ml 80% sulfuric acid. This suspension was heated at 170 ± 5°C in an oil bath for 3 h. The dark, blackish-colored solution was cooled and poured into 150 ml cold $H_2O$. The precipitate was collected, washed with $H_2O$, and recrystallized from EtOH and benzene to give 1.1 g (34%) of **11Φ**, mp: 336–338°C. IR (KBr): 3400, 3130 (br), 1720, 1645, 1625, 1600 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.40 (d, 6H, 6,7-$CH_3$), 7.01 (s, 1H, 3-CH), 7.80–8.15 (m, 5H, aromatic protons). MS: *m/z* 337. Anal $C_{19}H_{15}NO_5 \cdot 0.25\ H_2O$ (C, H, N).

The 8-carboxamide analogue (**11Φ**) was converted to the 8-carboxylic acid analogue (**2Φ**) by the standard procedure as described for **2a** *(Method A)*. The product was recrystallized from EtOH and benzene to give 91% yield of pure diacid **2Φ**, mp: 381–383°C. IR (KBr): 2750 (br), 1710, 1690, 1600 $cm^{-1}$. $^1$H-NMR ($Me_2SO$–$d_6$): $\delta$ 2.40 (s, 6H, 6,7-$CH_3$), 7.10 (s, 1H, 3-CH), 7.85–8.12 (m, 5H aromatic protons). MS: *m/z* 338. Anal $C_{19}H_{14}O_6 \cdot 1/6\ H_2O$ (C, H).

*Biological methods*

*B16 melanoma assay*

B16 melanoma was maintained by biweekly serial transplantation subcutaneously in syngeneic female C57B1/6 mice. For therapeutic trials, tumors from donor mice were pooled at 2–3 wk post-implant and homogenized in 10 vol Hank's balanced salt solution in a loose-fitting teflon-glass homogenizer. One-half ml of this tumor brei was inoculated intraperitoneally in female B6D2$F_1$ mice (17–21 g). These mice were randomized to treatment groups of 7 mice each, and treatment was initiated 24 h after tumor implantation. The drugs prepared as water-soluble sodium salts were delivered ip in a

vol of 0.5 ml per mouse on d 1–8 over a dosage range of 160–10 mg/kg/d. Animals were monitored daily for survival and were maintained for 60 d. In each experiment, there were 3 groups of untreated mice as controls. The median survival time of the control mice in 7 separate experiments varied from 15–25 d. A positive control, FAA, was also included in each of the 7 experiments. Antitumor activity was assessed by comparison of median survival time of treated groups with that of the untreated control. A prolongation of survival of > 40% was considered an indication of significant antitumor effect.

*Colon carcinoma 38 assay*
Fragments (2–3 mm) of colon carcinoma 38 were implanted sc in the right flank of female $B6D2F_1$ mice which were randomized to groups of 7 (3 groups of untreated controls). Drugs were administered ip weekly for 4 wk beginning 3 d after tumor implantation. Tumors were measured in perpendicular diameters on d 36 when all control animals had palpable tumors. Tumor volume was calculated by length x $width^2$ x 0.5. Mice were maintained until d 93 when the experiment was terminated.

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## References

1 Atassi G, Briet P, Berthelon JJ, Collonges F (1985) *Eur J Med Chem* 20, 393–402
2 Plowman J, Narayanan VL, Dykes D, Szarvasi E, Briet P, Yoder OC, Paull KD (1986) *Cancer Treat Rep* 70, 631–635
3 O'Dwyer PJ, Showmaker D, Zaharko DS, Grieshaber C, Plowman J, Corbett T, Valeriote F, King SA, Cradock J, Hoth DF, Leyland-Jones B (1987) *Cancer Chemother Pharmacol* 19, 6–10
4 Corbett TH, Bissery MC, Wozniak A, Plowman J, Polin L, Tapazoglou E, Dieckman J, Valeriote F (1986) *Invest New Drugs* 4, 207–220
5 Corbett TH, Roberts BJ, Leopold WR, Peckham JC, Wilkoff LJ, Griswold DP, Schabel FM Jr (1984) *Cancer Res* 44, 717–726
6 Bibby MC, Double JA, Phillips RM, Loadman PM (1987) *Br J Cancer* 55, 159–163
7 Kerr DJ, Kaye SB, Graham J, Cassidy J, Harding M, Setanoians A, McGrath JC, Vezin WR, Cunningham D, Forrest G, Soukop M (1986) *Cancer Res* 46, 3142–3146
8 Bibby MC, Double JA, Loadman PM, Duke CV (1989) *J Natl Cancer Inst* 81, 216–220
9 Zwi LJ, Baguley BC, Gavin JB, Wilson WR (1989) *J Natl Cancer Inst* 81, 1005–1013
10 Hill S, Williams KB, Denekamp J (1990) *Eur J Cancer* 25, 1419–1424
11 Smith GP, Calveley SB, Smith MJ, Baguley BC (1987) *Eur J Cancer Clin Oncol* 23, 1209–1212
12 Kerr DJ, Kaye SB (1989) *Eur J Cancer Clin Oncol* 25, 1271–1272
13 Kerr DJ, Maughan T, Newlands E, Rustin G, Bleehan NM, Lewis C, Kaye SB (1989) *Br J Cancer* 60, 104–106
14 Kaye SB, Clavel M, Dodion P, Monfardini S, ten Bokkel-Huinink W, Wagener DT, Gundersen S, Storer G, Smith J, Renard J, van Glabbeke M, Cavalli F (1990) *Invest New Drugs* 8, S95–S99
15 Halvin KA, Kuhn JG, Craig JB, Boldt DH, Weiss GR, Koeller J, Harman G, Schwartz R, Clark GN, Von Hoff DD (1991) *J Natl Cancer Inst* 83, 124–128
16 Ching LM, Baguley BC (1988) *Eur J Cancer Clin Oncol* 24, 1521–1525
17 Thomsen LL, Ching LM, Jospeh WR, Baguley BC, Gavin JB (1992) *Biochem Pharmacol* 43, 2401–2406
18 Ching LM, Baguley BC (1987) *Eur J Cancer Clin Oncol* 23, 1047–1050
19 Mace KF, Hornung RL, Wiltrout RH, Young HA (1990) *Cancer Res* 50, 1742–1747
20 Atwell GJ, Rewcastle GW, Baguley BC, Denny WA (1989) *Anti-Cancer Drug Des 4*, 161–169
21 Gamage SA, Rewcastle GW, Atwell GJ, Baguley BC, Denny WA (1992) *Anti-Cancer Drug Des* 7, 403–414
22 Nixon WJ Jr, Garland JJ, Blanton CD Jr (1980) *Synthesis* 56–58
23 Vaidya NA, Blanton CD Jr (1982) *J Org Chem* 47, 1777–1779
24 Vaidya NA, Nixon WJ Jr, Fatmi AA, Blanton CD Jr (1982) *J Org Chem* 47, 2483–2484
25 Vaidya NA, Panos CH, Kite A, Iturrian WB, Blanton CD Jr (1983) *J Med Chem* 26, 1422–1425
26 Fatmi AA, Vaidya NA, Iturrian WB, Blanton CD Jr (1984) *J Med Chem* 27, 772–778
27 Gewald K (1966) *Chem Ber* 99, 1002–1007
28 Cutler SJ, El-Kabbani FM, Keane C, Fisher-Shore SL, Blanton CD Jr (1990) *Heterocycles* 31, 651–661
29 Geran RI, Greenburg NH, MacDonald MM, Schumachar AM, Abbott BJ (1972) *Cancer Chemother Rep* Part 3 3, 1–188
30 Weislow OW, Kiser R, Fine DL, BaderJ, Shoemaker RH, Boyd MR (1989) *J Natl Cancer Inst* 81, 577–586
31 Boyd MR (1989) *Princ Pract Oncol Updates* 3, 1–12
32 Rewcastle GW, Atwell GJ, Zhuang L, Baguley BC, Denny WA (1991 ) *J Med Chem* 34, 217–222
33 Rewcastle GW, Atwell GJ, Palmer BD, Boyd PDW, Baguley BC, Denny WA (1991) *J Med Chem* 34, 491–496
34 Rewcastle GW, Atwell GJ, Baguley BC, Boyd M, Thomsen LL, Zhuang L, Denny WA (1991) *J Med Chem* 34, 2864–2870