Refinement and Evaluation of a Pharmacophore Model for Flavone Derivatives Binding to the Benzodiazepine Site of the $GABA_A$ Receptor

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To further develop and evaluate a pharmacophore model previously proposed by Cook and co-workers (*Drug Des. Discovery* **1995**, *12*, 193−248) for ligands binding to the benzodiazepine site of the $GABA_A$ receptor, 40 new flavone derivatives have been synthesized and their affinities for the benzodiazepine site have been determined. Two new regions of steric repulsive interactions between ligand and receptor have been characterized, and the receptor region in the vicinity of 6- and 3′-substituents has been mapped out. 2′-Hydroxy substitution is shown to give a significant increase in affinity, which is interpreted in terms of a novel hydrogen bond interaction with the previously proposed hydrogen bond-accepting site A2. On the basis of the results of these studies and the refined pharmacophore model, 5′-bromo-2′-hydroxy-6-methylflavone, the highest affinity flavone derivative reported so far (K_i = 0.9 nM), was successfully designed. A comparison of the pharmacophore model with a recently proposed alternative model (Marder; et al. *Bioorg. Med. Chem.*, **2001**, *9*, 323−335) has been made.

Introduction

One of the quantitatively most important neurotransmitters in the central nervous system (CNS) is γ-aminobutyric acid (GABA).[1] It is estimated that depending on the brain region, 20−50% of all CNS synapses use GABA as their transmitter.[2,3] GABA exerts its physiological effects by binding to three major classes of receptors: two types of ligand-operated chloride channels, $GABA_A$ and $GABA_C$ receptors, and $GABA_B$ receptors, which are coupled to G-proteins. When the GABA receptors are activated, the communication between the different cells in the CNS decreases. $GABA_A$ receptors are transmembrane proteins that are assembled from subunits to form a pentameric structure. The various subunits that belong to eight classes with multiple isoforms have been identified by their sequence homology[4] ($\alpha1-\alpha6$, $\beta1-\beta4$, $\gamma1-\gamma4$, δ, ϵ, π, θ, and $\rho1-\rho3$). Most $GABA_A$ receptors are composed of α-, β-, and γ-subunits. The so far most common combination is two α-, two β-, and one γ-subunit. Experiments with site-directed mutagenesis have shown that changes in the α- and γ-subunits alter the activity of the benzodiazepine receptor-mediated response,[5] and the benzodiazepine site is believed to be situated between an α- and a γ-subunit. It is also believed that different subunits mediate different physiological effects, that sedation and anterograde amnesia are mediated by $\alpha1$-containing receptors, and that anxiolytic activity is mediated by $\alpha2$-, $\alpha3$-, or possibly $\alpha5$-containing receptors.[6,7] The $GABA_A$ receptor possesses binding sites for compounds that allosterically modify the chloride channel-gating of GABA, such as benzodiazepines, β-carbolines, barbiturates, ethanol, and certain steroids[1]. The pharmacological effects of the benzodiazepines (anxiolytic, anticonvulsant, muscle relaxant, and sedative-hypnotic) make them the most important $GABA_A$ receptor-modulating drugs in clinical use.[8] It has been shown that many classes of compounds bind to the benzodiazepine receptor with high affinity, e.g., β-carbolines, triazolopyridazines, pyrazoloquinolinones, cyclopyrrolones, pyridodiindoles, and quinolines.[9,10] In addition, it has been found that flavones, naturally occurring and synthetic derivatives, bind with high affinity to the benzodiazepine site of the $GABA_A$ receptor.[11,12] Unified pharmacophore models (including agonists, antagonists, and inverse agonists) have been developed for the benzodiazepine site,[13,5] and the model by Cook and co-workers[5] has been selected for fitting flavonoids into a pharmacophore model and for further development by us[14] and others.[15] A quantitative structure−activity relationship (QSAR) model that confirms the interactions proposed by us[14] has been developed by Huang et al.[16] In the present investigation, 40 new flavone derivatives have been synthesized and tested and used to further refine the previously proposed pharmacophore model. A comparison of this model with a recently suggested alternative is made.

Results and Discussion

Chemistry. The structures of the flavones investigated in this study are shown in Chart 1. They were synthesized according to procedures that previously have been described for other flavones.[17] The synthesis involves an acetylation, a Fries rearrangement, a modified Schotten−Baumann reaction, and a Baker Venkataraman rearrangement followed by a cyclization catalyzed by acid (see Scheme 1). Aminoflavones were

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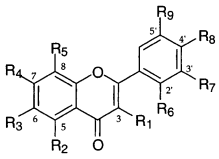
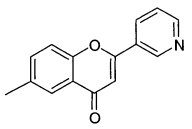
Chart 1



1: R_1=R_2=R_3=R_4=R_5=R_6=R_7=R_8=R_9=H
2: R_3=CH_3; R_1=R_2=R_4=R_5=R_6=R_7=R_8=R_9=H
3: R_3=CH_2CH_3; R_1=R_2=R_4=R_5=R_6=R_7=R_8=R_9=H
4: R_3=$(CH_2)_2CH_3$; R_1=R_2=R_4=R_5=R_6=R_7=R_8=R_9=H
5: R_3=$CH(CH_3)_2$; R_1=R_2=R_4=R_5=R_6=R_7=R_8=R_9=H
6: R_5=CH_3; R_1=R_2=R_3=R_4=R_6=R_7=R_8=R_9=H
7: R_3=R_8=CH_3; R_1=R_2=R_4=R_5=R_6=R_7=R_9=H
8: R_3=R_7=R_8=CH_3; R_1=R_2=R_4=R_5=R_6=R_9=H
9: R_3=R_7=R_9=CH_3; R_1=R_2=R_4=R_5=R_6=R_8=H
10: R_3=CH_3; R_6=NH_2; R_1=R_2=R_4=R_5=R_7=R_8=R_9=H
11: R_3=CH_3; R_7=NH_2; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
12: R_3=CH_3; R_8=NH_2; R_1=R_2=R_4=R_5=R_6=R_7=R_9=H
13: R_3=CH_3; R_6=OCH_3; R_1=R_2=R_4=R_5=R_7=R_8=R_9=H
14: R_3=CH_3; R_6=OH; R_1=R_2=R_4=R_5=R_7=R_8=R_9=H
15: R_3=CH_3; R_7=OCH_3; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
16: R_3=CH_3; R_7=OH; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
17: R_3=CH_3; R_7=$O(CH_2)_3CH_3$; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
18: R_3=CH_3; R_7=$OCH_2CH(CH_3CH_3)_2$;
R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
19: R_3=R_9=CH_3; R_6=OCH_3; R_1=R_2=R_4=R_5=R_7=R_8=H
20: R_3=R_9=CH_3; R_6=OH; R_1=R_2=R_4=R_5=R_7=R_8=H
21: R_3=CH_3; R_7=CO_2CH_3; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
22: R_3=CH_3; R_7=CO_2H; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
23: R_3=CH_3; R_7=$CO_2CH(CH_3)_2$;
R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
24: R_3=CH_3; R_7=$CO_2CH_2CH(CH_3CH_3)_2$;
R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
25: R_3=CH_3; R_7=CO_2CH_3; R_9=OCH_3;
R_1=R_2=R_4=R_5=R_6=R_8=H
26: R_3=CH_3; R_7=CO_2CH_3; R_9=OH; R_1=R_2=R_4=R_5=R_6=R_8=H
27: R_3=CH_3; R_7=CO_2H; R_9=OCH_3; R_1=R_2=R_4=R_5=R_6=R_8=H
28: R_3=CH_3; R_7=CO_2H; R_9=OH; R_1=R_2=R_4=R_5=R_6=R_8=H
29: R_3=CH_3; R_7=OCH_3; R_9=CH_2OH;
R_1=R_2=R_4=R_5=R_6=R_8=H
30: R_3=CH_3; R_7=R_9=CO_2CH_3; R_1=R_2=R_4=R_5=R_6=R_8=H
31: R_3=CH_3; R_7=R_9=CO_2H; R_1=R_2=R_4=R_5=R_6=R_8=H
32: R_3=CH_3; R_7=CO_2CH_3; R_9=CH_2OH;
R_1=R_2=R_4=R_5=R_6=R_8=H
33: R_3=CH_3; R_7=R_9=CH_2OH; R_1=R_2=R_4=R_5=R_6=R_8=H
34: R_3=CH_3; R_7=CO_2CH_3; R_9=NO_2; R_1=R_2=R_4=R_5=R_6=R_8=H
35: R_3=CH_3; R_7=CO_2H; R_9=NO_2; R_1=R_2=R_4=R_5=R_6=R_8=H
36: R_3=CH_3; R_7=CO_2CH_3; R_9=NH_2; R_1=R_2=R_4=R_5=R_6=R_8=H
37: R_3=CH_3; R_7=$CO_2CH_2CH_3$; R_9=NH_2;
R_1=R_2=R_4=R_5=R_6=R_8=H
38: R_3=CH_3; R_7=CF_3; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
39: R_3=CH_3; R_7=Br; R_1=R_2=R_4=R_5=R_6=R_8=R_9=H
40: R_3=CH_3; R_6=OCH_3; R_9=Br; R_1=R_2=R_4=R_5=R_7=R_8=H
41: R_3=CH_3; R_6=OH; R_9=Br; R_1=R_2=R_4=R_5=R_7=R_8=H



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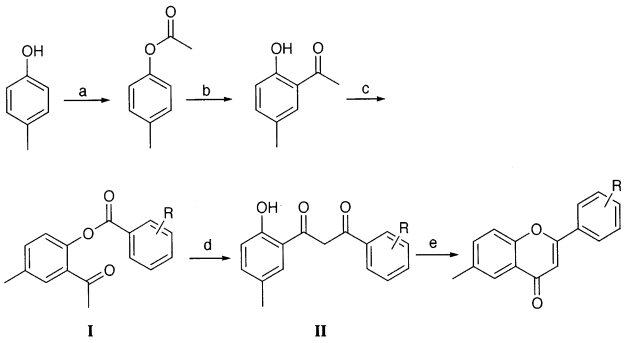
Scheme 1[a]



[a] Reagents: (a) Ac_2O, pyridine. (b) $AlCl_3$. (c) Substituted or nonsubstituted benzoyl chloride, pyridine. (d) KOH, pyridine. (e) H_2SO_4.

prepared by reduction of the corresponding nitro compound.[18] Esters were hydrolyzed with LiOH in tetrahydrofuran (THF) and water 1:1. Esters of benzoic acids were prepared by an acid-catalyzed reaction with the alcohol in THF or by the reaction of the alkyl halide in dimethyl formamide (DMF)/K_2CO_3. The latter reaction was also used for preparing ethers. 2-Methoxy-5-methylbenzoic acid was prepared from the corresponding aldehyde,[19] instead of from 2-bromo-4-methylanisol.[20] Ethers were cleaved by exposing them to BBr_3 in CH_2Cl_2 at −78 °C. Esters were reduced to alcohols with $LiAlH_4$ in anhydrous THF at 0 °C. The ether **18** and the ester **24** were made from **16** and **22**, respectively, under Mitsonobu conditions.[21]

Receptor Binding. As shown in Table 1, the tested flavonoids inhibit ^{3}H-Ro 15-1788 binding with a range of affinities from low nanomolar (**14**, **20**, and **41**) to micromolar concentrations. Compound **41** displays the highest affinity in the present series with a K_i value of 0.9 nM, and it is the highest affinity flavone derivative reported so far.

Table 1. K_I Values of Flavones Tested on ^{3}H-Ro 15-1788 Binding In Vitro to Rat Cortical Membranes

no.	name	K_i value (nM)
1	flavone	4200 ± 30
2	6-methylflavone	180 ± 40
3	6-ethylflavone	180 ± 40
4	propylflavone	480 ± 80
5	6-(1-methylethyl)flavone	720 ± 140
6	8-methylflavone	>1700
7	4′,6-dimethylflavone	4400
8	3′,4′,6-trimethylflavone	>1700
9	3′,5′,6-trimethylflavone	>1500
10	2′-amino-6-methylflavone	700 ± 46
11	3′-amino-6-methylflavone	1200
12	4′-amino-6-methylflavone	2600
13	2′-methoxy-6-methylflavone	820 ± 110
14	2′-hydroxy-6-methylflavone	6.2 ± 0.2
15	3′-methoxy-6-methylflavone	99 ± 15
16	3′-hydroxy-6-methylflavone	390 ± 130
17	3′-butoxy-6-methylflavone	710 ± 150
18	3′-(2-ethylbutyloxy)-6-methylflavone	1000 ± 210
19	2′-methoxy-5′,6-dimethylflavone	>1500
20	2′-hydroxy-5′,6-dimethylflavone	7.8 ± 0.9
21	methyl 3′-(6-methylflavone)carboxylate	220 ± 30
22	3′-(6-methylflavone)carboxylic acid	>1700
23	isopropyl 3′-(6-methylflavone)carboxylate	180 ± 10
24	2-ethylbutyl 3′-(6-methylflavone)carboxylate	610 ± 170
25	methyl 3′-(5′-methoxy-6-methylflavone)-carboxylate	3000
26	methyl 3′-(5′-hydroxy-6-methylflavone)-carboxylate	>1600
27	3′-(5′-methoxy-6-methylflavone)carboxylic acid	>1600
28	3′-(5′-hydroxy-6-methylflavone)carboxylic acid	>1600
29	5′-hydroxymethyl-3′-methoxy-6-methylflavone	>1600
30	dimethyl 3′,5′-(6-methylflavone)dicarboxylate	>1600
31	3′-5′-(6-methylflavone)dicarboxylic acid	>1600
32	methyl 3′-(5′-hydroxymethyl-6-methylflavone)-carboxylate	>1500
33	3′-5′-bis(hydroxymethyl)-6-methylflavone	>1500
34	methyl 3′-(6-methyl-5′-nitroflavone)carboxylate	>2100
35	3′-(6-methyl-5′-nitroflavone)carboxylic acid	>1700
36	methyl 3′-(5′-amino-6-methylflavone)-carboxylate	>1900
37	ethyl 3′-(5′-amino-6-methylflavone)carboxylate	3600
38	3′-(trifluoromethyl)−6-methylflavone	9.8 ± 2.7
39	3′-bromo-6-methylflavone	12 ± 2
40	5′-bromo-2′-methoxy-6-methylflavone	>1500
41	5′-bromo-2′-hydroxy-6-methylflavone	0.9 ± 0.2
42	6-methyl-2-(3-pyridyl)−4H-1-benzopyran-4-one (3′-aza-6-methylflavone)	130 ± 12

Pharmacophore Model. The pharmacophore model developed by Cook and co-workers[5] and previously employed by us for structure−activity analysis of a series of flavonoids[14] is shown in Figure 1. The proposed binding mode of compound **2** is shown. H1 and A2 are hydrogen bond donor and acceptor sites, respectively, whereas H2/A3 is a bifunctional hydrogen bond donor/acceptor site. L1−L3 are three lipophilic pockets and S1−S3 denote regions of steric repulsive ligand−receptor interactions (receptor-essential volumes).

The main conclusions in our previous structure−activity analysis on flavonoids were that in order to bind to the benzodiazepine site, the flavone skeleton should be planar or close to planar. Small substituents such as methyl and bromine in the 6-position significantly increase the affinity, whereas a 4′-NO_2 group significantly decreases the affinity. A 3′-NO_2 or 3′-methyl

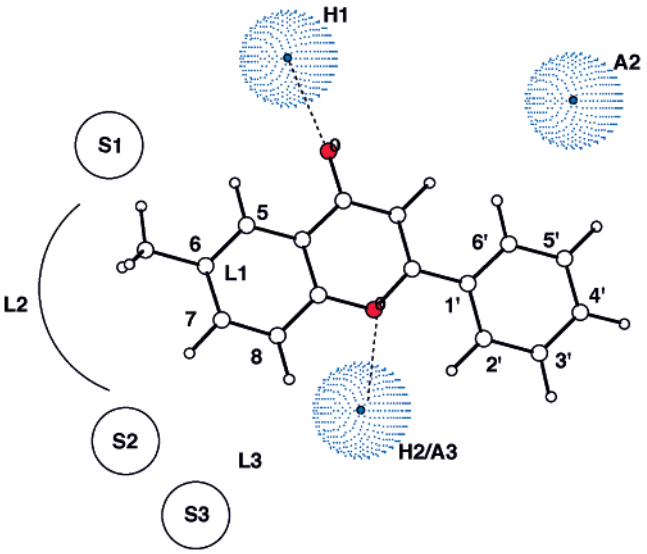


Figure 1. Proposed binding mode of compound **2** in the pharmacophore model developed by Cook and co-workers.

group (directed "downwards" in Figure 1) strongly increases the affinity making this substituent position of great interest for further investigations. None of the previously studied flavonoids was able to interact with the hydrogen bond accepting site A2, although this has been found to be an important interaction site for compounds that display potent inverse agonism.[5] Despite this, 6-methyl-3′-nitroflavone was shown to be a high affinity ligand with a K_i of 5.6 nM and displaying inverse agonism.[14]

The present series of flavonoids was designed to explore the dimensions of the lipophilic pocket L2 including the regions in the vicinity of L2 (compounds **3**−**6**) and the nature of the previously observed repulsive interactions between a 4′-substituent and the receptor (compounds **7**, **8**, and **12**). Furthermore, a series of 3′-substituted compounds were synthesized and tested in order to map out the properties of the region in the vicinity of a 3′-substituent (compounds **11**, **15**−**18**, **22**−**24**, **38**, and **39**). The 3′,5′-disubstituted compounds **9** and **25**−**37** were studied in order to obtain information on the effects of substitution in both of the equivalent 3′- and 5′-positions. Finally, to probe the possibility of obtaining favorable interactions with the hydrogen bond acceptor site A2, the 2′(6′)-aminoflavone **10** and the 2′(6′)-hydroxyflavone **14** were prepared together with the 2′,5′-disubstituted compounds **20** and **41**. The corresponding O-methylated compounds **13**, **19**, and **40** were prepared for comparisons.

Conformational Analyses. As mentioned above, a coplanar or close to coplanar arrangement of the two ring systems in the flavones is required for binding to the receptor. As steric hindrance to coplanarity may occur for the 2′-substituted compounds **10**, **13**, **14**, **19**, **20**, **40**, and **41**, the energy difference between the lowest energy minimum (with a twisted 2-phenyl ring) and the coplanar conformation was calculated for each of these compounds. The calculations were performed as previously described by using the MM3(92) force field.[14,22] The 2′-amino-substituted compound **10** displays a conformational energy difference of 3.5 kcal/mol between the coplanar conformation and the lowest energy one, whereas the 2′-methoxy compounds **13**, **19**, and **40** display a conformational energy difference of 1.7 kcal/mol and the 2′-hydroxy compounds **14**, **20**, and **41** display an energy difference of 1.3 kcal/mol. All compounds without a 2′-substituent display conformational energies for adopting a planar conformation of less than 0.2 kcal/mol.

Dimensions of the L2 Region. Methyl and ethyl substitution in the 6-position give essentially identical affinities (Table 1). However, on increasing the size of the 6-substituent from ethyl (**3**) to propyl (**4**) and isopropyl (**5**), the affinity decreases although the lipophilicity of the substituent increases. This indicates the presence of steric repulsions with the receptor for substituents of the size of a propyl group or larger.

Compound **6**, in which the methyl group in **2** is moved to the 8-position, displays a decreased affinity as compared to **2**. The decreased affinity of the 8-methyl compound **6** may be due to a steric conflict with the H2/A3 site (Figure 1).

4′-Substituted Flavones. In our previous study, we observed that a 4′-NO_2 group decreased the affinity by a factor of about 60.[14] However, it was not clear from the available data if the strong decrease in affinity is due to steric repulsive interactions or electrostatic repulsions between the 4′-NO_2 group and the receptor. Compounds **7** and **12** were prepared and tested in order to obtain more information on this interaction. The 4′-methyl-substituted compound **7** displays an affinity decrease as compared to **2** by a factor of 24, similar to that of the 4′-NH_2 compound **12** and slightly lower than the corresponding factor displayed by the 4′-NO_2-substituted compound. The 3′,4′-dimethyl-substituted compound **8** displays a reduced affinity by a factor of >59 as compared to the corresponding 3′-methyl-substituted compound (K_i = 29 nM).[14] Thus, the major part of the affinity decrease caused by 4′-substitution is most likely due to steric repulsions with the receptor although additional electrostatic or hydrogen-bonding interactions for the 4′-NO_2- and the 4′-NH_2-substituted compounds cannot be excluded.

Properties of the Receptor Region in the Vicinity of the 3′-Substituent. This region is of particular interest as we[14] and others[23−25] have shown that 3′-substituents as methyl, nitro, and bromo significantly increase the affinity. In the context of the pharmacophore model shown in Figure 1, we have previously concluded that a 3′-substituent should be directed "downwards".[14] The low affinity of the 3′,5′-dimethyl compound **9** (Table 1) clearly shows that a methyl group in the alternative equivalent position (5′) suffers strong steric repulsive interactions. Furthermore, all other 3′,5′-substituted compounds in Table 1 (compounds **25**−**37**) display K_i values higher than 1500 nM. Thus, it is concluded that the sterically repulsive region in the vicinity of a 4′-substituent discussed above extends to the region about the 5′-position.

For compound **2** and six compounds with small 3′-substituents NH_2 (**11**), OMe (**15**), OH (**16**), Me,[14] CF_3 (**38**), and Br (**39**), an excellent correlation between logK_i values and Hansch's π-values[26] can be obtained (logK_i = -0.960π + 1.984, R^2 = 0.97, data not shown) indicating that the affinites of these compounds may be understood in terms of a favorable transfer from the aqueous phase to a receptor phase with properties

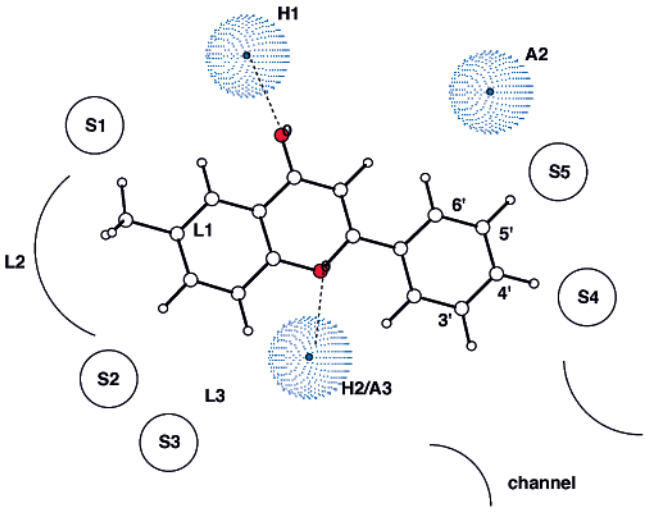


Figure 2. Steric repulsive interaction sites S4 and S5 identified in the present work and the proposed properties of the receptor region in vicinity of the 3′-position in flavones.

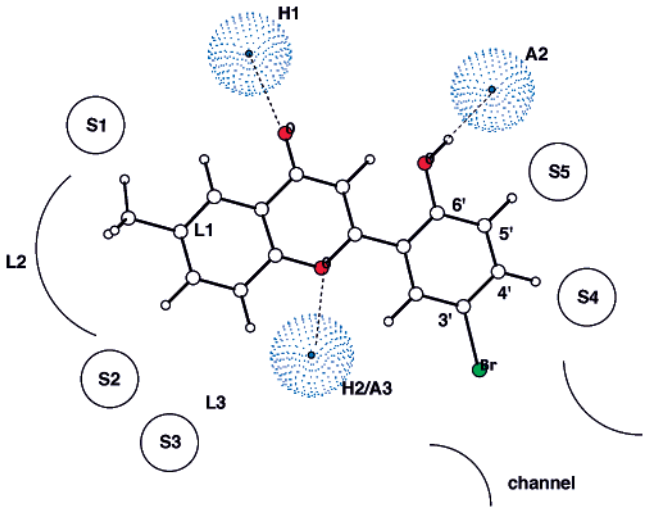


Figure 3. Proposed binding mode of compound **41** in the pharmacophore model displaying the proposed novel interaction between a 2′ (6′)-OH-substituted flavone and the A2 site.

similar to 1-octanol. The 6-methyl,3′-nitro-subtituted compound with a K_i of 5.6 nM[14] is about 30 times more active than predicted from the regression equation indicating that additional favorable interactions, e.g., hydrogen bonding, are available for this strongly polar substituent. The presence of a hydrogen-bonding environment about the 3′-position is also supported by the affinity of the pyridyl compound **42**. Although the desolvation energy of the pyridyl group is significantly higher than that of a phenyl group, compounds **2** and **42** have virtually identical affinities (Table 1). The low affinity of compound **22** is most probably due to the very high desolvation energy of a carboxylate group, which cannot be sufficiently compensated for by interactions in the receptor region about the 3′-position.

The affinities of the methyl and isopropyl esters, ester **21**, and **23** are slightly lower than that of the 3′-methoxy compound **15**. With larger alkyl ester groups and alkyl ether groups as in compound **24** and compounds **17** and **18**, the affinity slowly decreases with increasing size of the alkyl substituent. In the alkyl ester series, all affinites are within a factor of 3, whereas the corresponding factor for the alkyl ether series is 10.

Our interpretation of the affinity data for the 3′-substituted compounds is that the receptor region close to the 3′-position is a partly lipophilic region with possibilities for hydrogen bonding and that the extension of this region is channel-like in which the larger substituents of compounds **17**, **18**, and **24** are only partly desolvated. Such a channel may exist at the interface between an α- and a γ-subunit in the $GABA_A$ receptor where the benzodiazepine site most probably is located.[27]

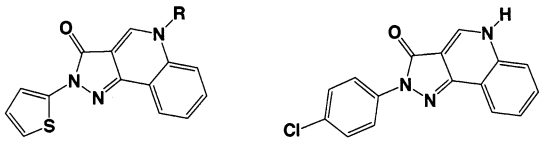
Figure 2 summarizes our conclusions on the properties of the receptor regions about the 3′-, 4′-, and 5′-positions.

Interactions with the Hydrogen Bond-Accepting A2 Site. As shown by the low affinities of the 3′,5′-disubstituted compounds discussed above, favorable interactions with the A2 site by substituents in the 5′-position ("upwards" in Figure 2) are not possible due to strong steric repulsions in this region. To investigate if interactions with the A2 site may be obtained by substituents in the 2′(6′)-position, compounds **10** and **14** were synthesized and tested. As shown in Table 1, a 2′(6′)-OH group (**14**) increases the affinity by a factor of 29, whereas a 2′(6′)-NH_2 group (**10**) decreases the affinity by a factor of 5 as compared to reference compound **2**. The lower affinity of compound **10** as compared to **14** by a factor of ca. 100 is most probably due to a combination of a higher energy required for **10** to adopt a coplanar arrangement of the ring systems (see above) and to the significant difference between the hydrogen-bonding energies involving a phenolic hydroxy group and an aniline type amino group, respectively. Halgren has calculated the phenol−water (with phenol as the hydrogen bond donor) and the aniline−water hydrogen bond interaction energies by high level ab initio quantum chemical methodology (MP2/6-31+G** basis set).[28] The calculations show that the phenol−water interaction is 3.5 kcal/mol stronger than the aniline−water interaction.

Introducing a 6′-hydroxy substituent to the 3′-methyl[14] and 3′-bromo[25] (**39**) compounds to give **20** and **41**, respectively, increases the affinity significantly as predicted on the basis of the high affinity of the 2′(6′)-hydroxy compound **14** and the pharmacophore model (Figure 1), which implies that the 6′-hydroxy group interacts with the A2 site and the 3′-substituent is directed "downwards" as shown in Figure 3. It should be noted that 3′-bromo-6′-hydroxy-6-methyl flavone (**41**) is the highest affinity flavone derivative reported so far with respect to binding to the benzodiazepine site of the $GABA_A$ receptor.

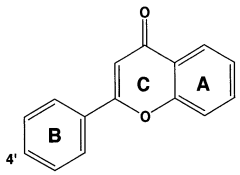
Methylation of the hydroxy group in **20**, **14**, and **41** to give **19**, **13**, and **40** significantly reduces the affinity due to the disruption of the hydrogen bond to A2, according to the pharmacophore model (Table 1, Figure 3). For the highest affinity 6′-hydroxy compound **41**, O-methylation decreases the affinity by more than a factor of 1500. This decrease in the affinity is similar to what is observed for compound **43** (Chart 2) as reported by Cook and co-workers.[5] The affinity decreases by more than a factor of 2500 on going from R═H to R═methyl in **43**. Compound **43** with R═H is a close analogue of CGS-9896 (**44**), which was used by

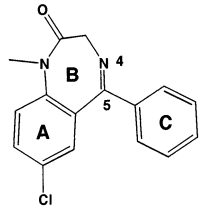
Chart 2



43 **44**

Chart 3





Cook and co-workers to establish the presence of a hydrogen-bonding site A2[5] and which superimposes extremely well with the flavones.[14] In this superimposition, the N−H bonds in **43** (R═H) and **44** superimposes with the OH bond in **41** and other 2′(6′)-hydroxy flavones. This strongly supports the superimpositions previously proposed by us and in particular the conclusion that the 2′(6′)-hydroxy group in the flavone series is directed "upwards" as in Figure 3 interacting with the A2 site and that the 3′-position consequently is directed "downwards".[14]

Comparisons with a Proposed Alternative Pharmacophore Model. Recently, Marder et al. proposed an alternative pharmacophore model in which the flavones are superimposed with diazepam.[15] According to the pharmacophore model developed by Cook and co-workers[5] and used as a starting point by us as well as by Marder et al., 1,4-benzodiazepines such as diazepam display interactions with the H1, L1, L2, and H2/A3 sites and, in addition, with the lipophilic region L3 (Figure 1).[5] It should be noted that the 1,4-benzodiazepines in this model do not interact with the A2 site and, according to our analysis, not with the site occupied by the 2-phenyl ring in the flavones.

As discussed above, it is well-established that a planar or close to planar structure is required for active flavones. To make the two aromatic rings in diazepam (Chart 3) reasonably coplanar, the seven-membered ring (B) in diazepam, which has a boat conformation in the lowest energy minimum, was flattened and the 5′-phenyl ring was reoriented with a total calculated conformational energy cost of 7−9 kcal/mol.[15] The superimposition of flavones and diazepam as suggested by Marder et al. is in our opinion highly problematic. The contrast between the high conformational energy calculated for the proposed bioactive conformation of diazepam and the very low one calculated for the flavones is hardly compatible with the high affinity of diazepam (IC_{50} = 8.1 nM).[5]

In the model of Marder et al., the flavones are fitted to diazepam so that the flavone C═O bond superimposes the C(5)═N(4) bond in diazepam and the A-ring in the flavones superimposes the 5-phenyl ring in diazepam (Chart 3). With this superimposition, the C═O group in the flavones is interacting with the H2/A3 site (Figure 1) and the 2-phenyl ring (B in Chart 3) is positioned close to the lipophilic region L2. In this model, there are no interactions between the flavones and the H1 and no possibility for interactions with A2. The drawback with the model suggested by Marder et al. is that it is not able to rationalize a number of observed important SAR for the flavones. The significantly increased affinity due to the 2′-OH group in compounds **14**, **20**, and **41** cannot be understood by the model, and the strong decrease in affinity due to the 4′-methyl group in **7** cannot be rationalized. This methyl group would in the model proposed by Marder et al. be positioned in the L2 lipophilic pocket with a predicted increase in affinity. The explanation provided for the low affinity of 4′-substituted flavones in terms of an electron rich site located close to L2 is not convincing as the methyl group in the 4′-position does not cause any significant changes of the electron distribution of the flavone molecule and the methyl group itself is certainly not a polar substituent.

Marder et al. cast doubts on our superimpositions of CGS-9896 (**44**, Chart 2) and the flavones[14] and argue for a superimposition of the flavones with diazepam on the basis that these compounds do not bind to the $\alpha_6\beta_3\gamma_2$-subtype, whereas CGS-9896 binds to this subtype. Affinity data for CGS-9896 on specific subtypes are to our knowledge not available, but the bromo analogue of **44** binds to the $\alpha_6\beta_3\gamma_2$-subtype with an affinity that is 31−222 times lower than the affinities for the $\alpha_{(1-5)}\beta_3\gamma_2$-subtypes.[29] Very few affinity data for flavones interacting with subtypes have been reported. Preliminary data for 6,8-dibromochrysin (5,7-dihydroxy-6,8-dibromoflavone) display a lower affinity for the $\alpha_6\beta_3\gamma_2$-subtype by a factor of >3−19.[15] Amentoflavone has a significant selectivity for the $\alpha_{(1-5)}\beta_3\gamma_2$-subtypes, but because this molecule is a biflavone, it must occupy other receptor sites than the simple flavones do and can therefore not be compared directly to the flavones studied by us in this and previous works. Thus, it is in our opinion premature to draw conclusions on the subtype selectivity of flavones. However, CGS-9896 used in our model is only one of several classes of compounds to which the flavones may be superimposed and which also superimposes with CGS-9896 exceedingly well as shown by Cook and co-workers.[5] For instance, dihydropyrido[3,4-b:5,4-b′]diindoles could equally well be used as a template as shown in our previous work.[14] According to Huang et al.,[29] these compounds show high selectivity for the $\alpha_{(1-5)}\beta_3\gamma_2$-subtypes. Thus, compounds that differ in their binding to a specific subtype do not necessarily require different binding modes in terms of the pharmacophore model developed by Cook and co-workers and employed by us for structure−activity analysis of flavones.

Conclusions

The pharmacophore model for the benzodiazepine site of the $GABA_A$ receptor originally proposed by Cook and co-workers[5] has been further validated and refined by the identification of two new regions of steric repulsive

interactions, S4 and S5. In terms of substituted flavones, the lipophilic L2 pocket optimally accommodates substituents of the size of an ethyl group. 2′-Hydroxy substitution has been shown to give a significant increase in affinity, which in terms of the pharmacophore model straightforwardly can be interpreted in terms of hydrogen bond interaction with a previously proposed hydrogen bond-accepting site A2. The receptor region in the vicinity of the 3′-position is most likely a partly lipophilic region with possibilities for hydrogen bonding and possibly close to a channel at the interface between an α- and a γ-subunit in the $GABA_A$ receptor. On the basis of the results of these studies and the refined pharmacophore model, we have designed and synthesized 5′-bromo-2′-hydroxy-6-methylflavone, with a K_i value of 0.9 nM, the highest affinity flavone derivative reported. As mentioned in the Introduction, different $GABA_A$ receptor subtypes mediate different pharmacological actions. It remains to be investigated if the compounds discussed in the present work display subtype selectivity.

Experimental Section

Chemistry. ^{1}H NMR and ^{13}C NMR were recorded at room temperature with a Bruker ARX300, Bruker DRX400, or Bruker ARX500 spectrometer. The spectra were recorded in $CDCl_3$, DMSO-d_6, and CD_3OD, and the solvent signals (7.27 and 77.23, 2.50 and 39.52, or 3.31 and 49.0 ppm, respectively) were used as reference. The raw data were transformed, and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). EI mass spectra were recorded at 70 eV with a JEOL SX102 spectrometer. Concentrations were made using rotary evaporation with bath temperatures at or below 40 °C. Anhydrous $MgSO_4$ was used as a drying agent for the organic extracts in the work up procedures. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck). Column chromatography was performed on SiO_2 (Matrex LC-gel: 60A, 35-70 MY, Grace) using the flash technique. Melting points (uncorrected) were determined with a Reichert microscope. Compound **5** (isopropylflavone) was purchased from Carbocore Inc.

2-Acetyl-4-ethylphenyl Bensoate (Scheme 1; I Leading to 3). To a stirred solution of 1-(5-ethyl-2-hydroxy-phenyl)-ethanone (1.59 g, 9.68 mmol) in anhydrous pyridine (1.94 mL), benzoyl chloride (1.63 g, 0.012 mol) was added dropwise over a period of 10 min. The solution was stirred at 50 °C for 30 min and then poured into a mixture of ice (9.7 g) and 1 M HCl(aq) (20 mL). The organic phase was extracted with diethyl ether and washed with brine, dried, and evaporated. The product was purified by chromatography (heptane:ethyl acetate 9:1) and obtained as a transparent oil in 85% yield. ^{1}H NMR ($CDCl_3$): 1.30 (t, 3H, J = 7.6), 2.55 (s, 3H), 2.74 (q, 2H, J = 7.6) 7.16 (d, 1H, J = 8.3), 7.42 (dd, 1H, J = 8.3, 2.3), 7.55 (m, 3H), 7.69 (d, 1H, J = 2.3), 8.23 (d, 2H, J = 8.2). ^{13}C NMR ($CDCl_3$): 15.9, 28.7, 30.4, 124.1, 129.1, 129.1, 129.3, 129.9, 130.7, 130.7, 131.0, 133.3, 134.2, 142.7, 147.8, 165.8, 198.3. MS *m*/*z* (% rel int): 268 (M^+, 10%), 105 (100%), 77 (43%).

1-(5-Ethyl-2-hydroxyphenyl)-3-phenylpropane-1,3-dione (Scheme 1; II Leading to 3). 2-Acetyl-1-benzoyloxy-4-ethylbenzene (2.68 g, 9.97 mmol) was dissolved in 10 mL of anhydrous pyridine and heated to 50 °C. Finely powdered potassium hydroxide (0.78 g, 14.0 mmol) was added in small portions over a period of 20 min, and stirring was continued for an additional 1.5 h at 50 °C. After the mixture was cooled to room temperature and 15 mL of 10% acetic acid(aq) was added, the precipitate formed was collected by filtration and recrystallized from EtOH to give slightly yellow-brown needles (yield 46%); mp 62−65 °C. In $CDCl_3$, the product is present as the enol form. ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, J = 7.3), 2.63 (q, 2H, J = 7.3), 6.84 (s, 1H), 6.93 (d, 1H, J = 8.5), 7.32 (dd, 1H, J = 8.5, 2.3), 7.53 (m, 3H), 7.84 (d, 2H, J = 8.1), 7.90 (d, 1H, J = 2.3), 11.94 (s, 1H), 15.58 (s, 1H). ^{13}C NMR ($CDCl_3$): 15.9, 28.1, 92.3, 118.6, 126.8, 126.8, 127.1, 128.7, 128.7, 128.8, 132.3, 133.7, 134.7, 135.8, 160.6, 177.4, 195.5. MS *m*/*z* (% rel int): 268 (M^+, 52%), 149 (24%), 105 (100%), 77 (42%).

6-Ethylflavone (3). Concentrated sulfuric acid (95−97%) (0.22 g, 2.22 mmol) was added to a suspension of the diketone **II** (0.56 g, 2.22 mmol) in 3 mL of glacial acetic acid and heated to 80 °C for 2 h, whereafter the mixture was poured onto 14 g of ice. After 30 min, the white crystals that formed were collected by filtration, washed with 30 mL of water, and recrystallized from acetone to give white needles (yield 54%); mp 96−98 °C. ^{1}H NMR ($CDCl_3$): 1.31 (t, 3H, J = 7.6), 2.78 (q, 2H, J = 7.6), 6.83 (s, 1H), 7.52 (m, 5H), 7.93 (d, 2H, J = 8.2), 8.06 (d, 1H, J = 2.2). ^{13}C NMR ($CDCl_3$): 15.4, 28.3, 107.4, 117.9, 123.7, 123.8, 126.2, 126.2, 129.0, 129.0, 131.4, 131.9, 133.9, 141.5, 154.6, 163.2, 178.6. MS *m*/*z* (% rel int): 250.1002 (M^+, 100, $C_{17}H_{14}O_2$ requires 250.0994), 249 (38%), 235 (52%), 133 (50%). Anal. ($C_{17}H_{14}O_2$) C, H.

2-Acetyl-4-propylphenyl Bensoate (Scheme 1; I Leading to 4). The compound was prepared according to the procedure described for **I** leading to **3**. 2-Acetyl-4-propylphenol was used instead of 2-acetyl-4-ethylphenol. The crude product was purified by chromatography (heptane:ethyl acetate 9:1) and obtained as a yellow oil in 93% yield. ^{1}H NMR ($CDCl_3$): 0.98 (t, 3H, J = 7.3), 1.69 (sext., 2H, J = 7.3), 2.55 (s, 3H), 2.66 (t, 2H, J = 7.3), 7.15 (d, 1H, J = 8.2), 7.39 (dd, 1H, J = 8.2, 2.3), 7.52 (m, 3H), 7.81 (d, 1H, J = 2.3), 8.23 (d, 2H, J = 8.1). ^{13}C NMR ($CDCl_3$): 14.2, 24.9, 30.3, 37.7, 124.0, 129.3, 126.3, 129.3, 130.5, 130.7, 130.7, 131.0, 134.0, 134.2, 131.2, 147.8, 165.7, 198.2. MS *m*/*z* (% rel int): 282 (M^+, 8%), 105 (100%), 77 (34%).

1-(2-Hydroxy-5-propylphenyl)-3-phenylpropane-1,3-dione (Scheme 1; II Leading to 4). The compound was prepared according to the procedure described for **II** leading to **3**. The product was purified by chromatography (heptane: ethyl acetate 15:1) to give yellow crystals in a yield of 58%; mp 58−60 °C. Only the enol form was obtained. ^{1}H NMR ($CDCl_3$): 0.97 (t, 3H, J = 7.3), 1.65 (sext., 2H, J = 7.3), 2.61 (t, 2H, J = 7.3), 6.84 (s, 1H), 6.96 (d, 1H, J = 8.3), 7.29 (dd, 1H, J = 8.3, 2.2), 7.52 (m, 3H), 7.86 (m, 3H), 11.91 (s, 1H), 15.60 (s, 1H). ^{13}C NMR ($CDCl_3$): 13.7, 24.7, 37.2, 92.3, 118.5, 126.8, 126.8, 127.7, 128.7, 128.7, 128.8, 132.3, 133.1, 133.7, 136.3, 160.6, 177.4, 195.7. MS *m*/*z* (% rel int): 282 (M^+, 49%), 105 (100%), 77 (38%).

6-Propylflavone (4). The compound was prepared according to the procedure described for **3**. The crude product was crystallized from acetone to give white needles in a yield of 48%; mp 97−99 °C. ^{1}H NMR ($CDCl_3$): 0.96 (t, 3H, J = 7.3), 1.71 (sext., 2H J = 7.3), 2.71 (t, 2H, J = 7.3), 6.82 (s, 1H), 7.53 (m, 5H), 7.92 (d, 2H, J = 8.1), 8.06 (d, 1H, J = 2.3). ^{13}C NMR ($CDCl_3$): 14.1, 24.8, 37.8, 107.9, 118.3, 124.1, 125.0, 126.7, 126.7, 129.5, 129.5, 131.9, 132.4, 134.9, 140.4, 155.2, 163.7, 179.1. MS *m*/*z* (% rel int): 264.1150 (M^+, 55, $C_{18}H_{16}O_2$ requires 264.1150), 236 (21%), 235 (100%), 133 (64%). Anal. ($C_{18}H_{16}O_2$) C, H.

2-Acetyl-6-methylphenyl Bensoate (Scheme 1; I Leading to 6). The compound was prepared according to the procedure described for **I** leading to **3**. 2-Acetyl-6-methylphenol was used instead of 2-acetyl-4-ethylphenol. The crude product was purified by chromatography (heptane:ethyl acetate 5:1) to give white crystals in 44% yield. Spectroscopic data were identical with those previously reported.[31]

1-(2-Hydroxy-3-methylphenyl)-3-phenylpropane-1,3-dione (Scheme 1; II Leading to 6). The compound was prepared according to the procedure described for **II** leading to **3**. Purification was made by recrystallization from EtOH to give yellow crystals in a yield of 35%. Spectroscopic data were identical with those previously reported.[32]

8-Methylflavone (6). The compound was prepared according to the procedure described for **3**. The crude product was recrystallized from acetone to give beige crystals in a yield of 69%. Spectroscopic data were identical with those previously reported.[33] Anal. ($C_{16}H_{12}O_2$) C, H.

2-Acetyl-4-methylphenyl 4-Methylbensoate (Scheme 1; I Leading to 7). The compound was prepared according to

the procedure described for **I** leading to **3**. 4-Methylbenzoyl chloride was made from 4-methylbenzoic acid. The acid (2.20 g, 16.2 mmol) was dissolved in $SOCl_2$ (1.77 mL, 24.3 mmol), a drop of DMF was added, and the solution was refluxed for 2.5 h. The acid chloride was purified by distillation using high vacuum. 2-Acetyl-4-methylphenol was used instead of 2-acetyl-4-ethylphenol. The product was purified by recrystallization from MeOH to give white crystals in a yield of 82%. Spectroscopic data were identical with those previously reported.[34]

1-(2-Hydroxy-5-methylphenyl)-3-(4-methylphenyl)propane-1,3-dione (Scheme 1; II Leading to 7). The compound was prepared according to the procedure described for **II** leading to **3**. Purification was made twice by recrystallization from EtOH to give yellow crystals in a yield of 96%. Spectroscopic data were identical with those previously reported.[34]

4′,6-Dimethylflavone (7). The compound was prepared according to the procedure described for **3**. The product was recrystallized from acetone to give beige crystals in a yield of 91%. Spectroscopic data were identical with those previously reported.[34] Anal. ($C_{17}H_{14}O_2$) C, H.

2-Acetyl-4-methylphenyl 3,4-Dimethylbensoate (Scheme 1; I Leading to 8). The compound was prepared according to the procedure described for **I** leading to **7**. The crude product was purified by recrystallization from MeOH to give white crystals in a yield of 93%; mp 67−69 °C. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H), 2.36 (s, 3H), 2.41 (s, 3H), 2.52 (s, 3H), 7.10 (d, 1H, J = 8.2), 7.29 (d, 1H, J = 8.5), 7.38 (dd, 1H, J = 8.2, 2.3), 7.65 (d, 1H, J = 2.3), 7.95 (m, 2H). ^{13}C NMR ($CDCl_3$): 20.0, 20.4, 21.1, 30.2, 123.8, 126.9, 128.1, 130.2, 130.7, 131.2, 131.5, 134.2, 136.1, 137.4, 143.7, 147.6, 165.8, 198.2. MS *m*/*z* (% rel int): 282 (M^+, 3%), 150 (12%), 133 (100%), 105 (34%), 77 (16%).

1-(2-Hydroxy-5-methylphenyl)-3-(3,4-dimethylphenyl)-propane-1,3-dione (Scheme 1; II Leading to 8). The compound was prepared according to the procedure described for **II** leading to **3**. Purification of the crude product was made twice by recrystallization from EtOH to give yellow needles in a yield of 74%; mp 113−115 °C. The product was present as the enol form in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.36 (s, 3H), 2.36 (s, 3H), 2.37 (s, 3H), 6.80 (s, 1H), 6.92 (d, 1H, J = 8.4), 7.28 (m, 2H), 7.56 (d, 1H, J = 2.2), 7.70 (dd, 1H, J = 8.1, 2.2), 7.74 (d, 1H, J = 2.1), 11.97 (s, 1H), 15.57 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.3, 20.6, 21.0, 92.1, 118.9, 119.1, 124.9, 128.3, 128.5, 128.6, 130.5, 131.6, 137.1, 137.6, 142.4, 160.7, 178.5, 195.7. MS *m*/*z* (% rel int): 282 (M^+, 40%), 135 (13%), 133 (100%), 105 (18%), 77 (13%).

3′,4′,6-Trimethylflavone (8). The compound was prepared according to the procedure described for **3**. The product was recrystallized from acetone to give light yellow crystals in a yield of 94%; mp 142−144 °C. ^{1}H NMR ($CDCl_3$): 2.33 (s, 3H), 2.35 (s, 3H), 2.46 (s, 3H), 6.77 (s, 1H), 7.24 (d, 1H, J = 8.2), 7.47 (m, 2H), 7.67 (m, 2H), 8.00 (d, 1H, J = 2.1). ^{13}C NMR ($CDCl_3$): 19.9, 20.0, 20.9, 106.7, 117.8, 123.6, 123.8, 124.9, 127.2, 129.3, 130.2, 134.8, 135.0, 137.4, 140.9, 154.5, 163.6, 178.6. MS *m*/*z* (% rel int): 264.1148 (M^+, 100, $C_{18}H_{16}O_2$ requires 264.1150), 249 (28%), 236 (23%), 134 (42%), 115 (14%). Anal. ($C_{18}H_{16}O_2$) C, H.

2-Acetyl-4-methylphenyl 3,5-Dimethylbensoate (Scheme 1; I Leading to 9). The compound was prepared according to the procedure described for **I** leading to **7**. The crude product was purified by chromatography (heptane:ethyl acetate 8:1) to give white crystals (yield 50%); mp 115−117 °C. ^{1}H NMR ($CDCl_3$): 2.41 (s, 6H), 2.43 (s, 3H), 2.54 (s, 3H), 7.10 (d, 1H, J = 8.2), 7.29 (s, 1H), 7.39 (dd, 1H, J = 8.2, 2.1), 7.66 (d, 1H, J = 2.1), 7.96 (m, 2H). ^{13}C NMR ($CDCl_3$): 21.0, 21.4, 21.4, 30.1, 123.8, 128.2, 128.2, 129.3, 130.7, 131.2, 134.2, 135.7, 136.1, 138.6, 138.6, 147.5, 165.9, 198.4. MS *m*/*z* (% rel int): 282 (M^+, 7%), 134 (10%), 133 (100%), 105 (18%).

1-(2-Hydroxy-5-methylphenyl)-3-(3,5-dimethylphenyl)-propane-1,3-dione (Scheme 1; II Leading to 9). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was purified by chromatography (heptane:ethyl acetate 12:1) to give yellow crystals (yield 95%); mp 99−101 °C. Only the enol form was present in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 2.42 (d, 6H, J = 0.5), 6.81 (s, 1H), 6.92 (d, 1H, J = 8.4), 7.21 (s, 1H), 7.29 (dd, 1H, J = 8.4, 2.2), 7.57 (m, 3H), 11.95 (s, 1H), 15.66 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.8, 21.5, 21.5, 92.5, 118.8, 124.9, 124.9, 128.3, 128.4, 134.0, 134.4, 134.4, 137.0, 138.7, 138.7, 160.6, 178.4, 195.7. MS *m*/*z* (% rel int): 282 (M^+, 45%), 135 (15%), 134 (13%), 133 (100%), 105 (18%).

3′,5′,6-Trimethylflavone (9). The compound was prepared according to the procedure described for **3**. The crude product was purified by chromatography (heptane:ethyl acetate 5:1) to give white crystals in a yield of 75%; mp 143−145 °C. ^{1}H NMR ($CDCl_3$): 2.42 (s, 6H), 2.48 (s, 3H), 6.79 (s, 1H), 7.18 (s, 1H), 7.53 (m, 4H), 8.03 (d, 1H, J = 2.0). ^{13}C NMR ($CDCl_3$): 21.2, 21.6, 21.6, 107.6, 118.1, 123.9, 124.3, 124.3, 125.2, 132.1, 133.4, 135.1, 135.3, 138.9, 138.9, 154.8, 163.9, 178.8. MS *m*/*z* (% rel int): 264.1151 (M^+, 100, $C_{18}H_{16}O_2$ requires 264.1151), 265 (19%), 236 (20%), 134 (52%). Anal. ($C_{18}H_{16}O_2$) C, H.

2′-Amino-6-methylflavone (10). Sn (89 mg, 0.749 mmol) was added to a solution of 6-methyl-2′-nitroflavone[14] (40 mg, 0.142 mmol) in EtOH (2 mL), and the solvent was heated to reflux. A 12 M amount of HCl (0.64 mL, 7.68 mmol) was added to the solution, and after 20 min, the mixture was cooled to room temperature. The solution was made weakly basic with 2 M NaOH(aq), extracted with diethyl ether, and washed with brine. The organic phase was dried and evaporated. The crude product was purified by chromatography (toluene:acetone 5:1) to give yellow-white crystals in 70% yield; mp 173−175 °C. ^{1}H NMR ($CDCl_3$): 2.49 (s, 3H), 4.46 (bs, 2H), 6.66 (s, 1H), 6.81 (d, 1H, J = 8), 6.87 (t, 1H, J = 8), 7.29 (t, 1H, J = 8), 7.40 (d, 1H, J = 8.4), 7.47 (d, 1H, J = 8), 7.50 (dd, 1H, J = 8.4, 2.2), 8.02 (d, 1H, J = 2.2). ^{13}C NMR ($CDCl_3$): 20.9, 110.3, 117.1, 117.1, 117.6, 118.5, 123.5, 125.1, 129.6, 132.1, 134.9, 135.3, 145.2, 154.5, 164.7, 178.3. MS *m*/*z* (% rel int): 251.0948 (M^+, 44, $C_{16}H_{13}NO_2$ requires 251.0946), 117 (100%). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

3′-Amino-6-methylflavone (11). 6-Methyl-3′-nitroflavone[14] was reacted according to the procedure described for 6-methyl-2′-nitroflavone leading to **10**. The compound was purified by chromatography (toluene:acetone 5:1) to give yellow-white crystals in 65% yield; mp 194−198 °C. ^{1}H NMR (DMSO-d_6): 2.42 (s, 3H), 5.41 (bs, 2H), 6.76 (s, 1H), 6.79 (ddd, 1H, J = 7.2, 2.1, 2.1), 7.20 (m, 3H), 7.59 (d, 1H, J = 8.6), 7.62 (dd, 1H, J = 8.6, 1.8), 7.82 (d, 1H, J = 1.8). ^{13}C NMR (DMSO-d_6): 20.5, 106.4, 110.9, 113.8, 117.2, 118.1, 123.1, 124.1, 129.7, 131.8, 135.0, 135.3, 149.3, 153.9, 163.5, 177.0. MS *m*/*z* (% rel int): 251.0948 (M^+, 100, $C_{16}H_{13}NO_2$ requires 251.0946), 117 (36%). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

4′-Amino-6-methylflavone (12). 6-Methyl-4′-nitroflavone[14] was reacted according to the procedure described for 6-methyl-2′-nitroflavone leading to **10**. The crude product was purified by chromatography (toluene:acetone 5:1) to give yellow-white crystals in 76% yield; mp 194−197 °C. ^{1}H NMR ($CDCl_3$): 2.47 (s, 3H), 4.16 (bs, 2H), 6.70 (s, 1H) 6.77 (d, 2H, J = 8.9), 7.44 (d, 1H, J = 8.5), 7.49 (dd, 1H, J = 8.5, 2.1), 7.76 (d, 2H, J = 8.9), 8.02 (bs, 1H). ^{13}C NMR ($CDCl_3$): 21.1, 105.0, 105.1, 114.9, 117.8, 121.3, 123.8, 125.1, 125.2, 128.2, 134.8, 135.0, 150.0, 154.6, 164.1, 178. MS *m*/*z* (% rel int): 251.0946 (M^+, 100, $C_{16}H_{13}NO_2$ requires 251.0946), 117 (47%). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

2-Acetyl-4-methylphenyl 2-Methoxybensoate (Scheme 1; I Leading to 13 and 14). The compound was prepared according to the procedure described for **I** leading to **7**. The acid chloride was commercially available. The product was purified by chromatography (heptane:ethyl acetate 6:1) as white crystals in 87% yield; mp 72−74 °C. ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H), 2.56 (s, 3H), 3.94 (s, 3H), 7.05 (d, 1H, J = 8), 7.08 (dt, 1H, J = 1.5, 8), 7.13 (d, 1H, J = 8.2), 7.37 (dd, 1H, J = 8.2, 2.3), 7.57 (ddd, 1H, J = 8.4, 7.6, 1.9), 7.64 (d, 1H, J = 2.3), 8.10 (dd, 1H, J = 7.7, 1.9). ^{13}C NMR ($CDCl_3$): 21.0, 30.1, 56.2, 112.4, 119.0, 120.6, 123.9, 130.6, 131.4, 132.8, 134.1, 134.7, 135.9, 147.5, 160.2, 164.5, 198.3. MS *m*/*z* (% rel int): 284 (M^+, 2%), 136 (7%), 135 (100%), 92 (12%), 77 (18%).

1-(2-Hydroxy-5-methylphenyl)-3-(2-methoxyphenyl)-propane-1,3-dione (Scheme 1; II Leading to 13 and 14). The compound was prepared according to the procedure

described for **II** leading to **3**. The product was isolated in 76% yield as yellow crystals, mp 102−104 °C, in $CDCl_3$, as the enol isomer. ^{1}H NMR ($CDCl_3$): 2.34 (s, 3H), 4.00 (s, 3H), 6.92 (d, 1H, J = 8.4), 7.04 (m, 2H), 7.23 (s, 1H), 7.28 (dd, 1H, J = 8.4, 1.8), 7.51 (m, 2H), 7.80 (dd, 1H, J = 7.8, 1.9), 11.98 (s, 1H), 15.61 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.9, 56.1, 97.7, 112.0, 118.6, 119.1, 121.1, 122.9, 128.2, 128.6, 130.2, 133.3, 136.8, 158.7, 160.6, 175.4, 196.1. MS *m*/*z* (% rel int): 284 (M^+, 21%), 235 (100%), 77 (18%).

2′-Methoxy-6-methylflavone (13). The compound was prepared according to the procedure described for **3**. The crude product was recrystallized from acetone to give white crystals in 96% yield; mp 108−110 °C. ^{1}H NMR ($CDCl_3$): 2.47 (s, 3H), 3.94 (s, 3H), 7.05 (d, 1H, J = 8.4), 7.11 (m, 2H), 7.47 (m, 3H), 7.90 (dd, 1H, J = 7.8, 1.7), 8.03 (bs, 1H). ^{13}C NMR ($CDCl_3$): 21.1, 55.9, 112.0, 112.7, 112.7, 118.0, 120.9, 121.2, 123.7, 125.2, 129.5, 132.5, 134.0, 155.0, 158.2, 160.9, 179.2. MS *m*/*z* (% rel int): 266.0956 (M^+, 80, $C_{17}H_{14}O_3$ requires 266.0943), 135 (100%), 134 (33%), 132 (19%), 131 (26%), 78 (17%). Anal. ($C_{17}H_{14}O_3$) C, H.

2′-Hydroxy-6-methylflavone (14). BBr_3, 3.6 mL (1 M in CH_2Cl_2), was added to **13** (0.20 g, 0.75 mmol) dissolved in freshly distilled CH_2Cl_2 (30 mL) at −78 °C and under inert conditions (N_2). After the addition, the cooling bath was removed and a beige precipitate was formed. The reaction mixture was left at room temperature for 48 h whereafter MeOH (4 mL) was added slowly. The reaction mixture was poured into a separatory funnel with 40 mL of 1 M NaOH(aq) and washed four times with CH_2Cl_2 to remove starting material. The mixture was acidified with concentrated HCl, extracted with CH_2Cl_2, and washed with brine. After the organic phase was dried and concentrated, the residue was purified by flash chromatography (heptane:ethyl acetate 2:1, pure ethyl acetate) to give the product as white crystals in a yield of 63%; mp 264−266 °C. ^{1}H NMR (CD_3OD): 2.46 (s, 3H), 6.95 (d, 1H, J = 8.3), 6.98 (ddd, 1H, J = 8, 7, 1.1), 7.32 (ddd, 1H, J = 8.3, 7.4, 1.7), 7.36 (s, 1H), 7.50 (d, 1H, J = 8.6), 7.55 (dd, 1H, J = 8.6, 2.0), 7.89 (dd, 1H, J = 7.9, 1.7), 7.92 (d, 1H, J = 2.0). ^{13}C NMR (CD_3OD): 21.2, 111.9, 117.5, 118.7, 118.9, 120.3, 123.6, 125.1, 129.4, 133.2, 135.9, 136.0, 155.6, 157.5, 163.1, 181.0. MS *m*/*z* (% rel int): 253 (36%), 252.0786 (M^+, 100, $C_{16}H_{12}O_3$ requires 352.0790), 135 (27%), 134 (54%). Anal. ($C_{16}H_{12}O_3$) C, H.

2-Acetyl-4-methylphenyl 3-Methoxybensoate (Scheme 1; I Leading to 15−18). The compound was prepared according to the procedure described for **I** leading to **7**. The product was purified by recrystallization in 80% MeOH(aq) to give white crystals. The mother liquor was purified by chromatography (heptane:ethyl acetate 3:1). The product was isolated as white needles in 91% yield; mp 72−75 °C. ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H), 2.52 (s, 3H), 3.88 (s, 3H), 7.11 (d, 1H, J = 8.2), 7.19 (dt, 1H, J = 1.5, 8), 7.39 (dd, 1H, J = 8.2, 2.3), 7.65 (d, 1H, J = 0.9), 7.70 (d, 1H, J = 2.3), 7.81 (d, 1H, J = 8.2). ^{13}C NMR ($CDCl_3$): 21.2, 30.3, 55.9, 114.9, 119.6, 120.8, 123.1, 124.0, 130.1, 131.0, 133.6, 134.4, 136.4, 147.6, 160.1, 165.6, 199.4. MS *m*/*z* (% rel int): 284 (M^+, 11%), 135 (100%), 107 (21%), 92 (16%), 77 (20%).

1-(2-Hydroxy-5-methylphenyl)-3-(3-methoxyphenyl)-propane-1,3-dione (Scheme 1; II Leading to 15−18). The compound was prepared according to the procedure described for **II** leading to **3**. The product was purified by chromatography (heptane:ethyl acetate 10:1) to give yellow crystals in 85% yield; mp 89−91 °C. In $CDCl_3$, the product is a 9:1 enol:keto mixture. Data are given for the major enol form. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H), 3.90 (s, 3H), 6.82 (s, 1H), 6.92 (d, 1H, J = 8.5), 7.11 (dd, 1H, J = 8.2, 2.3), 7.29 (dd, 1H, J = 8.5, 2.3), 7.41 (t, 1H, J = 8), 7.48 (dd, 1H, J = 2.3, 1.9), 7.53 (m, 2H), 11.90 (s, 1H), 15.63 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.8, 55.7, 92.8, 112.3, 118.3, 118.3, 118.8, 119.4, 128.4, 130.0, 135.3, 137.2, 137.2, 160.1, 160.6, 177.4, 195.8. MS *m*/*z* (% rel int): 284 (M^+, 40%), 135 (100%), 107 (18%), 77 (23%).

3′-Methoxy-6-methylflavone (15). The compound was prepared according to the procedure described for **3**. The crude product was purified with recrystallization from acetone to give white crystals. The mother liquor was purified by chromatography (heptane:ethyl acetate 6:1) to give the product in 85% yield; mp 120−122 °C. ^{1}H NMR ($CDCl_3$): 2.46 (s, 3H), 3.89 (s, 3H), 6.79 (s, 1H), 7.06 (dd, 1H, J = 8.3, 2.4), 7.48 (m, 5H), 8.01 (d, 1H, J = 2.4). ^{13}C NMR ($CDCl_3$): 21.1, 55.6, 107.9, 111.9, 117.3, 118.0, 118.9, 123.8, 125.1, 130.3, 133.4, 135.2, 135.4, 154.7, 160.2, 163.2, 178.7. MS *m*/*z* (% rel int): 267 (18%), 266.0937 (M^+, 100, $C_{17}H_{14}O_3$ requires 266.0943), 265 (12%), 134 (30%). Anal. ($C_{17}H_{14}O_3$) C, H.

3′-Hydroxy-6-methylflavone (16). The compound was prepared according to the procedure described for **13** leading to **14**. BBr_3, 1.1 equiv, was added. The reaction time was 18 h before addition of MeOH. Under the work up procedure, the organic phase was removed and the water phase was acidified with 2 M HCl(aq). New diethyl ether was added, and the organic phase was washed with water. The organic phase was dried and evaporated. White crystals were obtained in a yield of 92%; mp 196−198 °C. ^{1}H NMR (DMSO-d_6): 2.41 (s, 3H), 6.86 (s, 1H), 7.00 (dd, 1H, J = 8.1, 2.4), 7.36 (t, 1H, J = 8), 7.41 (t, 1H, J = 2), 7.48 (d, 1H, J = 7.8), 7.62 (m, 2H), 7.81 (d, 1H, J = 2.4), 9.89 (s, 1H). ^{13}C NMR (DMSO-d_6): 20.5, 106.8, 112.8, 117.1, 118.2, 118.8, 123.0, 124.1, 130.2, 132.5, 135.0, 135.3, 153.9, 157.9, 162.5, 177.0. MS *m*/*z* (% rel int): 253 (18%), 252.0795 (M^+, 100, $C_{16}H_{12}O_3$ requires 352.0786), 251 (14%), 224 (18%), 135 (15%), 134 (37%). Anal. ($C_{16}H_{12}O_3$) C, H.

3′-Butoxy-6-methylflavone (17). K_2CO_3 (134 mg, 0.97 mmol) followed by 1-brombutane (1.0 mL, 8.33 mmol) was added to a solution of **16** (175 mg, 0.69 mmol) in DMF (6 mL) at room temperature. After 48 h, the mixture was extracted with diethyl ether and washed with brine, dried, and evaporated. The crude product was purified by chromatography (heptane:ethyl acetate 6:1) to give a yield of 88%; mp 87−90 °C. ^{1}H NMR ($CDCl_3$): 1.00 (t, 3H, J = 7.3), 1.53 (sext., 2H, J = 7), 1.82 (quint. 2H, J = 7), 2.47 (s, 3H), 4.04 (t, 2H, J = 6.5), 6.79 (s, 1H), 7.05 (dd, 1H, J = 8.1, 2.1), 7.47 (m, 5H), 8.01 (d, 1H, J = 2.1). ^{13}C NMR ($CDCl_3$): 14.2, 19.6, 21.2, 31.6, 68.4, 108.0, 112.7, 118.0, 118.3, 118.9, 123.6, 125.4, 130.4, 133.6, 135.4, 135.6, 154.9, 160.0, 163.6, 179.0. MS *m*/*z* (% rel int): 308.1412 (M^+, 100, $C_{20}H_{20}O_3$ requires 308.1412), 308 (60%), 252 (100%), 224 (15%), 134 (21%). Anal. ($C_{20}H_{20}O_3$) C, H.

3′-(2-Ethylbutyloxy)-6-methylflavone (18). A solution of 2-ethyl-1-butanol (15 mg, 0.149 mmol) and triphenylphosphine (39 mg, 0.149 mmol) in dry THF (5 mL) was added dropwise to a solution of diethyl azodicarboxylate (26 mg, 0.149 mmol) and **16** (25 mg, 0.099 mmol) in dry THF (1 mL) at room temperature under N_2. The mixture was stirred for 16 h before 20 mL of 1 M NaOH(aq) was added, and the mixture was extracted with diethyl ether. The organic phase was dried and evaporated. The crude product was purified by chromatography (heptane:ethyl acetate 6:1), and the product was obtained as white crystals in a yield of 45%; mp 93−95 °C. ^{1}H NMR ($CDCl_3$): 0.97 (t, 6H, J = 7.4), 1.52 (m, 4H), 1.73 (m, 1H), 2.48 (s, 3H), 3.94 (d, 2H, J = 5.7), 6.82 (s, 1H), 7.08 (dd, 1H, J = 8.1, 2.2), 7.40 (d, 1H, J = 7.8), 7.49 (m, 4H), 8.03 (d, 1H, J = 2.2). ^{13}C NMR ($CDCl_3$): 11.4, 21.1, 23.6, 41.1, 70.6, 107.8, 112.5, 117.9, 118.1, 118.7, 123.8, 125.3, 130.2, 133.4, 135.2, 135.4, 154.8, 160.0, 163.4, 178.8. MS *m*/*z* (% rel int): 336.1726 (M^+, 37, $C_{22}H_{24}O_3$ requires 336.1725), 253 (42%), 252 (100%), 224 (15%). Anal. ($C_{22}H_{24}O_3$) C, H.

2-Methoxy-5-methylbenzaldehyde (Leading to 19 and 20). K_2CO_3 (2.84 g, 20.6 mmol) and MeI (1.28 mL, 20.6 mmol) were added to a solution of 2-hydroxy-5-methylbenzaldehyde (2.0 g, 14.7 mmol) in DMF (100 mL). After it was stirred for 20 h at room temperature, 100 mL of 0.5 M NaOH(aq) was added to the solution and the reaction mixture was extracted with diethyl ether and washed with brine, dried, and evaporated. The product was obtained as a yellowish oil in 98% yield. Spectroscopic data were identical with those previously reported.[35]

2-Methoxy-5-methylbenzoic Acid (Leading to 19 and 20). A solution of 2-methoxy-5-methylbenzaldehyde (2.19 g, 14.6 mmol) in MeOH (125 mL) was warmed to 60 °C whereafter 2 M KOH(aq) (30 mL) followed by 35% H_2O_2(aq) (40 mL)

was added. Gas evolution was observed. After 2 h of reflux, the solution was cooled and the solvent was evaporated. The residue was extracted with diethyl ether. The aqueous phase was acidified with diluted HCl(aq) and extracted with dichloromethane, which was dried and evaporated to give 2-methoxy-5-methylbenzoic acid as white crystals in 88% yield. The spectroscopic data were identical with those previously reported.[36]

2-Acetyl-4-methylphenyl 2-Methoxy-5-methylbensoate (Scheme 1; I Leading to 19 and 20). The compound was prepared according to the procedure described for **I** leading to **7**. The crude product was purified by chromatography (heptane:ethyl acetate 5:1−2:1) to give white crystals in 66% yield; mp 74−76 °C. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 2.42 (s, 3H), 2.56 (s, 3H), 3.91 (s, 3H), 6.95 (d, 1H, J = 8.5), 7.12 (d, 1H, J = 8.2), 7.36 (m, 2H), 7.64 (d, 1H, J = 1.9), 7.89 (d, 1H, J = 2.4). ^{13}C NMR ($CDCl_3$): 20.5, 21.0, 30.1, 56.3, 112.5, 118.6, 123.9, 129.9, 130.5, 131.5, 132.9, 134.1, 135.3, 135.9, 147.5, 158.2, 164.7, 198.4. MS *m*/*z* (% rel int): 298.1207 (M^+, 65, $C_{18}H_{18}O_4$ requires 298.1205), 150 (100%), 149 (100%), 106 (72%), 91 (100%), 78 (59%).

1-(2-Hydroxy-5-methylphenyl)-3-(2-methoxy-5-methylphenyl)propane-1,3-dione (Scheme 1; II Leading to 19 and 20). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was recrystallized from acetone to give yellow crystals. The yield was 88%; mp 91−94 °C. In $CDCl_3$, the product is a 2.5:1 enol:keto mixture. Data are given for the major enol form. ^{1}H NMR ($CDCl_3$): 2.33 (s, 3H), 2.37 (s, 3H), 3.97 (s, 3H), 6.91 (d, 1H, J = 8.4), 6.93 (d, 1H, J = 8.4), 7.22 (s, 1H), 7.29 (m, 2H), 7.50 (brs, 1H), 7.76 (brs, 1H), 11.98 (s, 1H), 15.64 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.4, 20.6, 56.0, 97.5, 111.9, 118.4, 118.9, 122.3, 127.9, 128.4, 129.9, 130.2, 133.6, 136.5, 156.6, 160.3, 175.4, 195.8. MS *m*/*z* (% rel int): 298.1208 (M^+, 37, $C_{18}H_{18}O_4$ requires 298.1205), 267 (10%), 150 (10%), 149 (100%), 135 (8%).

2′-Methoxy-5′,6-dimethylflavone (19). The compound was prepared according to the procedure described for **3**. The crude product was purified by chromatography (heptane:ethyl acetate 3:1) and isolated in 98% yield as yellow crystals; mp 101−103 °C. ^{1}H NMR ($CDCl_3$): 2.38 (s, 3H), 2.46 (s, 3H), 3.90 (s, 3H), 6.93 (d, 1H, J = 8.5), 7.13 (s, 1H), 7.27 (dd, 1H, J = 8.4, 2.3), 7.44 (d, 1H, J = 8.4), 7.49 (dd, 1H, J = 8.4, 2.1), 7.69 (d, 1H, J = 2.1), 8.02 (d, 1H, J = 2.3). ^{13}C NMR ($CDCl_3$): 20.7, 21.1, 55.9, 112.0, 112.6, 118.0, 120.7, 123.6, 125.1, 129.7, 130.2, 133.0, 134.9, 135.0, 155.0, 156.2, 161.1, 179.2. MS *m*/*z* (% rel int): 281 (20%), 280.1093 (M^+, 100, $C_{18}H_{16}O_3$ requires 280.1099), 146 (34%), 145 (22%), 135 (100%). Anal. ($C_{18}H_{16}O_3$) C, H.

2′-Hydroxy-5′,6-dimethylflavone (20). The compound was prepared according to the procedure described for **15** leading to **16**. BBr_3, 3 equiv, was added. The reaction time was 20 h before addition of MeOH. The crude product was purified by recrystallization from acetone to give white crystals in a yield of 61%; mp 270−272 °C. ^{1}H NMR (DMSO-d_6): 2.29 (s, 3H), 2.43 (s, 3H), 6.96 (d, 1H, J = 8.2), 7.11 (s, 1H), 7.20 (dd, 1H, J = 8.2, 1.9), 7.63 (dd, 1H, J = 8.5, 1.8), 7.67 (d, 1H, J = 8.5), 7.71 (d, 1H, J = 1.9), 7.83 (d, 1H, J = 1.8), 10.49 (bs, 1H). ^{13}C NMR (DMSO-d_6): 20.1, 20.5, 110.9, 117.0, 117.4, 118.3, 122.9, 124.0, 128.1, 128.4, 133.2, 134.8, 135.1, 154.2, 154.4, 160.7, 177.2. MS *m*/*z* (% rel int): 267 (22%), 266.0945 (M^+, 100, $C_{17}H_{14}O_3$ requires 266.0943), 135 (28%), 134 (21%), 132 (12%). Anal. ($C_{17}H_{14}O_3$) C, H.

3-(Methoxycarbonyl)benzoic Acid (Leading to 21 and 22). Concentrated H_2SO_4 (0.5 mL) was added to a solution of isophthalic acid (2.00 g, 12.0 mmol) in MeOH (15 mL) and THF (45 mL). The mixture was heated to 65 °C. The reaction was followed on TLC (CH_2Cl_2:MeOH:acetic acid 1:1:0.01) giving a mixture of mono- and dimethylated acids. The solvents were evaporated after 1.5 h, and the crude product was purified by chromatography (pentane:diethyl ether 3:1 → 2:3) to give the product as white crystals in 42% yield. Spectroscopic data were identical with those previously reported.[37]

2-Acetyl-4-methylphenyl Methyl Isophthalate (Scheme 1; I Leading to 21 and 22). The compound was prepared according to the procedure described for **I** leading to **7**. The product was purified by flash chromatography (heptane:ethyl acetate 5:1) to give white crystals in a yield of 79%; mp 104−106 °C. ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H), 2.54 (s, 3H), 3.95 (s, 3H), 7.12 (d, 1H, J = 8.2), 7.40 (dd, 1H, J = 8.2, 2.0), 7.62 (t, 1H, J = 8), 7.68 (d, 1H, J = 2.0), 8.31 (d, 1H, J = 7.9), 8.39 (d, 1H, J = 7.8), 8.85 (t, 1H, J = 2). ^{13}C NMR ($CDCl_3$): 20.9, 29.5, 52.4, 123.6, 128.9, 129.9, 130.5, 130.8, 130.8, 131.9, 134.1, 134.6, 136.2, 146.9, 158.7, 164.6, 166.1, 197.4. MS *m*/*z* (% rel int): 312 (M^+, 9%), 163 (100%), 135 (16%).

Methyl 3-[3-(2-Hydroxy-5-methylphenyl)-3-oxopropanoyl]benzoate (Scheme 1; II Leading to 21 and 22). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was purified by chromatography (heptane:ethyl acetate 3:1) to give yellow crystals in a yield of 91%; mp 121−123 °C. Only the enol form was present in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 3.99 (s, 3H), 6.88 (s, 1H), 6.93 (d, 1H, J = 8.5), 7.31 (dd, 1H, J = 8.5, 2.1), 7.58 (d, 1H, J = 1.9), 7.59 (t, 1H, J = 8), 8.16 (d, 1H, J = 7.9), 8.21 (d, 1H, J = 7.8), 8.59 (t, 1H, J = 2), 11.84 (s, 1H), 15.59 (s, 1H). ^{13}C NMR ($CDCl_3$): 21.0, 52.9, 93.1, 118.9, 119.0, 128.3, 128.7, 128.7, 129.5, 131.3, 131.5, 133.4, 134.6, 137.7, 160.9, 166.8, 176.3, 196.4. MS *m*/*z* (% rel int): 312 (M^+, 43%), 163 (100%), 135 (48%), 134 (15%), 77 (20%).

Methyl 3′-(6-Methylflavone)carboxylate (21). The compound was prepared according to the procedure described for **3**. The product was recrystallized from acetone to give white crystals in a yield of 96%; mp 192−194 °C. ^{1}H NMR ($CDCl_3$): 2.48 (s, 3H), 3.99 (s, 3H), 6.87 (s, 1H), 7.52 (m, 2H), 7.61 (t, 1H, J = 8), 8.02 (d, 1H, J = 2.3), 8.10 (d, 1H, J = 7.8), 8.20 (d, 1H, J = 7.9), 8.60 (t, 1H, J = 2). ^{13}C NMR ($CDCl_3$): 21.4, 52.9, 108.4, 118.4, 124.0, 125.5, 127.8, 129.7, 130.7, 131.6, 132.7, 132.8, 135.6, 135.9, 154.9, 162.5, 166.6, 178.8. MS *m*/*z* (% rel int): 295 (19%), 294.0894 (M^+, 100%, $C_{18}H_{14}O_4$ requires 294.0892), 266 (29%), 263 (18%), 134 (43%), 106 (16%). Anal. ($C_{18}H_{14}O_4$) C, H.

3′-(6-Methylflavone)carboxylic Acid (22). LiOH (30.7 mg, 1.28 mmol) was added to a solution of **21** (179 mg, 0.609 mmol) in a mixture of THF:H_2O (1:1) (10 mL), and the reaction was stirred at room temperature for 16 h. The solution was acidified with 2 M HCl(aq), extracted with diethyl ether, and washed with brine. The organic phase was dried and evaporated. The crude product was purified by chromatography (ethyl acetate) and isolated as white crystals in 67% yield; mp 249−252 °C. ^{1}H NMR (CD_3OD): 2.35 (s, 3H), 6.77 (s, 1H), 7.41 (d, 1H, J = 8.5), 7.45 (dd, 1H, J = 8.5, 2.0), 7.50 (t, 1H, J = 8), 7.84 (d, 1H, J = 2.0), 8.01 (dd, 1H, J = 7.9, 1.9), 8.09 (dd, 1H, J = 7.8, 1.7), 8.50 (t, 1H, J = 2). ^{13}C NMR ($CDCl_3$): 20.5, 107.1, 117.8, 122.9, 124.5, 124.5, 127.4, 129.0, 130.1, 131.7, 132.6, 135.4, 135.5, 154.4, 162.8, 167.5, 179.1. MS *m*/*z* (% rel int): 280.0734 (M^+, 100%, $C_{17}H_{12}O_4$ requires 280.0736), 252 (20%), 134 (41%). Anal. ($C_{17}H_{12}O_4$) C, H.

3-(Isopropoxycarbonyl)benzoic Acid (Leading to 23). The compound was prepared according to the procedure described for 3-(methyl carboxy)benzoic acid from isophthalic acid. *i*-Propanol was used instead of MeOH. After 44 h, the mixture was washed with diethyl ether to remove the diester. The water phase was acidified with 2 M HCl(aq) and extracted with diethyl ether. The organic phase was washed with brine, dried, and evaporated. The crude product was purified from the diester and the starting material by chromatography (toluene:acetone 7:3) as white crystals in a yield of 47%; mp 131−134 °C. ^{1}H NMR ($CDCl_3$): 2.82 (d, 6H, J = 6.3), 5.31 (sept., 1H, J = 6.3), 7.59 (t, 1H, J = 8), 8.31 (dd, 2H, J = 7.8, 1.7), 8.39 (dd, 2H, J = 7.9, 1.9), 8.77 (t, 1H, J = 2). ^{13}C NMR ($CDCl_3$): 22.1, 69.3, 128.9, 129.8, 131.5, 131.8, 134.4, 134.9, 165.4, 171.6. MS *m*/*z* (% rel int): 208 (M^+, 4%), 167 (40%), 149 (100%), 121 (17%), 65 (23%), 43 (16%).

2-Acetyl-4-methylphenyl Isopropyl Isophthalate (Scheme 1; I Leading to 23). The compound was prepared according to the procedure described for **I** leading to **7**. The crude product was purified by chromatography (heptane:ethyl acetate 6:1) as a colorless oil in 60% yield. ^{1}H NMR ($CDCl_3$): 1.42 (d, 6H, J = 6.3), 2.46 (s, 3H), 2.56 (s, 3H), 5.32 (sept., 1H, J = 6.3), 7.15 (d, 1H, J = 8.2), 7.42 (dd, 1H, J = 8.2, 1.9), 7.63

(t, 1H, $J = 8$), 7.69 (d, 1H, $J = 1.9$), 8.34 (d, 1H, $J = 7.8$), 8.40 (d, 1H, $J = 7.8$), 8.86 (t, 1H, $J = 2$). ^{13}C NMR ($CDCl_3$): 21.1, 22.1, 29.8, 69.2, 123.8, 128.4, 129.0, 129.2, 130.0, 130.8, 131.5, 131.9, 134.4, 134.8, 136.4, 147.2, 164.9, 165.3, 197.8. MS *m/z* (% rel int): 340 (M^+, 8%), 281 (15%), 191 (100%), 149 (92%), 121 (22%), 104 (25%), 76 (28%), 43 (15%).

Isopropyl 3-[3-(2-Hydroxy-5-methylphenyl)-3-oxopropanoyl]benzoate (Scheme 1; II Leading to 23). The compound was prepared according to the procedure described for **II** leading to **3**. Purification was made by chromatography (heptane:ethyl acetate 6:1) to give red crystals in a yield of 84%; mp 88−90 °C. In $CDCl_3$, the product is a 9:1 enol:keto mixture. Data are given for the major enol form. ^{1}H NMR ($CDCl_3$)**:** 1.43 (d, 6H, $J = 6.3$), 2.36 (s, 3H), 5.32 (sept., 1H, $J = 6.3$), 6.88 (s, 1H), 6.92 (d, 1H, $J = 8.4$), 7.30 (d, 1H, $J = 8.4$), 7.56 (m, 2H), 8.14 (d, 1H, $J = 7.2$), 8.21 (d, 1H, $J = 7.3$), 8.59 (s, 1H), 11.80 (s, 1H), 15.62 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.8, 22.1, 22.1, 69.3, 92.8, 118.7, 118.8, 127.9, 128.0, 128.5, 129.1, 131.0, 131.8, 133.0, 134.3, 137.4, 160.7, 165.6, 176.3, 196.1. MS *m/z* (% rel int): 340 (M^+, 60%), 298 (17%), 281 (32%), 191 (48%), 149 (100%), 135 (52%), 134 (18%).

Isopropyl 3′-(6-Methylflavone)carboxylate (23). The compound was prepared according to the procedure described for **3**. The crude product was purified by chromatography (heptane:ethyl acetate 6:1) to give white crystals in a yield of 81%; mp 147−149 °C. ^{1}H NMR ($CDCl_3$): 1.42 (d, 6H, $J = 6.3$), 2.48 (s, 3H), 5.32 (sept. 1H, $J = 6.3$), 6.88 (s, 1H), 7.50 (d, 1H, $J = 8.3$), 7.54 (dd, 1H, $J = 8.3, 1.9$), 7.61 (t, 1H, $J = 8$), 8.03 (d, 1H, $J = 1.9$), 8.10 (dd, 1H, $J = 7.9, 1.9$), 8.20 (dd, 1H, $J = 7.8, 1.8$), 8.58 (t, 1H, $J = 2$). ^{13}C NMR ($CDCl_3$): 21.2, 22.1, 22.1, 69.3, 108.1, 108.2, 118.1, 123.8, 125.3, 127.5, 129.3, 130.3, 132.1, 132.4, 135.4, 135.6, 154.7, 162.4, 165.4, 178.7. MS *m/z* (% rel int): *m/z* 323 (22%), 322.1206 (M^+, 100, $C_{20}H_{18}O_4$ requires 322.1205), 280 (33%), 263 (47%), 236 (18%), 134 (23%). Anal. ($C_{20}H_{18}O_4$) C, H.

2-Ethylbutyl 3′-(6-Methylflavone)carboxylate (24). The compound was prepared according to the procedure described for **18**, from **22**. The crude product was purified twice by chromatography (heptane:ethyl acetate 4:1) to give white crystals in a yield of 58%; mp 82−84 °C. ^{1}H NMR ($CDCl_3$): 0.99 (t, 6H, $J = 7.4$), 1.50 (m, 4H), 1.73 (m, 1H), 2.49 (s, 3H), 4.33 (d, 2H, $J = 5.8$) 6.87 (s, 1H), 7.50 (d, 1H, $J = 8.5$), 7.55 (dd, 1H, $J = 8.5, 2.1$), 7.63 (t, 1H, $J = 8$), 8.04 (d, 1H, $J = 2.1$), 8.11 (dd, 1H, $J = 7.9, 1.9$), 8.21 (dd, 1H, $J = 7.8, 1.8$), 8.61 (t, 1H, $J = 2$). ^{13}C NMR ($CDCl_3$): 11.4, 11.4, 21.2, 23.8, 23.8, 40.7, 67.8, 108.1, 118.1, 123.8, 125.3, 127.6, 129.4, 130.4, 131.8, 132.5, 132.6, 135.4, 135.7, 154.7, 162.4, 166.1, 178. MS *m/z* (% rel int): 364.1682 (M^+, 71, $C_{23}H_{24}O_4$ requires 364.1675), 281 (76%), 280 (100%), 263 (75%). Anal. ($C_{23}H_{24}O_4$) C, H.

Dimethyl 5-Methoxyisophthalate (Leading to 25). 5-Hydroxy-isophthalic acid (2 g, 10.98 mmol), methyl iodide (7.79 g, 54.9 mmol), and potassium carbonate (6.07 g, 43.9 mmol) were dissolved in 100 mL of *N*,*N*-dimethylformamide. The mixture was heated to 40 °C, and after 7 h, more methyl iodide (4.68 g, 32.9 mmol) was added. After it was stirred for 14 h, 200 mL of water was added and the mixture was extracted three times with 100 mL of diethyl ether. The organic phase was washed with 50 mL of 0.1 M NaOH(aq) followed by 50 mL of water, dried, and evaporated to give white crystals (yield 92%). Spectroscopic data were identical with those previously reported.[38]

3-Methoxy-5-(methoxycarbonyl)benzoic Acid (Leading to 25). The compound was prepared according to the procedure described for **22**. The reaction was stirred at room temperature for 2.5 h until TLC showed that diacid was formed. The product was purified by chromatography (heptane: ethyl acetate 4:1 with 1% acetic acid) and obtained as an oil (yield 33%, 62% starting material was recovered). ^{1}H NMR ($CDCl_3$ + 5%CD_3OD): 3.86 (s, 3H), 3.91 (s, 3H), 7.72 (dd, 1H, $J = 2.7, 1.5$), 7.74 (dd, 1H, $J = 2.7, 1.5$), 8.27 (dd, 1H, $J = 1.5, 1.5$). ^{13}C NMR ($CDCl_3$ + 5%CD_3OD): 52.5, 55.9, 119.5, 119.7, 123.5, 123.5, 131.8, 159.8, 166.6, 167.9. MS *m/z* (% rel int): 194 (M^+, 5%), 149 (100%), 191 (33%).

2-Acetyl-4-methylphenyl Methyl 5-Methoxyisophthalate (Scheme 1; I Leading to 25). The compound was prepared according to the procedure described for **I** leading to **7**. The compound was purified by chromatography (heptane: ethyl acetate 4:1) to give white crystals (yield 73%); mp 89−91 °C. ^{1}H NMR ($CDCl_3$): 2.44 (s, 3H), 2.54 (s, 3H), 3.93 (s, 3H), 3.96 (s, 3H), 7.13 (d, 1H, $J = 8.2$), 7.41 (dd, 1H, $J = 8.2, 2.2$), 7.67 (d, 1H, $J = 2.2$), 7.84 (d, 1H, $J = 2.7, 1.4$), 7.90 (dd, 1H, $J = 2.7, 1.5$), 8.46 (dd, 1H, $J = 1.5, 1.4$). ^{13}C NMR ($CDCl_3$): 21.1, 29.8, 52.7, 56.1, 120.0, 120.3, 123.7, 123.8, 130.8, 131.0, 131.3, 132.3, 134.3, 136.4, 147.2, 160.1, 164.7, 166.2, 197.7. MS *m/z* (% rel int): 342 (M^+, 10%), 194 (11%), 193 (100%), 165 (13%), 150 (10%).

Methyl 3-[3-(2-Hydroxy-5-methylphenyl)-3-oxopropanoyl]-5-methoxybenzoate (Scheme 1; II Leading to 25). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was purified by chromatography (heptane:ethyl acetate 6:1 → 4:1) to give yellow crystals in a yield of 67%; mp 145−147 °C. Only the enol form was present in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 3.94 (s, 3H), 3.99 (s, 3H), 6.87 (s, 1H), 6.93 (d, 1H, $J = 8.5$), 7.31 (dd, 1H, $J = 8.5, 2.1$), 7.59 (d, 1H, $J = 2.1$), 7.69 (dd, 1H, $J = 2.6, 1.6$), 7.74 (dd, 1H, $J = 2.6, 1.4$), 8.18 (dd, 1H, $J = 1.6, 1.4$), 11.81 (s, 1H), 15.62 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.8, 52.8, 56.1, 93.1, 117.4, 118.0, 118.7, 118.8, 120.4, 128.5, 128.6, 132.2, 135.7, 137.5, 160.2, 160.7, 166.5, 176.0, 196.1. MS *m/z* (% rel int): 342 (M^+, 42%), 193 (100%), 135 (26%).

Methyl 3′-(5′-Methoxy-6-methylflavone)carboxylate (25). The compound was prepared according to the procedure described for **3**. The crude product was purified by chromatography (heptane:ethyl acetate 1:1 → 1:3) to give white crystals in a yield of 93%; mp 193−195 °C. ^{1}H NMR ($CDCl_3$): 2.49 (s, 3H), 3.95 (s, 3H), 3.99 (s, 3H), 6.86 (s, 1H), 7.50 (d, 1H, $J = 8.5$), 7.52 (dd, 1H, $J = 8.5, 2.0$), 7.60 (dd, 1H, $J = 2.3, 1.7$), 7.70 (dd, 1H, $J = 2.5, 1.3$), 8.01 (d, 1H, $J = 2.0$), 8.17 (dd, 1H, $J = 1.7, 1.3$). ^{13}C NMR ($CDCl_3$): 21.2, 52.8, 56.0, 108.4, 117.0, 117.1, 118.2, 120.0, 123.9, 125.3, 132.6, 133.8, 135.4, 135.7, 154.7, 160.3, 162.2, 166.3, 178.6. MS *m/z* (% rel int): 324.1004 (M^+, 100, $C_{19}H_{16}O_5$ requires 324.0998), 325 (21%), 293 (18%), 134 (27%). Anal. ($C_{19}H_{16}O_5$) C, H.

Methyl 3′-(5′-Hydroxy-6-methylflavone)carboxylate (26). The compound was prepared according to the procedure described for **14**. BBr_3, 2 equiv, was added and after 2 h, an additional 2 equiv of BBr_3 were added. After 6 more hours, the reaction was quenched with 15 mL of water. The compound was purified by chromatography (toluene:acetone 9:1) to give white crystals (yield 52%); mp 255−257 °C. ^{1}H NMR ($CDCl_3$ + 10%CD_3OD): 2.39 (s, 3H), 3.87 (s, 3H), 6.74 (s, 1H) 7.47 (m, 3H), 7.54 (s, 1H), 7.88 (s, 1H), 8.02 (s, 1H). ^{13}C NMR ($CDCl_3$ + 10%CD_3OD): 20.9, 52.5, 107.3, 117.5, 118.1, 118.6, 119.7, 123.3, 124.8, 132.2, 133.1, 135.7, 135.8, 154.7, 158.0, 163.1, 166.7, 179.5. MS *m/z* (% rel int): 310.0843 (M^+, 100, $C_{18}H_{14}O_5$ requires 310.0841), 282 (21%), 279 (19%), 134 (38%). Anal. ($C_{18}H_{14}O_5$) C, H.

3′-(5′-Methoxy-6-methylflavone)carboxylic Acid (27). The compound was prepared from **25** according to the procedure described for **22**. The crude product was purified by chromatography (ethyl acetate with 1% acetic acid) to give white-yellow crystals (yield 46%); mp 250−252 °C. ^{1}H NMR (CD_3OD): 2.46 (s, 3H), 3.91 (s, 3H), 6.87 (s, 1H), 7.61 (m, 3H), 7.72 (s, 1H), 7.89 (s, 1H), 8.19 (s, 1H). ^{13}C NMR (CD_3OD): 21.1, 56.3, 108.1, 115.7, 119.0, 119.5, 121.0, 124.4, 125.6, 125.7, 133.8, 137.1, 137.3, 156.2, 161.7, 165.1, 165.4, 180.7. MS *m/z* (% rel int): 310.0840 (M^+, 100, $C_{18}H_{14}O_5$ requires 310.0841), 282 (28%), 134 (51%), 106 (22%), 105 (20%), 78 (22%). Anal. ($C_{18}H_{14}O_5$) C, H.

3′-(5′-Hydroxy-6-methylflavone)carboxylic Acid (28). The compound was prepared from **26** according to the procedure described for **22**. The crude product was purified by flash chromatography (heptane:ethyl acetate 1:1, with 1% acetic acid) to give pure product (yield 94%); mp 320−322 °C. ^{1}H NMR (DMSO-d_6): 2.44 (s, 3H), 6.92 (s, 1H), 7.55 (dd, 1H, $J = 2.1, 1.6$), 7.66 (m, 2H), 7.71 (d, 1H, $J = 8.5$), 7.85 (s, 1H), 8.0 (t, 1H, $J = 2$). ^{13}C NMR (DMSO-d_6): 20.4, 107.3, 117.2, 117.7,

118.3, 118.9, 123.0, 124.1, 132.9, 133.0, 135.2, 135.4, 153.9, 158.1, 161.7, 166.6, 177.0. MS *m/z* (% rel int): 296.0685 (M^+, 100, $C_{17}H_{12}O_5$ requires 296.0685), 297 (20%), 268 (22%), 134 (38%). Anal. ($C_{17}H_{12}O_5$) C, H.

5′-Hydroxymethyl-3′-methoxy-6-methylflavone (29). $LiAlH_4$ (9.83 mg, 0.26 mmol) was suspended in 5 mL of anhydrous THF and cooled to 0 °C. 5′-Methoxy-6-methyl-3′-methylcarboxyflavone (84 mg, 0.26 mmol) dissolved in 15 mL of anhydrous THF was added dropwise to the suspension, and the mixture was allowed to reach room temperature. After 4 and 15 h, more $LiAlH_4$ (9.8 mg, 0.26 mmol and 20 mg, 0.53 mmol) was added. After the last addition of $LiAlH_4$, the solution was stirred for 4 h, and then, 10 mL of 0.1 M NaOH(aq) was added and the mixture was extracted four times with 30 mL of diethyl ether. The organic phase was washed with 20 mL of 0.1 M hydrochloric acid(aq). The compound was purified by chromatography (heptane:ethyl acetate 1:1) to give white-yellow crystals (yield 27%); mp 170−172 °C. ^{1}H NMR ($CDCl_3$ + 10%CD_3OD): 2.43 (s, 3H), 2.89 (bs, 1H), 3.84 (s, 3H), 4.67 (s, 2H), 6.74 (s, 1H), 7.04 (dd, 1H, J = 2.4, 1.6), 7.28 (d, 1H, J = 2.5), 7.43 (d, 1H, J = 8.6), 7.46 (dd, 1H, J = 1.6, 1.4), 7.49 (dd, 1H, J = 8.6, 2.5), 7.92 (dd, 1H, J = 2.4, 1.4). ^{13}C NMR ($CDCl_3$ + 10%CD_3OD): 21.0, 55.6, 64.3, 107.4, 111.0, 115.5, 117.2, 118.0, 123.5, 125.0, 133.1, 135.4, 135.6, 144.2, 154.7, 160.3, 163.7, 179.3. MS *m/z* (% rel int): 296.1051 (M^+, 100, $C_{18}H_{16}O_4$ requires 296.1049), 297 (20%), 135 (16%), 134 (30%). Anal. ($C_{18}H_{16}O_4$) C, H.

Trimethyl 1,3,5-Benzenetricarboxylate (Leading to 30). The compound was prepared from 1,3,5-tribenzoic acid according to the procedure described for 5-methoxy-1,3-dimethylbenzdioat. The product was isolated as white crystals (yield 84%). Spectroscopic data were identical with those previously reported.[39]

3,5-Bis(methoxycarbonyl)benzoic Acid (Leading to 30). The compound was prepared according to the procedure described for **22**. The reaction was stirred at room temperature for 2.5 h until TLC indicated that the triacid had been formed. The product was purified by chromatography (heptane:ethyl acetate, 2:1, with 1% acetic acid) to give white crystals (yield 49% + 46% starting material). Spectroscopic data were identical with those previously reported.[40]

1-(2-Acetyl-4-methylphenyl) 3,5-Dimethyl Benzene-1,3,5-tricarboxylate (Scheme 1; I Leading to 30). The compound was prepared according to the procedure described for **I** leading to **7**. The crude product was purified by chromatography (heptane:ethyl acetate 3:1) to give white crystals (yield 58%); mp 130−132 °C. ^{1}H NMR ($CDCl_3$): 2.45 (s, 3H), 2.54 (s, 3H), 4.00 (s, 6H), 7.13 (d, 1H, J = 8.2), 7.41 (dd, 1H, J = 8.2, 1.8,), 7.68 (d, 1H, J = 1.8), 8.94 (t, 1H, J = 1.6), 9.02 (d, 2H, J = 1.6). ^{13}C NMR ($CDCl_3$): 21.1, 29.5, 52.9, 52.9, 123.7, 130.4, 130.9, 131.2, 131.2, 131.7, 134.4, 135.4, 135.4, 135.4, 136.6, 147.0, 164.1, 165.5, 165.5, 197.6. MS *m/z* (% rel int): 370 (M^+, 10%), 339 (7%), 222 (12%), 221 (100%), 193 (9%).

Dimethyl 5-[3-(2-Hydroxy-5-methylphenyl)-3-oxopropanoyl]isophthalate (Scheme 1; II Leading to 30). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was purified by chromatography (heptane:ethyl acetate 4:1) to give yellow crystals (yield 59%); mp 182−184 °C. Only the enol form was present in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.38 (s, 3H), 4.02 (s, 6H), 6.90 (s, 1H), 6.92 (d, 1H, J = 8.5), 7.31 (dd, 1H, J = 8.5, 2.1), 7.59 (d, 1H, J = 2.1), 8.75 (d, 2H, J = 1.5), 8.82 (t, 1H, J = 1.5), 11.76 (s, 1H), 15.60 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.7, 52.9, 52.9, 93.4, 118.6, 118.9, 128.6, 128.7, 131.7, 131.9, 131.9, 133.8, 135.0, 135.0, 137.7, 160.8, 165.8, 165.8, 174.7, 196.4. MS *m/z* (% rel int): 370 (M^+, 46%), 221 (100%), 193 (15%), 135 (27%), 134 (22%).

Dimethyl 3′,5′-(6-Methylflavone)dicarboxylate (30). The compound was prepared according to the procedure described for **3**. The crude product was recrystallized from acetone to give white crystals (yield 85%); mp 204−206 °C. ^{1}H NMR ($CDCl_3$ + 10%CD_3OD): 2.50 (s, 3H), 4.03 (s, 6H), 6.95 (s, 1H), 7.57 (m, 2H), 8.04 (bs, 1H), 8.78 (d, 2H, J = 1.5), 8.83 (t, 1H, J = 1.5). ^{13}C NMR ($CDCl_3$ + 10%CD_3OD): 21.0, 52.9, 52.9, 108.4, 118.2, 123.5, 125.1, 131.3, 131.3, 131.8, 133.0, 133.1, 135.7, 135.7, 136.0, 154.7, 161.5, 165.6, 165.6, 178.8. MS *m/z* (% rel int): 352.0946 (M^+, 100, $C_{20}H_{16}O_6$ requires 352.0947), 324 (18%) 321 (23%), 134 (35%). Anal. ($C_{20}H_{16}O_6$) C, H.

3′,5′-(6-Methylflavone)dicarboxylic Acid (31). The compound was prepared from **30** according to the procedure described for **22**. The product was purified by chromatography (THF:CH_2Cl_2 2:3, with 1% acetic acid) to give white crystals (yield 80%); mp 332−334 °C. ^{1}H NMR (DMSO-d_6): 2.45 (s, 3H), 3.33 (bs, 2H), 7.11 (s, 1H), 7.68 (dd, 1H, J = 8.6, 1.9), 7.78 (d, 1H, J = 8.6), 7.87 (d, 1H, J = 1.9), 8.62 (t, 1H, J = 1.5), 8.72 (d, 2H, J = 1.5). ^{13}C NMR (DMSO-d_6): 20.9, 108.6, 118.9, 123.5, 124.6, 124.6, 130.9, 131.1, 131.1, 132.8, 133.0, 135.8, 136.0, 154.5, 161.3, 166.4, 166.4, 177.4. MS *m/z* (% rel int): 324.0632 (M^+, 100, $C_{18}H_{12}O_6$ requires 324.0634), 325 (21%), 296 (31%), 134 (59%). Anal. ($C_{18}H_{12}O_6$) C, H.

Methyl 3′-(5′-Hydroxymethyl-6-methylflavone)carboxylate (32) and 3′,5′-Bis(hydroxymethyl)-6-methylflavone (33). The compounds were prepared from **30** according to the procedure described for **29**. The compounds were separated and purified by chromatography (heptane:ethyl acetate 1:9) to give light yellow crystals yield (22% monool and 19% diol). **32**: mp 202−204 °C. ^{1}H NMR ($CDCl_3$ + 5%CD_3OD): 2.42 (s, 3H), 3.44 (bs, 1H), 3.92 (s, 3H), 4.73 (s, 2H), 6.83 (s, 1H), 7.51 (m, 2H), 7.92 (d, 1H, J = 1.9), 8.10 (m, 2H), 8.44 (t, 1H, J = 1.7). ^{13}C NMR ($CDCl_3$ + 10%CD_3OD): 20.9, 52.5, 63.5, 107.7, 118.1, 123.4, 125.0, 126.3, 128.8, 130.7, 131.3, 132.4, 135.7, 135.8, 143.4, 154.7, 162.8, 166.6, 179.2. MS *m/z* (% rel int): 324.1000 (M^+, 100, $C_{19}H_{16}O_5$ requires 324.0998), 325 (21%), 293 (18%), 135 (16%), 134 (49%). Anal. ($C_{19}H_{16}O_5$) C, H. **33**: mp 203−205 °C. ^{1}H NMR ($CDCl_3$ + 5%CD_3OD): 2.41 (s, 3H), 3.50 (bs, 2H), 4.66 (s, 4H), 6.76 (s, 1H), 7.47 (m, 3H), 7.77 (bs, 2H), 7.91 (m, 1H). ^{13}C NMR ($CDCl_3$ + 10%CD_3OD): 20.9, 64.1, 64.1, 107.1, 118.0, 123.4, 123.7, 123.7, 124.9, 128.6, 131.9, 135.5, 135.6 142.8, 142.8, 154.8, 164.1, 179.5. MS *m/z* (% rel int): 296.1041 (M^+, 100, $C_{18}H_{16}O_4$ requires 296.1049), 294 (27%), 135 (41%), 134 (62%). Anal. ($C_{18}H_{16}O_4$) C, H.

3-(Methoxycarbonyl)-5-nitrobenzoic Acid (Leading to 34 and 35). The compound was prepared according to the procedure described for isophthalic acid leading to **21**. The product was purified by chromatography (pentane:diethyl ether 3:1 → 2:3) to give the product in 29% yield; mp 167−169 °C. ^{1}H NMR (CD_3OD): 4.00 (s, 3H), 8.76 (s, 1H), 8.91 (s, 1H), 8.92 (s, 1H). ^{13}C NMR (CD_3OD): 52.5, 127.6, 128.0, 132.7, 133.7, 135.5, 148.9, 164.7, 165.4. MS *m/z* (% rel int): 225 (M^+, 21%), 195 (34%), 194 (100%), 148 (31%), 120 (24%), 75 (31%), 74 (21%).

2-Acetyl-4-methylphenyl Methyl 5-Nitroisophthalate (Scheme 1; I Leading to 34 and 35). The compound was prepared according to the procedure described for **I** leading to **7**. The product was purified by flash chromatography (heptane:ethyl acetate 5:1) to give white crystals in a yield of 59%; mp 112−114 °C. ^{1}H NMR ($CDCl_3$): 2.44 (s, 3H), 2.53 (s, 3H), 4.01 (s, 3H), 7.13 (d, 1H, J = 8.2), 7.42 (dd, 1H, J = 8.2, 2.2), 7.70 (d, 1H, J = 2.2), 9.07 (dd, 1H, J = 2.2, 1.6), 9.11 (dd, 1H, J = 1.6, 1.6), 9.16 (dd, 1H, J = 2.2, 1.6). ^{13}C NMR ($CDCl_3$): 21.0, 29.0, 53.2, 123.7, 128.7, 128.9, 129.5, 131.5, 132.2, 132.8, 134.6, 136.5, 136.8, 146.6, 148.6, 162.9, 164.2, 197.4. MS *m/z* (% rel int): 357 (M^+, 20%), 208 (100%), 162 (22%), 134 (21%), 75 (15%).

Methyl 3-[3-(2-Hydroxy-5-methylphenyl)-3-oxopropanoyl]-5-nitrobenzoate (Scheme 1; II Leading to 34 and 35). The compound was prepared according to the procedure described for **II** leading to **3** and purified by recrystallization from EtOH two times to give yellow needles in a yield of 64%; mp 173−175 °C. In DMSO, the product is a 9:1 enol:keto mixture. Data are given for the major enol form. ^{1}H NMR (DMSO-d_6): 2.32 (s, 3H), 3.96 (3H), 7.08 (d, 1H, J = 8.3), 7.46 (dd, 1H, J = 8.3, 2.1), 7.61 (s, 1H), 8.04 (d, 1H, J = 2.1), 8.62 (brs, 1H), 8.67 (bs, 1H), 8.71 (bs, 1H), 11.97 (s, 1H), 15.61 (s, 1H). ^{13}C NMR (DMSO-d_6): 20.0, 53.0, 101.1, 118.4, 120.3, 123.9, 124.9, 125.4, 130.9, 131.4, 132.2, 137.0, 145.6, 148.0, 148.0, 155.7, 164.4, 190.4. MS *m/z* (% rel int): 358 (17%), 357

(M^+, 84%), 340 (23%), 208 (84%), 177 (18%), 162 (20%), 135 (72%), 134 (100%), 106 (16%), 77 (19%).

Methyl 3′-(6-Methyl-5′-nitroflavone)carboxylate (34). The compound was prepared according to the procedure described for **3**. The product was recrystallized from acetone to give white crystals in a yield of 95%; mp 210−212 °C. ^{1}H NMR (CD_3OD): 2.28 (s, 3H), 3.85 (s, 3H), 6.79 (s, 1H), 7.40 (d, 1H, J = 8.6), 7.42 (dd, 1H, J = 8.6, 2.0), 7.75 (d, 1H, J = 2.0), 8.68 (t, 1H, J = 2), 8.75 (t, 1H, J = 2), 8.77 (t, 1H, J = 2). ^{13}C NMR (CD_3OD): 20.5, 52.9, 108.5, 118.0, 123.0, 124.6, 124.7, 126.4, 132.2, 132.8, 134.0, 136.0, 136.2, 148.8, 154.4, 159.9, 164.2, 178.7. MS *m*/*z* (% rel int): 340 (22%), 339.0749 (M^+, 100, $C_{18}H_{13}N_1O_6$ requires 339.0743), 311 (29%), 134 (45%), 106 (25%), 105 (16%), 78 (22%). Anal. ($C_{18}H_{13}NO_6$) C, H, N.

3′-(6-Methyl-5′-nitroflavone)carboxylic Acid (35). The compound was prepared according to the procedure described for **22**. The crystals that precipitated by acidification were filtered off, washed with water, and dried. The product was obtained as white crystals in a yield of 94%; mp 310−313 °C. ^{1}H NMR (DMSO-d_6): 2.44 (s, 3H), 7.24 (s, 1H), 7.65 (dd, 1H, J = 8.5, 2.0), 7.75 (d, 1H, J = 8.5), 7.83 (brs, 1H), 8.73 (brs, 1H), 8.82 (brs, 1H), 8.96 (brs, 1H). ^{13}C NMR (DMSO-d_6): 20.5, 108.9, 118.6, 123.1, 124.1, 124.7, 126.0, 132.3, 133.7, 134.0, 135.6, 135.8, 148.7, 154.0, 159.4, 165.0, 177.1. MS *m*/*z* (% rel int): 325.0594 (M^+, 100, $C_{17}H_{11}NO_6$ requires 325.0586), 297 (15%), 134 (30%). Anal. ($C_{17}H_{11}NO_6$) C, H, N.

Methyl 3′-(5′-Amino-6-methylflavone)carboxylate (36) and Ethyl 3′-(5′-Amino-6-methylflavone)carboxylate (37). The compounds were prepared according to the procedure described for **10**. The compounds were separated and purified by chromatography on silica gel, with petroleum ether:ethyl acetate (1:1) and with toluene:acetone (2:1) to give both the methyl ester **36** (33%) and the ethyl ester **37** (23%). Compound **36** was obtained as an oil. ^{1}H NMR ($CDCl_3$): 2.48 (s, 3H), 3.96 (s, 3H), 4.01 (bs, 2H), 6.81 (s, 1H), 7.38 (dd, 1H, J = 1.8, 1.8), 7.50 (m, 2H), 7.53 (dd, 1H, J = 8.6, 2.0), 7.97 (dd, 1H, J = 1.8, 1.5), 8.02 (brs, 1H). ^{13}C NMR ($CDCl_3$): 21.2, 52.6, 108.1, 116.3, 117.8, 118.1, 118.7, 123.9, 125.3, 132.3, 133.5, 135.3, 135.6, 147.4, 154.7, 162.8, 166.7, 178.7. MS (70 eV): *m*/*z* 310 (21%), 309.0995 (M^+, 100, $C_{18}H_{15}NO_4$ requires 309.1001), 278 (12%), 175 (13%). Anal. ($C_{18}H_{15}NO_4$) C, H, N. Compound **37** was obtained as an oil. ^{1}H NMR ($CDCl_3$): 1.42 (t, 3H, J = 7.0), 2.48 (s, 3H), 4.03 (bs, 2H), 4.42 (q, 2H, J = 7.0), 6.80 (s, 1H), 7.36 (dd, 1H, J = 1.8, 1.8), 7.49 (m, 2H), 7.52 (dd, 1H, J = 8.6, 2.0), 7.97 (dd, 1H, J = 1.8, 1.5), 8.03 (brs, 1H). ^{13}C NMR ($CDCl_3$): 14.6, 21.2, 61.6, 108.1, 116.2, 117.7, 118.1, 118.7, 123.9, 125.3, 132.7, 133.5, 135.3, 135.6, 147.3, 154.7, 162.8, 166.2, 178.8. MS (70 eV): *m*/*z* 324 (22%), 323.1145 (M^+, 100, $C_{19}H_{17}NO_4$ requires 323.1158), 309 (26%), 278 (19%), 251 (24%), 250 (17%). Anal. ($C_{19}H_{17}NO_4$) C, H, N.

2-Acetyl-4-methylphenyl 3-(Trifluoromethyl)bensoate (Scheme 1; I Leading to 38). The compound was prepared according to the procedure described for **I** leading to **3**. The acid chloride was commercially available. The crude product was purified by chromatography (heptane:ethyl acetate 5:1) to give white crystals (yield 94%); mp 71−73 °C.^{1}H NMR ($CDCl_3$): 2.45 (s, 3H), 2.55 (s, 3H), 7.13 (d, 1H, J = 8.2), 7.42 (dd, 1H, J = 8.2, 2.2), 7.68 (m, 2H), 7.91 (d, 1H, J = 7.9), 8.41 (d, 1H, J = 7.9), 8.48 (brs, 1H). ^{13}C NMR ($CDCl_3$): 21.1, 29.5, 123.8, 123.8 (quart, J = 271), 127.4 (quart, J = 4), 129.6, 130.3 (quart, J = 4), 130.7, 131.1, 131.6 (quart, J = 33), 133.7, 134.4, 134.4, 136.5, 147.1, 164.4, 197.6. MS *m*/*z* (% rel int): 322 (M^+, 22%), 173 (100%), 145 (30%), 135 (9%).

1-(2-Hydroxy-5-methylphenyl)-3-(3-trifluoromethylphenyl)propane-1,3-dione (Scheme 1; II Leading to 38). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was purified by chromatography (heptane:ethyl acetate 10:1) to give yellow crystals in a yield of 67%; mp 87−89 °C. Only the enol form was present in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 6.86 (s, 1H), 6.94 (d, 1H, J = 8.4), 7.33 (dd, 1H, J = 8.4, 1.8), 7.57 (d, 1H, J = 1.8), 7.65 (dd, 1H, J = 7.9, 7.8), 7.82 (d, 1H, J = 7.8), 8.14 (d, 1H, J = 7.9), 8.20 (brs, 1H), 11.79 (s, 1H), 15.58 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.7, 93.1, 118.6, 118.9, 124.0 (quart J = 271), 123.8 (quart, J = 4), 128.5, 128.6, 128.8 (quart, J = 4), 129.6, 130.1, 131.6 (quart, J = 33), 134.9, 137.6, 160.8, 175.3, 196.3. MS *m*/*z* (% rel int): 322 (M^+, 72%), 173 (100%), 145 (30%), 135 (30%), 134 (35%).

3′-Trifluoromethyl-6-methylflavone (38). The compound was prepared according to the procedure described for **3**. The compound was purified by flash chromatography (heptane: ethyl acetate 8:1) to give light-yellow crystals (yield 87%); mp 147−149 °C. ^{1}H NMR ($CDCl_3$): 2.51 (s, 3H), 6.85 (s, 1H), 7.53 (m, 2H), 7.67 (dd, 1H, J = 7.9, 7.8), 7.80 (d, 1H, J = 7.8), 8.02 (brs, 1H), 8.09 (d, 1H, J = 7.9), 8.19 (bs, 1H). ^{13}C NMR ($CDCl_3$): 21.2, 108.5, 118.1, 123.3 (quart J = 4), 123.9, 123.9 (quart J = 271), 125.4, 128.2 (quart J = 4), 129.6, 129.9, 132.0 (quart J = 33), 133.2, 135.5, 135.8, 154.7, 161.7, 178.5. MS *m*/*z* (% rel int): 304.0711(M^+, 100, $C_{17}F_3H_{11}O_2$ requires 304.0711), 305 (18%), 276 (29%), 134 (52%). Anal. ($C_{17}F_3H_{11}O_2$) C, H.

2-Acetyl-4-methylphenyl 3-Bromobensoate (Scheme 1; I Leading to 39). The compound was prepared according to the procedure described for **I** leading to **3**. The acid chloride was (purchased from Aldrich) commercially available. The product was purified by chromatography (heptane:ethyl acetate 6:1) to give white crystals (yield 89%); mp 71−73 °C.^{1}H NMR ($CDCl_3$): 2.44 (s, 3H), 2.54 (s, 3H), 7.11 (d, 1H, J = 8.2), 7.41 (m, 2H), 7.67 (d, 1H, J = 2.2), 7.78 (ddd, 1H, J = 8.0, 2.0, 1.0), 8.15 (ddd, 1H, J = 7.8, 1.7, 1.0), 8.35 (dd, 1H, J = 2.0, 1.7). ^{13}C NMR ($CDCl_3$): 21.1, 29.7, 122.9, 123.7, 129.0, 130.4, 130.7, 131.0, 131.6, 133.4, 134.3, 136.4, 136.8, 147.1, 164.3, 197.7. MS *m*/*z* (% rel int): 332 (M^+, 20%), 334 (20%), 185 (96%), 183 (100%), 157 (21%), 155 (21%).

1-(2-Hydroxy-5-methylphenyl)-3-(3-bromophenyl)propane-1,3-dione (Scheme 1; II Leading to 39). The compound was prepared according to the procedure described for **II** leading to **3**. The crude product was purified by chromatography (heptane:ethyl acetate 12:1) to give yellow crystals (yield 69%); mp 122−124 °C. The product was present as the enol form in $CDCl_3$. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 6.80 (s, 1H), 6.93 (d, 1H, J = 8.5), 7.32 (dd, 1H, J = 8.5, 2.2), 7.39 (t, 1H, J = 8), 7.56 (d, 1H, J = 2.2), 7.69 (ddd, 1H, J = 8.0, 2.0, 1.1), 7.89 (ddd, 1H, J = 7.9, 1.7, 1.1), 8.09 (dd, 1H, J = 2.0, 1.7), 11.83 (s, 1H), 15.53 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.8, 93.0, 118.7, 118.9, 123.2, 125.6, 125.7, 128.5, 129.9, 130.5, 135.3, 136.0, 137.5, 160.7, 175.5, 196.1. MS *m*/*z* (% rel int): 332 (M^+, 66%), 334 (66%), 185 (98%), 183 (100%), 135 (51%), 134 (38%).

3′-Bromo-6-methylflavone (39). The compound was prepared according to the procedure described for **3**. The crude product was purified by chromatography (heptane:ethyl acetate 5:1) to give white crystals (yield 75%). Spectroscopic data were identical with those previously reported.[25] Anal. ($C_{16}H_{11}BrO_2$) C, H.

2-Acetyl-4-methylphenyl (5-Bromo-2-methoxy)bensoate (Scheme 1; I Leading to 40 and 41). The compound was prepared according to the procedure described for **I** leading to **7**. The crystals were filtered off, rinsed with water and dried, and obtained as white crystals in 88% yield; mp 110−112 °C. ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H), 2.55 (s, 3H), 3.92 (s, 3H), 6.94 (d, 1H, J = 8.9), 7.11 (d, 1H, J = 8.2), 7.38 (dd, 1H, J = 8.2, 2.2), 7.65 (m, 2H), 8.21 (d, 1H, J = 2.2). ^{13}C NMR ($CDCl_3$): 21.1, 29.8, 56.6, 112.6, 114.3, 120.8, 123.9, 130.8, 131.0, 134.2, 135.1, 136.2, 137.2, 147.1, 159.2, 163.3, 198.0. MS *m*/*z* (% rel int): 364 (5%), 362.0154 (M^+, 5, $C_{17}H_{15}Br_1O_4$ requires 362.0154), 216 (7%), 215 (98%), 214 (7%), 213 (100%), 172 (5%), 170 (5%).

1-(2-Hydroxy-5-methylphenyl)-3-(5-bromo-2-methoxyphenyl)propane-1,3-dione (Scheme 1; II Leading to 40 and 41). The compound was prepared according to the procedure described for **II** leading to **3**. The product was isolated in 84% yield as yellow crystals; mp 124−126 °C. In $CDCl_3$, the product is a 3:1 enol:keto mixture. Data are given for the major enol form. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H), 4.00 (s, 3H), 6.93 (m, 2H), 7.20 (s, 1H), 7.30 (dd, 1H, J = 8.3, 2.1), 7.49 (d, 1H, J = 1.6), 7.57 (dd, 1H, J = 8.9, 2.6), 8.08 (d, 1H, J = 2.1), 11.90 (s, 1H), 15.49 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.9, 56.5, 98.1, 113.5, 113.7, 118.6, 124.6, 128.3, 128.6, 130.0, 132.7, 135.6, 137.1, 157.7, 160.7, 173.4, 196.4. MS *m*/*z* (% rel int):

364 (60%), 362.0165 (M^+, 59, $C_{17}H_{15}BrO_4$ requires 362.0154), 333 (17%), 215 (100%), 213 (100%), 150 (48%), 135 (100%).

5′-Bromo-2′-methoxy-6-methylflavone (40). The compound was prepared according to the procedure described for **3**. The product was isolated in 91% yield as white crystals; mp 151−155 °C. ^{1}H NMR ($CDCl_3$): 2.47 (s, 3H), 3.93 (s, 3H), 6.93 (d, 1H, J = 9.0), 7.13 (s, 1H), 7.51 (m, 3H), 8.01 (m, 2H). ^{13}C NMR ($CDCl_3$): 21.1, 56.2, 113.1, 113.3, 113.8, 118.0, 122.8, 123.6, 125.2, 131.9, 134.9, 135.2, 135.3, 154.9, 157.2, 159.1, 179.0. MS *m*/*z* (% rel int): 346 (72%), 344.0048 (M^+, 74, $C_{17}H_{13}$-$^{79}BrO_3$ requires 344.0048), 267 (25%), 163 (27%), 162 (35%), 135 (100%), 134 (33%), 113 (26%). Anal. ($C_{17}H_{13}BrO_3$) C, H.

5′-Bromo-2′-hydroxy-6-methylflavone (41). The compound was prepared according to the procedure described for **14**. Only 2 equiv of BBr_3 were added. The reaction time was 4 h before addition of MeOH. A beige solid, insoluble in CH_2Cl_2 and brine, was filtered off and recrystallized from acetone to give white crystals in 72% yield; mp 285−287 °C. ^{1}H NMR (DMSO-d_6): 2.43 (s, 3H), 7.02 (d, 1H, J = 8.6), 7.12 (s, 1H), 7.55 (dd, 1H, J = 8.6, 2.1), 7.63 (d, 1H, J = 8.5), 7.70 (d, 1H, J = 8.5), 7.81 (brs, 1H), 8.02 (d, 1H, J = 2.1). ^{13}C NMR (DMSO-d_6): 30.5, 120.6, 121.4, 128.4, 129.3, 129.8, 132.8, 133.9, 140.5, 144.9, 144.9, 145.3, 164.1, 165.9, 169.0, 187.2. MS *m*/*z* (% rel int): 333 (18%), 332 (98%), 331 (25%), 329.9888 (M^+, 100, $C_{16}H_{11}{}^{79}BrO_3$ requires 329.9892), 135 (28%), 134 (55%). Anal. ($C_{16}H_{11}BrO_3$) C, H.

2-Acetyl-4-methylphenyl Nicotinate (Scheme 1; I Leading to 42). The compound was prepared according to the procedure described for **I** leading to **3**. Nicotinoyl chloride·HCl was purchased from Aldrich. The product was purified by chromatography (heptane:ethyl acetate 5:1) to give white crystals in a yield of 79%; mp 85−87 °C. ^{1}H NMR ($CDCl_3$): 2.45 (s, 3H), 2.55 (s, 3H), 7.13 (d, 1H, J = 8.2), 7.41 (dd, 1H, J = 8.2, 2.2), 7.48 (dd, 1H, J = 8.0, 4.9), 7.68 (d, 1H, J = 2.2), 8.47 (ddd, 1H, J = 8.0, 2.2, 1.9), 8.87 (dd, 1H, J = 4.9, 1.9), 9.40 (d, 1H, J = 2.2). ^{13}C NMR ($CDCl_3$): 21.1, 29.6, 123.8, 125.8, 130.5, 131.2, 134.4, 136.6, 137.8, 138.0, 146.9, 151.7, 154.2, 164.4, 197.6. MS *m*/*z* (% rel int): 255 (M^+, 18%), 106 (100%), 78 (47%), 51 (16%).

1-(2-Hydroxy-5-methylphenyl)]-3-pyridin-3-ylpropane-1,3-dione (Scheme 1; II Leading to 42). The compound was prepared according to the procedure described for **II** leading to **3**. Purification was made by chromatography (heptane:ethyl acetate 3:1) to give yellow crystals in a yield of 44%; mp 112−114 °C. In $CDCl_3$, the product is a 10:1 enol:keto mixture. Data are given for the major enol form. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H), 6.88 (s, 1H), 6.93 (d, 1H, J = 8.5), 7.31 (dd, 1H, J = 8.5, 1.9), 7.45 (dd, 1H, J = 7.9, 4.9), 7.54 (bs, 1H), 8.22 (d, 1H, J = 7.9), 8.77 (dd, 1H, J = 4.9, 1.8), 9.16 (d, 1H, J = 1.8), 11.81 (s, 1H), 15.45 (s, 1H). ^{13}C NMR ($CDCl_3$): 20.5, 93.0, 93.1, 118.3, 118.7, 123.6, 128.3, 129.6, 134.1, 137.4, 148.0, 152.7, 160.5, 174.4, 196.0. MS *m*/*z* (% rel int): 255 (M^+, 100%), 238 (14%), 135 (45%), 134 (32%), 107 (25%), 106 (100%), 79 (35%), 78 (55%), 77 (32%), 51 (20%).

3′-Aza-6-methylflavone (42). The compound was prepared according to the procedure described for **3**. The crude product was recrystallized from acetone to give white crystals in a yield of 97%; mp 156−159 °C. ^{1}H NMR ($CDCl_3$): 2.41 (s, 3H), 6.76 (s, 1H), 7.42 (m, 2H), 7.47 (dd, 1H, J = 8.6, 2.2), 7.94 (d, 1H, J = 2.2), 8.13 (ddd, 1H, J = 8.1, 1.9, 1.5), 8.71 (dd, 1H, J = 4.8, 1.5), 9.11 (d, 1H, J = 1.9). ^{13}C NMR ($CDCl_3$): 21.0, 108.3, 117.9, 123.6, 123.7, 125.1, 128.0, 133.6, 135.4, 135.6, 147.6, 152.1, 154.5, 160.9, 178.1. MS *m*/*z* (% rel int): 237.0800 (M^+, 100, $C_{15}H_{11}NO_2$ requires 237.0790), 209 (13%), 134 (38%), 106 (13%). Anal. ($C_{15}H_{11}NO_2$) C, H, N.

Tissue Preparation. Preparations were performed at 0−4 °C unless otherwise indicated. Cerebral cortex from male Wistar rats (150−200 g) was homogenized for 5−10 s in 20 mL of Tris-HCl (30 mM, pH 7.4) using an Ultra-Turrax homogenizer. The suspension was centrifuged at 27 000*g* for 15 min, and the pellet was washed three times with buffer (centrifuged at 27 000*g* for 10 min). The washed pellet was homogenized in 20 mL of buffer and incubated on a water bath (37 °C) for 30 min to remove endogenous GABA and then centrifuged for 10 min at 27 000*g*. The final pellet was resuspended in 30 mL of buffer, and the preparation was frozen and stored at −20 °C.

^{3}H-Ro 15-1788 Binding Assay. The membrane preparation was thawed and centrifuged at 0−4 °C for 10 min at 27 000*g*. The pellet was washed twice with 20 mL of Tris-citrate (50 mM, pH 7.1) using an Ultra-Turrax homogenizer and centrifuged for 10 min at 27 000*g*. The final pellet was resuspended in Tris-citrate (50 mM, pH 7.1, 500 mL buffer per gram of original tissue), and then used for binding assays. Aliquots of 0.5 mL of membrane suspension were added to 25 μL of test solution and 25 μL of ^{3}H-Ro 15-1788 (0.7−1.5 nM, final concentration in assay), mixed, and incubated for 40 min at an ice-bath (0−4 °C). Nonspecific binding was determined using Clonazepam (1 μM, final concentration) added to separate samples. Following incubation, the samples were added to 5 mL of ice-cold buffer and poured direcly onto Whatman GF/C glass fiber filters under suction and immediately washed with 5 mL of ice-cold buffer. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting. Specific binding was total binding minus nonspecific binding.

Calculations. The IC_{50} values were calculated by (applied test sustance concentration, μM) × 1/(C°/Cx − 1), where C° is specific binding in control assay and Cx is the specific binding in the test assay. K_i values were calculated from IC_{50} values using the binding affinity constant, K_d = 1.6 nM, for 3H-Ro 15-1788 binding.

Computational Methods. Conformational analysis of the flavones was performed by using the molecular mechanics program MM3(92) developed by Allinger and co-workers.[41,42]

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