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A unified strategy for the synthesis of amorfrutins A and B and evaluation of their cytotoxicity

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Abstract: 3,5-Dimethoxy-benzaldehyde was used as a starting material to synthesize a central intermediate, 2-hydroxy-4-methoxy-6-phenethylbenzoic acid that was converted very quickly and with good yields into amorfrutins A and B. Furthermore, this compound was also used as a starting material to synthesize a piperazinyl-rhodamine B conjugate. The latter compound showed good cytotoxicity (EC_{50} = 2.3–5.1 μM) and promising selective cytotoxicity (S = 2.1–4.6) for human tumor cell lines as compared to non-malignant fibroblasts (NIH 3T3).

Keywords: Amorfrutin A; Amorfrutin B; synthesis; cytotoxicity.

1. Introduction

The vital role of secondary natural products for the development of new drugs is undisputed [1-3]. For thousands of years, people have been using the almost inexhaustible reservoir of plant ingredients [4] of the so-called "God's pharmacy" [5]. For example, in infectious diseases and cancer 75 and 60% of new drugs originate from natural sources [1-3]. The global market for pharmaceuticals is about 1.1 trillion US$; thereby, 35% of the medicines have developed from natural products [1-3]. Cancer, infectious diseases, and complex non-communicable diseases are still the most frequent causes of death worldwide [6]. Of particular interest are phenolic compounds; they are widely dispersed throughout the plant kingdom, and more than 10.000 different phenolic structures have been isolated so far. For many of them, cytotoxic or anticancer activity has been reported. But also for cardiovascular diseases, which are often also associated with type II diabetes mellitus and obesity, there is an unsustainable burden on society [7]. A new strategy for early invention and prevention consists of the timely application of antidiabetic and lipid-lowering compounds such as the 2-hydroxybenzoic acid-derived amorfrutins [8-10].

Amorfrutins A (**1**) and B (**2**) (Fig. 1) were initially isolated from parts of the bastard indigo-bush *Amorpha fruticosa* [11,12]. Still, these and other amorfrutins have also been found in other plants, such as the licorice species *Glycyrrhiza foetida* [13-16]. *A. fruticosa* is an indigenous American shrub, while *G. foetida* is a photoautotrophic plant in the family of Fabaceae. The physiological effects of amorfrutins can be attributed, in part, to selective activation of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) [14,17-21]. PPARγ regulates genes of glucose and fatty acid metabolism. However, the complex also appears to be important in treating cancer [12,16,22], inflammations [23,24,] and for impending the age-related decline of metabolism [13,15,25-27].

Several syntheses have been published to access amorfrutins A and B whereby the former has been the focus of scientific interest. In contrast, the number of syntheses for the latter has remained small [28-34]. However, of particular interest are synthetic strategies that allow in principle to synthesize as many as possible of the previously known amorfrutins and, if necessary, analogs in a unified manner [31,33].

A most recently published synthesis of amorfrutin B from amorfrutin A methyl ester seems particularly worth mentioning since it holds a critical step in a Johnson-Claisen rearrangement reaction [33,35]. However, this method's elegance is diminished by the available length of the synthesis and the sometimes only moderate yields.

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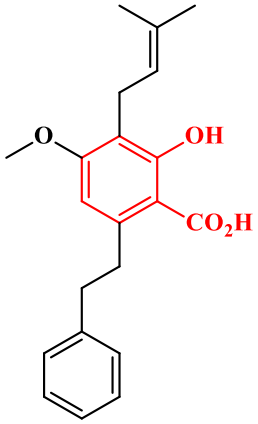
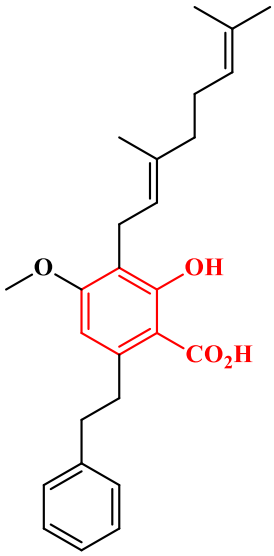


Amorfrutin A (1)



Amorfrutin B (2)

Figure 1. Structure of amorfrutins A (**1**) and B (**2**) highlighting the 2-hydroxybenzoic acid core structure

2. Results and discussion

In the course of our syntheses, we could show that compound **3** (Scheme 1) is easily accessible in large quantities from commercial 3,5-dimethoxy-benzaldehyde in only 6 steps in a total yield of 63% [34]. Thus, **3** seems to be an ideal starting material for synthesizing the two amorfrutins A and B.

Regarding the synthesis of amorfrutins, **3** was converted into methyl ester **4** by reaction with MeI in the presence of Cs_2CO_3 in 98% yield. The reaction of **4** [34,36] with K.H. and prenyl chloride gave a mixture of **5** (as a product of C-alkylation) [34,37], and **6** (as an etherification product) [13]. Whereas, from the reaction with geranyl chloride, a mixture of **7** (from a C-alkylation) [17] and **8** (C- and O-alkylation) was obtained. Both mixes were easily separated by column chromatography. Hydrolysis of **5** furnished amorfrutin A (**1**) while from the hydrolysis of **7**, amorfrutin B (**2**) was obtained in 84% isolated yield. The side products of the former reactions, **6** and **8** were transformed by their reaction with $CeCl_3$ in acetonitrile in the presence of NaI very easily into starting material **4** and amorfrutin B **2**, respectively.

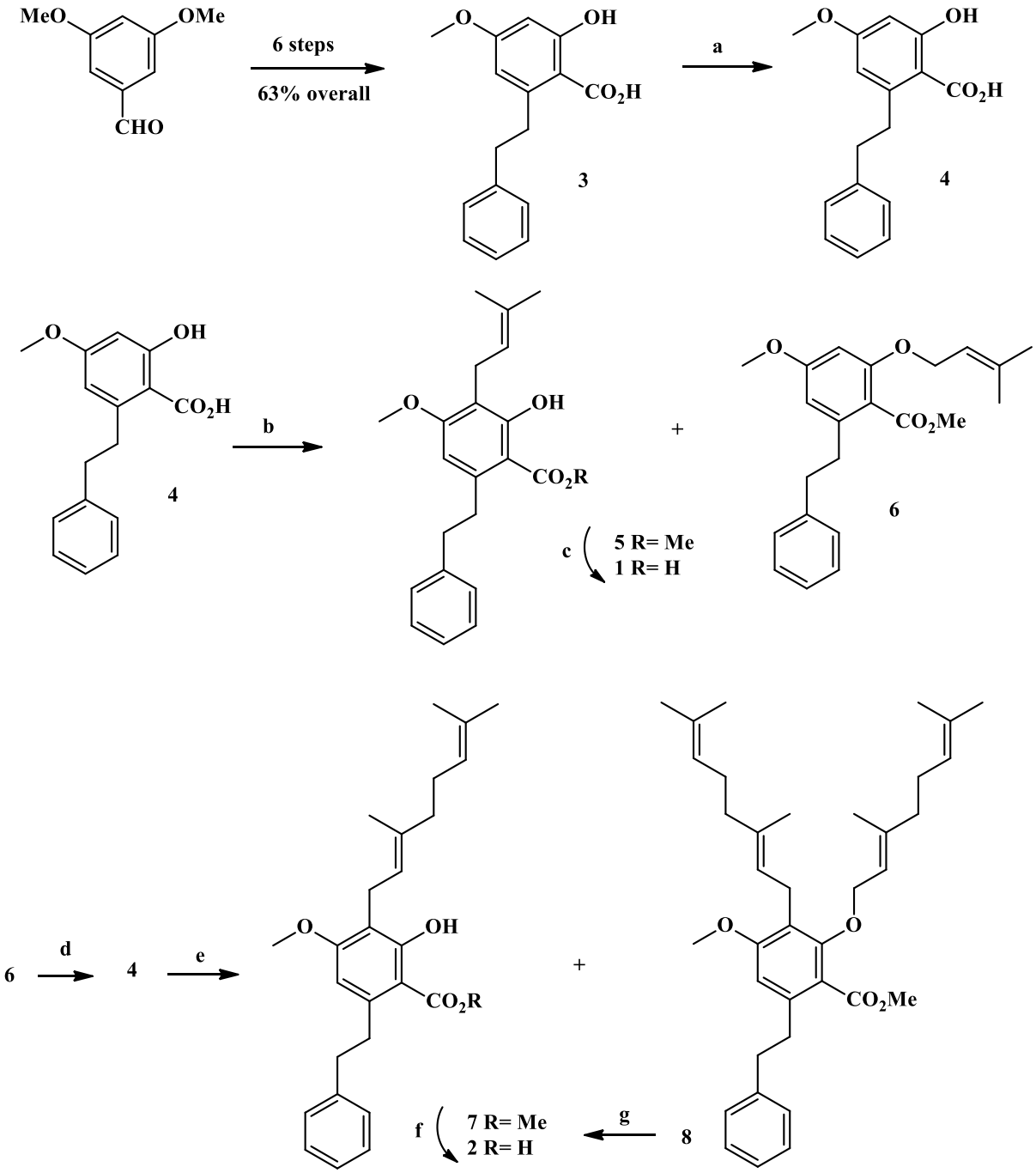
Table 1. Cytotoxicity of compounds **1-10** and rhodamine B (**Rho**) [a].

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
Rho	> 30	> 30	> 30	> 30	> 30	> 30
1	> 30	> 30	> 30	> 30	> 30	> 30
2	23.8 ± 2.0	> 30	26.2 ± 1.4	25.0 ± 2.0	27.1 ± 0.9	> 30
3-5	> 30	> 30	> 30	> 30	> 30	> 30
6	> 30	> 30	> 30	22.9 ± 1.8	> 30	> 30
7	> 30	> 30	28.9 ± 3.8	20.5 ± 1.9	> 30	> 30
8	19.6 ± 2.3	> 30	12.0 ± 1.2	19.8 ± 2.1	22.0 ± 2.3	> 30
9	> 30	> 30	17.8 ± 3.9	26.4 ± 2.1	> 30	> 30
10	4.7 ± 0.3	5.1 ± 0.2	3.4 ± 0.5	2.3 ± 0.3	3.7 ± 0.2	1.1 ± 0.1
STA	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.05	0.008 ± 0.001	0.2 ± 0.02

[a] (EC_{50} values in µM from SRB assays after 72h of treatment, the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error, cut-off 30 µM). Human cancer cell lines: A375 (epithelial melanoma), HT29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (squamous cell carcinoma); non-malignant: NIH 3T3 (mouse fibroblasts). Staurosporine (**STA**) was used as a positive standard;

Selectivity S = $EC_{50\ \text{tumor cell line}}$ / $EC_{50\ \text{NIH 3T3}}$.

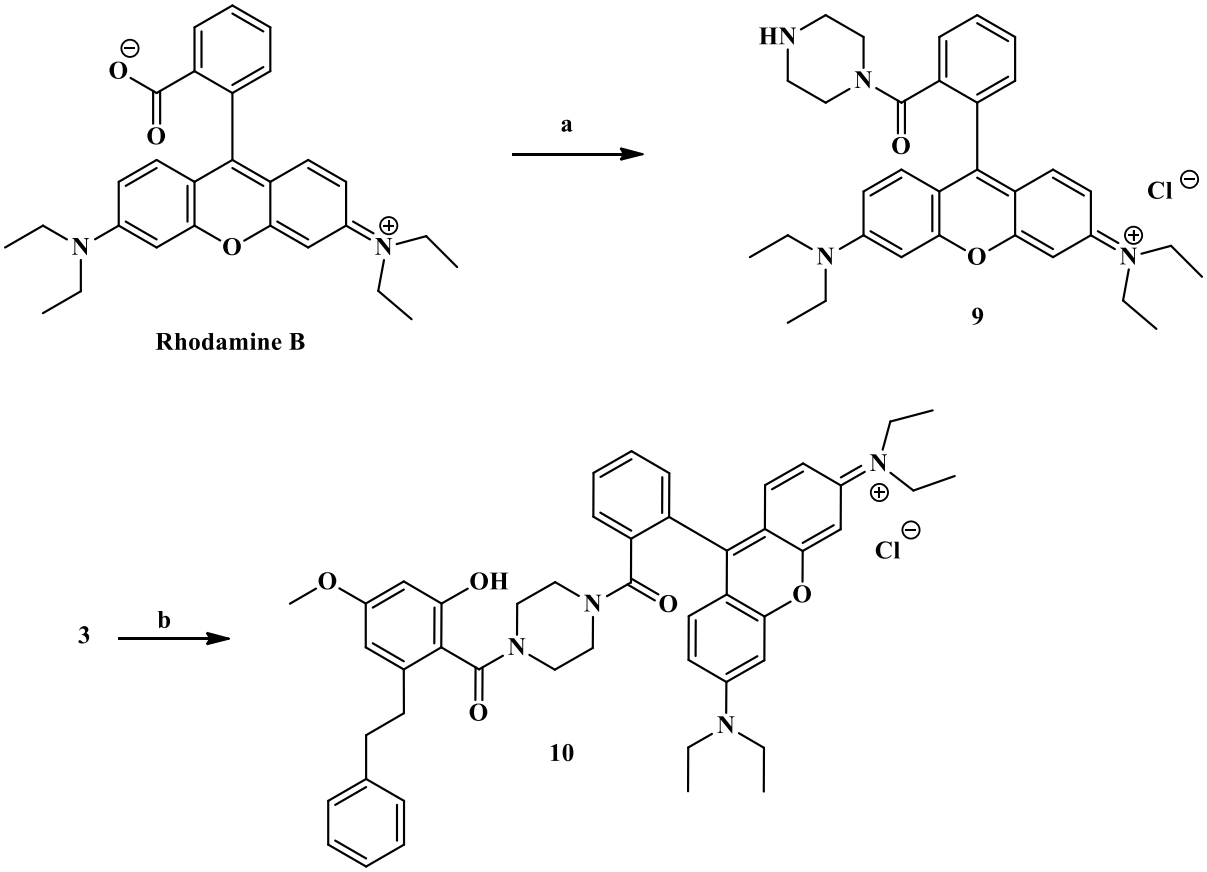
Relatively little is known about the possible cytotoxicity of amorfrutins A and B. Thus, these two compounds were investigated in an SRB assay. This showed amorfrutin A (**1**, Table 1) not to be cytotoxic for several human tumor cell lines as well as for non-malignant mouse fibroblasts (NIH 3T3). EC_{50} values between 23.8 µM (for A375 cells) and 27.1 µM (for FaDu cells), however, were observed for amorfrutin B (**2**).



Scheme 1. Synthesis of amorfrutin A (**1**) and amorfrutin B (**2**) from central intermediate **4**. Reactions and conditions: a) Cs_2CO_3, DMF, MeI, 25°C, 12 h, 96%; b) KH, toluene, prenyl chloride, 75°C, 2 h, 72% (of **5**) and 25% (of **6**); c) KOH, MeOH, H_2O, reflux, 8 h, 90%; d) $CeCl_3$ x 7 H_2O, ACN, NaI, 25°C, 6 h, 95%; e) KH, toluene, geranyl chloride, 70°C, 12 h, 74% (of **7**) and 11% (of **8**); f) KOH, MeOH, H_2O, reflux, 10 h, 84%; g) $CeCl_3$ x 7 H_2O, ACN, NaI, 25°C, 6 h, 84%

We were recently able to show several not cytotoxic (EC50 > 30 µM) di- and triterpene derived carboxylic acids and hydroxycinnamic acid derivatives. Their transformation into a piperazinyl amide and the latter's reaction with rhodamine B led to analogs of significantly increased cytotoxicity [38-46]. Hence, the reaction of **3** with the piperazinyl-rhodamine B conjugate **9** [38,47-49]gave **10**.

Hybrid compound **10** held good cytotoxicity (Table 1) in low micro-molar concentration for all human tumor cell lines, combined with promising selectivity.



Scheme 2. Synthesis of compounds **9** and **10**: reactions and conditions: a) DCM, $(COCl)_2$, DMF, piperazine, 24 h, 25°C, 67%; b) EDC x HCl, HOBt, DCM, **9**, 12 h, 25°C, 74%

3. Conclusion

From 3,5-dimethoxy-benzaldehyde, a central intermediate, 2-hydroxy-4-methoxy-6-phenethyl-benzoic acid is accessible in only 6 steps, which can be converted very easily and with good yields into the amorfrutins A and B. This intermediate, however, can also serve as a starting material for the synthesis of amides. In this case, piperazinyl-rhodamine B conjugate showed good cytotoxicity for several human tumor cell lines (A375, HT29, MCF-7, A2780, FaDu) as well as promising tumor/non-tumor cell selectivity.

Acknowledgments

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4. Experimental

Instrumentation was previously described [38–46]. Starting materials were obtained from local suppliers in bulk, and the solvents (technical grade) were re-distilled and dried according to usual procedures. All reactions were performed under argon using oven-dried glassware. The routine aqueous workup included the dilution of the reaction mixture with the solvent (used for the reaction), aqueous extraction, re-extraction of the aqueous phase (twice), drying of the combined organic phases ($MgSO_4$), and evaporation of the organic phase under reduced pressure.

4.1. Cytotoxic evaluation

The cell lines were obtained from the Department of Oncology (Martin-Luther-University Halle Wittenberg). Cultures were maintained as monolayers in RPMI 1640 medium with L-glutamine (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and penicillin/streptomycin (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) at 37°C in a humidified atmosphere with 5% CO_2. The compounds' cytotoxicity was evaluated using the sulforhodamine-B (Kiton-Red S, ABCR) micro-

culture colorimetric assay, as previously reported [38–46]. In short, the cells were seeded into 96 well plates on day zero at appropriate cell densities to prevent the confluence of the cells during the experiment. After 24 hours, the cells were treated with different concentrations (1, 3, 7, 12, 20, and 30 µM), but the final concentration of DMSO/DMF never exceeded 0.5%, which was non-toxic to the cells. After 72 h treatment, the supernatant media from the 96 well plates were discarded, then the cells were fixed with 10 % trichloroacetic acid and allowed to rest at 4°C. After 24 hours of fixation, the cells were washed in a strip washer and then dyed with SRB solution (200 µL, 10 mM) for 20 minutes. The plates were then washed four times with 1 % acetic acid to remove the dye's excess and allowed to air-dry overnight. Tris base solution (200 µL, 10 mM) was added to each well. The absorbance was measured with a 96 well plate reader from Tecan Spectra.

Methyl 2-hydroxy-4-methoxy-6-phenethylbenzoate (4)

From **3**: A suspension of **3** (2.00 g, 7.34 mmol) and Cs_2CO_3 (4.8 g, 7.8 mmol) in dry DMF (40 mL) was stirred for 10 min; iodomethane (0.72 mL, 10.8 mmol) was added, and the stirring was continued for 12 h. Usual workup gave **4** (2.02 g, 96%) as a colorless oil pure enough for the transformations to follow.

From **6**: To a solution of **6** (150 mg, 0.42 mmol) in acetonitrile (5 mL) $CeCl_3$ ×7 H_2O (190 mg, 0.51 mmol) and NaI (80 mg, 0.53 mmol) were added. The mixture was stirred at 25°C for 12 h. Usual aqueous work-up followed by chromatography (silica gel, *n*-hexane/ethyl acetate, 6:1) gave **4** (115 mg, 95%); m.p. 49-51°C (lit.: [34] 50-52°C); R_F = 0.45 (*n*-hexane/ethyl acetate, 12:1);
IR (ATR): $\tilde{\nu}$ = 2952w, 1650m, 1614m, 1575m, 1496w, 1434m, 1380w, 1325s, 1203m, 1253s, 1203s, 1156s, 1110m, 1047m, 956m, 802m, 748m, 698s, 638w, 599w cm^{-1};
UV/Vis ($CHCl_3$): λ_{max} (log ε) = 230 (4.30), 265 (4.19), 304 (3.82) nm;
^{1}H NMR (500 MHz, $CDCl_3$) δ = 11.75 (s, 1H, OH), 7.34 – 7.16 (m, 5H, 10-H, 11-H, 12-H), 6.38 (d, J = 2.6 Hz, 1H, 5-H), 6.29 (d, J = 2.6 Hz, 1H, 3-H), 3.96 (s, 3H, COOMe), 3.79 (s, 3H, OMe), 3.21 – 3.14 (m, 2H, 7-H_2), 2.88 – 2.82 (m, 2H, 8-H_2) ppm;
^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.9 (COOH), 165.8 (C-4), 164.2 (C-2), 146.8 (C-6), 142.1 (C-9), 128.6 (C-10), 128.5 (C-11), 126.1 (C-12), 111.0 (C-5), 104.8 (C-1), 99.4 (C-3), 55.4 (OMe), 52.2 (COOMe), 39.0 (C-7), 38.4 (C-8) ppm;
MS (ESI, MeOH): m/z = 287.1 (100, $[M+H]^+$);
analysis calcd for $C_{17}H_{18}O_4$ (286.32): C 71.31, H 6.34; found: C 71.11, H 6.60.

Methyl 2-hydroxy-4-methoxy-3-(3-methyl-2-buten-1-yl)-6-phenylethylbenzoate (5) and methyl 4-methoxy-2-[(3-methylbut-2-en-1-yl)oxy]-6-phenethylbenzoate (6)

A solution of **4** (1.0 g, 3.49 mmol) in dry toluene (30 mL) and K.H. (154 mg, 3.84 mmol; K.H. was obtained as a suspension in mineral oil. Before the reaction, the suspension was washed in a Schlenk-frit with dry *n*-hexane to remove the oil. The pure K.H. was dried in a stream of dry argon) was stirred at 25°C for 20 min, followed by 20 min at 70°C. At 25°C, prenyl chloride (440 mg, 4.21 mmol) was added, and the mixture was stirred at 75°C for 2 hours. Usual workup followed by chromatography (silica gel, *n*-hexane/ethyl acetate, 12:1) gave **5** (890 mg, 72%) and **6** (301 mg, 24%).

Data for **5**: colorless solid; m.p. 67-69°C (lit.: [34] m.p. 67.9 C); R_F = 0.58 (*n*-hexane/ethyl acetate, 12:1);
IR (ATR): $\tilde{\nu}$ = 2924m, 1655m, 1603m, 1573m, 1494w, 1435m, 1405m, 1288s, 1224s, 1154s, 1112s, 1004m, 962w, 804s, 773m, 737s, 700s, 656m, 616w, 558w, 522m cm^{-1};
UV/Vis (MeOH): λ_{max} (log ε) = 226 (4.37), 270 (3.99), 308 (3.55) nm;
^{1}H NMR (500 MHz, $CDCl_3$) δ = 11.72 (s, 1H, OH), 7.33 – 7.17 (m, 5H, 10-H, 11-H, 12-H), 6.21 (s, 1H, 5-H), 5.23 – 5.17 (m, 1H, 14-H), 3.96 (s, 3H, COOMe), 3.80 (s, 3H, OMe), 3.34 (d, J = 7.0 Hz, 2H, 13-H_2), 3.21 – 3.14 (m, 2H, 7-H_2), 2.88 – 2.82 (m, 2H, 8-H_2), 1.78 (s, 3H, CH_3), 1.68 (s, 3H, CH_3) ppm;
^{13}C NMR (126 MHz, $CDCl_3$): δ = 172.3 (COOH), 162.0 (C-4), 161.4 (C-2), 144.2 (C-6), 142.2 (C-9), 131.8 (C-15), 128.5 (C-10, C-11), 126.1 (C-12), 122.5 (C-14), 115.4 (C-3), 106.1 (C-5), 105.3 (C-1), 55.6 (OMe), 52.1 (COOMe), 39.4 (C-7), 38.6 (C-8), 25.9 (CH_3), 22.1 (C-13)), 17.9 (CH_3) ppm;
MS (ESI, MeOH): m/z = 355.1 (15, $[M+H]^+$), 377.2 (100, $[M+Na]^+$);
analysis calcd for $C_{22}H_{26}O_4$ (354.5): C 74.55, H 7.39; found: C 74.32, H 7.54.

Data for **6**: pale yellowish oil; R_F = 0.45 (*n*-hexane/ethyl acetate, 12:1);
IR (ATR): $\tilde{\nu}$ = 2963w, 1602m, 1495w, 1435m, 1364m, 1259s, 1218m, 1204m, 1159s, 1103s, 1032m, 955w, 883w, 850w, 797s, 748m, 725w, 697s, 604m cm^{-1};
UV/Vis ($CHCl_3$): λ_{max} (log ε) = 229 (4.25) nm;
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33 – 7.15 (m, 5H, 10-H, 11-H, 12-H), 6.34 (d, J = 2.2 Hz, 1H, 5-H), 6.23 (d, J = 2.2 Hz, 1H, 3-H), 5.43 (t, *J* = 6.3 Hz, 1H, 14-H), 4.53 (d, J = 6.5 Hz, 2H, 13-H_2), 3.89 (s, 3H, COOMe), 3.75 (s, 3H, OMe), 2.94 – 2.80 (m, 4H, 7-H_2, 8-H_2), 1.77 (s, 3H, CH_3), 1.72 (s, 3H, CH_3) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.9 (COOH), 161.4 (C-4), 157.6 (C-2), 141.9 (C-6), 141.8 (C-9), 137.5 (C-15), 128.6 (C-10), 128.5 (C-11), 126.1 (C-12), 119.9 (C-14), 117.0 (C-1), 106.2 (C-5), 98.1 (C-3), 66.1 (C-13)), 55.5 (OMe), 52.2 (COOMe), 37.8 (C-7), 36.4 (C-8), 25.7 (CH_3), 18.4 (CH_3) ppm; MS (ESI, MeOH): m/z = 355.0 (20, $[M+H]^+$), 377.1 (100, $[M+Na]^+$); analysis calcd for $C_{22}H_{26}O_4$ (354.5): C 74.55, H 7.39; found: C 74.31, H 7.58.

Amorfrutin A, 2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-phenethylbenzoic acid (1)

To a solution of KOH (760 mg, 13.56 mmol) in MeOH/H_2O (7:1, 14 mL) a solution of **5** (800 mg, 2.26 mmol) in MeOH (20 mL) was added. After heating under reflux for 8 h followed by usual aqueous work-up and chromatography (silica gel, n-hexane/ethyl acetate, 4:1) amorfrutin A (**1**, 696 mg, 91%) was obtained as an off-white solid; m.p. 111-113°C (lit.: [34] m.p. 113.7°C); R_F = 0.33 (*n*-hexane/ethyl acetate, 4:1);

IR (ATR): $\tilde{\nu}$ = 2925w, 1610s, 1495w, 1453m, 1435w, 1267s, 1228s, 1175s, 1115s, 1040m cm^{-1};

UV/Vis (MeOH): λ_{max} (log ε) = 224 (4.35), 265 (3.83), 305 (3.42) nm;

^{1}H NMR (500 MHz, $CDCl_3$): δ = 11.90 (s, 1H, OH), 7.35 –7.15 (m, 5H, 10-H, 11-H, 12-H), 6.25 (s, 1H, 5-H), 5.26 – 5.15 (m, 1H, 14-H), 3.82 (s, 3H, OMe), 3.38 (d, J = 7.0 Hz, 2H, 13-H_2), 3.30 – 3.23 (m, 2H, 7-H_2), 2.98 – 2.90 (m, 2H, 8-H_2), 1.81 (s, 3H, CH_3), 1.71 (s, 3H, CH_3) ppm;

^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.1 (COOH), 163.4 (C-4), 162.0 (C-2), 145.7 (C-6), 142.3 (C-9), 131.9 (C-15), 128.5 (C-10), 128.4 (C-11), 126.0 (C-12), 122.6 (C-14), 115.7 (C-3), 106.7 (C-5), 103.7 (C-1), 55.6 (OMe), 39.6 (C-7), 38.5 (C-8), 25.7 (CH_3), 22.0 (C-13), 17.9 (CH_3) ppm;

ESI-MS (MeOH): m/z = 341.0 (50, $[M+H]^+$), 363.1 (100, $[M+Na]^+$);

analysis calcd for $C_{21}H_{24}O_4$ (340.41): C 74.09; H 7.11; found: C 73.91; H 7.30.

Methyl (*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxy-6-phenethylbenzoate (7) and methyl 3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-(((*E*)-3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-methoxy-6 phenethylbenzoate (8)

To a suspension of K.H. (110 mg, 2.74 mmol) in dry toluene (100 mL), a solution of **4** (714 mg, 2.49 mmol) in dry toluene (50 mL) was slowly added, and the mixture was stirred at 23°C for 15 min followed by the addition of geranyl chloride (560 µL, 3.04 mmol). The stirring at 70°C was continued for 12 h. Usual aqueous workup followed by chromatography (silica gel, *n*-hexane/ethyl acetate, 12:1) gave **7** (778 mg, 74%) and **8** (114 mg, 8%) each as a colorless oil.

Data for **7**: R_F = 0.67 (*n*-hexane/ethyl acetate, 9:1);

IR (ATR): ν = 3085*vw*, 3061*vw*, 3027*w*, 2952*w*, 2916*w*, 2854*w*, 1725*w*, 1651*s*, 1609*m*, 1573*m*, 1496*w*, 1452*m*, 1436*m*, 1404*m*, 1376*m*, 1280*vs*, 1226*s*, 1194*m*, 1155*s*, 1112*s*, 1077*w*, 1043*w*, 1030*w*, 1007*m*, 962*w*, 912*w*, 882*w*, 836*w*, 807*m*, 770*m*, 748*m*, 699*s*, 659*w*, 608*m*, 557*w*, 488*w*, 470*w* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.73 (*s*, 1H, OH), 7.34 – 7.26 (*m*, 2H, 11-H), 7.25 – 7.17 (*m*, 3H, 10-H, 12-H), 6.22 (*s*, 1H, 5-H), 5.22 (*tq*, *J* = 7.0, 1.3 Hz, 1H, 17-H), 5.08 (*tq*, *J* = 7.0, 1.4 Hz, 1H, 21-H), 3.96 (*s*, 3H, CO_2Me), 3.80 (*s*, 3H, OMe), 3.40 –3.33 (*d*, *J* = 7.0 Hz, 2H, 16-H_2), 3.22 – 3.16 (*m*, 2H, 7-H_2), 2.90 – 2.83 (*m*, 2H, 8-H_2), 2.10 – 2.02 (*m*, 2H, 20-H_2), 2.00 – 1.94 (*m*, 2H, 19-H_2), 1.79 (*d*, *J* = 1.3 Hz, 3H, Me), 1.65 (*d*, *J* = 1.4 Hz, 3H, Me), 1.58 (*d*, *J* = 1.3 Hz, 3H, Me) ppm;

^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.1 (CO_2Me), 161.9 (C-4), 161.3 (C-2), 144.0 (C-6), 142.0 (C-9), 135.0 (C-18), 131.1 (C-22), 128.4 (C-10), 125.9 (C-11), 124.5 (C-12), 122.9 (C-21), 122.2 (C-17), 115.3 (C-3), 105.9 (C-1), 105.2 (-C5), 55.4 (OMe), 52 (CO_2Me), 39.8 (C-19), 39.3 (C-8), 38.4 (C-7), 26.7 (C-20), 25.7 (Me), 22.0 (C-16), 17.6 (Me), 16.1 (Me) ppm;

MS (ESI, MeOH): m/z = 391.2 (92%, $[M+H-MeOH]^+$), 423 (40%, $[M+H]^+$), 445.2 (80%, $[M+Na]^+$), 461.2 (40%, $[M+K]^+$), 477.1 (22%, $[M+Na+MeOH]^+$);

analysis calcd for $C_{22}H_{34}O_4$ (422.57): C 76.74, H 8.11; found: C 76.50, H 8.32.

Data for **8**: R_F = 0.46 (*n*-hexane/ethyl acetate);

IR (ATR): ν = 3086*vw*, 3062*vw*, 3026*vw*, 2931*w*, 2859*w*, 1725*s*, 1602*s*, 1584*m*, 1496*w*, 1453*m*, 1432*m*, 1382*w*, 1346*w*, 1317*m*, 1282*m*, 1260*s*, 1225*m*, 1195*s*, 1157*vs*, 1099*s*, 1081*m*, 1049*s*, 1030*m*, 993*w*, 949*w*, 923*w*, 881*w*, 827*m*, 817*m*, 788*w*, 748*m*, 700*s*, 639*w*, 609*w*, 574*w*, 561*w*, 490*w*, 461*w* cm^{-1};

UV/Vis ($CHCl_3$): λ_{max} (log ε) = 228 (4.52), 283 (3.71) nm;

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 – 7.24 (*m*, 2H, 11-H), 7.24 – 7.15 (*m*, 3H, 10-H, 12-H), 6.34 (*d*, *J* = 2.3 Hz, 1H, 5-H), 6.25 (*d*, *J* = 2.2 Hz, 1H, 3-H), 5.43 (*t*, *J* = 6.4 Hz, 1H, 17-H), 5.09 (*t*, *J* = 6.8 Hz, 1H, 21-H), 4.55 (*d*, *J* = 6.3 Hz, 2H, 16-H_2), 3.88 (*s*, 3H, CO_2Me), 3.74 (*s*, 3H, OMe), 2.93 – 2.78 (*m*, 4H, 8-H_2, 7-H_2), 2.14 – 2.01 (*m*, 4H, 20-H_2), 19-H_2), 1.71 (*s*, 3H, Me), 1.68 (*s*, 3H, Me), 1.60 (*s*, 4H, Me) ppm;

^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.8 (CO_2Me), 161.2 (C-4), 157.5 (C-2), 141.8 (C-6), 141.6 (C-18), 140.6 (C-9), 131.8 (C-22), 128.4 (C-10), 128.3 (C-11), 126 (C-12), 123.8 (C-21), 119.6 (C-17), 116.8 (C-1), 106.1 (C-5), 98 (C-3), 66 (C-16), 55.3 (OMe), 52.0 (CO_2Me), 39.5 (C-19), 39.3 (C-8), 38.4 (C-7), 26.3 (C-20), 25.6 (Me), 17.7 (Me), 16.7 (Me) ppm; MS (ESI, MeOH): m/z 581.4 (70%, $[M+Na]^+$), 559.2 (100%, $[M+H]^+$);

analysis calcd for $C_{37}H_{50}O_4$ (558.80): C 79.53, H 9.02; found: C 79.37, H 9.25.

Amorfrutin B, (*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxy-6-phenethyl benzoic acid (2)

From **7**: A solution of **7** (100 mg, 0.24 mmol) in MeOH (5 mL) was added to a 40°C solution of KOH (84 mg, 1.5 mmol) in MeOH/H_2O (7:1, 40 mL), and the mixture was heated under reflux for 10 h. Usual workup followed by chromatography (silica gel, *n*-hexane/ethyl acetate, 3:1) gave **2** (81 mg, 84%) as a colorless solid.

From **8**: Following the procedure given for the synthesis of **4** (from **6**) from **8** compound **2** (72%) was obtained. An analytical sample showed: m.p. 74-76°C

(lit: [50] 80.2-83.1°C); R_F = 0.23 (*n*-hexane/ethyl acetate, 3:1);
IR (ATR): ν = 3064*w*, 3028*w*, 2961*m*, 2925*b*, 2855*w*, 2671*w*, 2592*w*, 2537*w*, 1633*s*, 1607*s*, 1571*m*, 1496*m*, 1453*m*, 1430*m*, 1401*m*, 1380*m*, 1348*w*, 1266*vs*, 1221*s*, 1188*m*, 1170*m*, 1149*m*, 1113*s*, 1077*m*, 1046*w*, 1030*w*, 1003*w*, 983*w*, 923*w*, 902*m*, 858*w*, 837*m*, 819*m*, 804*m*, 773*m*, 749*s*, 734*m*,696*s*, 679*m*, 663*w*, 608*m*, 562*w*, 533*w*, 494*m* cm^{-1}; UV/Vis ($CHCl_3$): λ_{max} (log ε) = 229 (4.07), 277 (3.76), 313 (3.26) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.56 (*s*, 2H, COOH + OH), 7.33 – 7.27 (*m*, 2H, 11-H), 7.23 – 7.18 (*m*, 3H, 10-H, 12-H), 6.22 (*s*, 1H, 5-H), 5.21 (*t*, *J* = 5.9 Hz, 1H, 16-H), 5.08 (*t*, *J* = 6.9 Hz, 1H, 20-H), 3.79 (*s*, 3H, OMe), 3.36 (*d*, *J* = 7.0 Hz, 2H, 15-H_2), 3.32 – 3.23 (*m*, 2H, 7-H_2), 2.98 – 2.89 (*m*, 2H, 8-H_2), 2.11 – 1.93 (*m*, 4H, 19-H_2, 18-H_2), 1.81 – 1.76 (*m*, 3H, Me), 1.68 – 1.62 (*m*, 3H, Me), 1.60 – 1.56 (*m*, 3H, Me) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.6 (COOH), 162.9 (C-4), 162.2 (C-2), 145.8 (C-6), 141.9 (C-9), 135.2 (C-17), 131.1 (C-21), 128.5 (C-10), 128.4 (C-11), 125.9 (C-12), 124.5 (C-20), 122.1 (C-16), 115.5 (C-3), 106.5 (C-5), 103.7 (C-1), 55.5 (OMe), 39.8 (C-18), 39.2 (C-8), 38.1 (C-7), 26.8 (C-19), 25.7 (Me), 21.9 (C-15), 17.7 (Me), 16.1 (Me) ppm;
MS (ESI, MeOH): m/z = 391.2 (100%, $[M+H-H_2O]^+$), 409 (46%, $[M+H]^+$), 431.2 (98%, $[M+Na]^+$) and m/z = 363.2 (22%, $[M-H-CO_2]^-$), 407.2 (100%, $[M-H]^-$);
analysis calcd for $C_{26}H_{32}O_4$ (408.54): C 76.44, H 7.90; found: C 76.20, H 8.03.

3,6-Bis(diethylamino)-9-[2-(1-piperazinylcarbonyl)phenyl]-xanthylium chloride (9)

This compound was prepared as previously reported from rhodamine B, oxalyl chloride and piperazine [38] in 67% yield as a dark purple solid; m.p. > 350°C; R_F = 0.15 (chloroform/methanol, 8:2); λ_{max} (log ε) = 260 (0.23), 354 (0.06), 561 (0.82) nm;
IR (ATR) *v* = 3401*br*, 1589*m*, 1529*w*, 1411*s*, 1328*s*, 1275*s*, 1246*m*, 1180*s*, 1132*m*, 1074*m*, 1011*w*, 977*m*, 922*m*, 820*m*, 683*m*;
^{1}H NMR (500 MHz, CD_3OD): δ = 7.79 - 7.74 (*m*, 3H, 3-H + 4-H + 5-H), 7.52 (*m*, 1H, 6-H), 7.28 - 7.25 (*d*, 1H, 10-H), 7.10 - 7.09 (*m*, 1H, 11-H), 6.98 – 6.97 (*d*, 1H, 13-H), 3.72 – 3.59 (*m*, 6H, 15-H_a + 15-H_b + 17-H_a + 17-H_b + 20-H_a + 20-H_b), 3.08 – 3.05 (*t*, 4H, 18-H_a + 18-H_b + 19-H_a + 19-Hb), 1.33 – 1.30 (*t*, 3H, 16-H_a + 16-H_b + 16-H_c) ppm;
^{13}C NMR (126 MHz, CD_3OD): δ = 169.53 (C-1), 159.2 (C-8), 157.3 (C-12), 156.7 (C-14), 135.7 (C-7), 133.0 (C-10), 132.3 (C-2), 131.8 (C-6), 131.5 (C-5), 131.4 (C-4), 128.9 (C-3), 115.4 (C-11), 114.8 (C-9), 97.4 (C-13), 46.9 (C-15), 46.8 (C-17 + C-20), 44.5 (C-18 + C-19), 12.8 (C16) ppm;
MS (ESI, MeOH): *m/z* = 256.4 (24%, $[M+H]^{2+}$), 511.3 (100%, $[M]^+$);
analysis calcd for $C_{32}H_{39}ClN_4O_2$ (547.14): C 70.25, H 7.18, N 10.24; found: C 70.07, H 7.30, N 10.01.

***N*-(6-(Diethylamino)-9-(2-(4-(2-hydroxy-4-methoxy-6-phenethylbenzoyl)piperazine-1-carbonyl)phenyl)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium chloride (10)**

To a solution of **3** (181 mg, 0.66 mmol) in dry DCM (30 mL), EDC HCl (153 mg, 0.79 mmol), HOBt (120 mg, 0.79 mmol) and **9** (361 mg, 0.66 mg) were added; the mixture was stirred for 12h. Usual work-up followed by chromatography (silica gel, $CHCl_3$/MeOH, 1% → 15%) gave **10** (392 mg, 74%) as a pink amorphous solid; R_F = 0.2 ($CHCl_3$/MeOH, 9:1);
IR (ATR): ν = 3061*w*, 2974*w*, 2932*w*, 2869*w*, 1629*m*, 1584*vs*, 1558*m*, 1528*m*, 1506*m*, 1481*m*, 1464*m*, 1456*m*, 1410*s*, 1393*s*, 1333*vs*, 1271*s*, 1245*s*, 1196*m*, 1178*vs*, 1158*s*, 1130*s*, 1072*s*, 1048*m*, 1000*s*, 977*m*, 921*m*, 868*m*, 821*s*, 787*m*, 754*m*, 702*m*, 682*s*, 664*m*, 642*m*, 620*m*, 581*m*, 546*m*, 523*m*, 497*m* cm^{-1}; UV/Vis ($CHCl_3$): λ_{max} (log ε) = 229 (4.57), 261 (4.48), 357 (3.85), 563 (5.01) nm;
^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.08 (s, 1H, OH), 7.66 (s, 1H, 23-H), 7.60 (dd, *J* = 5.9, 2.9 Hz, 2H, 21-H, 24-H), 7.43 (s, 1H, 22-H), 7.33 – 7.26 (m, 2H, 11-H), 7.25 –7.20 (m, 2H, 28-H, 37-H), 7.20 – 7.12 (m, 3H, 10-H, 12-H), 6.78 (dd, *J* = 9.4, 2.4 Hz, 2H, 29-H, 36-H), 6.70 (d, *J* = 2.4 Hz, 2H, 31-H, 34-H), 6.66 (s, 1H, 3-H), 6.27 (d, *J* = 2.3 Hz, 1H, 5-H), 4.37 (s, 2H, 15a-H_2, 17a-H_2), 3.94 (s, 2H, 16a-H_2, 18a-H_2), 3.73 (s, 3H, OMe), 3.67 – 3.50 (m, 8H, 39-H_2, 41-H_2, 43-H_2, 45-H_2), 3.06 – 2.96 (m, 2H, 7-H_2), 2.89 (s, 4H, 15b-H_2, 16b-H_2, 17b-H_2, 18b-H_2), 1.32 (dt, *J* = 17.3, 7.0 Hz, 12H, 4 x Me) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.2 (CO), 168.3 (CO), 167.4 (C-4), 161 (C-2), 157.8 (C-27, C-38), 157.5 (C-25), 155.3 (C-30, C-35), 142 (C-6), 140.8 (C-9), 135.4 (C-20), 132.3 (C-28, C-37), 131.4 (C-21), 131.2 (C-26), 130.3 (C-10), 129.8 (C-22), 128.6 (C-23), 128.3 (C-11), 127.4 (C-24), 125.8 (C-12), 115.2 (C-1), 113.9 (C-32), 113.5(C-33), 113.3 (C-29, C-36), 107.6 (C-5), 99.1 (C-3), 96.1 (C-31, C-34), 55.3 (OMe), 45.9 (C-39, C-41, C-43, C-45), 41.8 (C-7), 37.5 (C-8), 35.4 (C-15, C-16, C-17, C-18), 12.6 (4 x Me) ppm;
MS (ESI, MeOH): m/z = 765.3 (100%, $[M-Cl]^+$;
analysis calcd for $C_{48}H_{53}N_4O_5Cl$ (801.43): C 71.94, H 6.67, N 6.99; found: C 71.77, H 6.81, N 6.67.

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