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Synthesis and Antiaggregant Properties of Stabilized Analogues of Polyunsaturated Fatty Acid Metabolites

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Abstract—New aromatic and heteroaromatic analogues of polyunsaturated fatty acid metabolites have been prepared using short and versatile strategies. Preliminary studies of their activity as inhibitors of platelet aggregation are reported.

Polyunsaturated fatty acid metabolites have very important regulatory functions in living systems and many of them have been associated with various diseases. 13HODE (also called 'coriolic acid') is a linoleic acid metabolite which has many potent biological properties: it is involved in tumor cell adhesion and possesses anti-TXA_2 activity among other biological activities. Its (*S*)-isomer is an inhibitor of RBL-1 (rat basophilic leukemia) 5-lipoxygenase . It acts as a self-defense agent against rice blast disease, displays unique calcium ionophoric properties, and it is also present in sera of patients with familial mediterranean fever and may have a role in its pathogenesis. Furthermore, it acts as a vessel wall chemorepellant, it is an inhibitor of platelet adhesion in human endothelium cell cultures and it appears to be involved in tumor cell adhesion and melatonin regulation of cancer growth.[1] Similarly the 12-HETE obtained from arachidonic acid via the 12 lipoxygenase pathway, has been implicated in angiogenesis, the viability and metastatic potential of tumor cells, atherogenesis, coronary thrombosis, type I diabetes induction, inflammation and psoriasis, and inhibition of apoptosis.[2] It is interesting to note that both 12-HETE and 13-HODE exhibit a good activity, in the micromolar range, in antagonizing TXA_2-induced platelet aggregation.[3] In each case, both enantiomers are active, even if the (*R*) derivatives are slightly more potent. These derivatives, like most of the polyunsaturated fatty acid metabolites, are relatively instable due to the presence of the double bonds. Therefore, it appears interesting to design more stable analogues. A straightforward, and classical strategy for that purpose is to replace the *E*,*Z* dienyl system by an aromatic ring in a cyclisation-aromatisation process.[4]

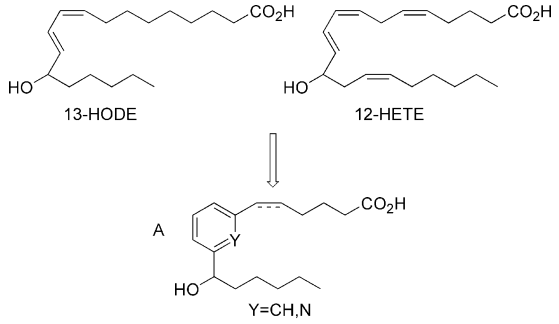

Figure 1.

As part of a general programme dealing with the synthesis and biological evaluation of stabilized analogues of polyunsaturated metabolites, we have designed the derivatives of general structure A which mimic the 13- HODE in the central and lower part of the molecules and have analogies to 12-HETE in the upper part of the skeleton (Fig. 1). Such derivatives could be useful to establish structure–activity relationships in these series

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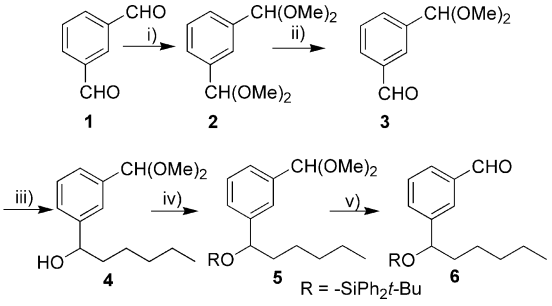
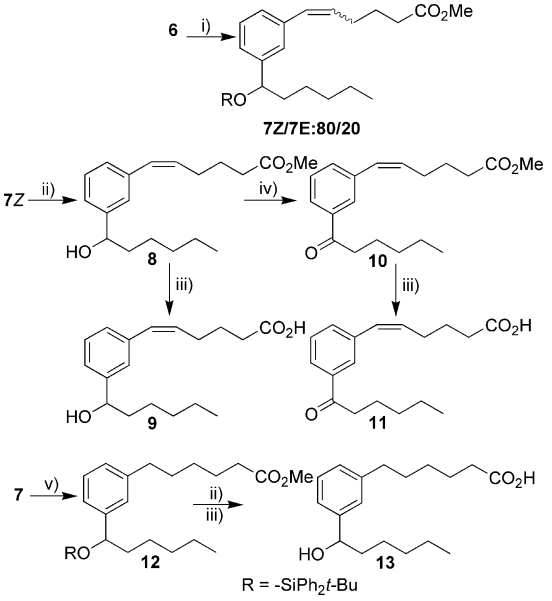
and to design derivatives with better agonist/antagonist properties. Of special interest was the nature of the aromatic or heteroaromatic ring, and the presence, or not, of the double bond in the upper chain of these analogues. The purpose of this publication is to disclose versatile and efficient synthesis for such derivatives and to report preliminary data on their antiaggregant properties.[5]

Synthesis of key intermediate 6. The isophtalaldehyde **1** is the obvious precursor for all the analogues with the aromatic ring in central position. The selective monodeprotection of its bisacetal **2** proved to be the best way for the preparation of the key aldehyde **3** (82% overall yield from **1** on a 40 mmol scale). Reaction with the pentyl Grignard reagent followed by protection of the secondary alcohol and deprotection of the aldehyde led to the key intermediate **6** (50% overall yield from **1**) (Scheme 1).

Synthesis of analogues with the acid function in position 1. Starting from **6**, a classical Wittig reaction led to a



Scheme 1. (i) $HC(OMe)_3$ (4 equiv), NH_4NO_3 (cat.), MeOH (reflux, 2 h), 93%; (ii) SiO_2, 1% H_2SO_4, CH_2Cl_2 (rt, 45 min), 88%; (iii) *n*-$C_5H_{11}MgBr$ (1.7 equiv), THF (−50 °C to rt), 78%; (iv) imidazole (2.5 equiv), *t*-$BuPh_2SiCl$ (1.2 equiv), DMF (rt, 24 h), 82%; (v) SiO_2, 2.5% H_2SO_4, CH_2Cl_2 (rt, 1.5 h), 96%.



Scheme 2. (i) $Ph_3P^+(CH_2)_4CO_2H\ Br^-$ (1.5 equiv), LiHMDS (3 equiv), HMPA/THF (−80 °C, 1.5 h) then Na_2CO_3, Me_2SO_4 (rt, 12 h), 74%; (ii) *n*-$Bu_4N^+F^-$ (1.3 equiv), THF (0 °C to rt, 14 h), 80%; (iii) LiOH, THF/H_2O (rt, 15 h), then acetic acid, 86%; (iv) Swern oxidation, 93%; (v) H_2−Pd/C, AcOEt, 98%.

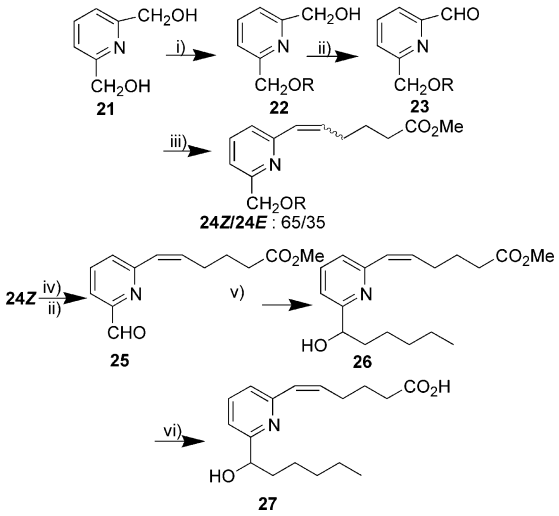
(8:2) mixture of the two styrene type derivatives **7*Z*** ($J = 11.6$ Hz) and **7*E*** ($J = 16$ Hz) separated by chromatography.[6] The first target molecule **9** was obtained in two steps from **7*Z*** by deprotection and saponification (8 steps, 25% overall yield from **1**).The saturated analogue **13** was prepared similarly from a mixture of **7*Z*** and **7*E*** after hydrogenation of the double bond (67% overall yield from **7**). In order to understand the possible role of the secondary alcohol function, it seemed of interest to prepare ketone **11**. This was best obtained by oxidation of **8** followed by saponification (80% overall yield)[7] (Scheme 2).

Synthesis of pyridine type analogues. In these series, the commercially available pyridine 2,6 diol **21** appeared as the most appropriate starting material. A selective monosilylation to **22** could be performed by using the conditions reported for monoprotection of linear diols.[8] Due to the presence of the pyridine nucleus, oxidation of **22** was not straightforward. Although the use of BBCP[9] gave aldehyde **23** in 50–68% yield, the Swern method proved to the best in this case and the key intermediate **23** was obtained in 90–97% yield. Then the same Wittig reaction as before led to the (65:35) mixture of olefins **24*Z*** and **24*E***, separated by chromatography.[10]

Table 1. Biological activities of compounds

Inhibition of platelet aggregation (IC_{50}, μM)[a]		
Compd	U 46619	Collagen
9	0.9	20
11	0.5	7
13	0.5	27
27	1.6	57
Bay u 3405	NT	0.05
12 (R) HETE	4	NT

NT: not tested.
[a]$n = 2$–5 in all experiments.



Scheme 3. (i) NaH (1 equiv), THF (50 °C, 6 h) then *t*-$BuPh_2SiCl$ (1 equiv), (rt, 2 h), 61%; (ii) Swern oxidation, 91%; (iii) $Ph_3P^+(CH_2)_4CO_2H\ Br^-$ (1.5 equiv), LiHMDS (3 equiv), HMPA/THF (−80 °C, 3 h) then Na_2CO_3, Me_2SO_4 (rt, 12 h), 86%; (iv) *n*-$Bu_4N^+F^-$, THF (0 °C to rt, 14 h), 86%; (v) *n*-$C_5H_{11}MgBr$ (1.5 equiv), THF (−50 °C, 1 h and then to rt), 46%; (vi) LiOH, THF/H_2O (rt, 15 h), then acetic acid, 87%.

Starting from the *cis* isomer **24*Z*** ($J = 11.8$ Hz), the deprotection of the alcohol followed by a second Swern oxidation gave the required aldehyde **25**. The addition of the pentyl Grignard reagent followed by a saponification led to the target molecule **27** (6 steps, 16% overall yield from **21**).

Biological tests. In order to improve the solubility of these acids in water, we have prepared the corresponding sodium salts[11] and used the latter derivatives for the biological tests. Preliminary data on the in vitro biological activity of representative examples are summarized in Table 1. The anti-platelet activity of the compounds was measured by studying their antagonist effects on human and rabbit washed platelets (WP) respectively aggregated with U 46619 (0.2 μM), a stable mimetic analogue of thromboxane A_2 or with collagen (2 μg/mL), a physiological agonist that causes endogenous thromboxane A_2 synthesis. The IC_{50} values are expressed in μM (Scheme 3).

All compounds described in Table 1 are antagonists of thromboxane (TP) receptors. The selective TP-receptor antagonist Bay u 3405, inhibits collagen-induced aggregation of human platelets illustrating that TP-receptors are implicated in this response. The arachidonate metabolite, 12(*R*) HETE, was studied against U-46619-induced aggregation of rabbit platelets and shown to be active. The most active compounds **9, 11** and **13** are potent TP-receptor antagonists in U-46619-induced platelet aggregation but the ketone derivative (**11**) is more active in collagen-induced platelet aggregation showing the crucial role of the secondary alcohol function. The pyridine type analogue (**27**) is less potent in both types of aggregation.

In conclusion, we have reported short and versatile sequences towards stabilized analogues of polyunsaturated fatty acid metabolites. Some of these derivatives are good inhibitors of platelet aggregation.

References and Notes

1. See for instance: Babubri, F.; Fiandanese, V.; Marchese, G.; Punzi, A. *Tetrahedron* **2000**, *56*, 327. Prakesch, M.; Gree, D.; Gree, R. *J. Org. Chem.* **2001**, *66*, 3146 and references cited therein.
2. See Han, X.; Corey, E. *J. Org. Lett.* **2000**, *16*, 2543 and references cited therein.
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4. For a recent example (L-6310333) see: Johnson, T. E.; Holloway, M. K.; Vogel, R.; Rutledge, S. J.; Perkins, J. P.; Rodan, G. A.; Schmidt, A. *J. Steroid Biochem. Mol. Biol.* **1997**, *63*, 1.
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6. These isomers were separated by SiO_2 chromatography using as eluent a 5:95 mixture of ether and low boiling (< 60 °C) petroleum ether. TLC **7*Z***, $R_f = 0.47$; **7*E***, $R_f = 0.42$, after three elutions.
7. Starting from **7*Z***, it was possible to replace the acidic function in position 1 by various functional groups. The synthesis of such analogues will be described in the full paper.
8. McDougal, P. G.; Rico, J. C.; Rico, J. G.; Oh, Y. I.; Condon, B. D. *J. Org. Chem.* **1986**, *51*, 3388.
9. Firouzabadi, H.; Saradarian, A. R.; Naderi, M.; Vessal, B. *Tetrahedron* **1984**, *40*, 5001.
10. These isomers were separated by SiO_2 chromatography using as eluent a 20:80 mixture of ether and low boiling (< 60 °C) petroleum ether. TLC **24*Z***, $R_f = 0.26$; **24*E***, $R_f = 0.21$.
11. All new compounds have spectral and analytical data in agreement with the indicated structures. We thank Drs. J. P. Volland and M. Amm (IdRS) for the microanalyses. Spectral and analytical data for key intermediate **6** as well as for the final products submitted to biological tests, as their sodium salts: **6**; IR (NaCl, film, ν cm^{-1}): 1701 (C=O); 1605 (C=C arom.); 1107 (Ph-Si). ^1H NMR (90 MHz, $CDCl_3$, δ): 9.92 (s, 1H, CHO); 7.81–7.04 (m, 14H, arom.); 4.72 (t, 1H, CHOSi, $J = 6.1$); 1.8–1.5 (m,2H, CH_2CHOSi); 1.14–0.99 (m, 6H, $CH_2(CH_2)_3CH_3$; 1.05 s, 9H, $C(CH_3)_3$); 0.82 (t, 3H, CH_2CH_3). ^{13}C NMR (22.5 MHz, $CDCl_3$, δ): 191.6 (CHO); 145.9; 136.2; 135.7; 133.9; 133.4; 132.1; 129.6; 129.4; 128.5; 128 ; 127.6; 127.5; 127.3 (arom.); 75.3 (CHOSi); 39.8 (CH_2CHOSi); 31.5 ($CH_2CH_2CH_3$); 27 ($C(CH_3)_3$); 24.2 ($CH_2(CH_2)_2CH_3$); 22.3 (CH_2CH_3); 19.2 ($C(CH_3)_3$); 13.8 (CH_2CH_3); anal. calcd for $C_{29}H_{36}O_2Si$: C 78.33, H 8.16 ; found: C 77.82, H 8.11. **9** ; white powder (mp 140 °C); IR (NaCl, nujol, ν cm^{-1}): 3310 (OH); 1560 (C=O). ^1H NMR (90 MHz, D_2O, δ): 7.29–7.08 (m, 4H, arom.); 6.40 (d, 1H, ArCH=CH ; $J = 11.6$); 5.70 (dt, 1H, ArCH=CH; $J = 11.3$, 6.3); 4.57 (t, 1H, CHOH, $J = 6.6$); 2.41–2.02 (m, 4H, $CH=CHCH_2$; CH_2CO_2Na); 1.85–1.45 (m, 4H, CH_2CHOH; $CH_2CH_2CO_2Na$); 1.28–0.85 (m, 6H, $CH_2(CH_2)_3CH_3$); 0.69 (t, 3H, CH_2CH_3, $J = 6$). ^{13}C NMR (22.5 MHz, D_2O, δ) 182.3 (CO_2Na); 145.2; 138.3; 133.5; 129.8; 129.0; 128.4; 127.3; 125.2 (4 C arom. and C=C); 74.8 (CHOH); 39.1 (CH_2CHOH); 37.2 (CH_2CO_2Na); 32.2 ($CH_2CH_2CH_3$); 29.0 ($CH=CHCH_2$); 26.8; 25.8 ($CH_2CH_2CO_2Na$; $CH_2(CH_2)_2CH_3$); 23.0 (CH_2CH_3); 14.4 (CH_2CH_3); anal. calcd for $C_{18}H_{25}O_3Na$: C 69.21, H 8.07; found: C 69.40, H 8.03. **11**; white powder (mp 174 °C); IR (NaCl, nujol, ν cm^{-1}): 1675 (ketone); 1550 (C=O carboxylate). ^1H NMR (90 MHz, D_2O, δ): 7.67–7.45 (m, 2H, arom.); 7.41–7.14 (m, 2H, arom.); 6.22 (d, 1H, ArCH = CH, $J = 11.7$); 5.65 (dt, ArCH=CH, $J = 11.5$, 7.5); 2.72 (t, 2H, $ArCOCH_2$, $J = 7.5$); 2.39–2.00 (m, $CH=CHCH_2$; CH_2CO_2Na); 1.85–1.37 (m, 4H, $CH_2(CH_2)_2CH_3$; $CH_2CH_2CO_2Na$); 1.30–1.04 (m, 4H, $CH_2(CH_2)_2CH_3$); 0.79 (t, 3H, CH_2CH_3). ^{13}C NMR (22.5 MHz, D_2O, δ) 202.2 (C=O); 183.1 (CO_2Na); 138.7; 137.3; 134.9; 133.9; 129.2; 128.9; 128.6; 126.8 (C arom. and C=C); 39.1 ($ArCOCH_2$; 38.0 (CH_2CO_2Na); 32.1 ($CH_2CH_2CH_3$); 29.1 ($CH=CHCH_2$); 27.0 ($CH_2CH_2CO_2Na$; $CH_2(CH_2)_2CH_3$); 23.1 (CH_2CH_3); 14.4 (CH_2CH_3); anal. calcd for $C_{18}H_{23}O_3Na$: C 69.66, H 7.47; found: C 69.69, H 7.38. **13**; white powder (mp 165 °C); IR (NaCl, nujol, ν cm^{-1}): 3350 (OH); 1560 (C=O). ^1H NMR (90 MHz, D_2O, δ): 7.24–6.94 (m, 4H, arom.); 4.53 (t, 1H, CHOH, $J = 7.0$); 2.72–2.43 (m, 2H, $ArCH_2CH_2$); 2.32–2.06 (m, 2H, CH_2CO_2Na); 1.95–1.29 (m, 8H, $CH_2(CH_2)_3CH_2CO_2Na$; CH_2CHOH); 1.29–0.96 (m, 6H, $CH_2(CH_2)_3CH_3$); 0.80–0.50 (m, 3H, CH_2CH_3). ^{13}C NMR (22.5 MHz, D_2O, δ) 185.8 (CO_2Na); 146.5; 145.4; 130.6; 129.8; 128.5; 125.8 (C arom.); 76.6 (CHOH); 40.7; 40.1; 37.8 (CH_2CHOH; $CH_2(CH_2)_3CH_2CO_2Na$); 33.8; 33.3; 31.3 ($CH_2CH_2CH_3$; $ArCH_2CH_2CH_2$); 28.3; 27.5 ($CH_2CH_2CO_2Na$; $CH_2(CH_2)_2CH_3$)); 24.5 (CH_2CH_3); 15.9 (CH_2CH_3); anal. calcd for $C_{18}H_{27}O_3Na$: C 68.77, H 8.66; found: C 68.71, H 8.53. **27**; IR (NaCl, nujol, ν cm^{-1}): 3450 (OH); 1570 (C=O). ^1H NMR (90 MHz, D_2O, δ): 7.95–7.69 (m, 1H, arom.); 7.45–7.22 (m, 2H,

arom.); 6.54 (d, 1H, ArCH=CH, $J=11.0$); 6.00 (dt, 1H, ArCH=CH, $J=11.0$, 6.9); 4.75 (under DOH) (1H, CH_2CHOH); 2.44–2.13 (m, 4H, $CH_2CH_2CH_2CO_2Na$); 1.95–1.59 (m, 4H, $CH_2CH_2CO_2Na$; CH_2CHOH); 1.47–1.02 (m, 6H, $CH_2(CH_2)_3CH_3$); 0.80 (t, 3H, CH_2CH_3). ^{13}C NMR (22.5 MHz, D_2O, δ) 182.2 (CO_2Na); 163.2; 155.7; 138.4; 136.8; 128.8; 123.4; 119.9 (C arom. and C=C); 74.6 (CHOH); 38.1; 37.3 (CH_2CHOH; CH_2CO_2Na); 32.2 ($CH_2CH_2CH_3$); 29.2 ($CH=CHCH_2$); 26.5; 25.3 ($CH_2CH_2CO_2Na$; $CH_2(CH_2)_2CH_3$); 22.9 (CH_2CH_3); 14.4 (CH_2CH_3); anal. calcd for $C_{17}H_{24}NO_3Na$: C 65.16, H 7.72, N 4.47; found: C 65.55, H 7.82, N 4.72.