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ARACHIDONATE EPOXYGENASE: INHIBITORS AND METABOLITE ANALOGUES

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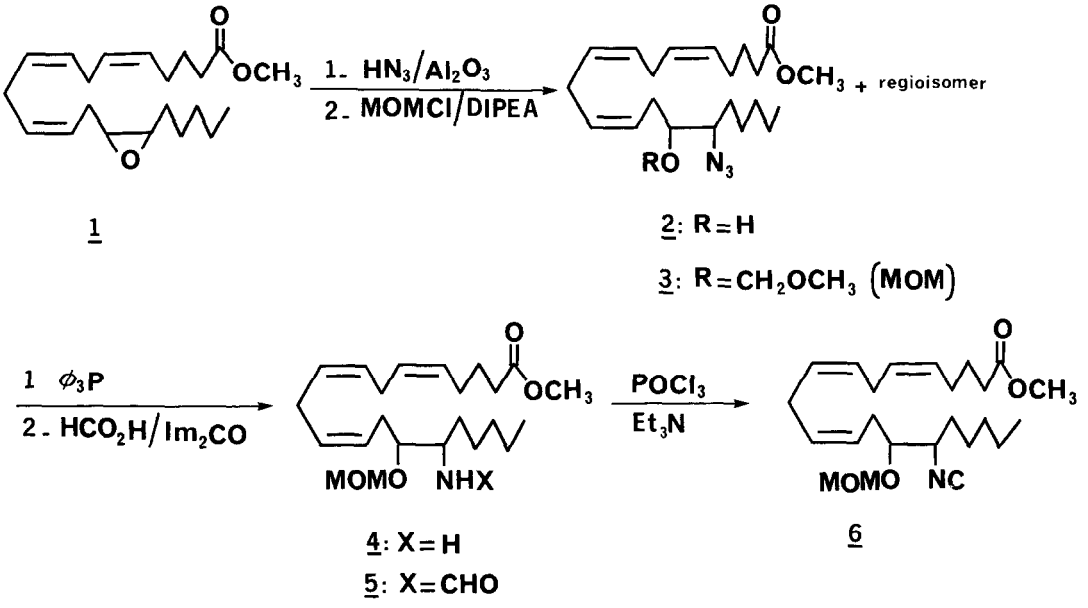
Summary: The preparation and initial in vitro evaluation of a series of potent arachidonate epoxygenase inhibitors and heteroatom analogues of epoxyeicosatrienoic acid are described.

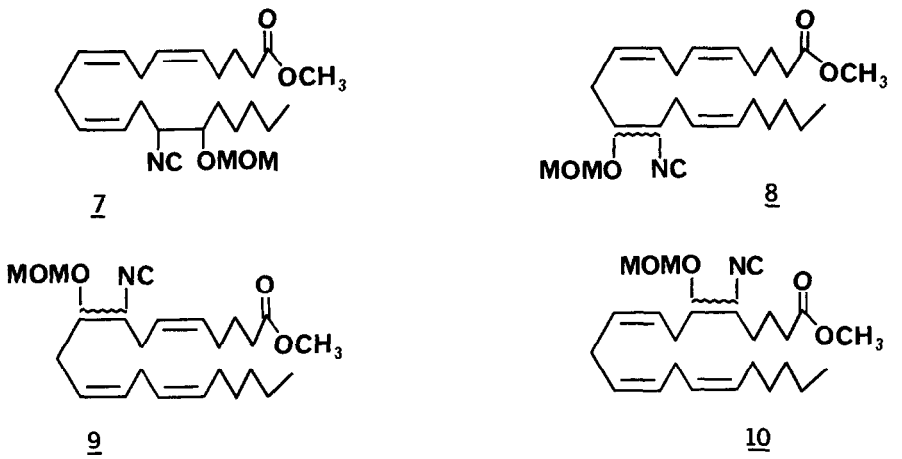
Recently, evidence has been presented for a novel mode of eicosanoid production mediated by cytochrome P-450 and requiring NADPH[1]. Designated the epoxygenase pathway, this route produces four regioisomeric epoxyeicosatrienoic acids (EETs). The EETs exhibit significant in vitro biological activity[2] and have been detected in mammalian tissue[3] and human urine[4]. In recognition of the need[5] to selectively intervene in epoxygenase metabolism and to unravel the physiological role of its metabolites, we report herein the preparation and in vitro evaluation of a series of potent epoxygenase inhibitors and EET heteroatom analogues[6].

Exposure of methyl 14,15-EET 1[7] to anhydrous hydrazoic acid (Et_2O/PhH 5:1, 10 min) in the presence of neutral alumina (Woelm 200) according to the general procedure of Posner and Rogers[8] furnished (Scheme I) a chromatographically separable mixture of azidohydrin 2[9,10] and its positional isomer (78%, 1.7:1 ratio). TLC: SiO_2, Et_2O/hexane 1:2, R_f ∿ 0.26 and 0.28, respectively. Treatment of 2 with diisopropylethylamine (5 equiv) and chloromethyl methyl ether (4.8 equiv) for 60 h in dry dichloromethane gave methoxymethyl (MOM) ether 3 (90%) after extractive isolation and chromatography (SiO_2, Et_2O/hexane 2:1, R_f ∿ 0.5). Selective azide reduction[11] by triphenylphosphine (2 equiv, THF, 24 h), hydrolysis of the intermediate iminophosphorane (THF/H_2O 7:1, 36 h), and purification (SiO_2, 10% MeOH/CH_2Cl_2, R_f ∿ 0.29) afforded amine 4 (86%) which was directly formylated[12] using carbonyldiimidazole (Im_2CO) and formic acid in THF for 1 h. The resultant formamide 5 (SiO_2:Et_2O, R_f ∿ 0.46) was dehydrated with $POCl_3$ (5 equiv) and Et_3N (20 equiv) in Et_2O/Petroleum ether (1:1) at 40°C for 1 h. Addition of the reaction mixture to ice cold 5% aqueous $NaHCO_3$, extractive isolation, and chromatography (SiO_2, Et_2O/hexane 1:1, R_f ∿ 0.45) provided isonitrile 6 in 85% yield from 4.

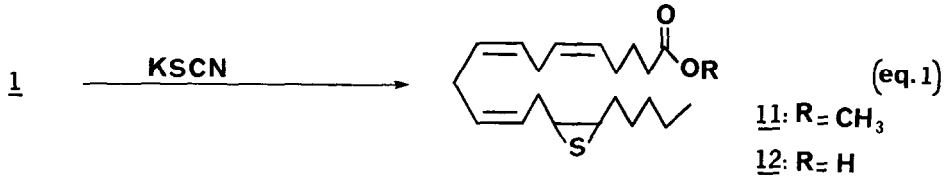


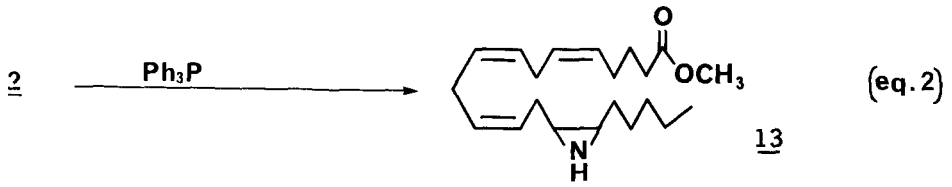
Scheme I

Repetition of the above sequence using the positional isomer of azidohydrin 2 afforded 7 in 67% overall yield. Isonitriles 8-10 were prepared analogously from the corresponding methyl EETs[7]. In the case of the latter compounds, however, no attempt was made to separate regioisomers. Prior to testing, 6-10 were saponified (NaOH, 10 h) in THF/H_2O (3:1) and the sodium salts isolated using BioRad SM-2 resin[13].



Physico-chemical considerations led to the working hypothesis that isonitriles 6-10 could function as powerful site specific inactivators of epoxygenase activity by coordination to the heme iron of cytochrome P-450[14]. Indeed, analysis of the isonitrile bound cytochrome P-450 difference spectrum of 6-10 revealed high binding affinity. Over a range of 0.1-100 μM 6-10, the spectral dissociation constant utilizing 3 μM hemoprotein was ∿ 7 μM. The metabolism of 100 μM arachidonic acid by liver microsomal fractions from phenobarbital induced rats was inhibited 75% by 6-10 at 5 μM. Significantly, arachidonate metabolism by PG synthetase purified from ram seminal vesicles, soybean lipoxygenase and by sonicated human platelets was unaffected by 6-10 at 5 μM; 100 μM 6-10 blocked metabolism in these systems by no more than 20%.





It was also deemed desirable to examine EET heteroatom analogues for epoxygenase inhibition. Treatment of 1 with excess potassium thiocyanate[16] in MeOH (65°C, 36 h) under argon (eq. 1), acidification (pH 4), and extractive isolation evolved cis-episulfides 11 (55%) and 12 (27%) (SiO_2, Et_2O/Hexane 1:2, R_f ∿ 0.55 and 0.24, respectively). When 2 was allowed to interact with triphenylphosphine (1 equiv, 18 h) in dry benzene (eq. 2), cis-aziridine 13[6b] was obtained (SiO_2, CH_3CN, R_f ∿ 0.14). Testing of the respective sodium salts showed 13 was comparable to the isonitriles in inhibiting arachidonate metabolism by liver microsomal fractions. In contrast, episulfide 12 was an order of magnitude less potent. Studies are in progress to assess the EET agonist/antagonist activity of EET heteroatom analogues.

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9. Satisfactory spectral data were obtained for all new compounds using chromatographically homogeneous samples.
10. Physical data. 2: NMR ($CDCl_3$, 90 MHz) δ 0.88 (3H,t), 1.08-2.52 (17H,m), 2.56-2.96 (4H,m), 3.04-3.60 (2H,m), 3.64 (3H,s), 5.12-5.64 (6H,m); IR ($CHCl_3$): 3580, 2100, 1730 cm^{-1}. 3: NMR (δ) 0.88 (3H,t), 1.08-2.48 (16H,m), 2.64-2.92 (4H,m), 3.08-3.64 (2H,m), 3.36 (3H,s), 3.64 (3H,s), 4.52-4.80 (2H,m), 5.12-5.52 (6H,m); IR ($CHCl_3$): 2120, 1735 cm^{-1}. 4: NMR (δ) 0.88 (3H,t), 1.08-2.44 (18H,m), 2.52-2.92 (5H,m), 3.20-3.48 (1H,m), 3.36 (3H,s), 3.64 (3H,s), 4.52-4.76 (2H,m), 5.08-5.52 (6H,m). 5: NMR (δ) 0.88 (3H,t), 1.08-2.44 (16H,m), 2.52-2.88 (4H,m), 3.36 (3H,s), 3.64 (3H,s), 3.88-4.20 (1H,m), 4.48-4.76 (2H,m), 5.12-5.50 (6H,m), 8.12-8.24 (1H,m); IR ($CHCl_3$) 1730, 1690 cm^{-1}. 6: NMR (δ) 0.88 (3H,t), 1.08-2.52 (16H,m), 2.64-2.96 (4H,m), 3.36 (3H,s), 3.64 (3H,s), 3.40-3.72 (2H,m), 4.52-4.80 (2H,m), 5.08-5.64 (6H,m); IR ($CHCl_3$) 2150, 1735 cm^{-1}. 11: NMR (δ) 0.88 (3H,t), 1.26-1.84 (10H,m), 2.06 (3H,t), 2.24-2.53 (4H,m), 2.70-3.00 (6H,m), 3.64 (3H,s), 5.27-5.94 (6H,m); PCI mass spec m/e: 351, 319, 287, 269. 13: NMR (δ) 0.88 (3H,t), 1.12-2.40 (19H,m), 2.60-2.92 (4H,m), 3.64 (3H,s), 5.12-5.52 (6H,m); mass spec m/e: 332, 318, 302, 262.
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